TWO ASPECTS OF THE BIOLOGY OF AN AFRICAN HONEYBEE, *APIS MELLIFERA SCUTELLATA* (HYMENOPTERA, APIDAE): LAYING WORKERS, AND COLONY DEFENCE BEHAVIOUR

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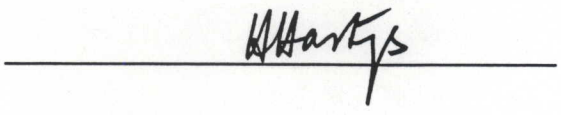
This thesis is my own work except where indicated to the contrary in the text. The thesis has not been submitted for a degree to any other University.

H. HASTINGS 

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ABSTRACT

In queenright, normal colonies of *Apis mellifera scutellata* Lepeletier the ovaries of all workers sampled were undeveloped. When such colonies were abruptly deprived of queen and brood, worker oviposition commenced after 5-6 days, a much faster rate than in European colonies. After 2-4 weeks of queenlessness slight development had occurred in the ovaries of *ca.* 25% of workers and very few (< 4%) had ovaries at the egg-laying stage. Workers of all ages underwent ovarian development, as in European honeybees. But, contrary to general opinion that age does not influence worker ovarian development, the proportions of workers that underwent such development in marked age groups were highest in those that were 3-6 days old at dequeening. It is therefore proposed that worker ovarian development be viewed as an age-based polyethism similar, e.g., to wax gland development. However, egg laying itself should be viewed as a "specialized polyethism", since it occurs in few workers at any one time.

Although worker eggs were laid many to a cell and were positioned haphazardly in both worker and drone cells, superfluous eggs were removed by nurse bees so that larvae were reared singly in their cells, resulting in many drones, both normal and undersized, in the hopelessly queenless colony. A distinct pattern of brood rearing emerged as an abundant initial oviposition, production of a large batch of drones, and then low-level brood rearing over 69-98 days, so that some of the queenless workers survived for 3-4 times the life span of queenright *A. m. scutellata* workers.

A profile of worker laying and brood rearing in the hopelessly queenless *A. m. scutellata* colony was drawn: (i) worker ovaries totally

undeveloped when queenright; (ii) rapid inception of laying after queen and brood loss; (iii) few workers lay eggs; (iv) large batch of drones reared; (v) extended survival of colony. Other races, notably *A. m. capensis* Escholtz and European bees, have different profiles.

The efficient production of many males by hopelessly queenless *A. m. scutellata* suggests a strong selection pressure for this trait, the function of which is proposed to be founded in propagation of the colony's genes. Implications of this concept for ecological and genetic-evolutionary studies are discussed.

In Part 2, colony defence behaviour (CDB) against humans is compared with other kinds of CDB and it is shown that the behaviour should be approached as a social phenomenon emerging at a level higher than the simple aggregate of individual behaviours. Factors contributing to variability in CDB against humans are reviewed and used in a critique of the published methods for quantifying the behaviour: many such methods appear unnecessarily confounded. A set of criteria for the design of methods to quantify CDB is drawn and it is proposed that different response-aspects (i.e. behavioural variables) of a measurement procedure should (i) be well correlated in repeated measurements on the same colony and on different colonies measured simultaneously at each repetition; (ii) consistently rank responsiveness of colonies that differ in CDB levels; and (iii) be well correlated with variation of relevant weather factors. A measurement method was designed in which nine behavioural variables were recorded in response to the presence of the observer at the hive and to human breath blown into the hive entrance. The measurements entailed the initial reaction to the breath stimulus, and the numbers of workers that guarded the hive entrance or flew about the observer and stung him, as he arrived at the hive and at various stages during the three minutes

following the administration of the breath stimulus. Recordings were made on a set of five colonies on 59 out of 62 consecutive days. Cluster analysis of correlation matrices between the behavioural variables within and between colonies, and between behavioural and weather variables, indicated three out of the nine behavioural variables (initial reaction to stimulus, and numbers that flew about the observer after the stimulus) as likely to be superior CDB measurements according to criteria (i) and (iii) above. The other six measurements, including changes in numbers of guards and some counts of workers flying about, and stinging, the observer, were indicated as likely to be poor measurements of CDB. Temperature was the only weather variable with a strong effect on CDB intensity. Criterion (ii) did not discriminate effectively between the variables although it might do better in colony sets with preselected CDB levels.

Responses to a "moving-lure bioassay" (commonly used in CDB analyses) were poorly correlated with ambient temperature and with the three good "breath-test" measurements mentioned above. Aspects of the lure bioassay were also shown by *a priori* argument to be flawed.

It is proposed that the methods of analysis developed in the study could be used to test and improve designs of other CDB measurement methods reported in the literature.

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CHAPTER 1

GENERAL INTRODUCTION

Although the honeybee (*Apis mellifera* Linnaeus) has been studied more extensively than any other insect and is among the best known of all animals (Michener, 1974, 1982b; Crane, 1980), the many thousands of scientific publications on it (Wheeler, 1923; Maa, 1953; Wilson, 1971) have been concentrated on European races, notably *A. m. ligustica* Spinola, *A. m. carnica* Pollmann and *A. m. mellifera* Linnaeus, the main commercial honeybees of Europe, Australia and North America (Ruttner 1975; 1988). Relatively little is known about the honeybee of the African savannahs, *A. m. scutellata* Lepeletier, as is reflected in Fletcher's (1978) review of scientific knowledge about the race, which cites about 40 serious investigations; and in Crane's (1978) bibliographies, which list several additional scientific reports, but which contain mainly anecdotal or practical beekeeping articles in their *ca.* 900 entries. Few studies have been published since these reviews (see e.g. recent surveys of Seeley, 1985; Winston, 1987; Fletcher, 1988).

A case in point is the biology of laying workers and the factors that control their ovarian development. While the topic has been extensively studied in European bees (reviewed in chapter 2), it was largely neglected in *A. m. scutellata* before Ruttner and Hesse (1981), Jackson (1982) and recently Allsopp (1988): no studies are reported in Fletcher's (1978) review, and only one (Jay, 1975) is listed by Crane (1978). The initial impetus for the laying worker studies in Part 1 of the present thesis was an observation by D.J.C. Fletcher (pers. comm.) that in newly queenless *A. m. scutellata* colonies workers may begin laying within a few days, very much earlier than European workers in similar circumstances.

Investigation of whether rapid ovarian development is a constant characteristic in *A. m. scutellata* revealed phenomena in the queenless colony which had not been properly studied in any honeybee race, and the present study was broadened to incorporate these.

While African honeybees have received relatively little attention from biologists in Africa, a population descended from them has been the subject of much interest in South America following the release of 26 *A. m. scutellata* swarms at Sao Paulo (Brazil) in 1956 and their subsequent spectacular colonization of that continent (reviewed by Michener, 1975; Needham *et al.*, 1988). As far as the South American public has been concerned, the most notable characteristic of these bees has been their propensity to attack humans and livestock *en masse* with little provocation. This, and the fact that these bees are likely to invade the southern USA in the 1990's (Taylor, 1977, 1984, 1985; Rinderer, 1986a,b; Needham *et al.*, 1988), has elicited substantial research in South America, one aim of which was to make objective measurements of defence responses of colonies, often with a view to breeding docile strains (e.g. Goncalves and Stort, 1978; Rinderer, 1982; reviewed in sections 8.4.5 and 8.5). No similar investigation on colony defence behaviour (CDB) in *A. m. scutellata* in Africa had been undertaken prior to the present study, even though the variable, often vigorous, defensive behaviour of this race has always been one of its most noted characteristics (Smith, 1953, 1966; Kerr, 1957; Fletcher, 1978; Rinderer, 1982; Anderson *et al.*, 1983). As a first step, the various methods that have been used to measure CDB were assessed and some were performed as pilot observations. Inadequacies in these methods quickly became apparent and the emphasis of the study shifted to a search for an ethologically sound method of measuring defence responses against the human intruder in whole colonies (Part 2).

Until recently the name *A. m. adansonii* Latreille 1804 was applied

to the honeybees of most of sub-Saharan Africa. It is now restricted to the bees of coastal West Africa whence the type-sample for *adansonii* was taken: the name *A. m. scutellata* Lepeletier 1836 has replaced *adansonii* for the honeybee of the savannahs of central, eastern and southern Africa (Ruttner 1975, 1982, 1988; Ruttner and Kauhausen, 1985). *Scutellata* has recently gained wide acceptance (e.g. by Winston, 1979b, 1987; West-Eberhard, 1981; Collins *et al.*, 1982, 1988; Michener, 1982a; Otis, 1982; Taylor and Spivak, 1984; Rinderer *et al.*, 1985; Seeley, 1985; Cornuet, 1986; Rinderer, 1986a,b; Whiffler *et al.*, 1988) and was adopted for the honeybees of the present study, which were from the Pietermaritzburg area of Natal, South Africa.

PART 1

WORKER OVARY DEVELOPMENT, OVIPOSITION, AND REARING OF WORKER BROOD IN QUEENLESS COLONIES OF *APIS MELLIFERA SCUTELLATA*

CHAPTER 2

INTRODUCTION TO PART 1

The life cycle of European races of *Apis mellifera* entails two long, "stationary" phases: hibernation during winter and a normal phase, from spring to autumn, in which foraging is possible. The normal phase is briefly interrupted by three queen rearing phases: colony reproduction by fission (swarming); supersedure of ageing or otherwise failing queens; and emergency replacement of healthy queens lost in accidents (Butler, 1974; Seeley, 1985). *A. mellifera* of sub-Saharan Africa do not hibernate but forage perennially and often migrate to resource-rich areas (Fletcher, 1978; Seeley, 1985).

In queenright European colonies, workers' ovaries are usually undeveloped, although there are various circumstances (reviewed below) in which their ovaries may develop partially. If the colony loses its queen at a stage when it has no immature females with which to raise a new queen, the colony is then *hopelessly queenless* and is usually doomed to dwindle and die (Millen, 1942; Butler, 1974; Seeley, 1985; Winston, 1987; *see* chapter 6). During hopeless queenlessness some workers may lay male (rarely, female) eggs which may be reared to adulthood. This form of ovarian development in the worker, the subject of the present study, is the result of parthenogenetic oogenesis (Soumalainen, 1950; White, 1973, p.682; Crozier, 1975), and is distinct from "ovary development" in the embryo (Velthius, 1970; as used e.g. by Haydak, 1943; Lensky *et al.*, 1978) and from "ovary development" as represented by the number of ovarioles (as used e.g. by Haydak, 1943; Levin and Haydak, 1951; Weaver, 1956).

The progression of ovarian development in the worker may be seen under X20 binocular magnification as swellings in the ovarioles, caused by

growth of the eggs within. Undeveloped ovaries bifurcate from the common oviduct as slender, undifferentiated tubules. Slight swelling and compartmentation of the ovarioles signifies early activation whereafter the ovarioles thicken and reveal the shapes of the eggs within them, which are at first spherical and then enlarge and elongate through an oval "bean shape" to the "sausage shape" of the mature egg (illustrated in Leuenberger, 1927; Maurizio, 1954; Sakagami, 1954; Snodgrass, 1956; Pain, 1968b; Velthius, 1970; Dade, 1977; Winston, 1987). Systems of classifying this process were devised by e.g. Perepelova (1926), Hess (1942), Altmann (1950), Butler (1956), Sakagami and Akahira (1958), Pain (1961a), Jay (1970) and Jackson (1982): all entail the delimitation of two to six "stages" of development, according to the appearance of the ovaries. But, regardless of the system employed, authors invariably speak of just three stages, namely undeveloped, partially developed and fully developed. In the literature various terms have been used to refer to these stages, some of which are confusing. The term *undeveloped* is to be preferred to "resting" or "inactive" as used by Velthius (1970) since, in ordinary dissection, ovaries can be seen to be undeveloped, whereas the state of their activity cannot be determined. Workers with *partially* developed ovaries were termed "potential laying workers" by Butler (1974, p.57) and "anatomical laying workers" by Perepelova (1926), to distinguish them from workers that actually lay eggs, which were termed "functional laying workers" by Butler and "physiological laying workers" by Perepelova. These terms are unnecessarily elaborate and it is confusing to call workers with partially developed ovaries "laying workers", particularly since authors often drop the qualifying adjectives "anatomical", "potential", etc. and so render their meaning obscure. Further, there are no biologically based reasons for designating a worker with partially developed ovaries a "potential laying worker" since any worker, including one with undeveloped

ovaries, may become a laying worker as far as is known (section 3.1); also, not all workers with partially developed ovaries in a queenless group do become layers and so not all of them are "potential laying workers". The term *laying worker* is in fact adequate in itself, meaning workers that lay eggs, and there is no need to qualify it with adjectives such as "functional" or "physiological". From this point of view the adjective "anatomical" as opposed to "physiological" (Perepelova, 1926; Costa Leonardo, 1985) is essentially meaningless as a qualifier of "laying worker". So too is Anderson's (1963, 1977a, 1981) use of the term "anatomical laying worker", which he extended to embrace workers with mature eggs as well as those with "medium" ovarian development. Ribbands' (1953) term "ovary-developed worker", erected in place of "anatomical laying worker", can be confusing because it may be taken to refer not only to workers with partially developed ovaries but also to laying workers, which are also "ovary-developed workers". In the present study a simple, unambiguous nomenclature was devised for stages of worker ovarian development (section 3.2.5; Fig. 3-4, p.29).

In the normal phase of the honeybee colony, production of queen pheromones* by the queen is at its highest (Butler, 1954, 1960b, 1970) and these pheromones then inhibit queen rearing and worker ovarian development (Butler, 1954; 1959b; Pain, 1961a; Butler and Fairey, 1963; Velthuis, 1977). Worker ovarian development is also inhibited by the presence of brood in the hive (discussed below). In the normal phase the queen

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* A complex array of substances in the mandibular, tergal and Arnhard glands (reviewed by Velthuis, 1985). An important component is *E*-9-oxo-2-decenoic acid (9ODA) which inhibits worker ovarian development (Butler *et al.*, 1961; Boch *et al.*, 1979; Free, 1987).

constantly lays female eggs which are raised as workers and are maintained in the colony as neuters. The only directly reproductive activities of the normal-phase colony are occasional matings by its drones, which are raised seasonally (Butler, 1974; Seeley, 1985).

When in the normal colony the supply of queen pheromones to the workers diminishes, as it does at the onset of swarming or supersedure (Butler, 1954, 1957b, 1960b; Simpson, 1959, 1974; Lensky and Slabezki, 1981; Seeley and Fell, 1981; Winston, 1987, pp. 139,194), or is cut off, as in accidental queen loss, the colony goes into a reproductive phase and the workers build and provision queen cells. Simultaneously, ovaries of some of the workers begin to develop (Butler, 1970, 1974). However, in European honeybees workers usually do not lay during queen production, even when the colony is queenless, such as during the eight days between the departure of the prime swarm and the emergence of the swarm-replacement queens (Millen, 1942; Butler, 1974; Simpson, 1974), or during the entire queen rearing process in emergency replacement. Some reasons for this are known. Brood, both sealed and unsealed, has on its own a strong inhibitory effect on worker ovarian development (Perepelova, 1928a; Millen, 1942; Mussbichler, 1952; Jay, 1970, 1972; Kropacova and Haslbachova, 1970, 1971; Jay and Nelson, 1973; Jay and Jay, 1976; Winston and Taylor, 1980; Kubisova *et al.*, 1982; Hepburn *et al.*, 1988). Queen rearing is invariably accompanied by a steady diminution of brood in the colony (Simpson, 1974; Seeley, 1985, p.45), but usually sufficient quantities last to inhibit laying by workers until the new queen hatches (Free, 1977, 1987; Seeley, 1985, pp.25, 37). If all the brood hatches out before the end of the queen rearing phase, partial ovarian development may occur in many workers in the presence of occupied queen cells (Jay, 1968, 1970), but development of laying workers may still be inhibited, first by the queen cells in the colony (Millen, 1942; Boch, 1979; Free and Ferguson, 1982; Hepburn *et al.*, 1988) and then

by the newly emerged virgin queen (Millen, 1942; Ribbands, 1953, p.279) who soon develops a supply of queen pheromones at a level of about one-fourth that of a mature, mated queen (Butler, 1960a; Butler and Paton, 1962). When the new queen has mated and commences laying, her pheromone production increases to maximum and the colony returns to normal (Butler and Paton, 1962; Butler, 1974).

A queen on a mating flight may be lost to her colony through misorientation, or she may succumb to predators; if, as often happens, her colony has no other queen, no occupied queen cell, and no young brood from which queen rearing can be restarted, hopeless queenlessness ensues - laying workers appear when the remaining brood in the colony diminishes below some critical level. Hopeless queenlessness may follow other kinds of accidents which render the colony queenless and broodless, e.g. when the queen is lost from a reproductive or migratory swarm, or when a predator attacks a hive and kills the queen and consumes the brood. The latter circumstance was simulated in the experiments of chapters 3, 4 and 5 by removing the brood and queen from the normal colony (discussed in chapter 6).

In some honeybee races workers may lay in circumstances that would inhibit European workers from doing so. Also, the time to onset of oviposition, after inhibitory factors are removed, varies according to race (*see* chapter 5).

Besides the removal of inhibitory factors, two other conditions are necessary for workers to develop their ovaries to their fullest potential. First, the workers must have an adequate supply of protein (pollen) in their diet otherwise little development will occur (Tuenin, 1929; Hess, 1942; Mussbichler, 1952; Maurizio, 1954; Pain, 1961b). Developed ovaries are resorbed when workers are starved (Ribbands, 1953; Pain, 1968b) or when the colony is requeened (Husing and Bauer, 1968). Second, the workers must

be in a group. The ovaries of well fed workers confined individually in separate cages do not develop, even after two to three weeks (Hess, 1942; Pain, 1961b), whereas they do develop slightly in workers kept in groups of only two or three (Pain, 1961b). The great variability that is commonly obtained between groups renders interpretation of experimental treatments of caged workers difficult (Voogd 1956): e.g. in identically treated groups of 50 workers, all of which emerged from a single comb over a short period, partial ovarian development ranged from 0-95% (Velthuis, 1970). Development of worker ovaries is in fact affected by the size of the queenless group (Allen, 1965; see also Wilson, 1971, p.298 and Moritz *et al.*, 1987b on "group effects"). It thus seems advisable to conduct experiments on normal-sized colonies wherever possible.

In the normal phase, queen pheromones also function in the maintenance of colony cohesion and order (Butler, 1973; Free, 1987) and influence the colony-maintaining activities of the workers (Free, 1969), e.g. attending the queen (Butler, 1973), nursing brood (Kuwabara, 1947), cell cleaning (Free and Williams, 1974), nest construction (Darchen, 1957, 1960; Chauvin *et al.*, 1961; Free, 1967), foraging (Showers, 1967; Jaycox, 1970; Youngs and Burgett, 1982) and hoarding (Free and Williams, 1972). These activities occur in an elaborate integrated division of labour which is manifested in the colony as polyethisms in groups of workers of similar age. The physiology of each worker, particularly glandular activities, changes according to the tasks it undertakes. The individual performs a variety of tasks appropriate to its age, and the proportions of workers of each age group that perform a particular task vary according to the needs of the colony (Ribbands, 1952; Lindauer, 1953; Free, 1965; Wilson, 1971; Michener, 1974; Seeley, 1982, 1985, p.31, 1986; Winston and Punnett, 1982; Nowogrodzki, 1984; Kolmes, 1985, 1986; Kolmes and Winston, 1988). In colonies in which queen rearing or worker laying is under way, most of the

routine day-to-day polyethisms that occur in the queenright colony are maintained and even the doomed, hopelessly queenless colony continues to function as a unit (Free, 1965; Gary, 1975). Aspects of this phenomenon were investigated in the present study (chapters 3 and 4).

Almost all the information about worker ovarian development in the above review is from experiments with European honeybees. Tropical Asian honeybee species differ from European honeybees in many respects (Butler, 1974; Seeley, 1985), including aspects of the biology of their laying workers (*see* chapter 5). *A. m. scutellata* is adapted to life in tropical and warm-temperate savannahs (Ruttner, 1986) and so differs from European races in many ways (Fletcher, 1978; Winston *et al.*, 1983; Seeley, 1985; Winston, 1987). This makes it possible, or likely, that laying workers of *A. m. scutellata* may differ in some respects from their European counterparts. The only African honeybee in which laying workers have been well studied is *A. m. capensis* Escholtz, which is unique among honeybee races in that its workers lay high proportions of female eggs, many of which are reared as workers which maintain the hopelessly queenless colony until a replacement queen is reared from a worker-laid female egg (Onions, 1912; Anderson, 1963; Ruttner, 1977c; Anderson *et al.*, 1983). *A. m. scutellata* is, in the southernmost extreme of its range, contiguous with *A. m. capensis*, so that information about *scutellata* laying workers will be useful in gaining perspective about the anomalous *capensis* laying worker system. This is discussed in chapters 5 and 6.

CHAPTER 3

OVARY DEVELOPMENT, FLIGHT ACTIVITY: RELATIONSHIP TO AGE IN QUEENLESS WORKER BEES

3.1 INTRODUCTION

The factors known to inhibit ovarian development in honeybee workers are reviewed in chapter 2. In the normal European honeybee colony the queen and her brood hold practically all workers' ovaries undeveloped with, at the most, only a small proportion of them slightly developed (Perepelova, 1926, 1928b; Hess, 1942; Groot and Voogd, 1954; Maurizio, 1954; Butler, 1957a; Verheijen-Voogd, 1959; Jay, 1968, 1970, 1972; Pain, 1968b; Jay and Jay, 1976; Kropacova and Haslbachova, 1969, 1970, 1971) (*see* section 3.2.5 for terminology of stages of ovarian development, e.g. "slightly developed"). However, proportions of ovarian development in the workers of a colony do not remain uniform when inhibitory factors are removed. No matter how a colony is treated, not all the workers will undergo ovarian development and, of those that do, not all will be laying workers at any one time (Perepelova, 1926, 1928a; Butler, 1956, 1957a, 1967; Sakagami, 1959). Most estimates of maximal proportions of workers with ovarian development (of all stages) in queenless or swarming colonies range from about 50% (Sakagami, 1954; Jay, 1968, 1970) through 60-80% (Perepelova, 1926; Ribbands, 1953, p.278; Butler, 1967, 1974; Kropacova and Haslbachova, 1969, 1970, 1971) to 87-90% (Hess, 1942; Kropacova and Haslbachova, 1970). Maximum proportions of laying workers were estimated as 22-25% in European bees (Perepelova, 1928a; Hess, 1942 *in* Anderson, 1963) and as 46% (mean 28%) in *A. m. capensis* (Anderson, 1963).

Explanations about which workers in a colony do or do not undergo

ovarian development are tentative. They fall into five main categories, discussed below.

(1) Interactions between workers

In honeybees, dominance-related aggressive interactions occur between queens and workers (Lensky *et al.*, 1970; Yadava, 1971; Yadava and Smith, 1971a,b; Weaver and Weaver, 1980). In newly queenless European colonies, laying workers and workers with highly developed ovaries were mauled by a faction of consistently aggressive workers which usually had undeveloped or slightly developed ovaries (Sakagami, 1954; Velthuis, 1976). However, this aggression did not prevent the submissive workers from laying (Velthuis, 1976). Queenless workers of two patrilines attacked half sisters more than full sisters - the effects of such attacks on egg laying by any individual was not determined (Evers and Seeley, 1986). In *A. m. capensis* older workers, with undeveloped ovaries, attack and often kill younger workers with developing ovaries. These attacks may be elicited by the small quantities of queen pheromones secreted by the victims (Crewe, 1984). In these cases the killings are, of course, effective in eliminating potential layers, although the functions of such interactions have not been determined. Thus, the effects of aggression on differential oogenesis among honeybee workers are as yet not understood.

In trophallaxis among small, queenless groups, workers that were habitually successful "askers" underwent ovarian development, while the "offerers" did not (Korst and Velthuis, 1982): trophallaxis may be a means by which various task differentiations (including ovary development) may be mediated. This possibility has yet to be examined in the full-sized colony.

Butler (1956, 1967, 1970) suggested that the workers whose ovaries

develop first might, by producing queen pheromones, partially inhibit those of others in the colony from doing so. This contention has never been rigorously tested in the normal-sized colony in the natural conditions under which workers develop ovaries, but three findings indicate that it is a possibility. First, ovarian development in groups of newly queenless workers is inhibited by the introduction of laying workers, both into small colonies (Jay and Nelson, 1973) and into cages of 50 workers (Velthuis *et al.*, 1965; Velthuis, 1970). However, the results of the cage experiments were equivocal: see Butler (1967) and Velthuis (1970) where ovarian development in control cages varied between 0-95%. Further, any brood laid by workers in such experiments will contribute to inhibition of worker oogenesis (Jay and Nelson, 1973) and so could partially confound the effects of the laying workers themselves.

Second, individuals in queenless groups of European bees in cages or small observation hives may attract retinues, as normally found around all fully functional queens (Sakagami, 1958; Milum, 1962; Velthuis *et al.*, 1965; Velthuis, 1970, 1976). These false queens often reduced ovarian development in their hivemates, either when they were introduced into groups (Velthuis, 1970), or when they developed whilst members of groups (Sakagami, 1958) - however, in both studies it was found that false queens were not always laying workers, and that some in fact had only partially developed ovaries (at stage 2 of Velthuis, 1970).

Third, some of the workers in queenless European colonies produce queen pheromones (Crewe and Velthuis, 1980; Saiovici, 1983), which explains how they may attract retinues and how they could inhibit ovary development in hivemates. In *A. m. capensis*, false queens in queenless colonies secrete relatively high amounts of queen substance (Ruttner *et al.*, 1976; Hemmling *et al.*, 1979; Crewe and Velthuis, 1980): Crewe (1981) suggested that they inhibit ovarian development in other members of their groups,

since ordinary workers from groups with false queens soon developed into false queens themselves when they were transferred to groups of either European or *scutellata* workers.

Thus, in queenless colonies the ovaries of some workers might be inhibited by queenlike pheromonal secretions by others. However, in most of the investigations cited above, workers that produced queen substance did not always have developed ovaries, egg layers did not always produce queen substance, and producers of queen substance did not always attract retinues. This lack of direct association between high ovarian development and production of queen pheromones, or elicitation of retinues, detracts from Butler's (1956, 1967, 1970) hypothesis that it is the workers that *first develop their ovaries* that produce inhibitory substances - the workers (non-laying and laying) that produce queen substance must do so in some system more elaborate than that envisaged by Butler and so may contribute to the differential ovarian development characteristic of honeybee workers, but probably are not the sole controlling factor. While worker interactions may mediate organization of the hopelessly queenless colony, explanation about which workers *first* undergo ovarian development in the newly queenless colony must be sought elsewhere, and may lie in one or more of the remaining four categories.

(2) Ontogenetic factors

Workers reared as larvae in a queenless colony underwent ovarian development more readily than workers reared similarly in a queenright colony (Williams and Free, 1975). Queenless worker larvae developed more rapidly, and developed more ovarioles, than queenright larvae (Kuwabara, 1947; Levin and Haydak, 1951; Weaver, 1956). Williams and Free (1975) suggested that these differences may arise through several factors

including quantity and/or quality of food, and lower levels of queen substances for larvae in queenless colonies. They noted Huber's (1814) hypothesis that worker larvae adjacent to queen cells may occasionally be fed queen-determining substances and so have a high propensity to develop ovaries when adult. These hypotheses may explain the advent of incipient laying workers in colonies during queen-rearing or queenless phases, but they cannot explain the differential propensities for ovarian development found in queenless workers that were reared in normal, queenright colonies.

The current consensus on queen - worker differentiation is that differential feeding of the zero to three day old female larva by nurse bees dictates whether it will develop into a queen or into a worker. The quality of the nutrient initiates the course of caste differentiation by instigating one of two hormonal responses in the larva, which initiate different metabolic and developmental process (Rembold and Ulrich, 1982; Beetsma, 1985; Rembold, 1985). Worker larvae older than three days grow into intercastes when given a queen larva diet, as do young female larvae fed with inferior royal jelly (Haydak, 1943; Weaver, 1955, 1957; Woyke, 1971; Brian, 1979). Although intercastes are very uncommon in nature (Jackson, 1982) the purely physiological basis of caste differentiation allows the possibility that subtle differences in nutrition of queenright worker larvae may produce workers with different propensities for ovarian development. Thus modern findings on caste differentiation do not negate the applicability of the hypothesis of Williams and Free (1975) to queenright larvae, although the question awaits rigorous analysis.

(3) Genetic factors

The possiblity that individual propensities for oogenesis among workers may have a genetic basis has not been investigated. Genetic factors

undoubtedly underlie racial variation in various aspects of worker oogenesis, including propensity to undergo ovarian development (section 5.1). Weaver (1956) and Chaud-Netto and Bueno (1979) suggested that variation in ovariole numbers among workers of a colony could have a genetic basis. If this were true it might obliquely suggest that individual propensities for oogenesis might be under some form of genetic influence, since Velthuis (1970) found that workers with an ovariole number near the mean for their colony have a greater chance of undergoing ovarian development than those with a higher number of ovarioles. However, the likelihood of such an association has recently been reduced by Allsopp (1988) who found no association between ovariole number and ovarian development in workers of *A. m. scutellata* and *A. m. capensis*.

(4) Association with polyethisms

A false queen may drop the activities that workers of her age normally exhibit (Sakagami, 1958) but ordinary laying workers, and workers with partially developed ovaries, undertake normal worker duties (Perepelova, 1926, 1928a; Leuenberger, 1927; Pain and Verge, 1950; Sakagami, 1959; Hoffmann, 1961). Nurse bees may develop their ovaries more readily than foragers (Maurizio, 1954; Sakagami, 1959; Engels, 1974), although this distinction is not always apparent (Hoffmann, 1961). Thus, the tendency for or against oogenesis in the worker is not, as far as is known, specifically associated with any other activity.

Amongst beekeepers there is a common conception that laying workers cannot or do not fly, so that they can be eliminated by hive displacement methods (e.g. Root, *et al.*, 1972; Taylor, 1974), even though Haydak (1940) has specifically stated that such methods are ineffective. Hoffmann (1961) and Mobus (1983) showed experimentally that laying workers may fly from the

hive and forage.

(5) Association with age

Practically every worker activity and glandular development is associated with age (chapter 2). However, many experiments have indicated no relationship between age and worker ovarian development either by showing that ovary development may occur in workers of all ages, from nurse bees to very old, overwintered individuals (Leuenberger, 1927; Perepelova, 1928a; Husing and Ulrich, 1938; Hess, 1942; Pain and Verge, 1950; Park, 1949; Ribbands, 1953, p.281; Sakagami, 1959; Verheijen-Voogd, 1959; Pain, 1968b; Kropacova and Haslbachova, 1970; Jay, 1972; Ruttner and Hesse, 1981), or by showing that ovarian development is always very variable amongst groups of workers of the same age (Hess, 1942; Butler, 1957a; Verheijen-Voogd, 1959; Allen, 1965). From such evidence Butler (1959a) concluded that age is not associated with the variation in oogenesis that always occurs amongst workers. In groups aged zero days at dequeening, Kropacova and Haslbachova (1969, 1971) and Jay (1972) found circumstances in which there was slightly more ovarian development in the groups when they attained four weeks of age than there was when the groups were two weeks old, although in most circumstances there were no differences between these two age groups. In mixtures of young and old workers (< 24 h and > 39 days old at dequeening), both age groups laid and produced equal numbers of drones per worker in three out of six experimental colonies: the young bees produced significantly more drones in the three other colonies (Delaplane and Harbo, 1987a). Jay (1968) and Jackson (1982) found that ovary development occurred more readily in bees that were nurses (i.e. up to three weeks old) when the colony was dequeened, than in workers that were older at dequeening: the important factor was the workers' age at dequeening rather

than their total age. This was also implied for *A. m. capensis* by Crewe (1984). Allsopp (1988) disagreed with Crewe's interpretation that it is the very young workers which develop their ovaries at queen loss because, in newly emerged workers placed in newly queenless colonies, highly developed ovaries appeared only after 15 days in an *A. m. capensis* colony, and after 14-20 days in an *A. m. scutellata* colony: laying workers usually appear after much shorter periods when normal colonies of these races lose their queens so that, in Allsopp's view, it must be older workers (~~7-10~~ about 15 days old) that first undergo ovarian development in the newly queenless colony.

None of the abovementioned studies directly investigated whether there is any relationship between age and ovarian development with the characteristics of a typical polyethism, i.e. whether oogenesis might occur in typical proportions in groups of workers of various ages. This possibility was examined in the present study.

3.2 METHODS AND MATERIALS

3.2.1 Experimental procedure

Colonies in single-chamber Langstroth hives were established at 10-20 m intervals in a row of trees in an orchard on the experimental farm of the University of Natal, Pietermaritzburg. The wide spacing of the colonies and the foliage between them reduced drifting (Jay, 1971) and robbing. A variety of flowering plants in the vicinity provided bee forage at all times. Colonies were placed in position at least three weeks before they were used in an experiment. Colonies selected for the experiment were queenright and healthy with a regular brood pattern and adequate food stores, and showed no signs of absconding, swarming, supersedure or

emergency queen replacement. Each colony had at least six frame sides carrying brood (including eggs), and eight or more frame sides covered with workers during the day, with foraging under way. Large, crowded colonies (with over 12 frame sides of brood and all frame sides covered with bees) were avoided, first, because this condition often presages swarming (Simpson, 1959, 1974; Butler, 1974) and second, to avoid management problems attendant on handling large colonies, particularly difficulty in finding the queen and mass colony defence actions. Before a colony was assigned to an experiment, 25 of its workers were dissected to ascertain that their ovaries were undeveloped. (Method of dissection is given in section 3.2.4.) Colonies in this essentially non-reproductive state (as regards queen production) were regarded as *normal* colonies (*see* chapter 2).

Distinctively marked groups of newly emerged worker bees (method: section 3.2.2) were introduced into a set of four queenright colonies (I, II, III and IV) at intervals of three to seven days over a period of six weeks, so that each colony ultimately had eight colour-groups of known age (Fig. 3-1). The initial groups comprised approximately 300 workers; thence, progressively smaller groups were used, until the last-placed ones comprised approximately 200 workers (Appendix Table 3-1). The colonies were then dequeened ~~by the~~ (hive displacement method) and housed on broodless combs (method: section 3.2.3). On the day of dequeening, 10 workers from each colour-group, and 25 unmarked workers, were taken from each colony and later dissected to determine the developmental states of their ovaries. A marked group was introduced into the newly queenless colony. After dequeening the colonies were left to settle down for four days. Every day thereafter, the colonies were scrutinized for the appearance of worker eggs (reported in chapter 5). At each examination all queen cells were cut out and discarded. Two marked groups were placed in each colony *after* it had been dequeened (Fig. 3-1).

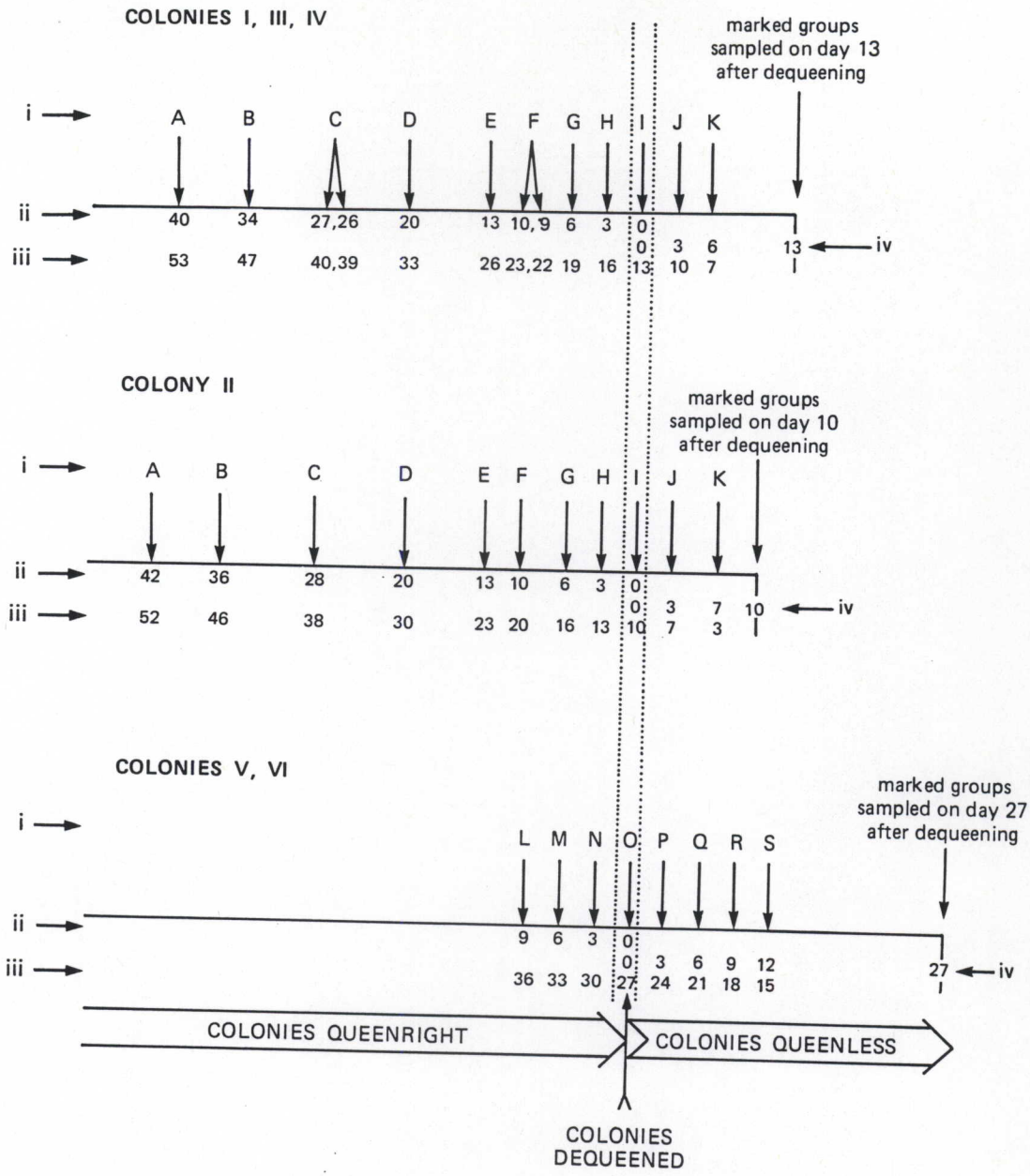


FIGURE 3-1. Timing of introduction and sampling of marked groups in relation to dequeening of experimental colonies I, II, III, IV, V and VI. Ages of marked groups are given in days.

i → designations of marked groups
ii → ages of marked groups at dequeening
iii → ages of marked groups when sampled
← iv number of days after dequeening

After 13 days of queenlessness colonies I, III and IV were killed with ethyl acetate vapour. Colony II was killed after 10 days of queenlessness, a discrepancy owing to the interference of inclement weather during colony manipulations. On the day on which they were killed, the colonies were separated into field bees and house bees by hive displacement (method: section 3.2.3). The colonies were killed at sundown, after all flights had stopped. Each dead colony thus contained several marked age-groups of workers which had either been members of the queenright, then queenless, colony, or had been placed in the queenless colony. The marked bees were retrieved, sorted into age-groups, and dissected (section 3.2.4) to determine the development of their ovaries. Field bees and house bees were kept separate. The bees were preserved for dissection by freezing or in Bouin's fluid.

The second set of colonies, V and VI, were subjected to procedures similar to those described above but with different timing in the addition of the marked groups, and in the queenless period (Fig. 3-1). These colonies were not displaced before they were killed.

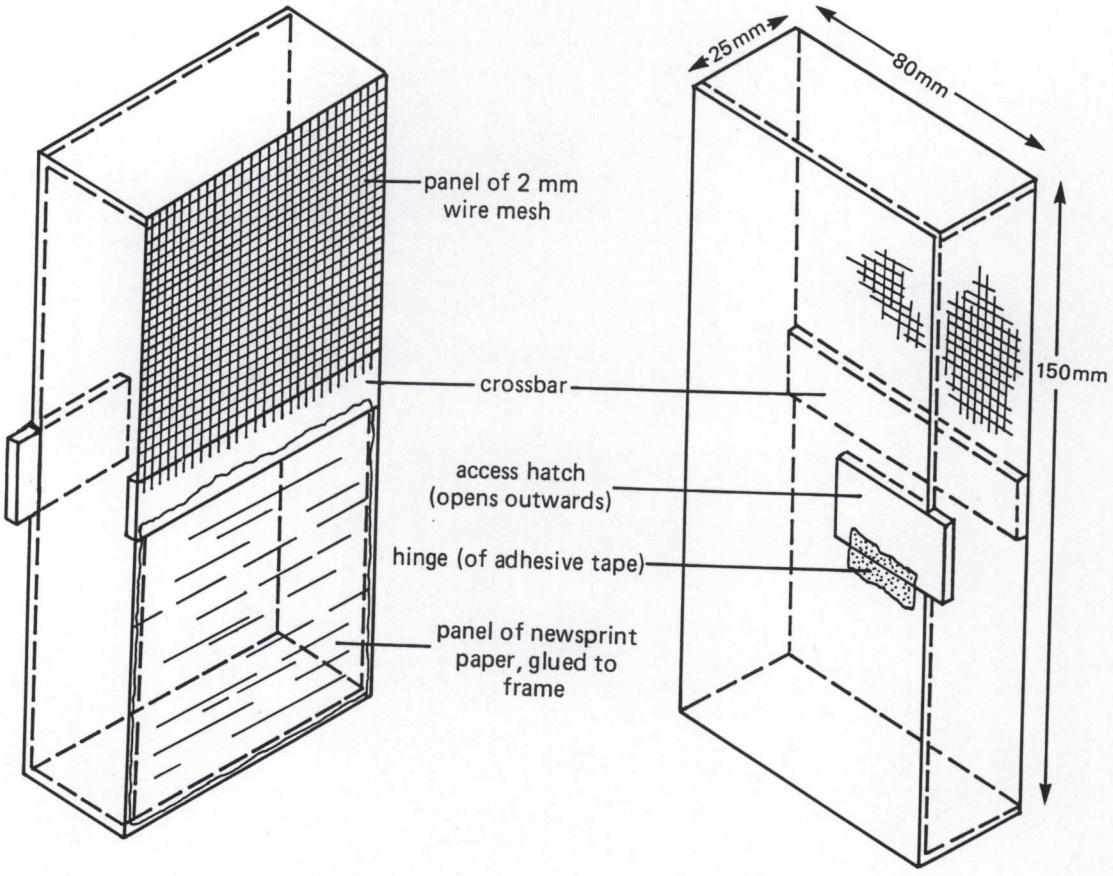
It should be noted that, apart from the different queenless periods in the two sets of colonies (I-IV) and (V, VI), all colonies were treated similarly. The aim was to observe events following queen and brood removal from colonies that had been established as queenright and normal, i.e. in which there initially were queenright brood patterns and in which workers' ovaries were undeveloped. Since these features of normal colonies are commonplace it was not necessary to incorporate normal colonies as experiment controls. Events in the queenless colonies were simply referred to the known conditions in queenright colonies. This approach has been used in several studies on queenless colonies, e.g. Perepelova (1926), Hess (1942), Anderson (1963), Kropocova and Haslbachova (1969, 1970, 1971) and is fundamentally different from the experimental treatment approach of e.g.

Jay (1968, 1970, 1972), Jay and Nelson (1973) and Jay and Jay (1976) in which controls were essential.

3.2.2 Production of marked workers and introduction into colonies

A method was devised to mark about 300 newly emerged workers per hour, without narcotizing them at any stage. The workers emerged in a constant temperature chamber (34°C) from brood combs taken within 24 h from colonies other than the experimental ones. Each worker was held in soft forceps while a spot of quick drying paint (acetone based) was applied to its dorsal thorax or abdomen through a hypodermic syringe with the point of the needle filed flat. This method proved superior to application of paint by a fine brush (as by von Frisch, 1967; Smith, 1972) or by a small pipette (as by McDonald and Levin, 1965), in that the paint in the syringe did not dry prematurely and could be insinuated amongst the cuticular hairs in controlled amounts, enabling rapid production of a neat, durable spot.

Painted workers were placed in an "introduction box" (Fig. 3-2). When the required number had accumulated the box was placed on the top bars of the experimental colony, papered side downmost. A 10 mm high spacer was placed on the rim of the hive, which allowed the hive cover to be replaced with the introduction box in place. The workers of the colony and the imprisoned workers chewed through the paper before mixing directly (see Johansson and Johansson, 1976 on newspaper method of uniting colonies). The wire gauze panel adjacent to the paper panel was designed to enhance odour exchange and trophallaxis (Free and Butler, 1958) between the colony bees and the introduced bees before the paper was penetrated, thus reducing the fighting that may arise when bees of different colonies are suddenly mixed (Johansson and Johannson, 1976).



Under-surface of container, which was placed downmost on top-bars of the experimental hive.

Container in vertical position, as when provisioned with marked workers in the laboratory.

FIGURE 3–2. Container for introducing marked worker bees into a hive.

3.2.3 Removal of queen and brood

Before a colony was dequeened a separate "substitute" hive was made up with frames devoid of bees and brood but containing pollen and honey. These frames were obtained by taking brood frames from queenright colonies and killing the brood in them by exposure to cold. The frames were then replaced in their colonies for a few hours, until all dead brood had been removed by the bees. The broodless frames were then stored in a freezer until they were required for the experiment. When a substitute hive was assembled the broodless frames placed in it were numbered sequentially across the brood chamber and were kept in order throughout the experiment. To facilitate manipulation, 9 frames instead of the usual 10 were placed in each brood chamber. (According to Jay, 1972 and Jay and Jay, 1976, introduction of empty combs, previously used for brood rearing, does not affect worker ovarian development so that their use in the present study did not constitute a confounding factor.)

Queens of *A. m. scutellata* are known for their habit of running and hiding when the frames of a colony are manipulated (Anonymous, 1934). The excessive manipulations required to find a queen may disrupt the colony organization completely and, particularly in *A. m. scutellata*, may provoke mass attacks. Some of these difficulties occurred in the first set of colonies to be dequeened, in which a failsafe method for finding the queen was instituted by shaking all hive inmates onto the ground in front of the substitute hive. This, combined with an initial heavy smoking of the colony, had the effect of "demoralizing" the inmates and reducing colony defence behaviour. Once the bees had entered the substitute hive, the queen was removed from the wire queen excluder (Root *et al.*, 1972) that had been placed under the substitute hive when it was assembled. Each colony was then queenless and broodless, but had ample food stores. Within three

days of dequeening by this method two of the colonies absconded. This was considered to be in response to the sudden, massive disruption to these colonies in the dequeening procedure. Consequently a less disruptive method was devised in which the hive to be dequeened was displaced five metres to one side and replaced with a substitute hive, made up as described above. Foragers left the displaced hive and returned to the substitute hive. After a day of displacement the occupancy of the displaced hive was reduced to house bees among which the queen was easily found by examination of the combs. The queenless house bees were then shaken into the substitute hive and thus reunited with the field bees. In this way queenless, broodless colonies were obtained with relatively little disruption and with the avoidance of mass defence attacks. This method of dequeening was used for all colonies in the experiments reported in the present chapter, and for colonies A-D in the experiments reported in chapter 4. No absconding occurred in any of these colonies.

3.2.4 Dissection to expose the ovaries

Dissection of the worker to expose the ovaries is labour intensive and can be a limiting factor in the execution of experiments requiring the assessment of ovarian development in many individuals. Methods for this dissection are given by Weaver (1956), Velthuis (1970), Dade (1977) and Jackson (1982). A rapid method was devised which entailed pinning the specimen by running a slender, flexible entomological pin under the abdominal tergites. The specimen was then fixed in a wax-bottomed dish, ventral surface uppermost (Fig. 3-3), and dissected at X18 binocular magnification under a 50% ethanol solution. The membrane between the 7th and 6th abdominal sternites was severed with fine forceps, leaving the 7th sternite in place. The 6th sternite was levered upwards and the lateral

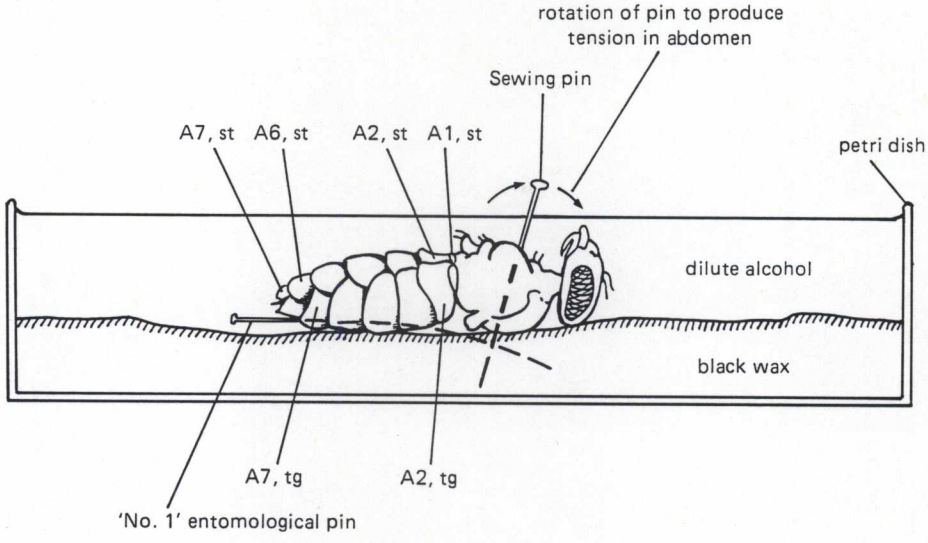


FIGURE 3-3. Pinning of honeybee worker for dissection to expose ovaries.

A1, st = 1st abdominal sternite
A2, st = 2nd abdominal sternite
A6, st = 6th abdominal sternite
A7, st = 7th abdominal sternite
A2, tg = 2nd abdominal tergite
A7, tg = 7th abdominal tergite

membranes between the sternites and tergites were severed so that the 6th to 3rd sternites were removed as a unit, exposing the ovaries at either side of the ventriculus. If the ovaries were not immediately visible the oviducts at the point of their bifurcation from the median oviduct were searched for by manipulating the 7th abdominal sternite, and the 6th and 7th abdominal ganglia. In the infrequent instances in which no ovaries were found the individuals were recorded as having undeveloped ovaries. In most individuals both ovaries were found. Where there was unequal development the state of the more highly developed ovary was recorded.

3.2.5 Assessment of ovarian development in the worker

It was found that ovarian development (*see* chapter 2) was most easily classified according to Velthuis' (1970) system) and that a subdivision could be made in his stage 2, as listed in Table 3-1. The three main

TABLE 3-1. Classification of worker ovarian development used in the present study, as modified from Velthuis (1970).

Categories of Velthuis (1970)		Modification of Velthuis' categories	
Symbol	Category	Symbol	Category
1	ovaries resting and almost inactive	0	ovaries undeveloped with little or no visible compartmentation
2	early stages of development in which the eggs have still more or less round to bean-shaped form	1	subdivided into 1a and 1b:
		1a	slight development, with swelling in the ovarioles and eggs spherical, visible
		1b	at least one bean-shaped egg present
3	eggs having the sausage form	2	at least one sausage-shaped egg present

classes, 0, 1 and 2 represent ovaries *undeveloped*, *partially developed*, and *fully developed*, which is the classification by which ovarian development is usually conceived, irrespective of the underlying system of notation employed. The division of class 1 into two subdivisions, 1a and 1b, allows a finer analysis of partially developed ovaries to be made. Or, 1b can be combined with 2 to give a class of "highly developed ovaries", i.e. "ovaries with well to fully developed eggs", constituting all the workers most likely to be involved in the actual laying of eggs within a few days of sampling time. The coincidence of these symbolic and verbal categories, as used in the present study, is shown in Fig. 3-4.

3.3 RESULTS AND DISCUSSION

No ovary development was found in any of the workers sampled from the queenright colonies at the beginning of the experiment, nor in those sampled at the time of dequeening, which confirmed that the colonies had fully functional queens at the time of dequeening. Jackson (1982) also found all worker ovaries undeveloped in queenright *scutellata* colonies.

3.3.1 Mortality in marked age-groups

The total number of workers (field bees and house bees) recovered in each marked age-group when the colonies were killed was expressed as a percentage of the original number in the group (Fig. 3-5). Highest survival was 86% in several of the younger groups (J and K, in colonies II, III and IV) which reflected an initial loss of 14% from the introduction boxes: the highest loss in a newly introduced group was 37% in group K, colony I.

Mortality in the marked groups of colony I was consistent with that

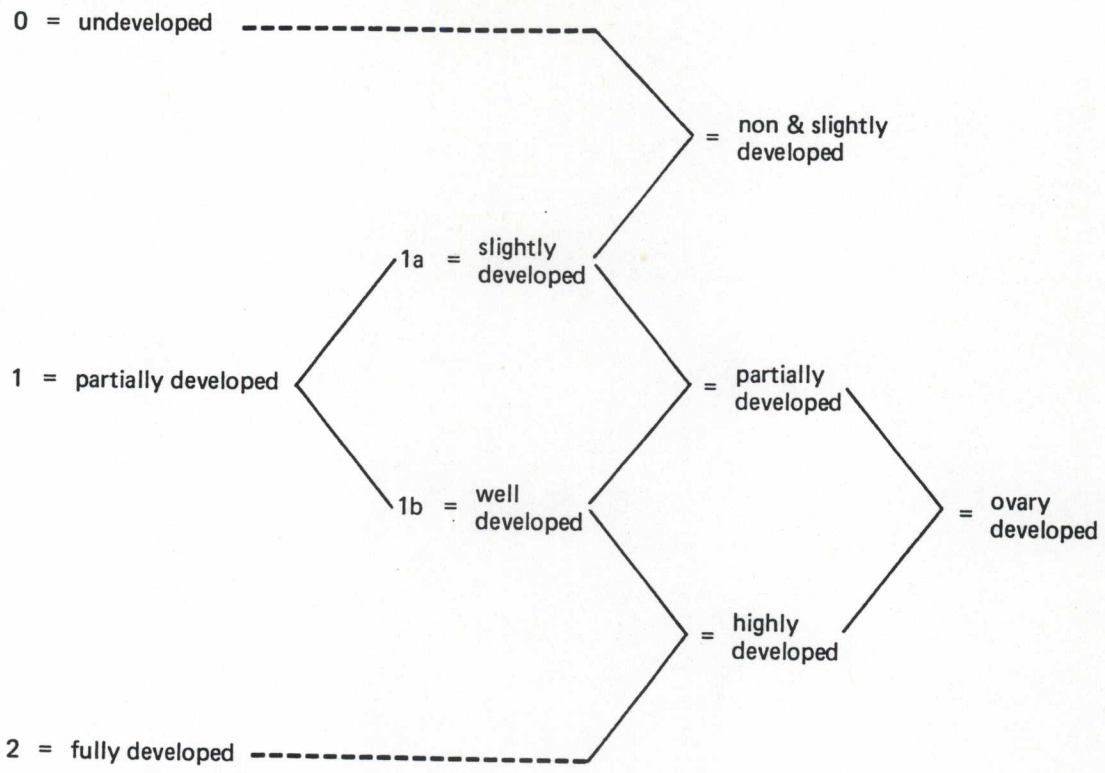


FIGURE 3-4. The coincidence of symbolic and verbal categories of the stages of worker ovarian development devised in this study.

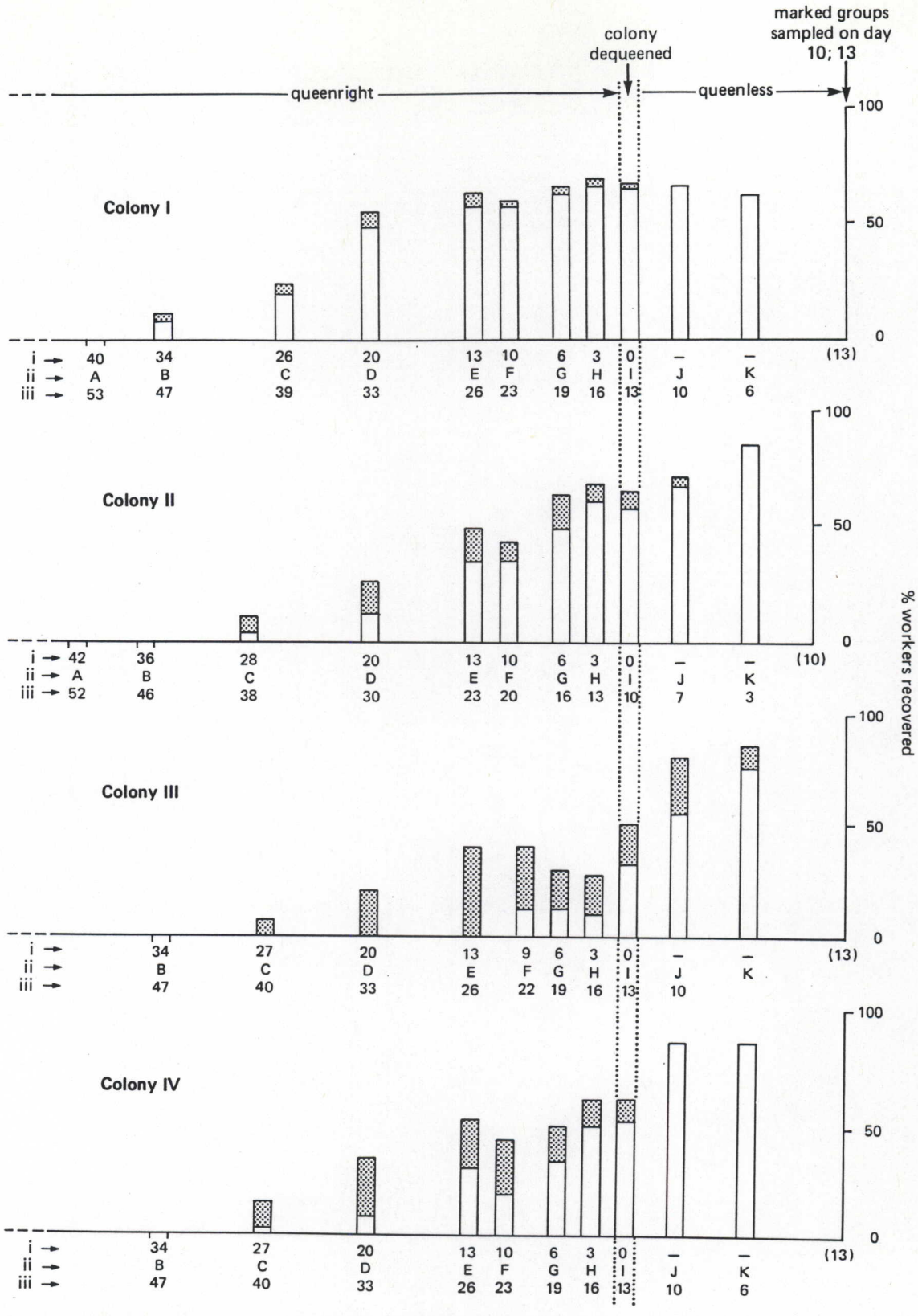


FIGURE 3-5. Colonies I, II, III and IV. Relationship between age of marked worker groups of field bees (stippled bars) and house bees (white bars) and proportions of them recovered at sampling time, 10 or 13 days after dequeening. The total height of each bar represents the percentage of marked workers recovered. The ratio between field and house workers that were separated by hive displacement on the day of sampling is shown within each bar. Ages of marked groups are given in days. Data from Appendix Table 3-1.

i → ages of marked groups at dequeening
ii → designations of marked groups
iii → ages of marked groups when sampled

expected if most of the marked workers had a lifespan of 30-40 days, the normal life span of active workers (Smith, 1960; Anderson *et al.*, 1983) that is, there was an approximately level rate of survival until the end of the lifespan at between 33 and 39 days, whereupon mortality increased sharply. In colonies II, III and IV survivorship in the marked groups decreased with age in a generally linear fashion, to below 15% after 38 to 40 days of age and to zero or near-zero in older groups. This indicated a steady rate of mortality in the marked groups of all ages, the causes of which were not determined in this study.

The emergence of an extremely long-lived group of workers in hopelessly queenless colonies left to run their course, is reported in chapter 4.

3.3.2 Separation of field bees from house bees by hive displacement

The progression of age-based polyethisms in honeybee workers in queenright colonies entails a transition from activities performed exclusively within the hive during early life, to foraging activities performed predominantly in later life (Seeley, 1982). The first flights of workers are undertaken at 3-17 days of age (Free, 1965) and are concerned with orientation, in which the landmarks associated with the hive are learnt (von Frisch, 1967). The age at which workers commence foraging varies between 9 and 35 days (Ribbands, 1952), although most start at about 20 days of age (Butler and Free, 1952; Free, 1965). By the method of hive displacement (section 3.2.3) workers that fly (field bees) can be partially separated from house bees (Free, 1958). The field bees that gather in the substitute hive must fly there from the displaced hive, either when returning from a regular foraging flight, or perhaps by drifting from the displaced hive in an orientation or defence flight. The workers that remain in the displaced

hive will consist of the young that have yet to fly and of experienced flyers that did not fly during the period of hive displacement. The proportion of field bees that fly on a particular day is greatly influenced by the weather and to lesser extents by various internal-colony factors (Ribbands, 1953; Seeley, 1985).

Figs. 3-5 and 3-6 show that not all the older workers (groups F to B) flew on the day of displacement. Colony I was displaced on a cool, overcast day, which is reflected in the low proportions of workers that flew, in comparison to colonies II, III and IV, which were displaced on warm, sunny days more conducive to foraging. However, all four colonies showed a distinct, steady trend towards more field bees in the older groups (Fig. 3-6), starting with very small proportions in some groups 6-7 days old, and rising with age in colonies II, III and IV to 100% in some groups 23 days old and older. These trends show that in *A. m. scutellata* colonies 2 weeks queenless an age-based polyethistic division was maintained between field bees and house bees in a form generally similar to that in queenright colonies, i.e. the queenright form of social order in this polyethism is carried into the queenless colony. This transposition was determined in European bees by Hoffmann (1961). Also, this distinct, age-based division of labour indicates that the marked groups were fully assimilated into the colonies.

3.3.3 Ovary development and age in queenless workers

Although over a hundred workers were retrieved in many of the marked field bee and house bee groups of colonies I-IV, a maximum of 25 workers per group was dissected in order to accommodate the frequent instances in which the field bee portion in a group was small in relation to the house bee portion, or *vice versa* (Appendix Table 3-1). This gave a compromise

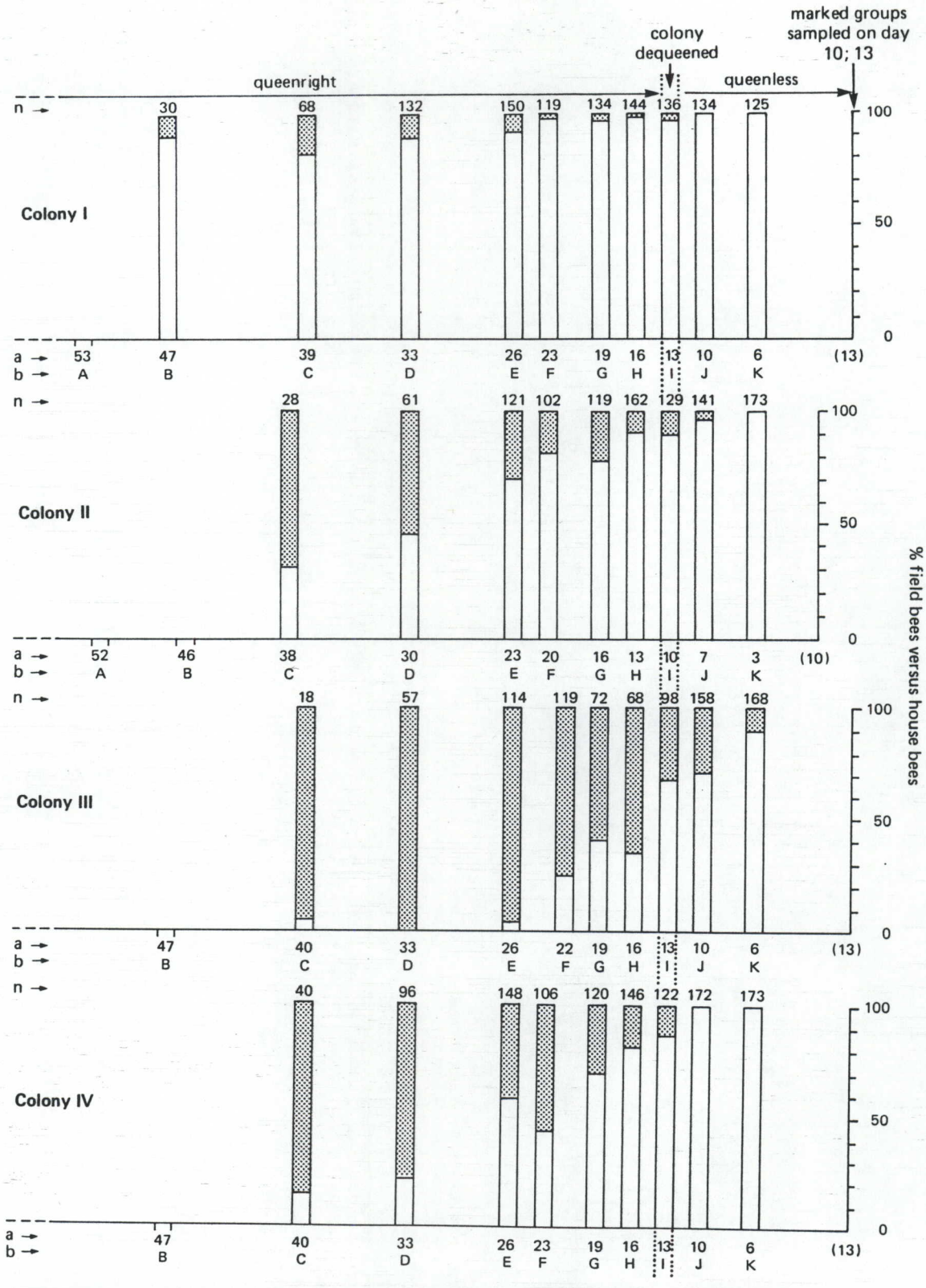


FIGURE 3-6. Colonies I, II, III and IV. Relationships between age of marked worker groups of field bees (stippled bars) and house bees (white bars) and proportions of them recovered at sampling time, 10 or 13 days after dequeening. The ratio between field and house bees that were separated by hive displacement on the day of sampling is shown within each bar as percentages of the total number of marked workers recovered in each age-group. Ages of marked groups are given in days. Data from Appendix Table 3-1.

a → ages of marked groups when sampled
b → designations of marked groups
n → number of workers recovered in group

between the need to base comparisons on numerous individuals and the need to compare as many groups as possible. In colonies V and VI, which were not displaced before they were killed, 50 workers from each marked group were dissected.

In analysing the effect of age upon ovarian development in workers several interrelated factors must be considered: age at dequeening; length of time that the colony was queenless; total age; the inherent longevity of the workers; the rate at which their ovaries develop; and the period over which they maintain their ovaries in a developed state. When the normal colony is dequeened, as in the present study, workers of all ages are present and the only factor completely under the control of the investigator is the queenless period that is imposed. A queenless period of approximately 2 weeks was chosen for colonies I-IV, to allow sufficient time for extensive ovarian development (rate of worker ovarian development is reviewed in chapter 5). At the same time, the 2 weeks' queenless period was sufficiently short to allow survival of most of the groups of workers that had been placed in the colonies prior to dequeening, assuming an average worker life span of about 4-6 weeks. Retrogression of developed ovaries (chapter 2) would presumably not have been important in this short queenless period. Thus in these colonies the important factors to be considered are the ages of the workers at the time of dequeening and their total ages when the queenless colonies were killed.

The ovarian development that obtained in the marked groups of the house bees of colony I (after 13 days of queenlessness) (Fig. 3-7) shows that, in the most general sense, ovary development may occur in workers of any age, as has been concluded by a number of authors, cited in section 3.1. However, closer scrutiny of the bars of Fig. 3-7 reveals that this interpretation, whilst true in itself, is inadequate in that it fails to reflect the existence of a relationship between the age of workers in a

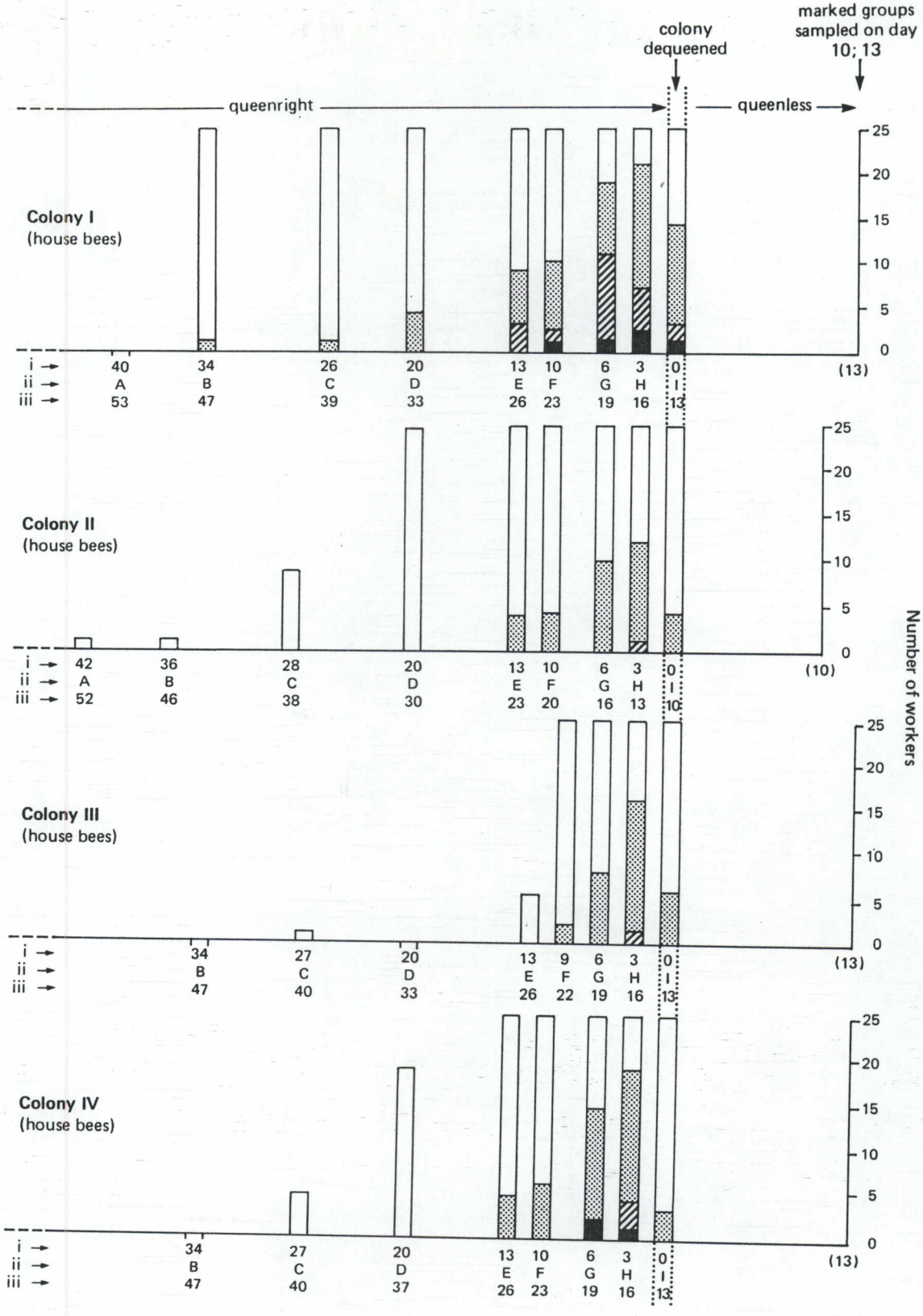


FIGURE 3-7. *Continued.*

i → ages of marked groups at dequeening

ii → designations of marked groups

iii → ages of marked groups when sampled

group and the *proportion* of them that underwent ovarian development. In the house bees of colonies I-IV, and the field bees of colonies II and IV (Fig. 3-8) the relationship between ovarian development and age can be interpreted as a bell-shaped curve which, over an age-range of 0-40 days at dequeening, is skewed to a peak of development in workers aged 3-6 days at dequeening (groups G and H). From this peak, percentages of ovarian development fell away sharply in workers older than about 13 days at dequeening. On the other side of the 3-6 day peak ovarian development was notably less in the workers placed in the colonies on the day of dequeening (group I). This age-based distribution was slightly different in the field bees of colony III, in which the aggregate ovarian development was highest in group I. In the field bees of colony I insufficient groups were recovered to permit characterization of an age-based distribution, although the proportions of ovarian development obtained in the three groups displayed in Fig. 3-8 do fit in with the distribution described above for the other colonies.

In general these results indicate that, after about 2 weeks of hopeless queenlessness, most ovarian development occurred in workers that were house bees at the time of dequeening, with a peak in workers that were 3-6 days old (i.e. young house bees) at dequeening. Further, this age-based pattern of ovarian development occurred in both field and house bees (2 weeks after dequeening) and was therefore independent of the polyethistic division between house bees and field bees described in section 3.3.2.

In colonies V and VI the proportions of ovarian development in marked groups aged 0-9 days at dequeening (groups L, M, N, O, Fig. 3-9) were similar to the patterns in groups of similar ages at dequeening in colonies I-IV (groups F, G, H, I, Fig. 3-8), with a peak of ovary development in workers that were 6 days old at dequeening. The fact that

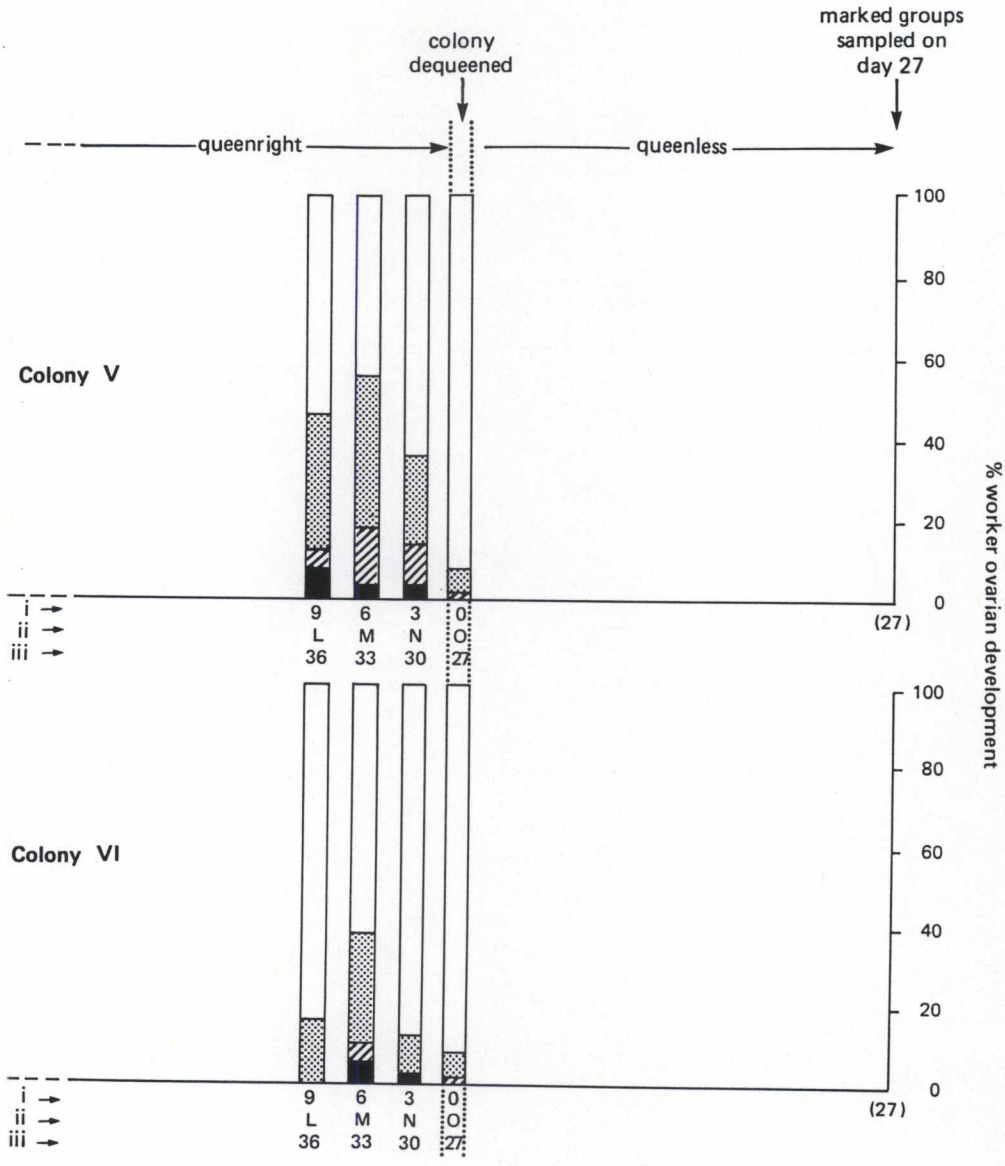


FIGURE 3-9. Colonies V and VI. Relationship between ovary development and age in workers sampled from marked age groups. Proportions of ovary development in each group are expressed as percentages of the total number of workers dissected (50 in each group). Ages of marked groups are given in days. Data from Appendix Table 3-3.

Stage 0	(ovaries undeveloped)	: white
Stage 1a	(ovaries slightly developed)	: stippled
Stage 1b	(ovaries well developed)	: diagonal hatching
Stage 2	(ovaries fully developed)	: black

i → ages of marked groups at dequeening
ii → designations of marked groups
iii → ages of marked groups when sampled

colonies V and VI were queenless for twice as long as colonies I-IV emphasized the strong influence of the *age of the workers at dequeening* upon the extent of their ovarian development when hopelessly queenless, rather than the queenless period, or the total age of the workers (which was 10-22 days in groups F-I in colonies I-IV but 27-36 days in groups N-O in colonies V and VI).

The 2-4 week queenless periods of the experiment were sufficient to allow ovarian development in workers that were newly emerged at dequeening, as specified by Allsopp (1988, *see* section 3.1, parag. 5), so that the present results do not indicate the ages of the very first layers in the newly queenless colony. It seems likely though, that many of the first layers would have been among those aged about 3-6 days at dequeening.

In all six colonies investigated the bulk of ovarian development in most age groups comprised workers with ovaries at stage 1a, the earliest stage of development in the scale used in this study. Most workers with ovaries developed beyond stage 1a, i.e. nearing or at the point of egg laying, were aged between 0-6 days at dequeening in colonies II, III, IV and VI, although in colonies I and V (house bees) this age range was somewhat wider, between 0-13 days old at dequeening. Thus the few workers that had advanced ovarian development were generally to be found in groups with a high proportion of workers with slight (1a) ovarian development.

In colony I workers with highly developed ovaries were found amongst the few workers that flew in the inclement weather of the day of displacement, as well as in the house bees (Fig. 3-7). In colonies II, III and IV there were fewer workers with highly developed ovaries: of these few, more occurred in the field bees than in the house bees in colonies II and III. These results indicated that worker flight activity, or lack of it, is not associated with highly developed ovaries. This interpretation applies only to proportions and distributions in age groups as a whole and

not to the proclivities of individuals. Thus, for instance, this interpretation does not preclude the possibility that amongst the house bees there may have been individuals temporarily specialized in egg laying that did not fly out of the hive, even though they might resume flight activity after their egg laying phase ended. Such workers would be equivalent, for example, to the "false queens" of Sakagami (1958).

3.3.4 Levels of worker ovarian development in the colony as a whole

Worker ovarian development in the colony as a whole has been expressed as proportions of ovarian development in samples of unmarked workers (i.e. in workers of all ages) by e.g. Anderson (1963), Allsopp (1988). An approximation of whole-colony samples was derived for the marked workers of colonies I-IV by summing the number of workers dissected in each colony (subdivided into field and house bees), from which the percentages of workers with ovaries in states 0, 1a, 1b and 2 were calculated and expressed in a bar chart (Fig. 3-10). Ovarian development of all stages ranged from 16% in field bees of colony I to 40% in house bees of colony IV, giving a mean for all four colonies of 28%. Most of the ovarian development that occurred was slight (mean of 25% workers with ovaries at stage 1a): few bees (2-10%) had well developed ovaries at stage 1b and fewer still (mean 1.5%) had fully developed ovaries at stage 2 (Appendix Table 3-2). Almost all of this ovarian development occurred in workers 13 days old or less at the time of dequeening, as discussed in the previous section. The total levels of ovarian development obtained in the groups placed in colonies V and VI zero to nine days before dequeening (groups O-L, Fig. 3-9) are thus relevant; in colony V they were 1a = 25%, 1b = 7%, 2 = 4% and in colony VI, 1a = 15%, 1b = 1.5%, 2 = 2% (Appendix Table 3-3). These levels were thus similar to those obtained in colonies I-IV.

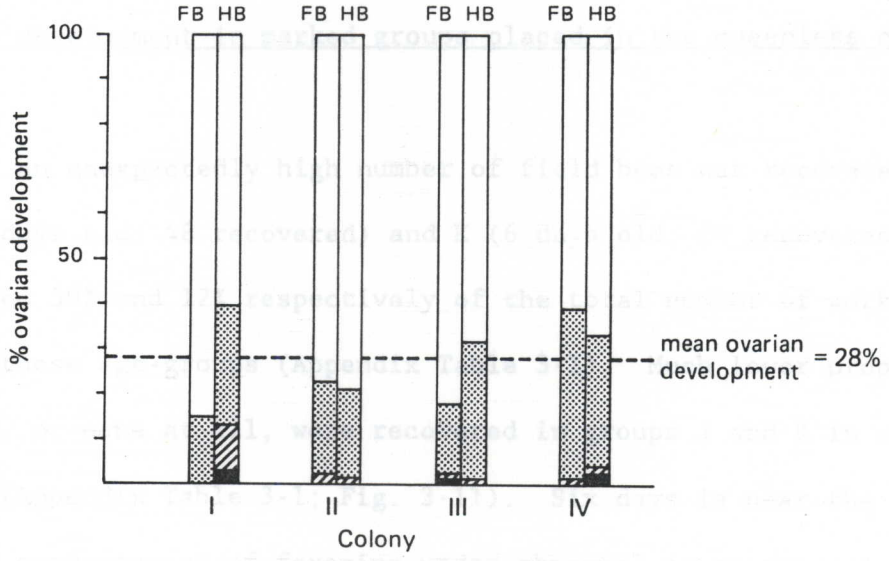


FIGURE 3-10. Colonies I, II, III and IV. Ovary development in field bees and house bees in each colony. Ovary development in marked age groups was summed and expressed as a percentage of the total number of workers dissected. Data from Appendix Table 3-2.

Stage 0	(ovaries undeveloped)	: white
Stage 1a	(ovaries slightly developed)	: stippled
Stage 1b	(ovaries well developed)	: diagonal hatching
Stage 2	(ovaries fully developed)	: black

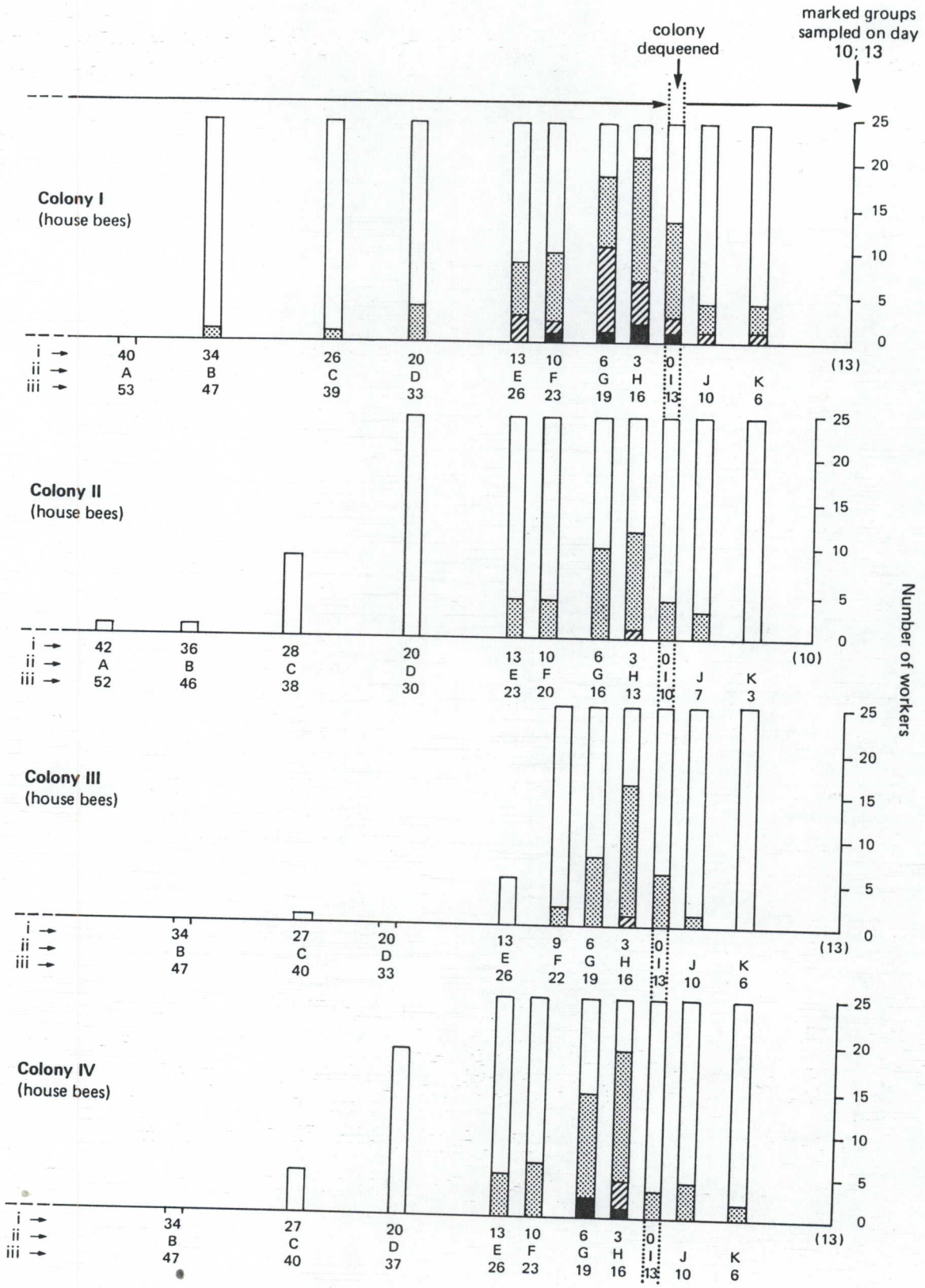


FIGURE 3-11. *Continued.*

i → ages of marked groups at dequeening

ii → designations of marked groups

iii → ages of marked groups when sampled

development in a few individuals) and (b) factors inhibitory to ovarian development that may have developed in the queenless colonies, such as queen pheromones secreted by some of the older workers, and/or the presence of brood laid by workers.

Relatively low proportions of ovarian development were obtained in groups P, Q, R and S in colonies V and VI (Fig. 3-12). These groups were placed in queenless colonies and lived in them for 24, 21, 18 and 15 days respectively. Their ages were roughly equivalent to the total ages of groups E-H in colonies I-IV, which were placed in the queenright colonies and then underwent 10-13 days' queenlessness. The percentage ovarian development in groups P-S in colonies V and VI ranged between 2-16% (mean 10.5%), whereas in groups E-H of colonies I-IV the proportion of development ranged between 0-85% (mean 40%). The relatively low proportions of ovarian development in groups P-S, which were old enough to have undergone more extensive ovarian development, indicates that inhibitory factors, such as those mentioned for groups J and K, may have been important in lowering the ovarian development in the groups introduced into queenless colonies V and VI.

3.4 CONCLUSION

The relationship between age and ovary development indicated in the present study bore the characteristics of a labile glandular development underlying a polyethism (chapter 2) analogous, for example, to wax gland development in queenright colonies (reviewed by Free, 1965 ; Wilson, 1971, p.123; Michener, 1974, p.125; Seeley, 1985, p.79) in which a proportion of workers 1-3 weeks old have wax glands developed to varying degrees, with a peak of development at 10-15 days and with lower proportions of younger and older individuals also with these glands developed (Turell, 1974). Workers with

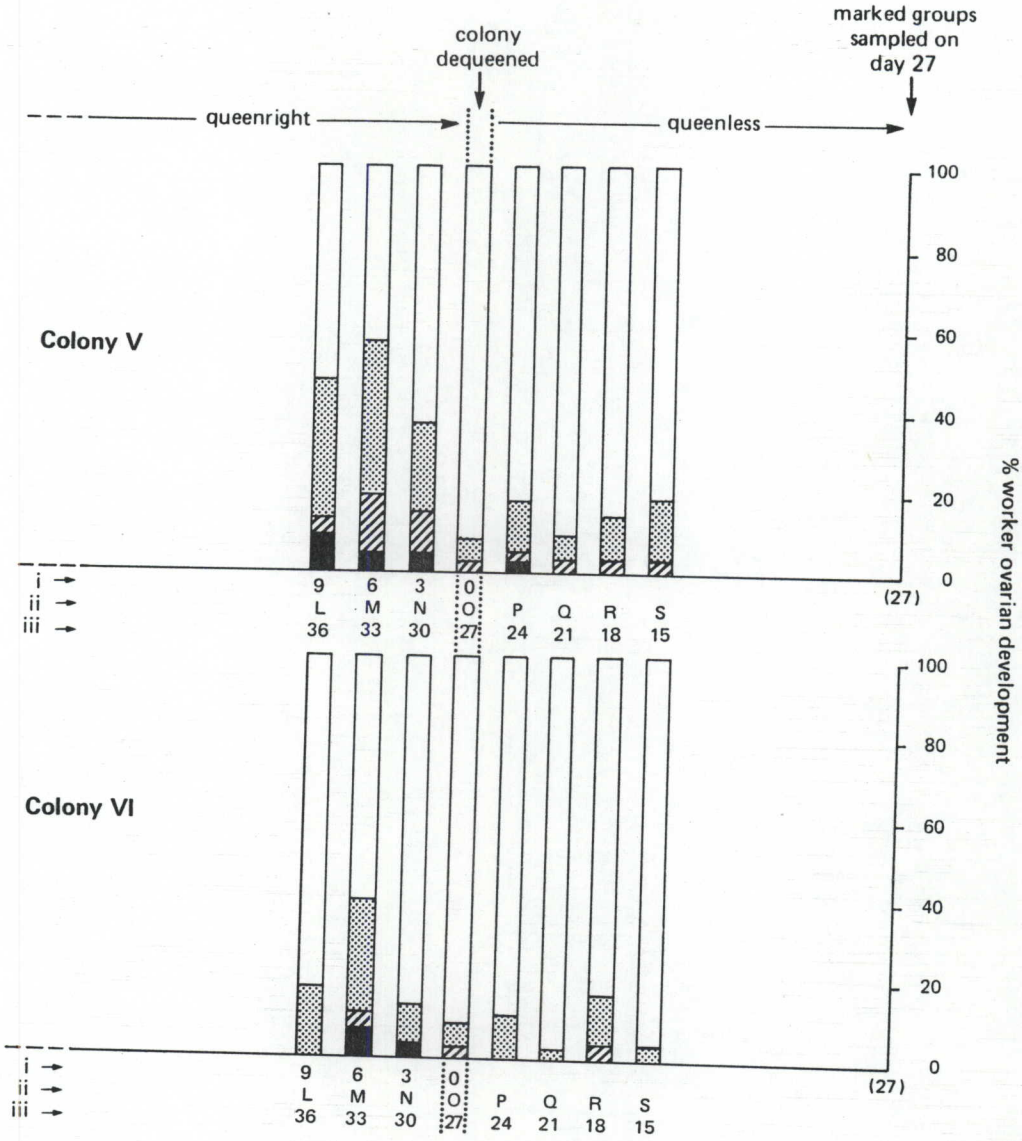


FIGURE 3-12. Colonies V and VI. Relationship between ovary development and age in workers sampled from marked age groups, including groups P, Q, R and S which were placed in the colonies after dequeening. Proportions of ovary development in each group are expressed as percentages of the total number of workers dissected (50 in each group). Data for groups placed in queenright colonies are the same as that shown in Figure 3-9. Ages of marked groups are given in days.

Stage 0	(ovaries undeveloped)	: white
Stage 1a	(ovaries slightly developed)	: stippled
Stage 1b	(ovaries well developed)	: diagonal hatching
Stage 2	(ovaries fully developed)	: black

i → ages of marked groups at dequeening
ii → designations of marked groups
iii → ages of marked groups when sampled

developed and semideveloped wax glands may undertake many duties besides comb building, just as queenless workers with ovaries at all stages of development undertake duties performed by workers in queenright colonies. While many workers come to use the products of their wax glands, relatively few ovary-developed workers appear to progress to lay eggs (see sections 3.1 and 3.3.3). In this respect worker laying resembles the "specialized" polyethisms normally performed by only a few individuals at a time, such as guarding (Butler and Free, 1952; Moore *et al.*, 1987), undertaker duty (Visscher, 1983) or water collecting (Robinson *et al.*, 1984). An extreme of specialization amongst laying workers is reached in the development of "false queens" (Sakagami, 1958). Viewed as a colony-level as opposed to an individual-level process (see e.g. Wilson, 1966, 1971, p.321; Crozier, 1977; Moritz, 1986a), worker oviposition and the ovarian development that underlies it may be characterised as a polyethism that arises in young (nurse) bees in response to the loss of the queen and the brood. The widespread view that worker ovarian development is not related to age because it is found in workers of all ages is thus rendered sterile in the light of the present study. Rather, worker ovarian development should be viewed as an age-related process that emerges when proportions of workers with ovarian development are compared among different age groups in the hopelessly queenless colony. This interpretation, of worker ~~ovary development~~ oviposition as an age-based polyethism, implies a higher level of social organization in this aspect of the hopelessly queenless colony than has hitherto been supposed and it conforms with the regular pattern of brood rearing in the hopelessly queenless colony, as determined in chapter 4 (*see* chapter 6 for further discussion of this point). This interpretation provides an additional perspective for future analysis of the reasons why not all workers undergo ovarian development and why relatively few actually lay eggs. Variable participation by workers is a feature of most honeybee

polyethisms (Seeley, 1982; Visscher, 1983; Winston, 1987). Analyses of the underlying causes for this have so far proven intractable but, as advances are made (see e.g. Winston and Punnett, 1982; Evers and Seeley, 1986; Winston, 1987; Breed, 1988; Frumhoff and Baker, 1988; Kolmes and Winston, 1988; Robinson and Page, 1988), the analytic methodology for polyethisms should prove useful in analyses of worker oogenesis and laying.

CHAPTER 4

DEMOGRAPHY OF WORKERS AND THEIR PROGENY AFTER REMOVAL OF THE QUEEN AND HER BROOD

4.1 INTRODUCTION

The circumstances in which honeybee workers may lay eggs are reviewed in chapter 2. The sudden removal of the queen and her brood from a normal, queenright colony induces rapid ovarian development in some of the workers. If the colony is then kept free of replacement queen cells, oviposition by some workers soon commences, and brood is reared.

Although brood rearing in the queenright colony and the process of queen rearing by queenless bees is well understood (Ribbands, 1953; Pain, 1968a,b; Butler, 1974, 1975; Michener, 1974; Gary, 1975; Winston, 1987), rearing of brood from laying workers in the hopelessly queenless colony has received cursory treatment in the literature: the aim of the present study was to describe this latter aspect more fully, in *A. m. scutellata*.

When honeybee workers lay they place their eggs haphazardly on the floor and walls of the cells, and commonly several eggs are found in each cell (Orosi-Pal, 1932; Sakagami, 1958; Smith, 1960; Butler, 1974; Tucker, 1978). In most honeybee races workers lay male eggs, which they place in both worker and drone cells. (*A. m. capensis* is an exception in that its workers lay predominantly female eggs: *see* chapter 2.) Brood reared from worker eggs is characteristically scattered irregularly on the combs and, owing to intermittent laying by workers in empty cells between occupied ones, the age of laying worker brood may vary greatly from one cell to the next (Millen, 1942; Park, 1949; Carlile, 1979).

In contrast to haphazard oviposition by workers, the queen honeybee

lays her eggs in a regular fashion, one to the floor of each cell, female eggs in worker cells and male eggs in drone cells, producing evenly aged ellipses of brood on the combs (Root *et al.*, 1972, p.103; Butler, 1974, 1975). Oviposition is normally restricted to the inner combs and to an elliptical area in the middle of each of these combs. The outermost brood combs usually have smaller ellipses of brood than the inner combs so that, in the three-dimensional fabric of the colony, the region of brood rearing forms an ellipsoid of alternating vertical layers of brood comb and nurse bees (Phillips, 1928; Smith, 1960, pp.16, 154). As each ellipse of brood within this ellipsoid emerges the vacated cells are cleaned by house bees and the queen lays in them again. The production of worker brood in the queenright colony is thus a repeated cycle that takes place within a specific region in the combs of the nest, and may continue uninterrupted for months until disrupted by swarming, supersedure, or accidental disturbance to the queen; or by hibernation, in temperate races (Free, 1969; Seeley, 1985); or by absconding, in tropical races (Fletcher, 1978).

Rearing of drone brood in queenright colonies generally occurs in batches over relatively short periods dictated by season, availability of food and the swarming cycle (Allen, 1965; Free, 1969; Free and Williams, 1975; Page, 1981; Lee and Winston, 1987), and so does not occur in the regular cycles of worker brood. Drone cells are normally situated at the outer margins of the worker brood-rearing ellipses (Phillips, 1928; Free, 1967; Frisch, 1974; Free and Williams, 1975; Winston, 1987) and so when in use form part of the ellipsoidal brood region of the colony.

During preliminary observations of hopelessly queenless colonies of *A. m. scutellata* it was noticed that although the first batches of eggs were indeed laid haphazardly, in both worker and drone cells, they nevertheless occupied the central areas of a few adjacent combs within the colonies. Eggs were laid in drone cells within the brood area and were not

scattered through every available patch of drone cells in the hive. This indicated the maintenance of a brood area, as in queenright colonies, and raised the question of the extent to which this organisation is transposed to the queenless colony - a topic not addressed in the literature, although it is known that queenless colonies maintain the basic social activities of the queenright colony (chapter 2).

The present study reports measurements of the course of brood rearing on the combs of normal colonies under natural conditions (section 3.1) that were made queenless and broodless: the demography of this form of brood rearing is characterised, from the inception of queenlessness to the natural demise of the colony. The pilot measurements that led to this study are described.

4.2 METHODS AND MATERIALS

4.2.1 Experimental procedure

Brood from laying workers was measured intermittently in four colonies (2, 3, V and VI) made hopelessly queenless (method: 3.2.3), and from these pilot observations detailed measurements were carried out on another four colonies, A-D. Days on which observations were made after dequeening are given in Figs. 4-2 and 4-3. At each observation the number of cells occupied by brood (eggs, young larvae, old larvae, sealed brood) was estimated (method: section 4.2.2). Queen cells and their contents were recorded, after which they were cut out of the combs and discarded.

4.2.2 Quantification of brood

In order to record the demography of laying worker brood a method was

required to count the number of cells that were sealed or that contained eggs or larvae. Because the recordings were made repeatedly during the queenless period of each colony the exposure of each frame in the field had to be brief, to avoid killing the brood and so disrupting the natural course of brood rearing; also, excessive disturbance to combs may cause live brood to be eaten (Woyke, 1977). Most brood measurement methods entail estimation of the easily visible sealed brood from areas obtained by grid-counts or from photographs or, most commonly, from measurements of axes of brood masses (Al-Tikrity *et al.*, 1974; Rogers *et al.*, 1983). None of these methods was suitable for counting the scattered brood of laying worker colonies. Scattered brood can be recorded accurately by tracing areas of cells on a transparent cellophane overlay for estimation by planimeter (Johannson and Johannson, 1971a,b), but in the present study it was found that the method took too long in the field to be practicable.

To quantify laying worker brood a grid was constructed (Fig. 4-1), the rhombs of which enclosed units of 100 cells, an intuitively manageable number for counting by eye. The frame to be examined was cleared of bees and the grid aligned over it, so that the sides of the rhombs coincided as far as possible with the cell rows of the comb. The grid and the frame were then held pressed together and could be angled to the light for a clear view of the contents of the cells. When occupied cells were few and scattered they were counted individually. When groups of cells with similar-aged brood were encountered, their numbers were estimated as decimal proportions of each rhomb, i.e. "1.0" for 100 cells occupied, "0.75" for three-quarters, "0.5" for half, "0.4" for about 40 cells occupied, and so forth.

For the counting of drone cells a grid similar to that shown in Fig. 4-1 was constructed with rhomb-sides of 61.5 mm, ten times the average width of the *A. m. scutellata* drone cell (Smith, 1961), so that each rhomb

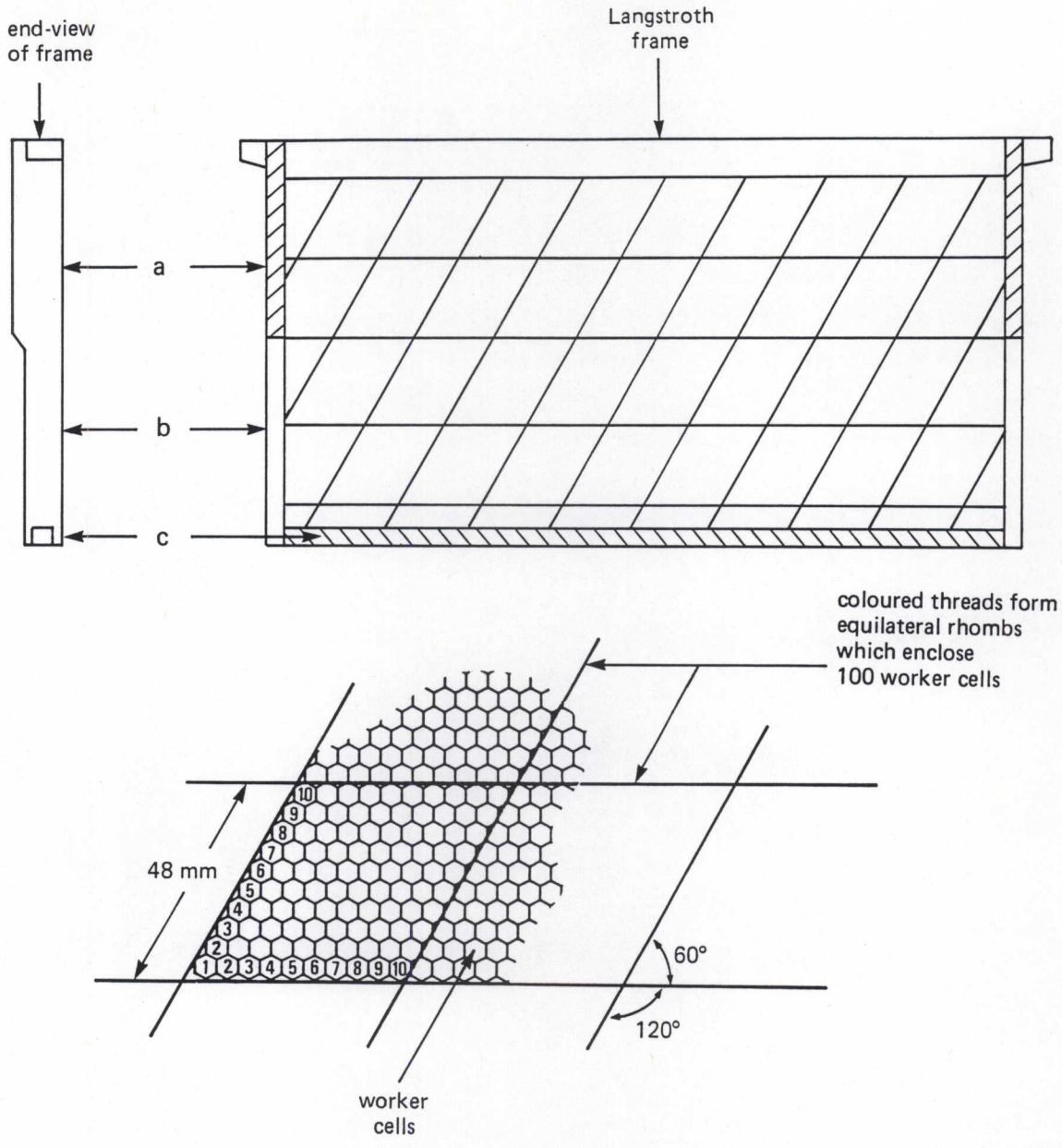


FIGURE 4-1. Grid apparatus for estimating numbers of comb cells occupied by brood. A standard Langstroth frame was modified to form an even surface for the attachment of the grid-threads, enabling the grid to be placed flush onto the comb during counting of cells. a = shoulder of end-bar pared to level of b. c = slat attached to side of bottom-bar, to raise its surface to coincide with that of the side-bar b. The side of each rhomb was ten times the mean width of the *A. m. scutellata* worker cell (Smith, 1960, p. 12). The accuracy of the rhombs as counting guides was enhanced by the fact that the area of an equilateral rhomb equals the area of an array of regular hexagons enclosed by it, provided the rhomb-side is a simple multiple of the centre-to-centre width of the hexagons.

enclosed 100 drone cells. On frames that had few drone cells the grid for worker cells was retained and used as a guide in counting drone cell occupancies individually.

A larva was classified as young when it did not completely cover the floor of its cell, a phase which lasts approximately three days (Smith, 1960). Old larvae, covering the floor of the cell, were taken to span four days: this distinction was based on the schedule of mean weights of drone larvae given by Jay (1963a), which show a sudden large increase from day three to day four in the life of the larva.

4.2.3 Quantification of worker numbers

In order to disturb the experimental colonies as little as possible, elaborate methods of counting workers in colonies (e.g. Bodenheimer, 1937; Farrar, 1937; Jeffree, 1951; Harbo, 1983) were avoided in favour of direct estimation of coverage of frames by workers (Winston, 1979a; Szabo, 1982) during certain brood-recording sessions. The proportion of each frameside covered by bees was estimated on a scale of 0.1 to 1.

4.3 RESULTS AND DISCUSSION

The time that elapsed between dequeening and onset of oviposition by workers varied between 5 and 7 days (chapter 5). In view of this regularity, all periods in brood rearing in the queenless colonies were given as "number of days after dequeening", except where otherwise specified.

4.3.1 Pilot observations

In colonies 2, 3, V and VI, early oviposition was restricted to a single frame, or to several adjacent ones (Figs. 4-2; 4-3). Thence, throughout the observation periods, oviposition and brood rearing occurred in sets of neighbouring combs. The outermost frames never carried brood of any kind.

The comb cells of colonies 2, 3, V and VI were initially occupied at a rate of 1000-1500 per day on days 5-10 after dequeening (Fig. 4-4). In colonies 2 and 3 this occupation reached an abrupt peak which in colony 2 attained over 6000 cells - more than twice the maximum in colony 3. This difference was maintained during the subsequent declines in the cell-occupancies of both colonies. In colonies V and VI the last two observations were too widely spaced to allow any inferences other than that, from day 20 after dequeening, colony V contained substantially more brood than colony VI. The total number of cells utilized in brood rearing in hopelessly queenless colonies thus appeared to vary considerably from colony to colony, although there were indications that the profile of cell occupancy through time might display some regularity.

In colony 2 eggs were initially present for five consecutive days without the appearance of larvae (Fig. 4-2). In colony 3 no larvae had appeared by day 4 after onset of oviposition and, in the ensuing 7 days, the larvae that did appear, between days 6-10 after onset of oviposition (Fig. 4-2), were in every instance insufficient in number to account for the quantities of eggs that had been in the colonies 3 days previously. The normal development period of European honeybee eggs of both sexes is 3 days (Jay, 1963) as it is for *A. m. scutellata* female eggs (Tribe and Fletcher, 1977). For the present study a development time of 3 days was assumed for male eggs of *A. m. scutellata*. The presence of eggs, but no

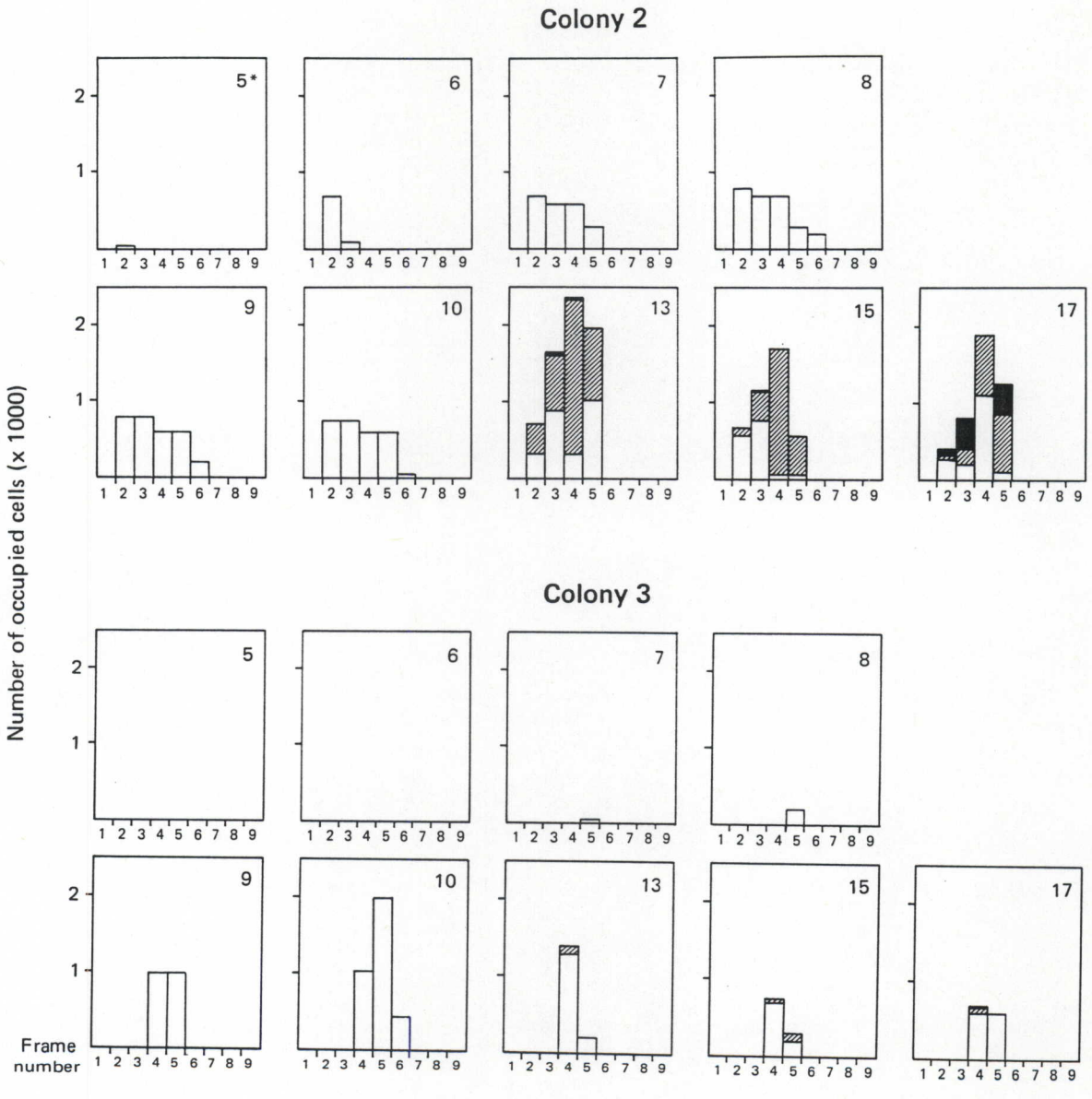


FIGURE 4-2. Pilot observations, colonies 2 and 3. Numbers of comb cells occupied by laying worker brood on observation days after dequeening. One bar represents the brood in both drone and worker cells on a single frame. (Data from Appendix Table 4-2.)

Cells with sealed brood: black
Cells with unsealed larvae: diagonal hatching
Cells with eggs: white
* number of days after dequeening

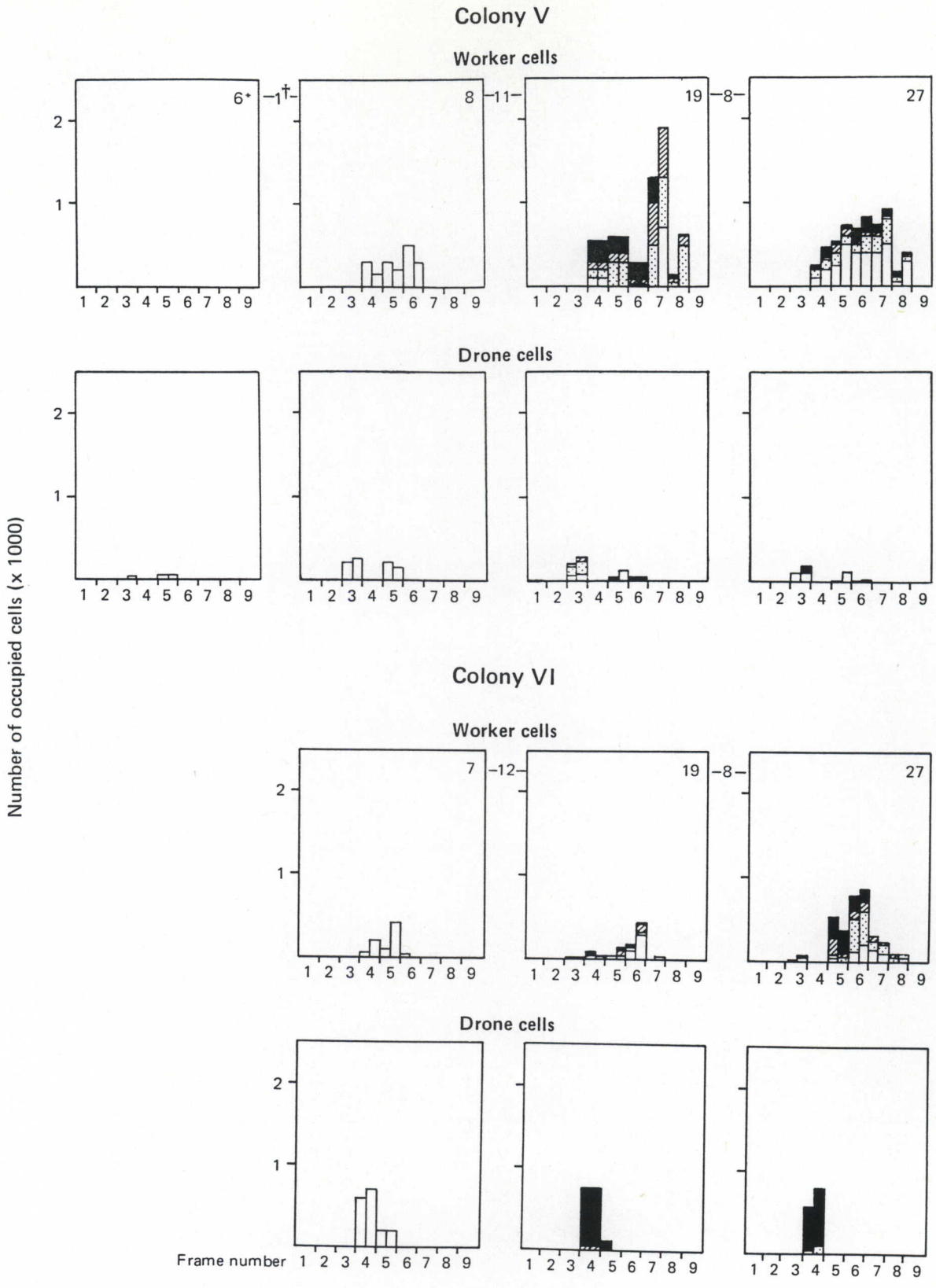


FIGURE 4-3. Pilot observations, colonies V and VI. Numbers of comb cells occupied by laying worker brood on observation days after dequeening. One bar represents the brood in drone or worker cells on one side of a frame. (Data from Appendix Table 4-5.)

Cells with sealed brood: black
Cells with old larvae (unsealed): diagonal hatching
Cells with young larvae: stippled
Cells with eggs: white
* number of days after dequeening
† number of days between observations

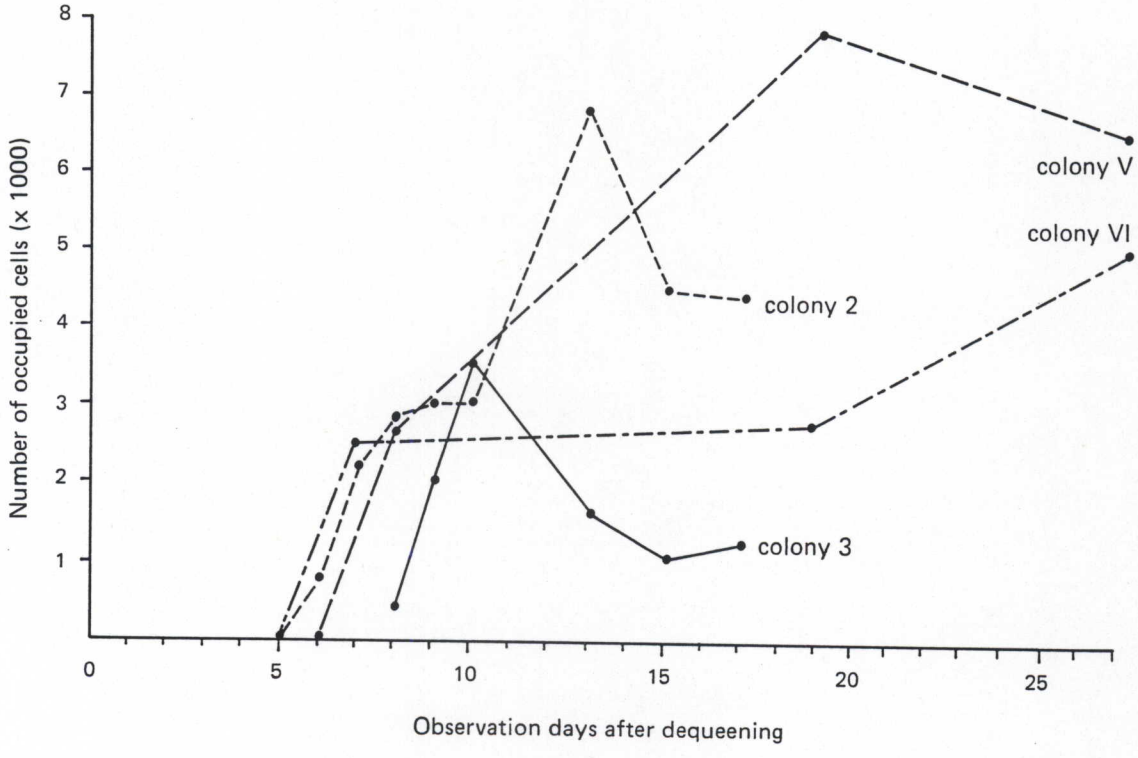


FIGURE 4-4. Pilot observations, colonies 2, 3, V and VI. Total number of cells occupied by laying worker brood on each observation day after dequeening.

larvae, beyond 3 days after the onset of oviposition in colonies 2 and 3 may have been the result either of inviability in the first-laid eggs, or of a slower development time in the eggs that were laid on the first and second days after commencement of oviposition, or of removal by nurse bees of eggs due to hatch on days 4 and 5 after the commencement of oviposition. The development period of honeybee eggs may vary between 2-6 days (Jay, 1963): the longer period was, however, artificially induced by chilling newly laid eggs for 3 days at 16-19°C below normal rearing temperature, a condition which was unlikely to have obtained in the large clusters of the newly queenless colonies of the present experiment. Eggs and other brood may die as a result of food deficiencies, diseases, inherent weaknesses, and adverse microclimatic conditions in the nest as determined especially by the density of workers covering the brood (Fukuda and Sakagami, 1968). Workers may eat or discard damaged or diseased brood; healthy brood may be removed in normal colonies, or in stressed circumstances (Woyke, 1977). Homozygous male eggs, produced by sibling mating, are routinely eaten (Woyke, 1963). In hopelessly queenless colonies, eggs and older brood may be thrown out of the hive (Butler, 1974). Thus there are various causes of death in honeybee brood, including circumstances in which nurse bees destroy healthy brood in colony-level processes of brood control. In the present study specific causes of the delays in advent of larvae from eggs were not determined.

With mortality the primary modifier, brood demography is fundamentally determined by the rate of oviposition and thence by the transitions from one development stage to the next, visually apparent in the combs as eggs, young larvae, old larvae, sealed cells (concealing the oldest larvae, and pupae) and emergence of adults. In a colony with brood at all stages of development the demography of the brood constitutes a multicomponent dynamic system, the interpretation of which is clarified

by reference to a model comprising a constant input of eggs and no brood mortality (Fig. 4-5). This graph resolves into three phases: first, an ascending profile of the broodless colony coming into brood production; second, the horizontal levels attained at "production equilibrium" by the various brood-stages; and third, a descending profile after the egg supply is cut off. (Fig. 4-5 is based on development times for drone brood. A similar model for worker brood would present a somewhat different profile owing to the different durations of the larval and pupal stages: see e.g. Smith, 1960; Fletcher, 1977b.) The equilibrium phase, in which input equals output at every brood-stage, is attainable only after brood rearing has been underway for the total development period of the brood and in normal colonies would be approached in worker brood during steady foraging conditions. This equilibrium would be less frequently attained in drone brood, which is produced more episodically than worker brood (section 4.1). The model is based on linear rates; curvilinear rates in real colonies would result in deviations from the relationships predicted by the linear model. Nevertheless, the linear model is adequate for preliminary interpretations of brood-rearing in hopelessly queenless colonies.

The brood demography of colony 2 (Fig. 4-6) may be interpreted as the production of a *batch* of brood in which an initial surge of oviposition diminished after the onset of rearing of larvae, from day 10 after dequeening onward. With reduction in egg production the quantity of cells with unsealed larvae declined as they were sealed, over days 13-17 after dequeening. In colony 3 there was also an initial surge of oviposition but, in contrast to colony 2, the quantity of unsealed larvae that was reared over days 10-17 after dequeening was relatively small (i.e. there was a higher mortality of eggs).

In colonies V and VI brood in drone cells was recorded separately from brood in worker cells, and young larvae were distinguished from old

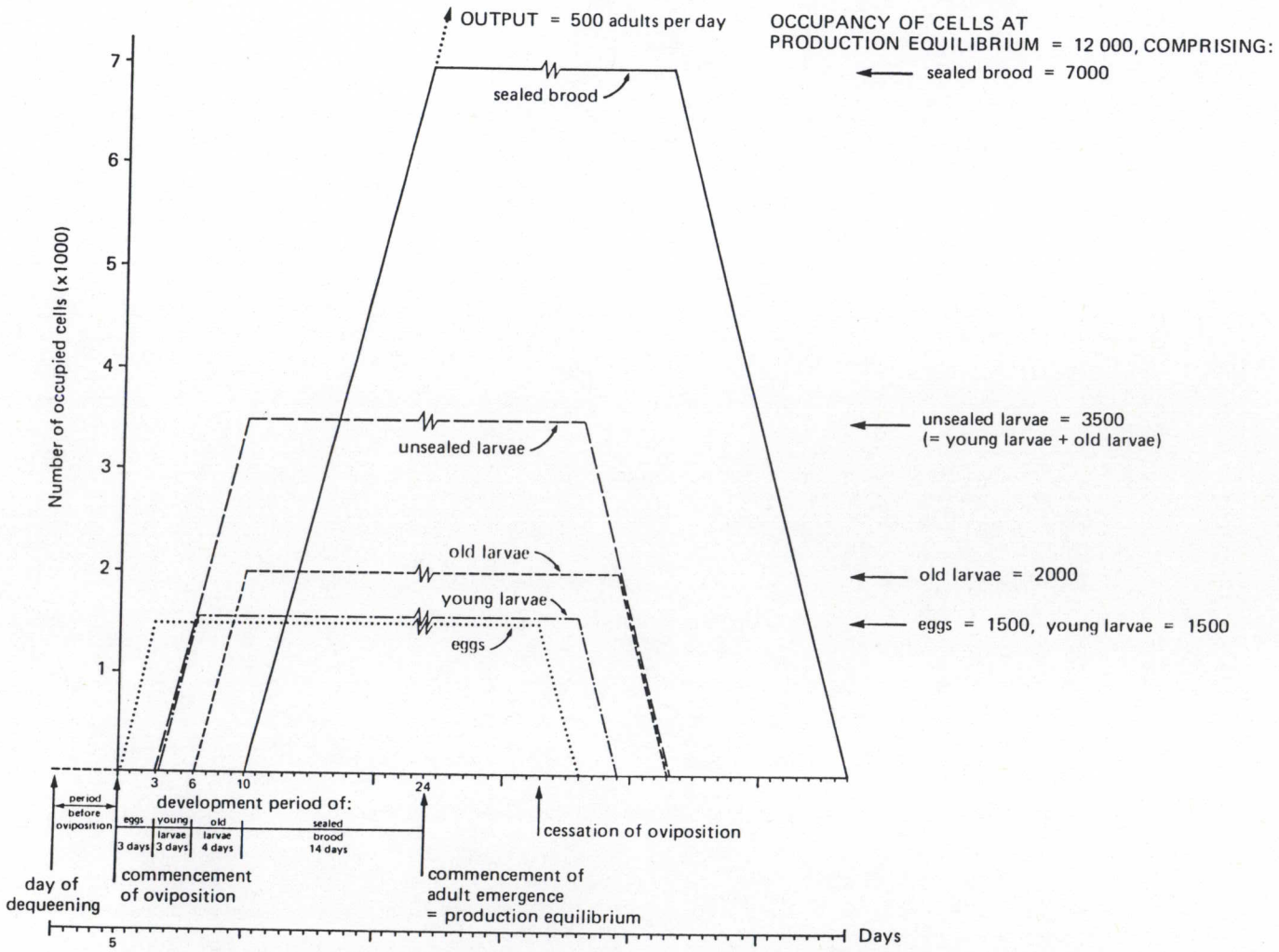


FIGURE 4-5. Hypothetical demography of drone brood production in an initially broodless colony, with an input of oviposition in 500 cells per day, and no mortality at any stage. Duration of life stages after Smith (1960) and Jay (1963).

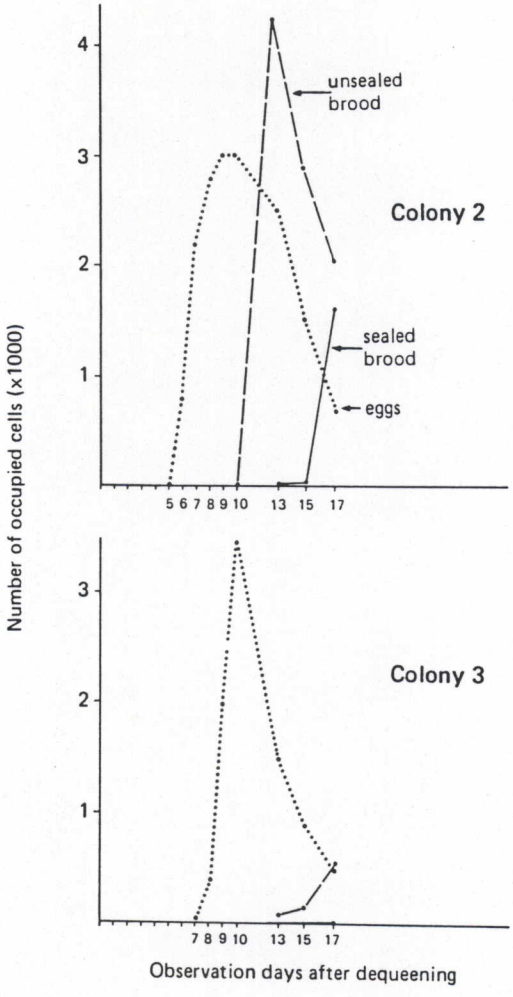


FIGURE 4-6. Pilot observations, colonies 2 and 3. Numbers of comb cells occupied by laying worker brood during 17 days after dequeening. (Data from Appendix Table 4-2.)

(Fig. 4-7). In the drone and worker cells of both colonies there were initial surges of oviposition, similar to those in colonies 2 and 3. In the worker cells of colony V, between days 19-27 after dequeening, a second surge of oviposition occurred, while occupancies of cells by unsealed and sealed brood declined together at approximately equivalent rates. In relation to the descending phase of the brood demography model (Fig. 4-5) the juxtaposition of these young and old unsealed larvae along the time axis was reversed, as was that between total quantities of unsealed brood and sealed brood. This indicates that the sealed worker brood of colony V was the product of a cohort of unsealed larvae previous to that recorded in the graph, and its descent can be attributed to a diminished input coupled with the emergence of adults. The decline of the unsealed brood must be attributed mainly to mortality in old and young larvae alike.

In the worker cells of colony VI (Fig. 4-7), between days 19-27 after dequeening, occupancy by unsealed larvae and sealed brood followed a different course to that in colony V. All stages were on the increase in what appeared to be the commencement of a brood-rearing surge, delayed by about 10 days in relation to the commencement of the initial surge of oviposition on day 5 after dequeening.

In colony VI on day 19 after dequeening, 1500 drone cells were occupied by sealed brood and 90 by old unsealed brood. This total approached the 1700 drone cells occupied by eggs on day 7 during the initial oviposition surge and represented a high survivorship of brood of all stages in this initial phase of brood rearing. In comparison to the simultaneous low survivorship of brood in worker cells, it appears that the main thrust in this colony at this stage was towards rearing of full-sized drones.

In colony V, 800 drone cells contained eggs by day 8 in the initial oviposition after dequeening. By day 19 all 800 of these cells were

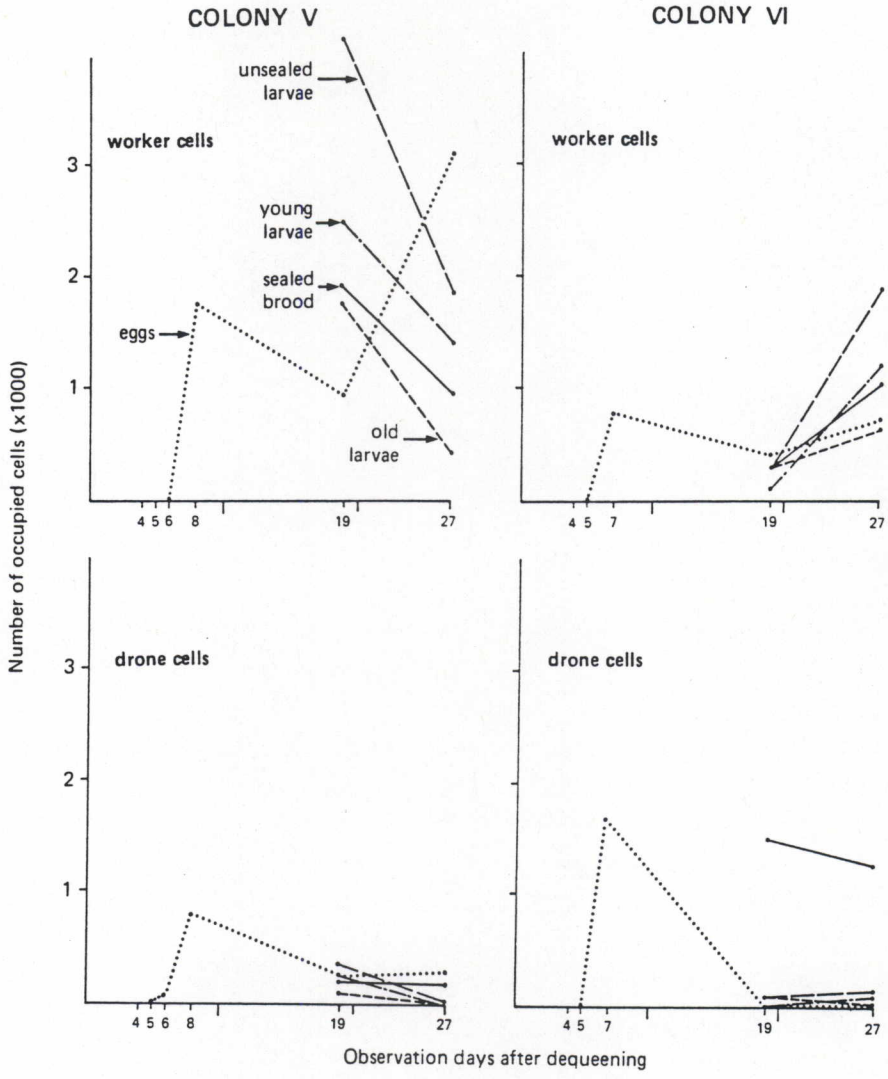


FIGURE 4-7. Pilot observations, colonies V and VI. Number of worker and drone cells occupied by laying worker brood during 27 days after dequeening. (Data from Appendix Table 4-4.)

occupied by a mixture of eggs, unsealed brood (young and old) and sealed brood. The production of this mixture must have been the result of the death of some of the original batch of eggs and larvae between days 8-19 after dequeening, and of the replacement of them by new eggs and larvae. Thus, in spite of mortalities, colony V reared many full-sized drones, as well as up to 2000 undersized drones in worker cells.

4.3.2 Detailed observations in colonies A, B, C and D

Four colonies (A-D) were dequeened and observed by the procedures developed for colonies V and VI (section 4.3.1) with the aim of recording the demography of each colony's brood, from the onset of oviposition through to the death of the colony. Observations were started 4-6 days after dequeening and were taken mostly at weekly intervals (*see* Appendix Table 4-4 for the actual intervals).

4.3.2.1 Eggs and brood in the individual cell

Eggs were placed irregularly on the cell walls, bases and rims; some were laid in cells half filled with pollen, but *none were seen in cells containing larvae*. A few eggs were shrivelled and smaller than normal. Occasional counts showed a wide variation in the number of eggs per cell, ranging from 1-10 in worker cells and up to 25 in drone cells. Generally, patches of cells with eggs tended to carry similar numbers of eggs per cell. In cells with bee milk there was invariably only one egg or young larva to a cell, which indicated that nurse bees removed badly placed and excess eggs: whatever the means, the untidy oviposition of the workers was brought to order as brood rearing commenced, resulting in the rearing of one individual per cell, as in the queenright colony. This process was

observed in the laying worker brood of *A. m. capensis* by Onions (1912).

Worker cells carried dome shaped cappings. Undersized drones were seen in all four colonies, from three to four weeks after the commencement of brood rearing, until the death of each colony.

4.3.2.2 Distribution of brood through the frame sides

The distribution of eggs and brood in worker cells through the frames of colonies A-D (Fig. 4-8) confirmed the pilot observation that initial oviposition and subsequent brood rearing was restricted to sets of neighbouring frames in the middle of the colony, indicating the maintenance of a brood area as in queenright colonies.

When the experimental colonies were made up, frames were assigned to the colonies at random. In cases such as colony D, which had very few drone cells, the distribution of drone brood was clearly restricted by the availability of drone cells (Fig. 4-8). The quantity and distribution of drone cells in experimental colonies is thus a factor that should be carefully considered in future studies of worker oviposition. In the two colonies in which drone cells were available in the outermost combs (A and B) brood rearing was restricted to those on the inner combs. Of these, all available cells were filled only once, on day 7 in colony A. In colony C, oviposition and brood rearing in the drone cells of frame 4 was consistently nil, or minimal, even though it was carried on apace in the adjacent frames (Fig. 4-8), indicating that occurrence of drone cells within the broodnest is not the only criterion for whether brood is reared in them (it was not possible to determine what discouraged brood rearing in those particular cells).

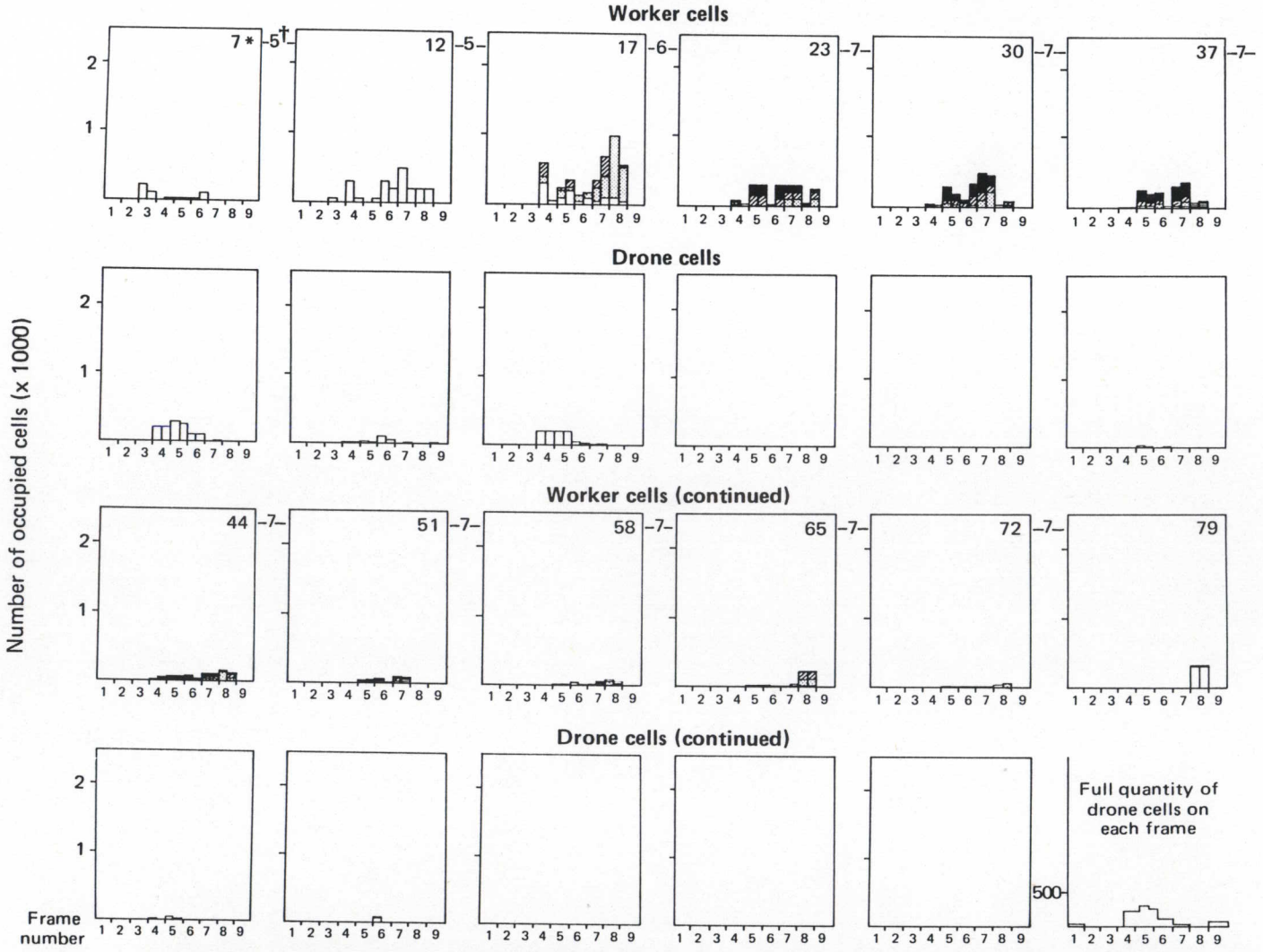


FIGURE 4-8. Colonies A, B, C and D. Numbers of worker and drone cells occupied by laying worker brood on observation days after dequeening. Each bar shows the occupancy of a single frame side. (Data from Appendix Tables 4-3 and 4-5.) *Above:* Colony A.
Cells with sealed brood: black; cells with old larvae (unsealed): diagonal hatching; cells with young larvae: stippled; cells with eggs: white.
* number of days after dequeening; † number of days between observations.

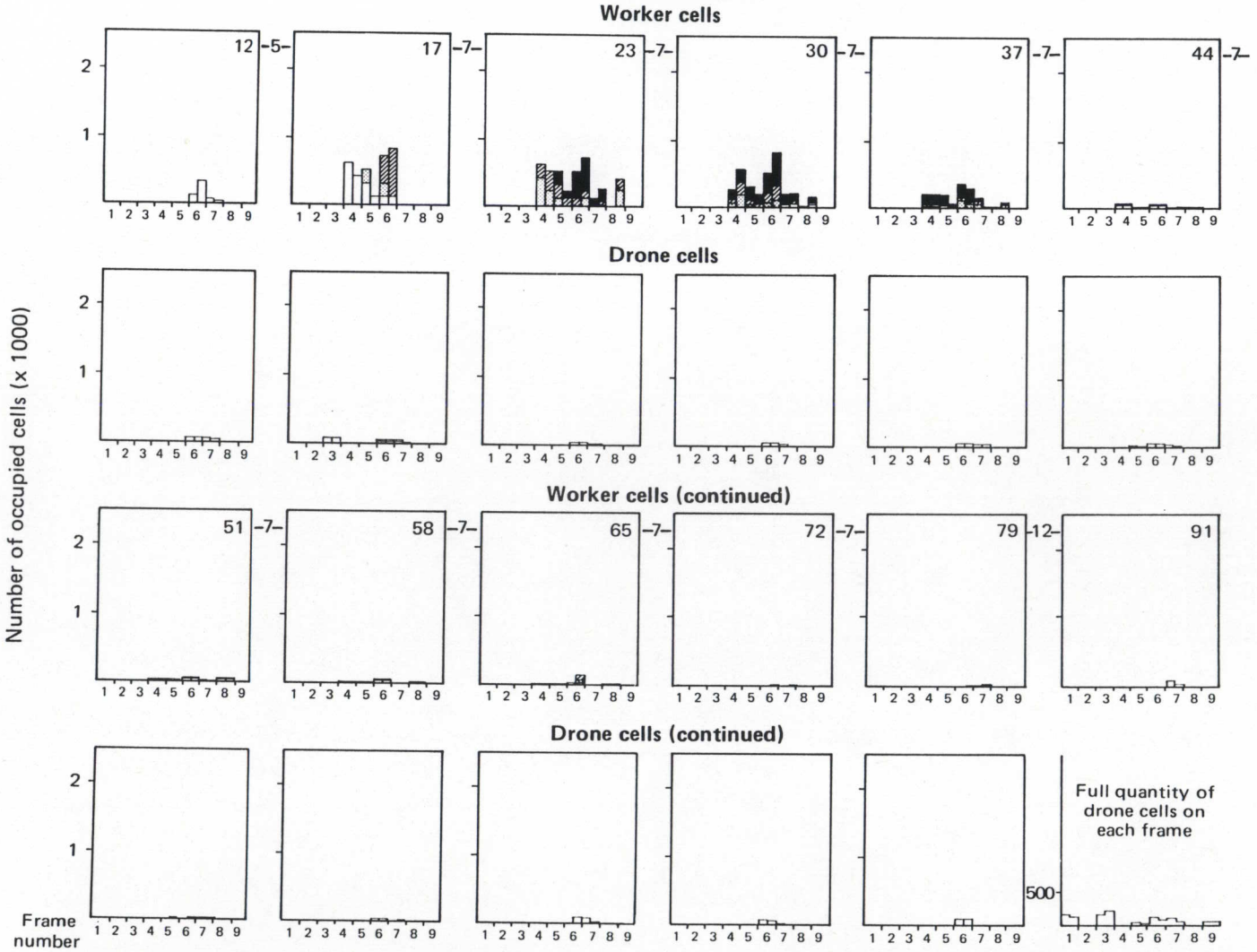


FIGURE 4-8. *Continued.* Colony B.

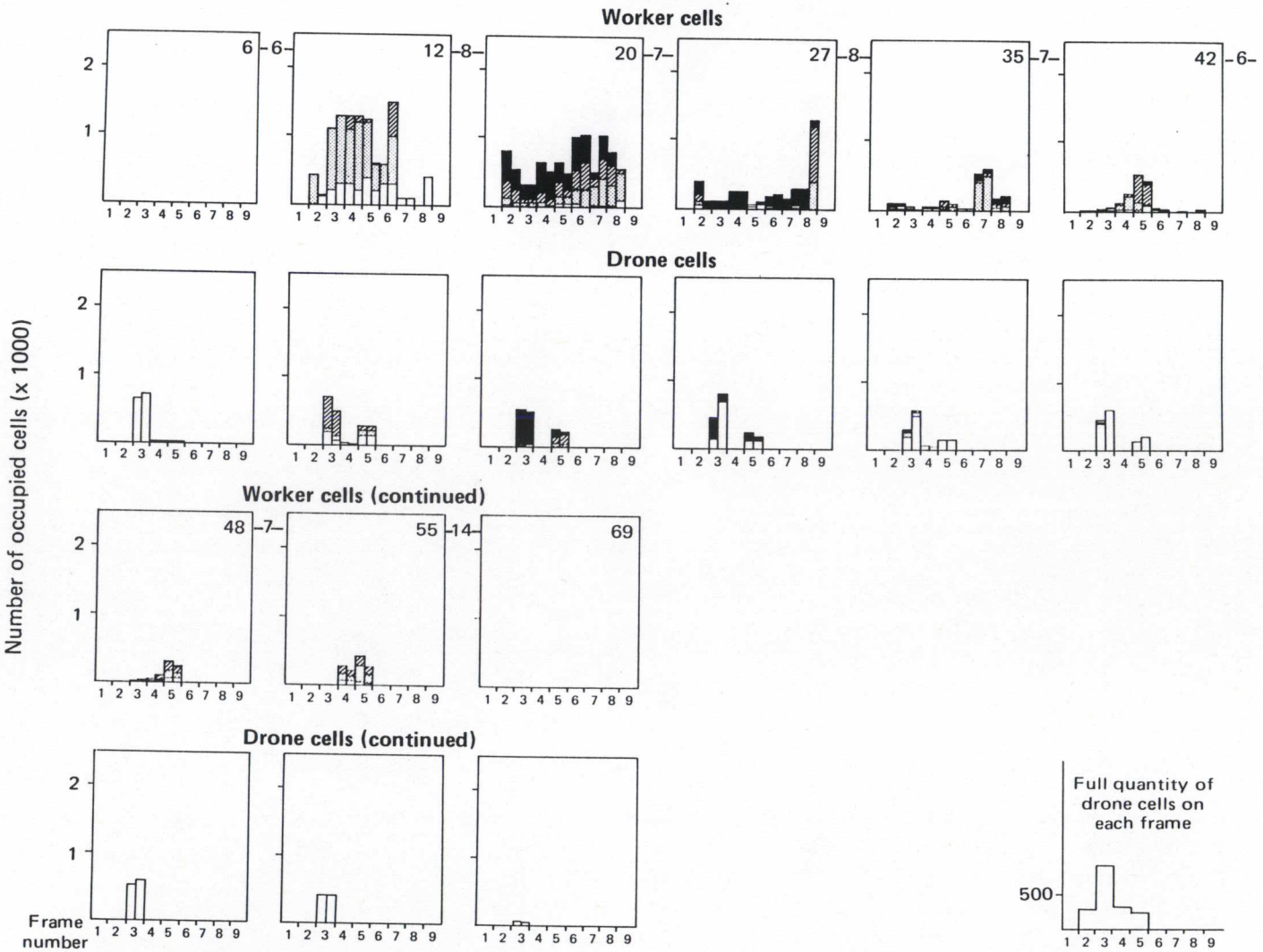


FIGURE 4-8. *Continued.* Colony C.

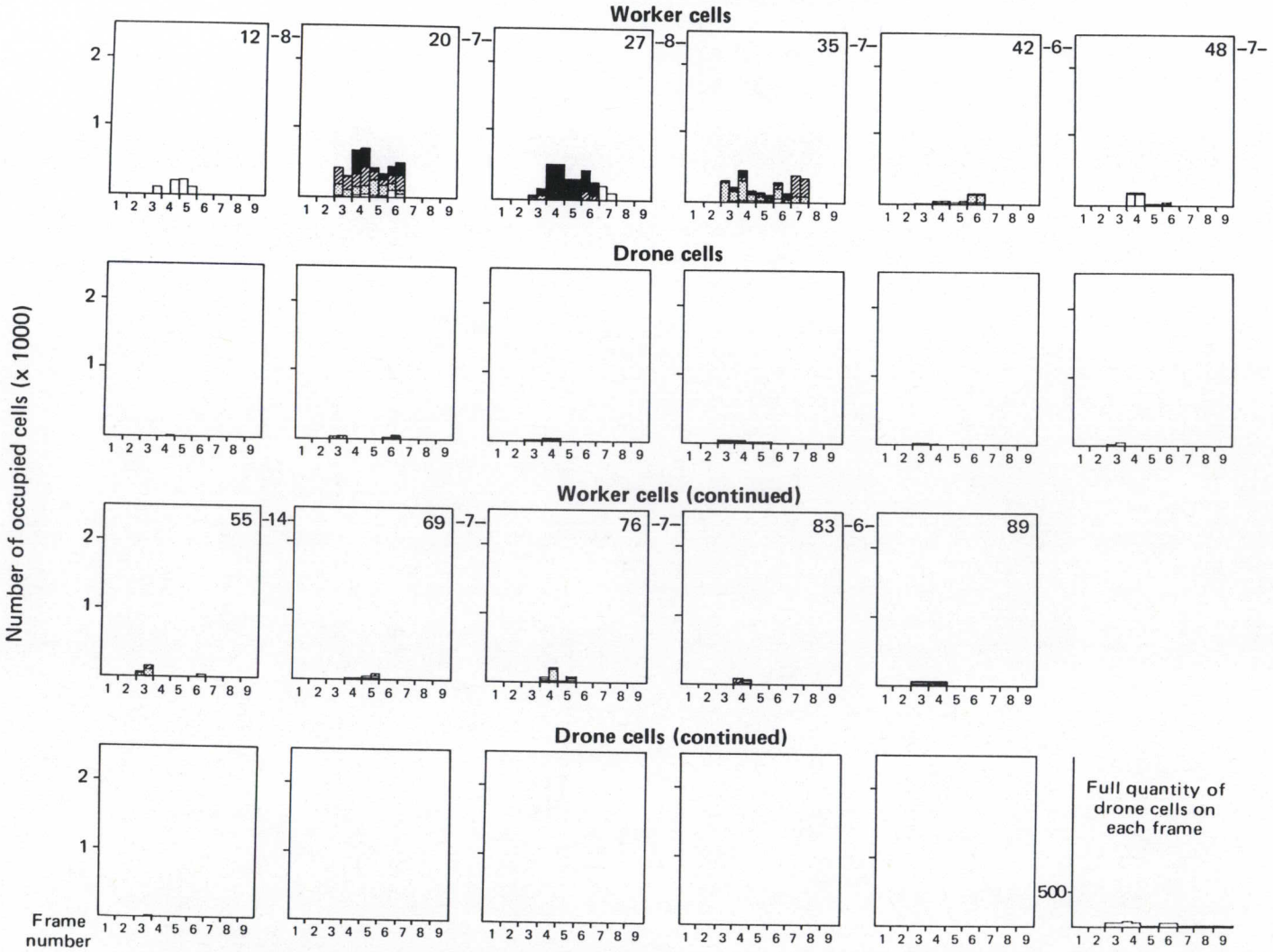


FIGURE 4-8. *Continued.* Colony D.

4.3.2.3 Total number of cells occupied at each observation

Occupation of worker cells in colonies A, B and D commenced between days 5-12 after dequeening and thereafter increased at rates of 300-500 cells per day until days 17-20, by which time totals of 2660-4274 cells were occupied (Fig. 4-9). Initial oviposition in colony C was more rapid and extensive, with 10 130 cells occupied between days 6-12, more than twice the maxima attained in the other three colonies. After the initial surges (between days 12-20 after dequeening) there was a levelling in cell occupancy in colonies B, C and D followed by a variable decline to a low occupancy of 1000 cells or less, from about day 47 after dequeening onwards. The occupancy of colony A followed a similar course, except for a marked decline (over days 16-23) before levelling out. After about day 47 after dequeening, brood rearing continued at a low level almost until the death of each colony.

Thus, although variable between colonies, a distinct form could be discerned in the occupancy of worker cells in the queenless colonies, constituting an initial surge of oviposition followed by rearing of a large batch of brood (as identified in the pilot observations); thence, brood was reared at a low level over a prolonged period until the colonies died out. The components of these curves are considered in the next section.

The uneven amounts of drone cells provided to the colonies precluded interpretation of the demography of the brood in them, other than the fact that oviposition generally commenced in the drone cells a few days before it did in the worker cells (see chapter 5). After commencement of oviposition, drone cells were occupied at fairly consistent levels in colonies B, C and D (Fig. 4-9).

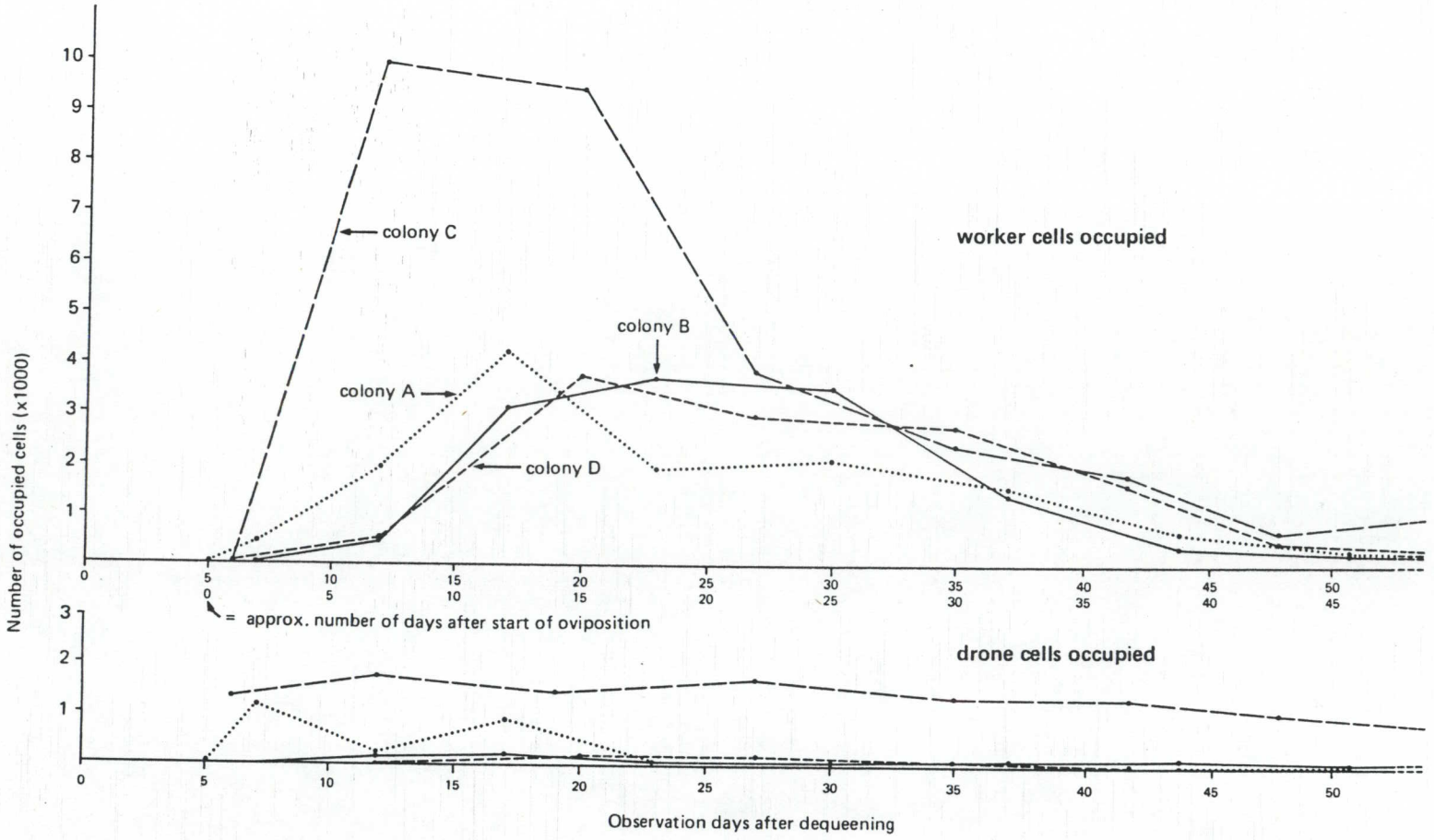


FIGURE 4-9. Colonies A, B, C and D. Numbers of comb cells occupied by laying worker brood (all stages together) on observation days after dequeening. (Data from Appendix Table 4-4.)

4.3.2.4 Occupation of cells by eggs, larvae and sealed brood

In the worker cells of colonies A-D the onset of oviposition and brood rearing (Fig. 4-10) was of a form similar to that indicated in the pilot observations: initial extensive oviposition was followed by production of a batch of sealed brood, ranging from approximately 1000 in colony A to 4000 in colony C, which then declined steadily over 15-20 days, as adults emerged, to low erratic levels over a prolonged period thereafter. In colonies C and D there was a resurgence of unsealed brood on days 30-40 after dequeening, but no further large batches of sealed brood were reared. This indicated that once the hopelessly queenless colony has reared an initial batch of offspring it will not repeat the process, even though a start may be made. The proximate causes of this decline in the initial ovipositional surge were not the concern of the present study: it may have been associated with exhaustion of the egg-laying capacities of the laying workers, or nurse bees could have destroyed excess eggs after the initial surge, or it could have arisen from inhibition of worker egg laying, by the accumulation of brood (chapter 2) in the colonies and by worker-produced queen substance (section 3.1).

A variety of survivorship patterns was evident in the worker brood. Thus although the initial oviposition curve in colony C was almost identical in shape and magnitude to those in colonies A and B, subsequent production of larvae and sealed brood was much higher in colony C than in colonies A and B: i.e. the survivorship of eggs in C was greater. High survivorship of eggs was seen also in colony D which reared larvae and sealed brood in amounts similar to colonies A and B, but from a much lower quantity of eggs. During the initial phase of brood rearing in colonies A and C, the highest quantities of young larvae were much higher than any quantities attained subsequently by old larvae and sealed brood. This

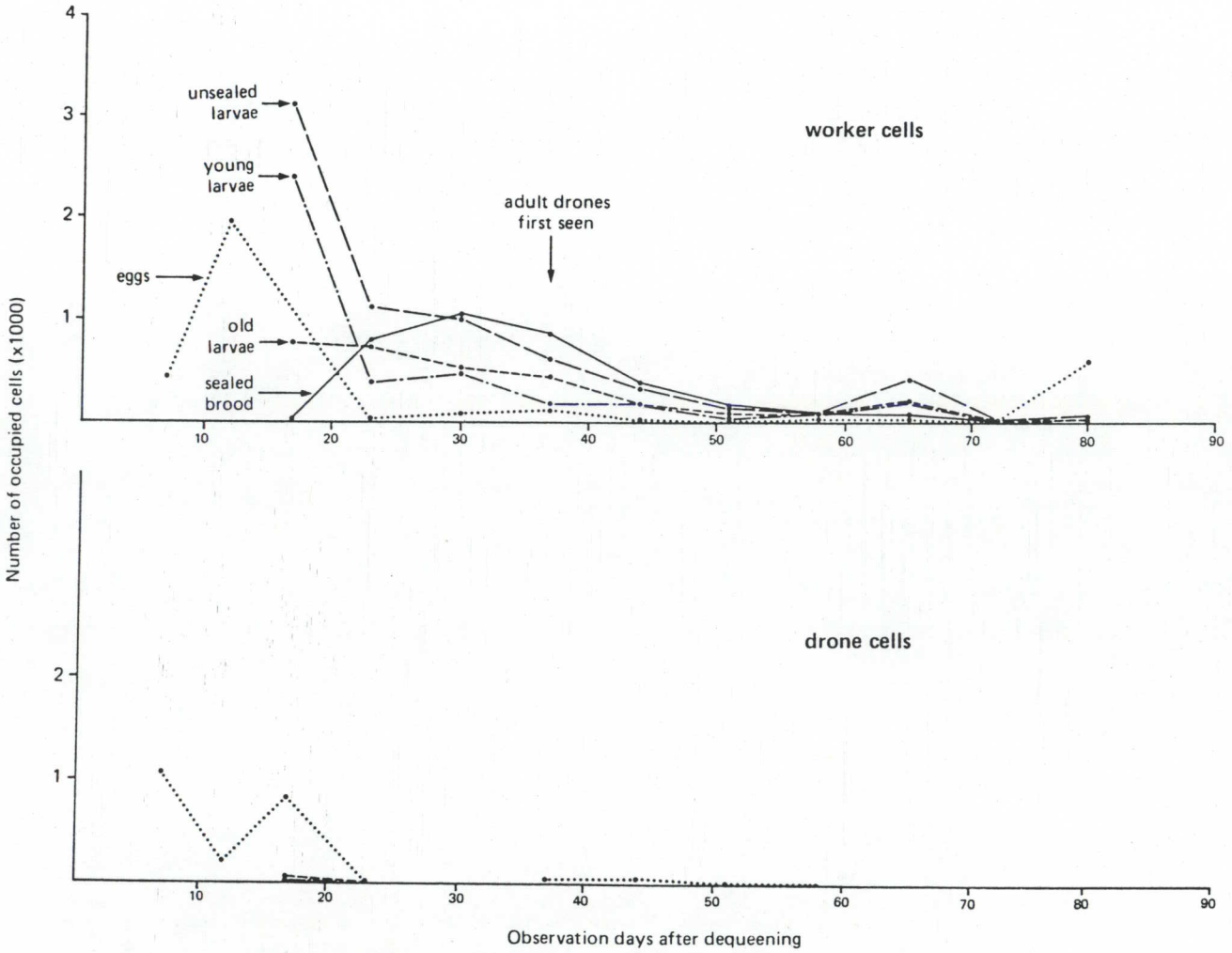


FIGURE 4-10. Colonies A, B, C and D. Numbers of worker and drone cells occupied by laying worker brood on observation days after dequeening. (Data from Appendix Table 4-4.) *Above:* Colony A.

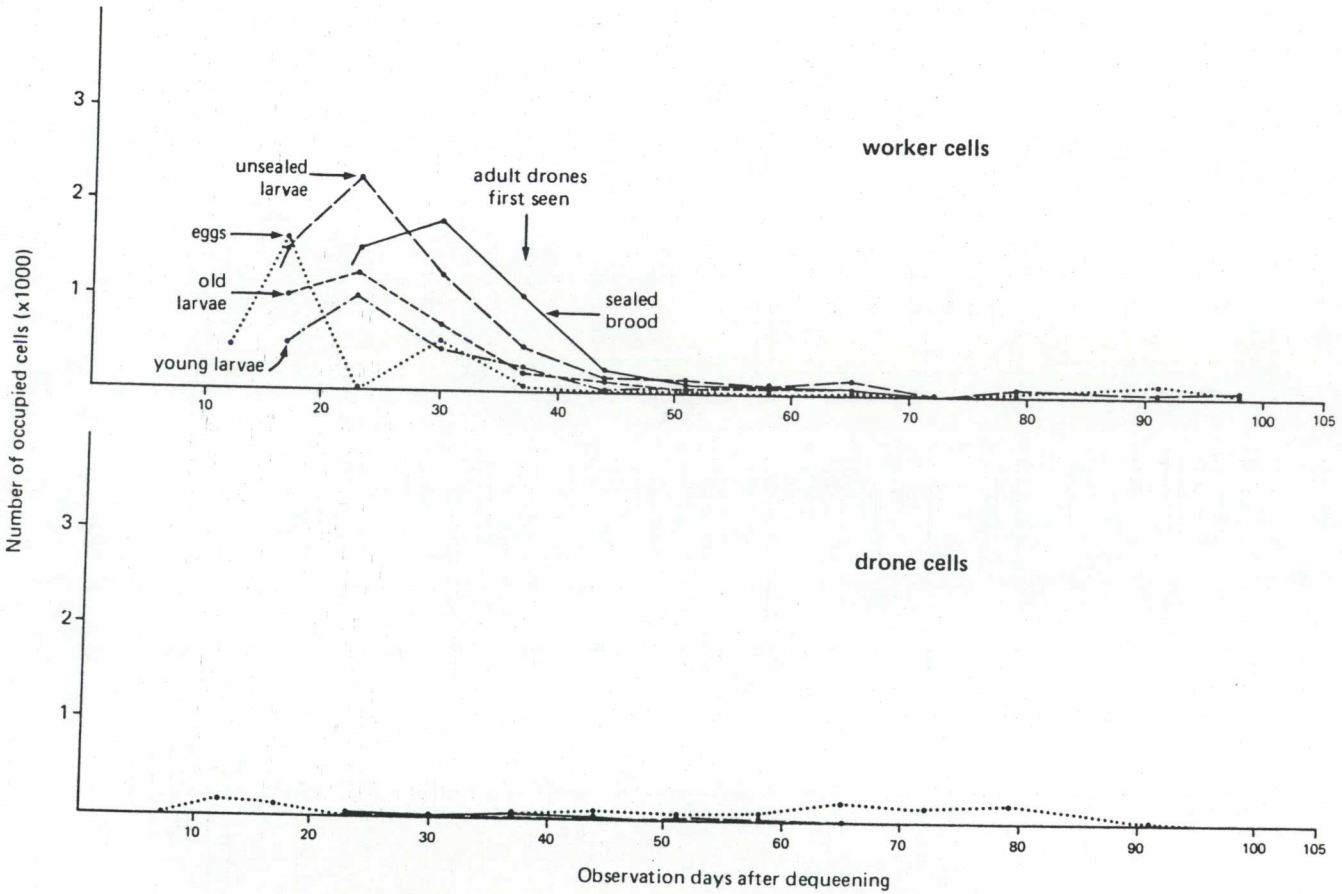


FIGURE 4-10. *Continued.* Colony B.

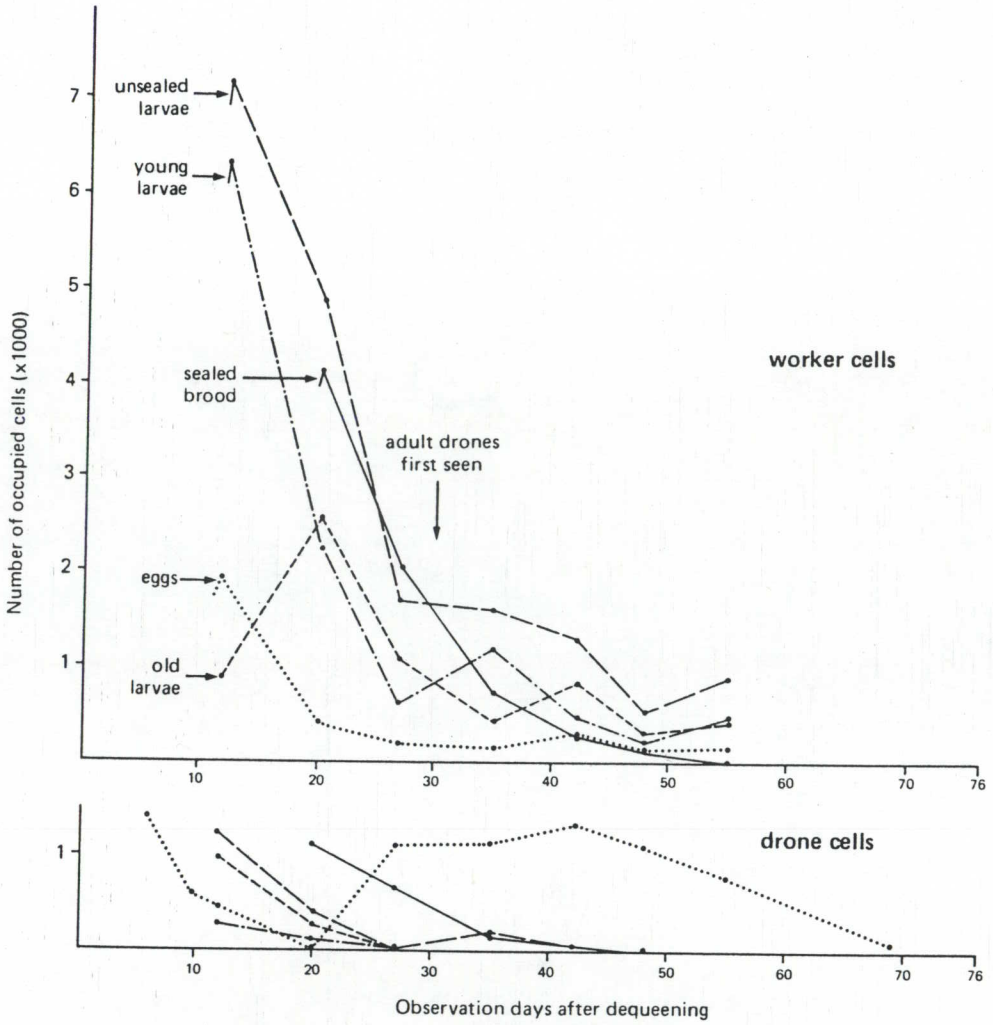


FIGURE 4-10. *Continued.* Colony C.

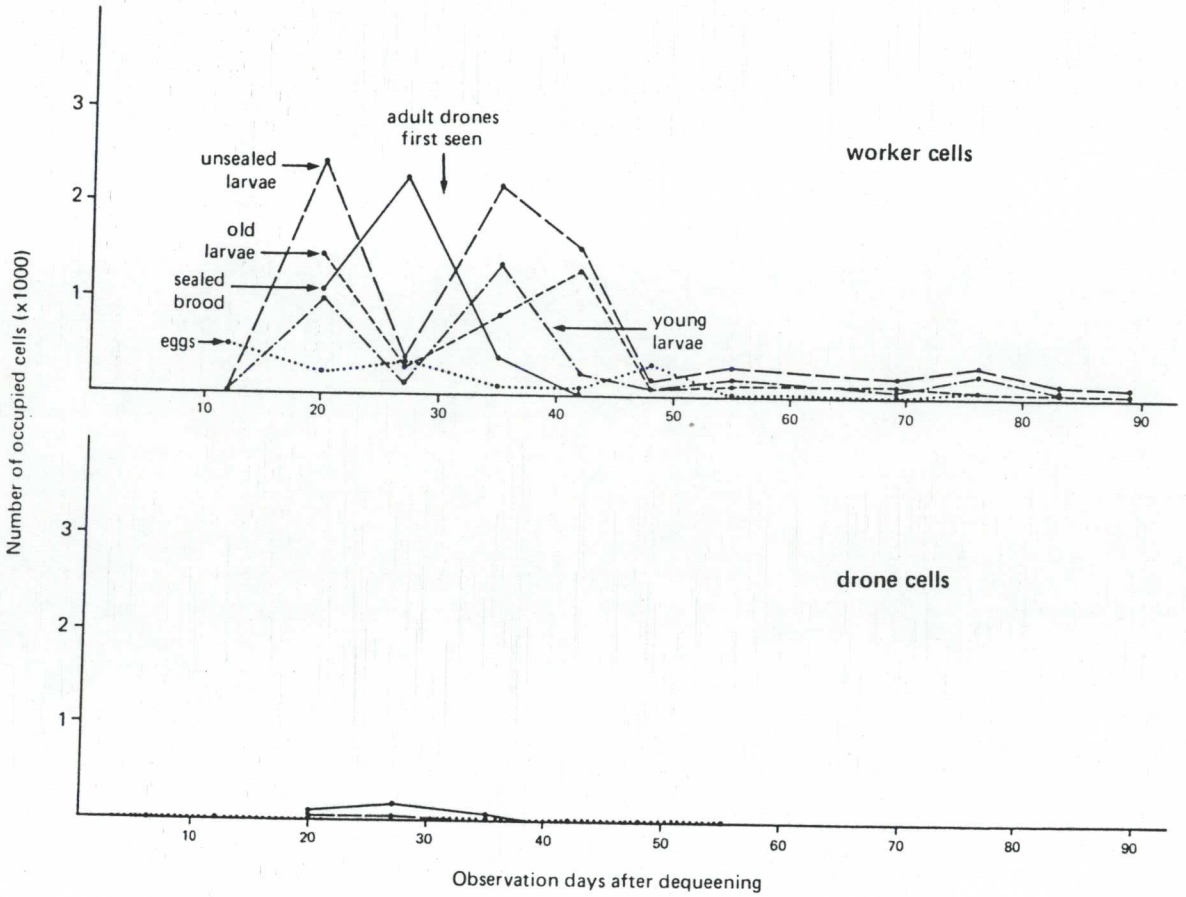


FIGURE 4-10. *Continued.* Colony D.

represented a marked reduction in brood quantity during the transition from young to old larvae. By contrast, in colonies B and D the high peaks of young larvae were topped by those of old larvae, indicating a *high* survival rate of young larvae in these colonies. In all four colonies there were high levels of survival in the transition from old larvae to sealed brood, as reflected by curves of roughly equivalent shape and magnitude for the two stages, the sealed cells following on from the old larvae.

The total number of drone cells supplied to each of the experimental colonies was: colony A 1364, colony B 1215, colony C 3744, colony D 485, distributed on the framesides as shown in Fig. 4-8. Choice of cell type at commencement of oviposition is discussed in chapter 5. When the experimental colonies were assembled (method: section 3.2.3) the combs were placed at random, so that the distribution of the drone comb did not approximate that of a natural nest. Within these limitations, considerable variation in drone brood demography was evident between the colonies (Fig. 4-10). Oviposition in the drone cells of colony C started with a surge, dropped away to near zero on day 20 after dequeening, and was resumed at a high level over the next 35 days. A single large batch of brood was reared between days 10-30, producing sealed brood from which at least 1000 full-sized drones could have emerged. This coincided with the high production of sealed brood in the worker cells. Thereafter, the levels of drone cells occupied by larvae and sealed brood dwindled away to almost nothing. A similar situation obtained in colony D, at a very much lower level (Fig. 4-10), where a batch of sealed brood was produced in drone cells over days 20-35 after dequeening, coinciding with the major production of sealed brood in the worker cells; after this, production of sealed brood continued in the drone cells at a very low level. In colony B there was a small initial surge of oviposition, followed by a prolonged production of sealed brood in drone cells at a low level right up to day 72 after dequeening.

Colony A had a large initial oviposition, but scarcely any brood was reared. From these results it is evident that queenless colonies of *A. m. scutellata* will in general produce drones in drone cells as well as drones in worker cells. In hopelessly queenless European colonies workers preferred to lay in drone cells and preferred the central brood rearing area within the hive (Page and Metcalf, 1984).

4.3.2.5 Drones in the colonies

The first appearance of drones in the queenless colonies (Fig. 4-10) coincided with the emergence times expected from a sealed-brood period of approximately two weeks. After their first appearance, drones of all sizes were always present until the demise of each colony and were occasionally seen flying to and from the hive entrance. No attempt was made to count the drones, since this procedure, rendered difficult by the presence of many worker-sized drones, would have caused excessive disturbance to the colonies. If the maximum number of sealed cells is taken as roughly representative of the number of drones that emerged into the colonies from the initial batches of brood, then peak production of worker-sized drones ranged from a minimum of 1060 in colony A to a maximum of 4120 in colony C; and peak production of full-sized drones (limited, as stated above, by the drone cells given to the colonies) ranged from 30 in colony A to 1500 in colony VI.

During summer European colonies usually contain several hundred drones and 2000-4500 over the main swarming period (Page, 1981; Lee and Winston, 1987). The demography of drones in queenright *A. m. scutellata* has not been studied, but the fact that colonies of this race may swarm and migrate throughout the year (Seeley, 1985, pp.145, 149) makes brief, large buildups in drone numbers unlikely (Lee and Winston, 1987). It is thus

possible that hopelessly queenless colonies of *A. m. scutellata* may at times contain many more drones than the queenright colonies in the neighbourhood.

4.3.2.6 Demography of workers in the queenless colonies

When colonies A-D were dequeened all brood was also removed, so that there was no replenishment of the workers in the colonies from this point onwards. (It is possible that drifters entered the colonies, although the colonies were kept in isolated positions in a row of trees to reduce drifting.) Workers survived in colony C for approximately 11 weeks; in D for 13 weeks; in A for 14 weeks; in B for 16 weeks (Fig. 4-11).

The estimations of the quantities of workers in the colonies in Fig. 4-11 were not particularly accurate (section 4.2.1) and were performed at midday, when some of the workers were out foraging. Further, the emergence of worker-sized drones into the colonies would have affected some of the estimations. These inaccuracies are reflected for instance in the curve for colony D, which was lower at week 4 than at weeks 6-8. Nevertheless the curves in Fig. 4-11 are all similar in form, and in each colony indicate a high rate of mortality in the first 3 weeks after dequeening (as found in queenless Africanized bees by Delaplane and Harbo, 1987b), after which the mortality slowed, then increased slightly at around week 7 (in colonies A, B and C), and then continued slowly and steadily until the death of each colony, 5-9 weeks later. In colony D the decline was somewhat more erratic than in the other 3 colonies.

In queenright colonies of European races the life span of a worker is 4-6 weeks in summer and 3 months or more during winter hibernations (Free and Spencer-Booth, 1959; Fukuda and Sekiguchi, 1966; Sakagami and Fukuda, 1968; Butler, 1975). No specific measurements of the life span of

A. m. scutellata workers in Africa were found in the literature consulted for the present study: Smith (1960) and Anderson *et al.* (1983) gave it as about 6 weeks. In South America the mean life span of queenright Africanized bees seldom exceeded 20 days (Winston, 1979b; Winston *et al.*, 1981; Winston and Katz, 1981); In Brazil no marked workers survived beyond 40 days in a queenright colony whereas some did in a queenless colony (Costa Leonardo, 1985) so that queenlessness may have induced a longer life span in some workers. The mean life span of *A. m. scutellata* workers in queenright colonies is thus likely to be fairly short, perhaps about a month with a maximum of 6 weeks. Thus if worker longevity were unaffected by the advent of hopeless queenlessness, colonies A-D would have died out after about 6 weeks of hopeless queenlessness. However, some of the workers of these colonies lived for 11-16 weeks, i.e. 3-4 times as long as queenright workers ordinarily do. This extended survival may be interpreted as an adjustment, or response, to hopeless queenlessness. Two colony-level effects of this were to prolong the survival of the drones reared in the initial batch and to prolong drone-brood rearing at a low level after production of the initial batch.

Worker longevity is increased by the free availability of protein (pollen) in early life and by exemption of workers from brood rearing duties (de Groot, 1953), as during hibernation in European bees but not, of course, in *A. m. scutellata*. Hence queenlessness, through diminishment in the quantities of brood to be reared, may result in increased worker longevity (Maurizio, 1950). Omholt (1988) has recently drawn a strong hypothesis that winter-bee longevity is induced in young bees that are absolved of heavy nurse duties when brood rearing is curtailed at the end of summer. Perhaps the longevity of the hopelessly queenless workers of the present study was induced in the young bees when their nurse duties were abruptly terminated with the removal of all brood from their colonies.

4.3.2.7 Decline and death of the colonies

In colony D the last surviving adults died or disappeared at the same time as brood rearing ceased, whereas adults survived the brood by three weeks in colony A, two weeks in colony B, and one week in colony C (Table 4-1).

On day 72 after dequeening the 46 remaining sealed cells in colony A were opened and examined. Thirty five cells contained live pupae; 7 had dead pupae, inverted in their cells with their tails facing the cell opening (a rare condition in queenright drone and worker brood: Jay, 1963b); and 4 had dead, normally aligned pupae. After a brief resurgence of oviposition (in 600 cells, on day 79), brood was absent from colony A by day 89. The cluster of workers and drones dwindled from 0.5 framesides on day 79 to 32 individuals 19 days later, on day 98. Wax moths (*Galleria mellonella* Linnaeus) contributed to the demise of the colony, but were kept at bay until the last two weeks of the colony's life.

Colonies B, C and D were left to decline intact. Colonies B and C dwindled slowly, in the same way as colony A. Colony D declined more rapidly: on day 89 it had 1037 adults and 139 worker cells with brood, but 4 days later all brood had died and the workers were reduced to several hundred, dead on the hive floor.

TABLE 4-1. Queenless colonies A, B, C and D. Duration of brood rearing and adult survival. (Data from Appendix Table 4-5).

Colony	(i) Duration of adult survival		(ii) Duration of brood rearing		(i) *minus* (ii) =	
	days	weeks	days	weeks	days	weeks
A	98	14.0	79	11.3	19	2.7
B	111	15.9	98	14.0	13	1.9
C	76	10.9	69	9.9	7	1.0
D	89	12.7	89	12.7	0	0.0

In the dying stages of all four colonies, 10-20 drones of all sizes were maintained until the last and the combs where the workers clustered were kept clean and free of wax moths and hive beetles *(Aethina tumida* Murray). However, hygiene deteriorated in combs outside the clusters and debris accumulated on the floors and entrance boards.

After colonies A and C had died out few dead bees remained in the hives, but there were several hundred in colonies B and D, from which samples of 50 individuals were collected and dissected to determine the extent of their ovarian development (method: section 3.2.4). All ovaries were undeveloped except in one individual from colony D, which had slight development. All the undeveloped ovaries were of the usual slender shape and it could not be determined whether any of them had retrogressed from a state of higher development. The muscles, connective tissues and organs of the abdomens of 70% of the specimens of both colonies (B and D) had a sooty black coating, which may have been symptomatic of "melanosis" (*see* Gilliam, 1978).

4.3.2.8 Emergency queen cells

When the queenless colonies were assembled no comb carried a queen cell. All queen cells were removed from the experimental colonies at each observation. Thus the queen cells recorded in Figs. 4-12, 4-13 and 4-14 had been drawn anew, or replaced, since the previous examination.

By day 2 after dequeening, 6 and 11 queen cells had been drawn in colonies 2 and 3 respectively (Fig. 4-12). One to 15 queen cells had been drawn by the first observation day (days 4-6 after dequeening) in colonies V, VI, and A-D (Figs. 4-13; 4-14). These first cells did not always contain eggs.

The rate at which queen cells were replaced between observations

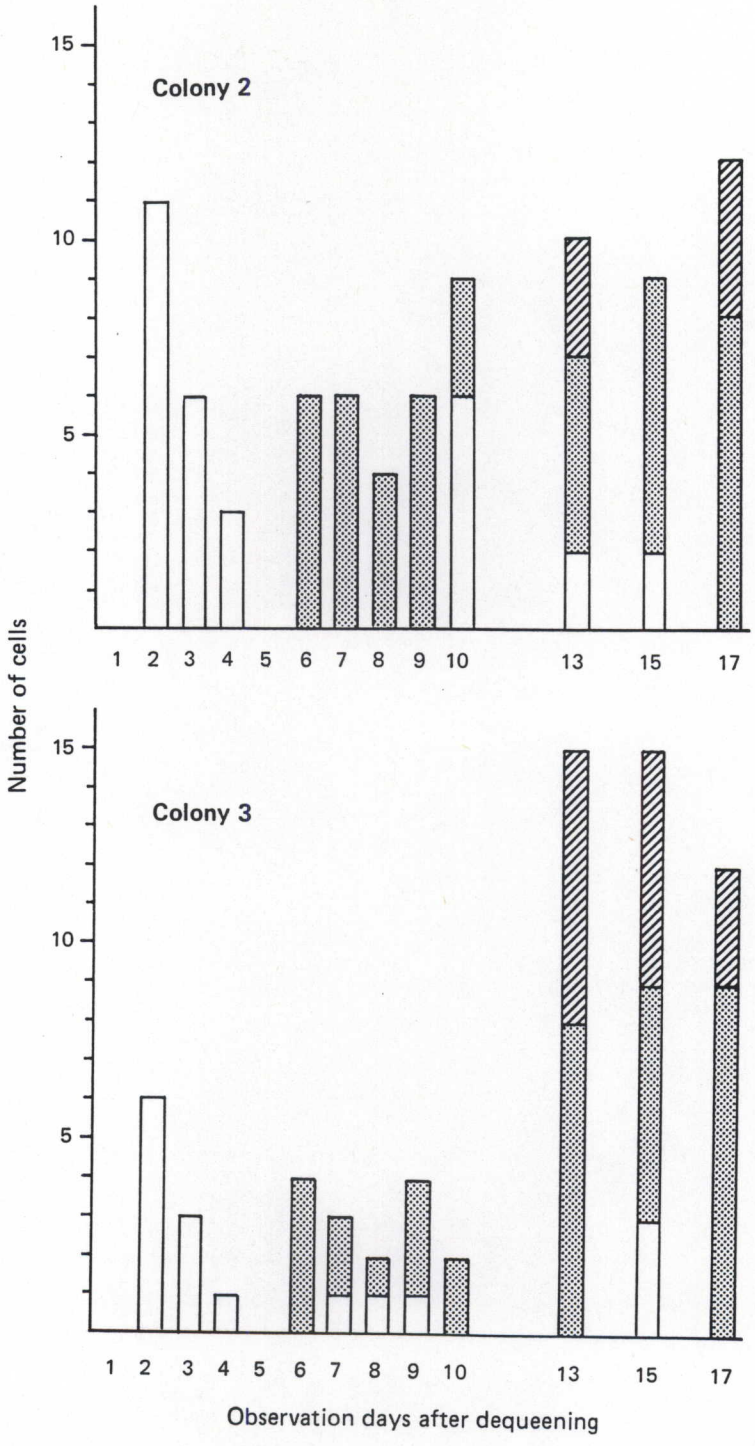


FIGURE 4-12. Pilot observations, colonies 2 and 3. Queen cells seen on observation days after dequeening. (Data from Appendix Table 4-1.)

Young larva in cell : diagonal hatching
Eggs in cell : stippled
Cell empty : white

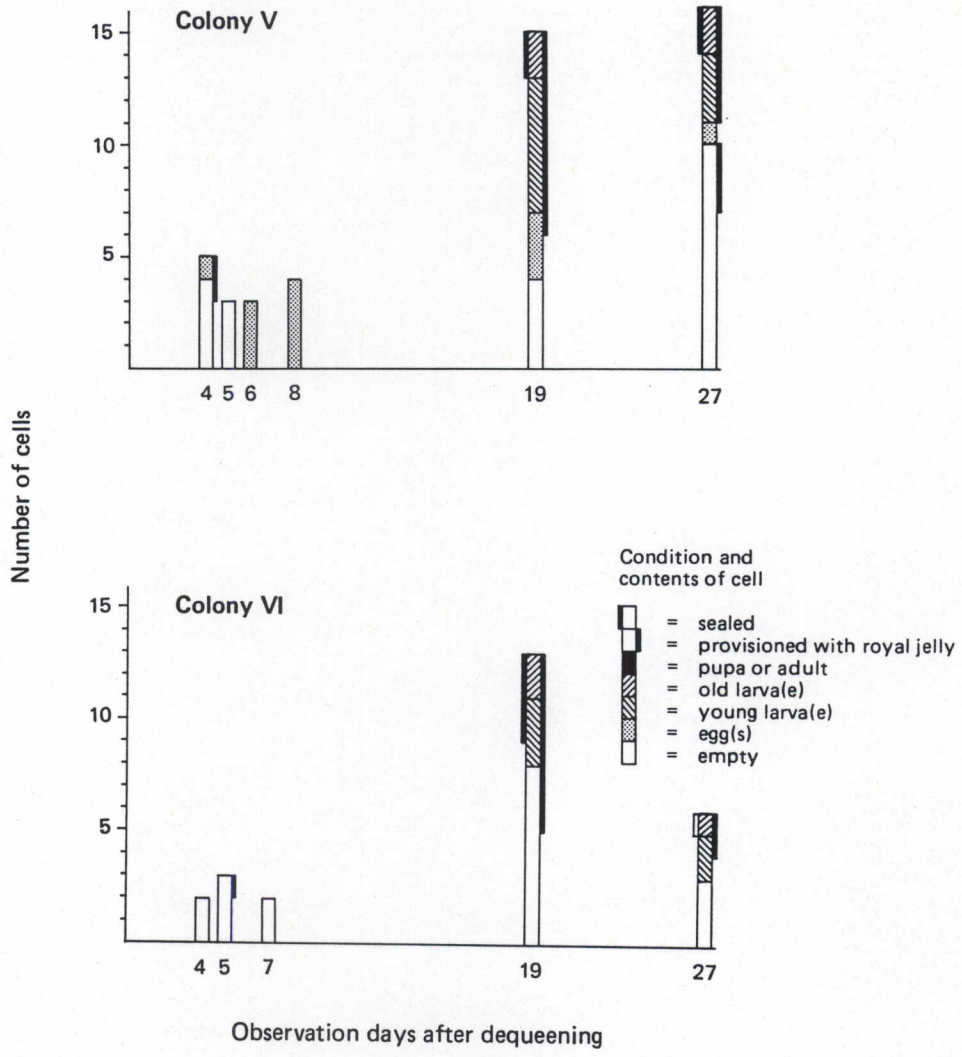


FIGURE 4-13. Pilot observations, colonies V and VI. Queen cells seen on observation days after dequeening. (Data from Appendix Table 4-5.)

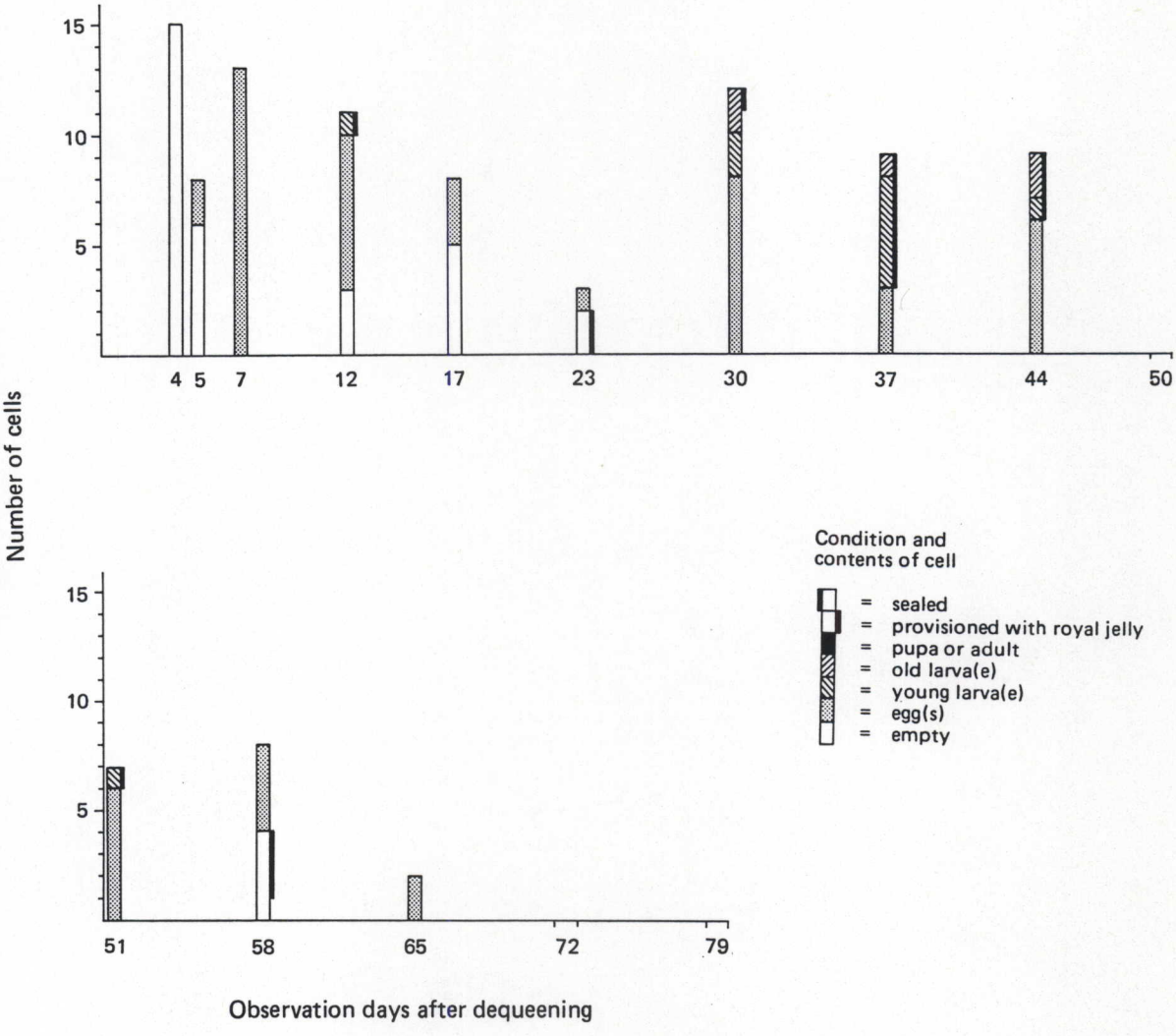


FIGURE 4-14. Colonies A, B, C and D. Queen cells seen on observation days after dequeening. (Data from Appendix Table 4-5.) *Above:* Colony A.

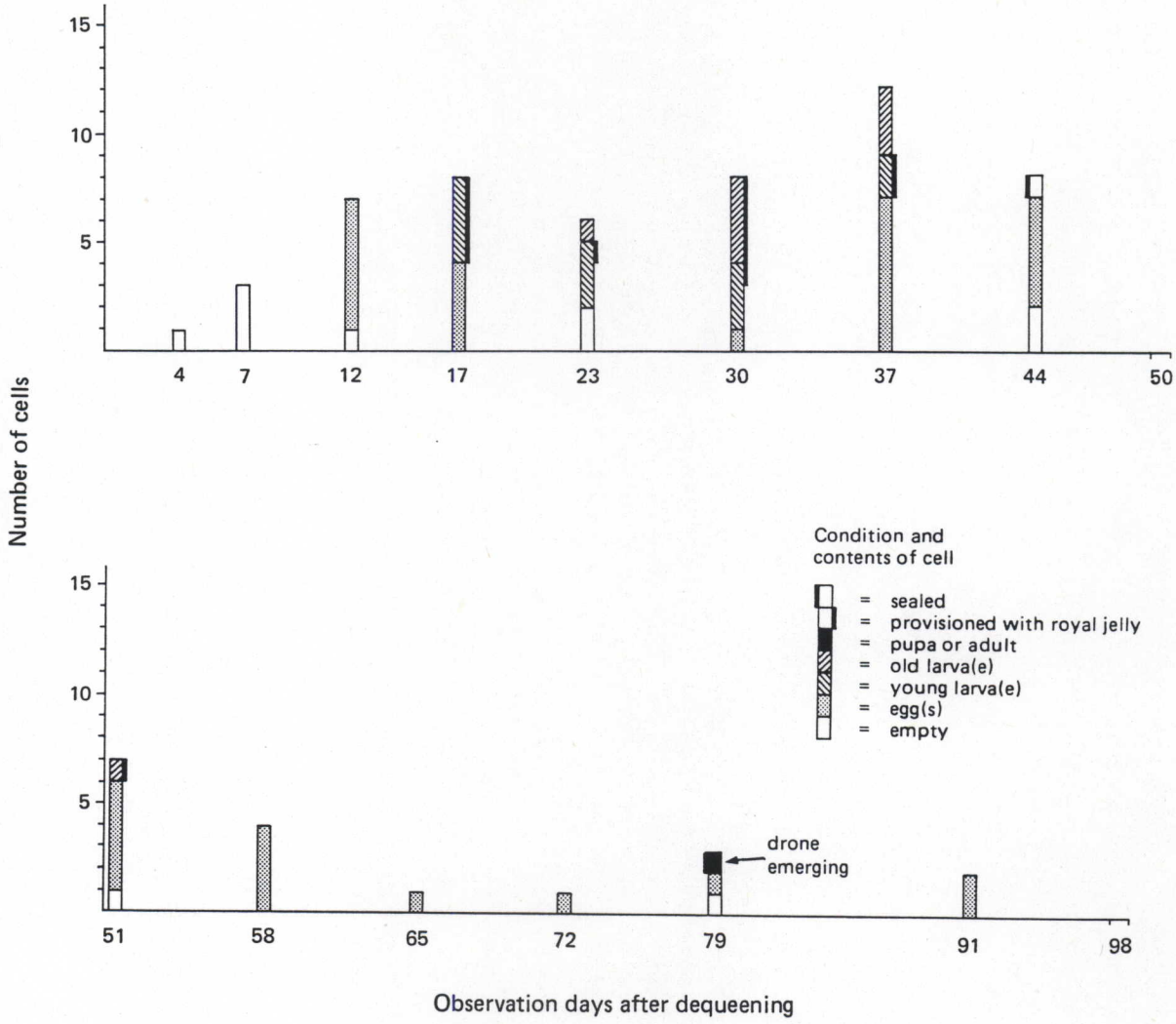


FIGURE 4-14. *Continued.* Colony B.

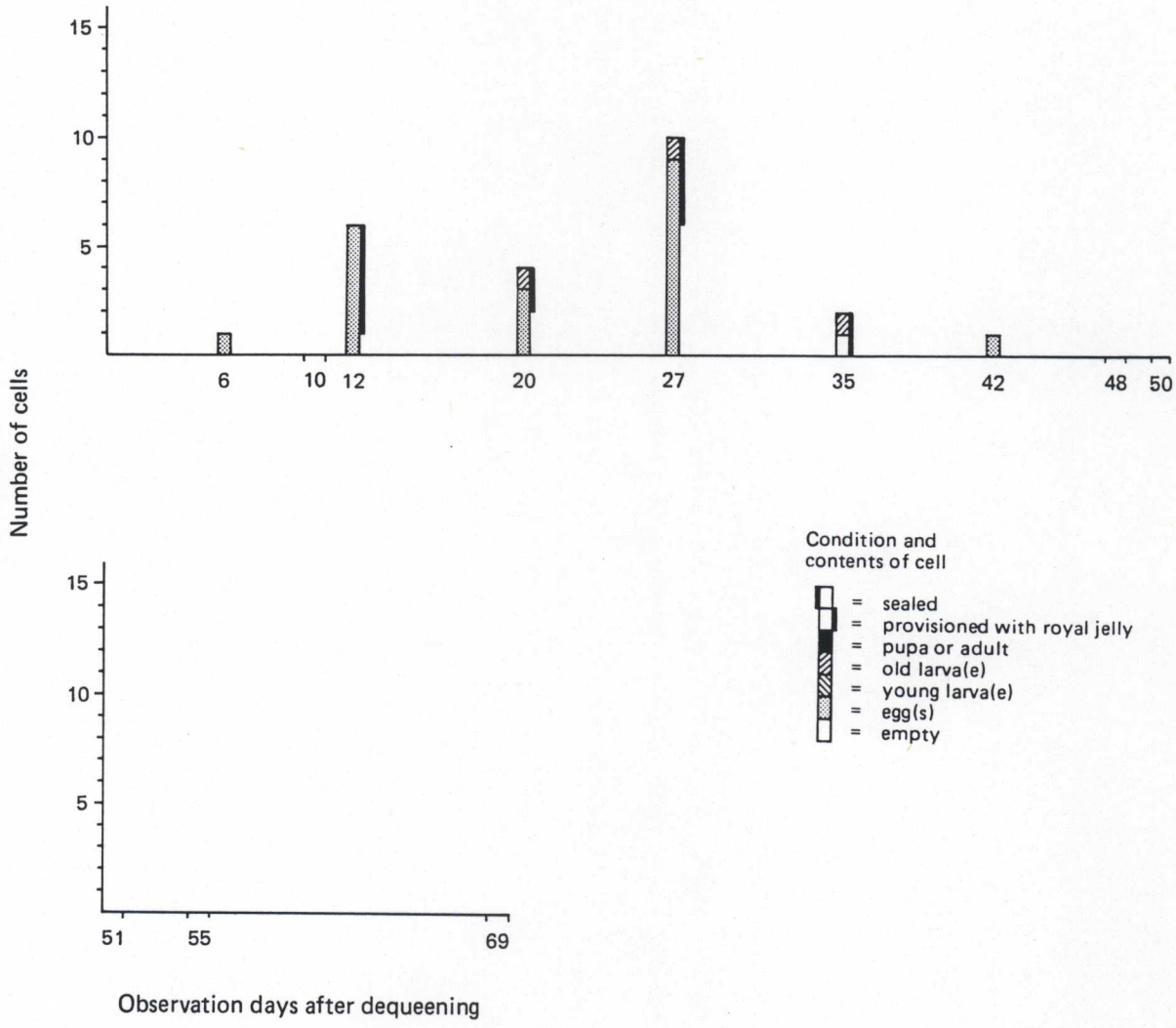


FIGURE 4-14. *Continued.* Colony C.

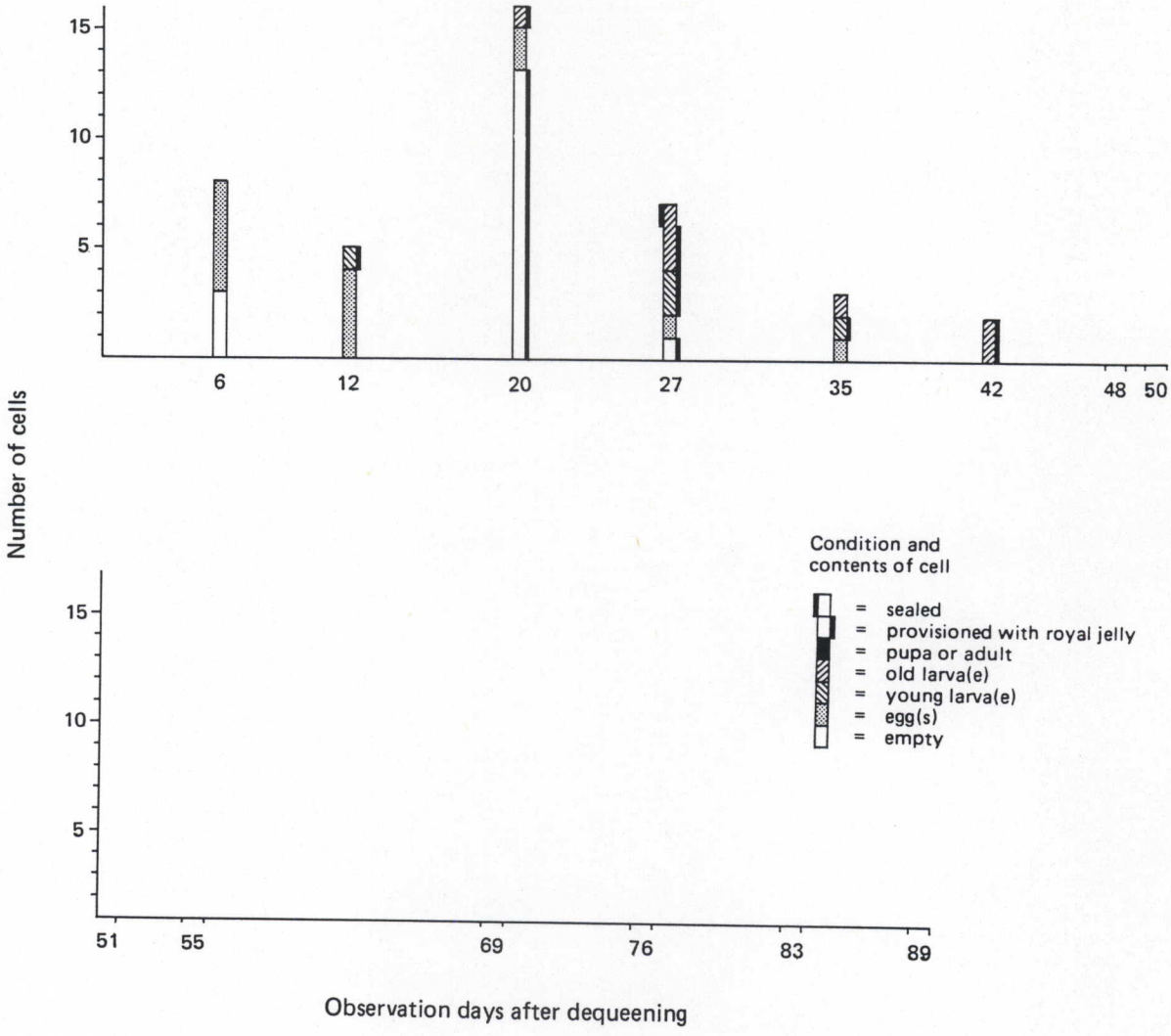


FIGURE 4-14. *Continued.* Colony D.

ranged from 0-9 per 24 h Fig. 4-12).

In colony V (Fig. 4-13) 3 or 4 queen cells were drawn during each one- or two-day hiatus between observations on days 5, 6 and 8 after dequeening, whereas 15 queen cells were drawn in the 10-day hiatus preceding observation day 19. This indicated a tendency to accumulate queen cells over time in the hopelessly queenless colony. A similar accumulation was seen between observation days 7 and 19 in colony VI.

In colonies A-D the drawing and provisioning of replacement queen cells persisted from 27 to 65 days after dequeening (Fig. 4-14): a total of 20 such cells was drawn in colony C; 39 in colony D; 65 in colony B; and 103 in colony A. Provisioning of these cells changed with time. Initially they were either empty or contained eggs. Later, approximately between days 17-51 after dequeening, the incidence of cells containing larvae and royal jelly increased. Thereafter drawing of queen cells either ceased (in colonies C and D) or diminished to relatively few cells, containing eggs (colonies A and B). The period of greatest rearing activity in the queen cells coincided with the rearing of the initial, large batch of brood in the combs.

The sexes of the larvae and pupae in the queen cells were not determined. However, on day 79 in colony A an undersized drone was found emerging from a sealed queen cell. All queen cells had been removed from the colony seven days previously and, since the development period of the drone is about 24 days, it is likely that this queen cell had been drawn over a comb cell that contained a drone larva approximately 17 days old. Associated with this were observations in colony A on days 4 and 5, and in colony B on day 23 after dequeening, in which clusters of 8, 13 and 25 emergency queen cells were drawn over drone cells. In colony A these cells were empty; in colony B they were sealed and contained old larvae, and presumably were drawn over drone cells occupied by brood. The clustered

conformation of these cells was very different from that of ordinary emergency queen cells, which are scarcely ever clustered.

In general the results indicate that the production of emergency queen cells followed a roughly similar course in each of the experimental colonies and that they were produced for considerable periods after the loss of the queen from the colony, in the absence of female brood.

4.4 CONCLUSION

Hopeless queenlessness is generally regarded as a moribund condition that follows loss of the queen from a broodless colony (reviewed in chapter 6). If the colony fails to produce a replacement queen it dwindles and dies (chapter 2). Hopeless queenlessness is often said to entail diminishment of the social organisation that preceded in the queenright colony: "when a colony loses its queen there is a lack of order in the hive, and . . . drones are reared in worker cells . . . we know that this happens because of the appearance of laying workers in such colonies." (Gary, 1975); "rather than rear these males as cooperatively and efficiently as possible, disharmony erupts in the nest as workers compete to provide the eggs . . . ." (Seeley, 1985). Butler (1974) said that haphazard oviposition by the laying workers and the discarding of many eggs and larvae by nurse workers are symptomatic of this disorder.

The present study indicated that in brood rearing in the hopelessly queenless colony, order is created from the haphazard oviposition, probably through removal of misplaced eggs or larvae by nurse workers, to the extent that substantial numbers of drones are efficiently reared to adulthood. Thus the throwing-out of laying worker brood may be regarded as a component of orderly brood rearing rather than a disorderly process in itself. The demography of brood rearing in the combs of the hopelessly queenless

colonies was characterised by an initial abundant oviposition, pruned subsequently to the production of a large batch of sealed brood in worker and drone cells (1000-4000 cells), followed by an extended period of brood rearing at a low level. Intrinsic to this process was a 3-4 fold extension in the life span of some of the workers in the hopelessly queenless colony.

From this perspective hopeless queenlessness may be interpreted as a distinct *phase* in the life of the honeybee colony, of irregular occurrence but with a characteristic form of social organisation rather than simply a moribund, disorderly condition. Other phases would include reproductive swarming, queen supersedure, emergency queen rearing, and the normal nonreproductive phase (*see* chapter 2). As with hopeless queenlessness, supersedure and emergency queen replacement are phases of irregular, rather than seasonal, occurrence. Functional interpretation and evolutionary aspects of hopeless queenlessness are considered in chapter 6.

CHAPTER 5

RATE OF ONSET OF WORKER OVIPOSITION AFTER LOSS OF QUEEN AND BROOD

5.1 INTRODUCTION

The factors that control worker ovarian development in honeybees are reviewed in chapter 2: in normal colonies of European races of *Apis mellifera* the ovaries of the worker bees are undeveloped and are held in that state by the influence of brood and queen pheromones. Ruttner and Hesse (1981) compared rates of onset of oviposition by newly queenless workers in seven honeybee races. Laying commenced after:

23-30 days in *A. m. mellifera, carnica*, and *ligustica* (Europe);

16 days in *adami* (Middle-East);

9.5 days in *scutellata*;

6.5 days in *capensis*;

5.6 days in *intermissa* (North Africa).

For these data Ruttner and Hesse introduced marked young workers of each race into European "carrier" colonies over 15 days, after which the carrier queen and workers were removed to produce queenless colonies of the introduced workers. Before their removal, the carrier queens were caged for five days so that there were no eggs or young larvae in the newly queenless hives from which replacement queens could be reared. The older brood was left in the newly queenless colonies. Non-normal conditions in this experiment included the small size of the queenless colonies (*ca.* 1500 workers) and their 15-day residence in a carrier colony of another race; also, the work was done in Germany, so that the non-European bees were in an exotic environment. Ruttner and Hesse argued that the results were

likely to be valid since they were consistent amongst the replications (two to four) for each race, and they agreed with rates of onset of oviposition measured in the bees' countries of origin (pers. comm. D.J.C. Fletcher for *scutellata*).

Some other measurements of rates of onset of worker oviposition, supplementary to Ruttner and Hesse, are reviewed below.

In *A. m. capensis*, Onions (1912) found that workers started laying 2-3 days after dequeening: if the brood was removed with the queen, they would start laying "in 24 hours". This process took longer in bees from Europe, and Rhodesia (i.e. *A. m. scutellata*), by "several days or probably weeks" (Onions, 1914). Also in *A. m. capensis*, Anderson (1963) recorded onset of oviposition at 4-8 days after dequeening, whereas the highest proportions of workers with highly developed ovaries (28%) occurred after 13 days. These colonies were normal when dequeened, after which their brood was left *in situ*, while all queen cells were removed daily. In European bees similarly treated, first oviposition was recorded at 14-30 days (Perepelova, 1926; Hess, 1942; Tucker, 1978). Unlike in *capensis*, maximal ovarian development (in 22-50% of workers) occurred *earlier* than first laying (Hess, 1942; Jay, 1968). Thus, under conditions of queenlessness but with queen-brood left in the colony, onset of oviposition by workers is markedly slower in European races than in *A. m. capensis*, even though maximal ovarian development in the colony seems to be attained at similar rates. Substantial variations in development times have been recorded in European bees, whereas rates appear to be fairly consistent in *A. m. capensis*.

In European colonies deprived of all brood at dequeening and subsequently kept free of queen cells (a combination of conditions likely to induce maximal ovarian development), Jay (1970) found no worker eggs by 21 days after dequeening, although some workers had well developed ovaries.

However, Millen (1942) found that European colonies in these circumstances produced laying workers after 10-26 days.

In *A. m. scutellata* no direct measurements have been reported of the rate at which laying workers appear in the *normal* colony after loss of queen and brood. In Angola, Kerr and Portugal-Araujo (1958) removed the queen and brood from a normal colony and found over 600 drone pupae in it upon examination 15 days later. This means that, at the latest, worker oviposition must have commenced at five days after dequeening, if Angolan *scutellata* drone brood pupates at about 10 days of age (*see* section 4.3.1). Crewe (1984) tabulated (but did not substantiate) first appearance of laying workers in *A. m. scutellata* as 6-15 days. In caged workers of a colony from Kenya there were "no significant difference in ovary development of workers of European and African origin" (Jay, 1975). In a small observation colony of *scutellata*, formed by adding groups of workers, workers "experienced substantial ovarial development" during the first two weeks of queenlessness but did not lay during the first six weeks after dequeening (Jackson, 1982). Results from small observation colonies and cages, while relevant to the particular circumstances in the experiments, cannot be extrapolated directly to normal colonies, since worker ovarian development in highly artificial conditions may be affected by many factors other than those under consideration in the experiment (chapter 2).

The present study reports the time to onset of worker oviposition following removal of the queen and her brood from full-sized normal colonies of *A. m. scutellata*, in South Africa (Pietermaritzburg, Natal).

5.2 METHODS AND MATERIALS

After dequeening and brood removal (method: section 3.2.3) the 12 colonies used in the experiments of chapters 3 and 4 were scrutinized for the first

appearance of laying worker eggs in the comb cells and in the emergency queen cells. The criteria for selecting normal colonies and the methods used to dequeen them, to remove their brood and to remove emergency queen cells drawn after dequeening, are described in chapter 3.

Only colonies 2 and 3 had their combs examined on each day following dequeening. In the others, examinations were delayed until day four after dequeening (or day six in colonies C and D). Examinations were made intermittently thereafter, as recorded in Appendix Tables 4-1 and 4-4.

5.3 RESULTS AND DISCUSSION

After removal of queen and brood from the 12 experimental colonies, an average of 5.8 days elapsed before the first eggs were seen (Table 5-1). In some colonies eggs were first encountered in large numbers and it was inferred that laying must have been under way at least on the preceding (non-observation) day. The earliest times at which eggs could be *inferred* to have been laid in the colonies averaged out at 4.7 to 5.4 days after dequeening, giving a median of approximately 5 days after dequeening, which is a rapid rate compared with other honeybee races. The commencement of oviposition in the 12 colonies was in fact remarkably regular, ranging as it did between 5-7 days after dequeening. The number of days between dequeening and the occupation of at least 50 cells by worker eggs was estimated to be between 5.6 and 6.5, giving an average of about 6 days. This represents a lapse of about a day between the first appearance of eggs in the colonies and the occupation of 50 or more cells by eggs. Thus in normal colonies of *A. m. scutellata*, from which queen and brood were removed abruptly, onset of oviposition at about 5 days was followed within 24 hours by the commencement of the rapid initial buildup of oviposition, described in section 4.3.6. The 5-6 day period for onset of worker laying

TABLE 5-1. Number of days to first oviposition after colonies were dequeened.

Colony	Days elapsed after dequeening until eggs were first seen				Inferred earliest day	
	worker cells	drone cells	queen cells	comb cells (drone *and* workers)	workers started laying	50 comb cells contained worker eggs
2	–	–	6	5	4–5	5–6
3	–	–	6[b]	7	5–6	7
I	–	–	7	5[c]	4–5	–
II	–	–	5	5	4–5	–
III	–	–	7	6[c]	5–6	–
IV	–	–	6	6	5–6	–
V	8	5[a]	4[b]	5	4	5–6
VI	7	7	7–19	7[c]	4–5	5–6
A	7	5[a]	5	5	4–5	5–6
B	7–12	7[a]	7–12	7[c]	7	7–8
C	7–12	6[a]	6	6	6	7–8
D	7–12	6[a]	6	6	4–5	4–5
Mean				5.8	4.7–5.4	5.6–6.5

– no observation

[a] eggs laid in drone cells before worker cells

[b] eggs laid in queen cells before comb cells

[c] eggs laid in comb cells before queen cells

(Data for colonies 2, 3, V, VI and A–D from Appendix Tables 4-1 and 4-4. Data for colonies I–VI not tabulated elsewhere.)

found in the present study is appreciably lower than the mean of 9.5 days determined for *scutellata* by Ruttner and Hesse (1981). However, the old brood that Ruttner and Hesse left in their newly queenless colonies may have retarded the onset of oviposition somewhat, since old brood inhibits worker ovarian development (chapter 2). It is thus possible that the rates reported by Ruttner and Hesse would be substantially reduced in colonies deprived of all brood at dequeening.

When a normal honeybee colony loses its queen through an accident, construction of queen replacement cells usually begins within a few hours, even if there is no brood suitable for producing queens. Thus when workers in hopelessly queenless colonies begin to lay they usually have an array of queen cells, as well as worker and drone cells, in which to place their eggs. In the present observations there appeared to be no consistent preference for queen cells over comb cells (worker and drone cells) as a site for initial oviposition: among the 12 colonies measured, queen cells had the first eggs in 2 colonies; comb cells had the first eggs in 5 colonies; and in the remaining 5 colonies oviposition commenced in both cell types at about the same time (Table 5-1). However, in the detailed observations in colonies V, VI and A-D, drone and queen cells together were initially preferred over worker cells in all but colony VI, in which drone and worker cells were laid in first (Table 5-1). In European laying workers a preference for drone and queen cells has been noted (Park, 1949, Free and Williams, 1974; Page and Metcalf, 1984).

Crewe (1982) postulated an association between the progressive increase in quantities of 9ODA (chapter 2) found in queens of *mellifera*, *scutellata* and *capensis* respectively, and the progressive increase in the rapidity with which these three races develop laying workers after queen loss. However, Crewe (1987, 1988) abandoned this interpretation when Ruttner and Hesse (1981) showed that *intermissa* (which has queens

pheromonally similar to *mellifera*) could develop laying workers faster than *capensis*. It is in fact apparent that several components of worker ovarian development and laying in *scutellata* are strikingly different from those in the two honeybee types whose laying worker biology is relatively well known, i.e. European races and *A. m. capensis*. Although both *capensis* and *scutellata* workers commence laying very soon after queen loss, the control of the *capensis* queen over her worker's ovaries seems to be weaker than in *scutellata*, since *capensis* workers usually have ovaries partially developed in the normal queenright colony (Anderson, 1963), whereas no ovarian development has been found in workers of normal *scutellata* colonies (section 3.3). In contrast, Fletcher and Ross (1985) stated that in queenright *scutellata* colonies laying workers often develop immediately above a queen excluder and that during emergency queen rearing they develop rapidly enough to produce drone pupae before the new queen emerges. However, these observations require experimental confirmation and at present should not be taken as indicating that laying workers are usually present in normal *scutellata* colonies, since worker laying does not occur immediately after a normal colony is suddenly rendered hopelessly queenless, as would be expected if laying workers usually occurred in normal colonies. (This reasoning also applies against the unsubstantiated observations of Gary (1975) and Taber (1980) that small proportions of workers usually lay in normal European colonies.) Rather, the circumstances of queenright worker laying specified by Fletcher and Ross (1985) may indicate that in *scutellata* the normally tight control of the queen over the worker ovaries is readily broken in workers that evade the full influence of the inhibitory factors of queen and brood in the hive. This might be a concomitant of the type of rapid worker ovarian development that occurs after queen loss in *scutellata*.

Attempts such as that of Crewe (1982, 1987, 1988), to clarify the

biological meaning of queen mandibular secretions in relation to laying worker characteristics, will be facilitated by the compilation of characterizations, or profiles, of the features of the hopelessly queenless phase that differ between the races and species of *Apis*. The basic profile of hopelessly queenless *scutellata* is: (i) although the ovaries of *scutellata* workers develop rapidly after loss of the queen and her brood, they are wholly undeveloped in the normal, queenright colony (chapters 3 and 4); (ii) the proportions of *scutellata* workers that attain slight ovarian development at the height of the laying phase are, at an average of 25%, much lower than the maximal proportions recorded for European races and *capensis* and very few (*ca.* 1-10%) have highly developed ovaries (section 3.3.4); (iii) a great many eggs are laid, and many drones are reared (chapter 4); (iv) the life span of the hopelessly queenless colony is three to four times longer than that expected if its workers maintained their queenright life span (chapter 4).

A first attempt to tabulate the profiles of the queenless phases of various honeybee types is given in Table 5-2 which shows that although the basic profiles of *scutellata, capensis* and European races can be drawn, our knowledge of this aspect of honeybee biology is at present inadequate. Better understanding will come from investigations in which hopeless queenlessness is approached as a distinct phase in the life of the honeybee, shaped in each race by the forces of natural selection, as discussed in chapter 6.

TABLE 5-2. Provisional profiles of laying-queen production of 90DA, worker ovarian development, oviposition and brood rearing in hopelessly queenless colonies of seven honeybee types.

Race, species	Quantity of 90DA in normal queen [a]	Workers undergo ovarian development in presence of		Worker ovarian development after removal of queen and brood			Quantities of worker progeny in queenless colony		Life span of hopelessly queenless colony	Arrhenotoky
		laying queen	swarming, emergency queen replacement, supersedure	onset of laying	maximum % of laying workers	maximum % of all stages	eggs	adults reared		
A.m. scutellata	intermediate	no [b]	yes [c] (lay)	intermediate [d] fast [e]	low [e]	low [e]	many [f]	many [f]	long [f]	yes
European	low	no [g] yes [h]	yes [i] (many, slight)	slow [j]	high [j]	high [j]	–	many [k]	–	yes
A.m. capensis	high	yes [l]	–	fast [l]	high [l]	high [l]	–	–	requeens	thelytoky
A.m. intermissa [d] (N. Africa)	low	no	–	fast	–	–	–	–	–	yes
A.m. adami [d] (Mid. East)	–	no	–	intermediate	–	–	–	–	–	yes
A. cerana	–	yes [m] no [n]	yes [o]	fast [m o]	high [n]	high [n]	many [n]	many [n]	–	yes
A. dorsata [p]	–	–	–	possibly fast	very low	low	–	many	–	yes

– = no information in literature consulted

a Crewe (1982, 1988)
b present study, chapter 3
c Fletcher and Ross (1985)
d Ruttner and Hesse (1981)
e present study, chapter 5
f present study, chapter 4
g reviewed in chapters 2 and 3
h Taber (1980)
i Perepelova (1926), Kropacova and Haslbachova (1970)
j *see* section 5.1
k Page and Metcalf (1984)
l Anderson (1963)
m Sakagami (1954, 1958), Butler (1970)
n Kasturi Bai and Reddy (1975)
o Millen (1942)
p Velthuis *et al.* (1971)

CHAPTER 6

DISCUSSION: FUNCTION OF LAYING WORKERS IN HONEYBEES

The literature reviews and experimental findings of chapters 2-5 raise several points concerning current understanding of ovary development, laying and brood rearing by *Apis* workers. Much research has centered on the queen pheromones that control worker oogenesis (reviewed in chapters 2 and 3, and by Pain, 1968a; Butler, 1973; Michener, 1974; Gary, 1974, 1975; Free, 1987). In most of this work, consideration of worker ovarian development was restricted to its use as an indicator of experimental treatments: its meaning in the life of the honeybee was not discussed. However, Smith (1960), Allen (1965) and Butler (1973) noted that hopeless queenlessness resulted in the production of useless, superfluous, often undersized drones. Velthuis (1970, 1976) suggested that because laying workers produce males that do not maintain the colony, the development of their ovaries is an atavism, i.e. a manifestation in the workers of vestigial queenlike characters not fully eliminated during the evolution of monogyny. Similarly, Sakagami (1958) said that the "adjustive responses" to queen loss (e.g. worker laying) "are not always adaptive from the standpoint of colony maintenance . . . ." Hamilton (1972, p. 194) noted that even though certain social insect species may normally produce males via workers, "this is certainly not true of some species (e.g. *Apis*)" and, at p. 210: "worker laying certainly contributes little to male production (10% in *A. mellifera adansoni* [sic], hardly any in

A. m. mellifera)."* Velthuis (1985) dismissed worker laying in the honeybee as "only an aberrant development without any actual consequences for the gene pool."

Thus the meaning of worker laying in the life of the honeybee was, when not ignored, often dismissed as inconsequential, essentially because it was seen as a symptom of a major irredeemable calamity for the colony: the queen is lost - if she is not replaced, the colony is then doomed to dwindle and die (e.g. Butler, 1974; Winston, 1987). Concomitantly with this view, it has been widely held that breakdowns in colony order occur during hopeless queenlessness, in the absence of the socially coordinating influence of the queen, and in the absence of the socially stabilizing processes that accompany the rearing of replacement queens. Symptoms of disorder have been said to include queenless roaring (Butler, 1954); ovary development in workers normally neutered by queen pheromones (chapter 2); fighting between workers (Seeley, 1985); haphazard oviposition by workers, of many eggs to a cell, or in inappropriate places (chapter 4); rearing of drones in worker cells (Gary, 1975); and the fact that many eggs and young larvae are thrown out of the hive by workers (Butler, 1974), or "wasted" (Velthuis, 1987). Thus Velthuis (1987) concluded, for honeybees and some other eusocial bees, that

> "The assumption that laying workers contribute to the gene pool, is superficial. If the reproductive system of a species includes worker participation one might expect efficiency . . . in the

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* The source for this statement is given by Hamilton as Kerr (1969), but the information is not given there, nor is it in any other paper by Kerr referred to by Hamilton, or in any other paper by Kerr consulted in the present study.

rearing [and] also in the timing of male production related to season . . . . In many bees, however, worker derived males are out of season . . . their reproductive potential approaches nil."

The foregoing dismissive opinions about arrhenotokous laying workers contrast sharply with opinions on the function of the predominantly thelytokous laying workers of *A. m. capensis*, in spite of the fact that production of laying worker brood in *capensis* colonies is just as "disorderly" as it is in other races (e.g. eggs are laid haphazardly, many to a cell: Anderson, 1977a). In fact, queenlessness might be regarded as even more disorderly in *capensis* than than in other races, since in newly queenless *capensis* extensive fighting breaks out among the workers whereas other races remain calm at this stage (Anderson, 1963, 1977b). Nevertheless the production of replacement queens by queenless *capensis* colonies has been widely noted (e.g. by Kerr, pers. comm. to Hamilton, 1964; Wilson, 1971; Butler, 1974; Michener, 1974; Crozier, 1975; Ruttner, 1977a,c) and the fact that the system is accessible to natural selection brooks no argument. The thelytokous capacity of *capensis* workers is positively selected if viable queens are ultimately produced from worker-laid eggs (Ruttner, 1977c). Tribe (1983) and Ruttner (1988) suggested that the trait evolved under an exceptionally high rate of queen loss (and hence hopeless queenlessness) owing mainly to the frequent high winds at the Cape peninsula (see also Ruttner, 1977c; Ruttner and Hesse, 1981). Onions (1912), Anderson (1963) and Ruttner (1977a,c) found that queen replacement does not inevitably follow the first production of female eggs in queenless *capensis* colonies. In fact, such colonies often remain queenless (with workers laying females) for up to four months until a queen is produced (Winston, 1987). Thus Hamilton (1964) and Ruttner (1988) suggested that thelytoky in *capensis* workers lessened the need to initiate queen rearing immediately after queen loss. Ruttner (1977c; see also Lundie, 1954;

Anderson, 1965; Kerr, 1969; Johannsmeier, 1983) suggested that *capensis* workers may expand the *capensis* population by their social parasitism of the adjoining race, *A. m. scutellata* (which has arrhenotokous laying workers): *capensis* workers may do this by joining *scutellata* colonies and laying in them when they become queenless. A further mechanism by which *capensis* gene frequencies may be maintained, or increased, in the face of inter-racial hybridization was shown by Ruttner (1977b,c): from *capensis* x *scutellata* hybrids, workers bred from laying workers had fully reconstituted *capensis* characteristics by the third or fourth generation.

Despite the reproductive advantages conferred by its thelytokous laying workers, *capensis* occurs in a relatively small area at the southern Cape and has not displaced the neighbouring *scutellata* to any great extent. These contiguous populations of *capensis* and *scutellata* thus constitute a point of focus for the following problem:

> "One of the least-understood aspects of honey bee biology is the absence of a high frequency of diploid egg production by workers among all races except for *A. m. capensis*. Production of female brood by workers should be highly favoured by selection, since it would ensure colony survival following queen loss . . . ."
> (Winston, 1987)

In European races queens are occasionally produced in hopelessly queenless colonies from the low proportions (approximately 2%: Woyke, 1962) of female eggs produced by workers that are otherwise arrhenotokous, as noted in Punic, Tunisian and Syrian races by Hewitt (1892; and see Anderson, 1918); and in about 5% of hopelessly queenless European colonies observed by Butler (1974). In *scutellata* (Tanzania) "laying workers rarely produce a queen, but I have observed it on two occasions." (Smith, 1961). Tucker (1958, 1978) found that although a few female larvae were produced in many European laying worker colonies, opportunities to rear queens from them

were mostly missed: either the nurse bees did not recognise the sex of the female larvae, or they were not inclined to rear queens from them. In *scutellata* (Angola), Kerr and Portugal-Araujo (1958) found that 0.63% of pupae reared from worker eggs were female, a similar proportion to that determined in eggs from European virgin queens by Mackensen (1943). It is thus evident that in several honeybee races with arrhenotokous laying workers the thelytokous alternative has been available, or exposed, to selection but has never been affected by it.

Arrhenotokous laying workers in Hymenoptera are capable of transmitting their genes (or, the genes of their colony) via matings by their sons (Hamilton, 1964). However,

> "Phenomena like worker oviposition , . . are traditionally glossed over in studies of highly integrated colonies . . . but . . . are likely to be fundamental to larger patterns of behavior and social organization." (West-Eberhard, 1981)

Worker laying has been mentioned as a minor reproductive factor in honeybees and as important in analyses of natural selection and evolution in some other hymenopteran societies (e.g. Alexander, 1974; Michener, 1974; Kerr, 1975; West-Eberhard, 1975, 1981; Wilson, 1975; Trivers and Hare, 1976; Crozier, 1975, 1977, 1979, 1982; Oster and Wilson, 1978; Owen, 1980, 1985, 1986; Owen and Plowright, 1982; Sakagami, 1982; Brian, 1983; Fletcher and Ross, 1985; Free, 1987). Bourke (1988) argued that worker reproduction is important in present-day systems of most of the higher eusocial Hymenoptera (Bombini, Meliponini, Apini, Vespini, Formicidae) and must have been of cardinal importance in the evolution of the female castes (queen, worker) in these animals. In honeybees Bourke noted the well-known reproductive capacities of the thelytokous workers of *A. m. capensis* and also the fact that hopelessly queenless colonies of races with arrhenotokous laying workers are capable of producing many males (6000 were

produced in a hopelessly queenless European colony observed by Page and Metcalf, 1984). Bourke cited several instances of social organization associated with male production in the hopelessly queenless colony, which may be founded in attempts by some workers to establish reproductive dominance in the colony: worker aggression, possibly between kin-factions; emergence of false queens; worker production of queen substance. The fact that European laying workers prefer to lay in drone cells (Free and Williams, 1974) thus producing full-sized, reproductively competitive drones, was cited as "additional evidence for the importance of worker male production in orphaned hives."

In assessing the performances of laying workers in various honeybee races, Ruttner and Hesse (1981) were generally cautious about the role of laying workers: "the reproductive function of the worker is never of significant importance in the undisturbed life-cycle of honeybee populations." They found that after queen loss, workers of European races began laying after a month and laid fewer eggs than races from hotter regions (Tunis and sub-Saharan savannah Africa), which began laying 5-10 days after queen loss. Ruttner and Hesse's opinion on the situation in tropical honeybees is worth quoting in full (translated from the German):

> "This [drone production by queenless colonies] will play a greater role in areas with a hot, extreme climate than in Europe, since there the survival strategy of the bees is directed towards excessive increase: in drought years, when 80% of the population may die, the deficit will soon be replaced in successive good years, through excessive swarming. The almost incredible ability of African bees to increase their numbers is demonstrated by their success after introduction to South America. In this high rate of increase - unknown in temperate climates - the males from queenless colonies will play a more significant role than in races with a

limited tendency to swarm. This could offer an explanation for the difference in [the rate of onset of oviposition in queenless colonies] between African and European races."

The results of the present study support and extend Ruttner and Hesse's suggestion that production of drones by hopelessly queenless colonies is well developed in races such as *A. m. scutellata*.

In the hopelessly queenless *scutellata* colonies of the present study a variety of social processes associated with efficient drone production were found, which were interpreted as components of a social organization of a high order rather than as disorderly by-products of the breakdown of social order, as it has usually been interpreted. In particular, there was:

(i) a strong association between worker age at dequeening and subsequent ovarian development, indicating a polyethism-type organization in worker oogenesis and oviposition (chapter 3);

(ii) maintenance of queenright age-based polyethisms such as foraging (chapter 3);

(iii) imposition of orderly brood rearing, in appropriate areas on the combs, by removal of haphazardly laid eggs and larvae, leaving one individual per cell in drone and worker cells (chapter 4);

(iv) regular, relatively rapid inception of oviposition after queen loss (chapter 5);

(v) a definite pattern of brood rearing, with an abundant initial oviposition and production of a large batch of drones (1000-4000), followed by low-level rearing over a prolonged period (chapter 4);

(vi) prolonged survival of the colony, including the worker-produced drones (chapter 4).

These findings are best interpreted in the same way in which Page and

Metcalf (1984) explained the preference of laying workers for drone cells (mentioned above): "worker egg-laying behaviour is [an] adaptation to queenlessness." In fact, extension of this approach for the present results gives an interpretation, based on the natural selection of the process, which has not been fully emphasized in the literature and which may be useful in future work on this aspect of honeybee biology. Thus, at first sight, the results listed above raise the question: "why should the hopelessly queenless colony expend such effort in the production of drones, many undersized?" Further, when queenright colonies eject drones in the adverse circumstances of rapidly diminishing worker numbers and food stores (Free, 1987; Winston, 1987), why do dwindling hopelessly queenless colonies tolerate them? Reasoning on these problems within the reductionist, mechanistic milieu in which worker oogenesis has traditionally been approached (i.e. the analysis of physiological control) produces little insight. However, when considered in the light of the possible *biological function* of the process, the following hypothesis may be drawn: *The function of arrhenotokous honeybee laying workers is to produce males to mate with queens*, or, more generally: *The function of hopelessly queenless honeybee colonies with arrhenotokous laying workers is to produce males to mate with queens*.

A corollary to this hypothesis is that the *reproductive activities of hopelessly queenless colonies are subject to natural selection*, as intimated by Ruttner and Hesse (1981) and Moritz (1986b) and as concluded by Page and Metcalf (1984), Hellmich *et al.* (1986) and Bourke (1988). The action of natural selection answers the questions posed in the previous paragraph and provides a heuristic basis for the consideration of all other aspects of the hopelessly queenless phase in honeybees.

For a biological entity to evolve by natural selection it must have three properties: variation, heritability and fitness differences

(Lewontin, 1970). These are present in the reproductive faculty of the hopelessly queenless colony. Specifically, two things are necessary for natural selection to affect the hopelessly queenless phase: (i) colonies in the phase must produce individuals that can mate; (ii) colonies must attain the phase often enough for mating successes and failures to change the gene frequencies of the traits that underlie worker production of reproductive colony members.

Moritz (1986b) emphasized that there are as yet no quantitative data concerning the "fitness parameters" (i.e. the reproductive capacities) of the males and females produced by honeybee laying workers (*see* point (i) above). However, there are several indications that laying worker drones are likely to function normally. Contrary to statements that laying worker colonies eject drones (e.g. Gary, 1975), queenless colonies normally nurture them (Butler, 1974; Page and Metcalf, 1984). (Gary's statement was an interpretation of Hoffmann, 1961, who worked with small observation colonies. Hoffmann in fact indicated that this finding was contrary to that of other authors.) Production of 6000 drones in a hopelessly queenless European colony has been mentioned above. Such colonies may eventually contain more drones than workers (Tucker, 1978) so that in the hopelessly queenless phase the usual queenright balance between season, number of workers, and number of drones (Free and Williams, 1975, and see Winston, 1987) no longer applies. In hopelessly queenless *A. m. scutellata* colonies drones of all sizes occurred, from the time of their first emergence through surprisingly extended periods of colony survival to the demise of the colonies (chapter 4). Laying worker drones of all sizes produce viable sperm (Butler, 1970, 1974) and fly from the hive (chapter 3).

Hopeless queenlessness has several causes in natural circumstances, some of which could operate frequently in relation to the rate of queen

production in a population (*see* point (ii) above). The most common of these is probably loss of the queen on a mating flight. Within her colony the queen is protected by her workers, but when flying she is vulnerable to predatory wasps and birds (*see* section 7.3); or she may lose her way, perhaps by being blown off course by strong winds (Guy, 1976; Tribe, 1983; Moritz, 1986b). When a queen fails to return from a flight, her colony will be hopelessly queenless if there are no other queens or queen cells in the colony, and no young brood from which another queen can be reared. Butler (1974), Michener (1974), and Winston (1987) review types of swarming and queen replacement, the processes which result in queens flying from the hive. In *emergency queen replacement* there is invariably no brood for further queen rearing by the time the replacement queen mates. However, in *supersedure* the old queen may sometimes continue to lay while new queens are reared (Butler, 1957b; 1974). In *reproductive swarming* the mother queen usually leaves the nest with her swarm (the prime swarm) just after the first queen cell is sealed (Simpson, 1974), leaving the colony queenless and with no young brood: the first-emerged replacement queen may kill the other queens in their cells (Butler, 1974; Winston, 1987), after which she undertakes about four mating flights* (Adams *et al.*, 1977; Thornhill and Alcock, 1983) on which she may be lost and so render the colony hopelessly queenless. Alternatively the first-emerged queen may not kill the other queens but may leave the colony with an "afterswarm" - several of these may issue until a single queen remains to head the mother

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* *Scutellata* queens undertook an average of six mating and orientation flights (Fletcher, 1977c); five queens observed closely undertook 1-2 mating flights and 4-12 (mean 7) orientation flights (Fletcher and Tribe, 1977b).

colony (Butler, 1974; Winston, 1980), which will become hopelessly queenless if that remaining queen is lost. After leaving the mother colony a prime swarm can become hopelessly queenless if the queen is accidentally lost while flying with the swarm, or is lost from the newly established hive, before she has laid the first female eggs; this is also the case with absconding swarms. Virgin queens of newly hived afterswarms undertake mating flights several days after moving into a new nest site (Lee and Winston, 1987) - afterswarms thus have a higher chance than prime swarms of becoming hopelessly queenless.

Another route to hopeless queenlessness entails the simultaneous loss of the queen and of brood suitable for queen replacement by the direct action of a predator such as man, honey badgers or ants (*Anomma* spp; *Dorylus* spp.) (Fletcher and Tribe, 1977c; Fletcher, 1978; *see* chapter 7). (This was the situation approximated in the experiments in chapters 3, 4 and 5.) If in such attacks, or as a result of disease, the queen is lost but emergency queen cells are drawn from surviving brood, hopeless queenlessness can follow if the queen cells are destroyed in a subsequent predator attack, or if the emergency replacement queen(s) are lost on mating flights, either from the queenless colony itself or from any afterswarms (Winston, 1979a) that issue from it.

Occasionally, virgin replacement queens are prevented from flying by inclement weather (Hayter, 1937) or by apivorous wasps (section 7.3). If held in the hive for long enough the virgin queen commences laying parthenogenetically and thereafter will not mate. A colony headed by a laying virgin is "doomed to extinction" in the same way as a laying worker colony, unless a new queen is somehow reared (Butler, 1974, p.157). If a laying virgin is lost the colony becomes hopelessly queenless, although a colony with a laying virgin itself constitutes a special case of hopeless queenlessness.

From the above review, it is clear that the occurrence of hopeless queenlessness is irregular and fortuitous and its frequency in a population is likely to be associated primarily with the frequency with which queens fly from the hive and are lost, either on mating flights or on flights at the head of a swarm.

No solid data exist for the frequency of hopeless queenlessness in nature (Winston, 1987). Unmanaged European colonies in Kansas produced 3.6 daughter colonies per established colony per year (Winston, 1980). In Louisiana, queens undertook three to five mating flights (Oertel, 1940; Roberts, 1944). Thus, in mid and southern USA, a single colony may in one year produce about four offspring colonies whose queens perform a total of 10-18 mating flights and so are at a high risk of hopeless queenlessness for that number of times. In European apiculture it is accepted that about 20-30% of colonies lose queens on mating flights (Tiesler, 1972; Ruttner, 1988). Thus it is likely that, even in temperate regions where predation is generally lower than in the tropics (chapter 7), hopeless queenlessness in natural conditions is not a rare occurrence (inferred also by Page and Metcalf 1984). In Italian colonies bred for seven years in South Africa, 30-40% of virgin queens were lost on mating flights (Beyleveld, 1935, 1939; and see Fletcher, 1978). By contrast, 20 *scutellata* queens observed by Fletcher (1977c) all survived their mating flights. Otherwise, data are lacking for survival of *scutellata* queens on mating flights. Loss of queens on mating flights through bird predation has been noted by South African beekeepers (e.g. Hayter, 1939; Mountain, 1972). A South African beekeeper estimated that 40% of mated *scutellata* queens were lost each year, for reasons unknown (Fletcher and Tribe, 1977c). Various factors point to a much higher incidence of hopeless queenlessness in *scutellata* than in temperate (European) races, primarily through a higher rate of mating flights and through the existence of more predators to kill the

flying queens:

(i) The frequency of swarming is much higher in *scutellata* than in European bees (Fletcher and Tribe, 1977c; Fletcher, 1978; Ruttner and Hesse, 1981; Seely, 1985) as is the rate of supersedure (Fletcher and Tribe, 1977a).

(ii) At each swarming session *scutellata* colonies produce more afterswarms than European colonies (Winston, 1987). "Queen-loss swarms" often issue after emergency queen replacement in *scutellata*, and do so more frequently than European bees (Fletcher and Tribe, 1977a,c; Punnett and Winston, 1983).

(iii) *Scutellata* colonies may often abscond - this source of swarms is practically absent in European races (Fletcher, 1978; Winston, 1987).

(iv) The variety, abundance and efficacy of honeybee natural enemies is higher in sub-Saharan Africa than elsewhere in the range of *Apis mellifera* so that *scutellata* queens on mating flights and in flying swarms will be at greater risk than their European counterparts (chapter 7). Indeed, the flight behaviour of *scutellata* queens may be adapted to a higher tempo of predation (Fletcher, 1977c, 1978; Seeley, 1985; Winston, 1987). Orientation and mating flights in *scutellata* are shorter than in European races and are made in the early afternoon, irrespective of prevalent weather conditions. This precise timing of flights was proposed to have evolved to avoid bird predators, whose peak feeding times are mostly in the morning and evening (Fletcher and Tribe, 1977b).

(v) The high incidence of whole-colony predation in Africa (chapter 7) will itself result in some hopeless queenlessness and will increase the number of emergency-replacement queens and queens of queen-loss swarms that must undergo risky flights.

From the foregoing evidence for (i) the viability of males produced by laying workers and (ii) the frequent occurrence of hopeless queenlessness in *A. m. scutellata*, it is likely that drone production by hopelessly queenless *scutellata* has been exposed to more intense natural selection than in European races (as intimated by Ruttner and Hesse, 1981). Some points about *A. mellifera* laying workers, as they relate to the findings of chapters 3-5 and supplementary to those drawn by Bourke (1988), are presented in the remainder of the present review.

Three reproductive alternatives in hopelessly queenless *A. mellifera*

Ruttner (1988) (see also Ruttner, 1985; Moritz, 1986b) reiterated two ways whereby death of the colony through hopeless queenlessness is avoided: (i) by worker thelytoky, commonly in *A. m. capensis* but rarely in other races (outlined above); (ii) by the maintenance of several virgin queens in the colony during the mating period until one mates successfully, after which monogyny is restored - this occurs only in four Mediterranean races. Ruttner was puzzled as to why the latter strategy, a simple and efficient solution, is not more common in *A. mellifera*, instead of the colony extinction that usually occurs in races with arrhenotokous laying workers. For these latter races the answer may lie in a third alternative in which hopelessly queenless colonies perpetuate their genotypes via matings by worker-produced males, as outlined above. If these matings maintain or increase the gene frequency of the trait, then it will be perpetuated at the expense of alternative systems. The inevitable loss of the fabric of the colony itself has worried many authors, e.g. Velthuis *et al.* (1971): ". . . in the genus *Apis* caste differentiation has gone so far that the workers are devoid of any reproductive function of significance for the survival of the colony . . . ." But survival of the colony need not be of

any account in the arrhenotokous laying worker system. Total self-sacrifice by the individual in order to maximize the chances of perpetrating its genotype is not uncommon in several insect reproductive systems (Thornhill and Alcock, 1983). In honeybees, the drone dies after blasting his genitalia into a queen. In some mantids, the male is devoured by the female during copulation - here, the death of the male is immaterial when weighed against the good chances of the propagation of his genes by his well-nourished spouse. If it is accepted that production of drones by arrhenotokous workers in queenless colonies has been selected as a reproductive strategy, then colony self-sacrifice is an inevitable adjunct in the system.

Arrhenotoky and thelytoky in laying workers are presently thought to be linked to one locus with a single pair of alleles - the "arrhenotoky allele" dominates the "thelytoky allele" so that heterozygous workers produce male offspring (Ruttner, 1986). Arrhenotoky is the primary condition in honeybees, being an integral component of their sex determination system; thelytoky is a condition derived from arrhenotoky (Soumalainen, 1950; Mackensen, 1951; Tucker, 1958; Wilson, 1971; White, 1973; Michener, 1974; Crozier, 1975, 1977; Adams *et al.* 1977; Verma and Ruttner, 1983).

In the South African Cape two honeybee races occur contiguously: *A. m. scutellata*, with arrhenotokous laying workers and *A. m. capensis*, with thelytokous laying workers. If, as is likely, *capensis* and southern *scutellata* share a common ancestry (Ruttner, 1988), then it is fair to assume that *capensis* worker thelytoky somehow evolved from an ancestor with arrhenotokous laying workers. Such evolution can be understood if the ancestral *capensis* population was geographically (spatially) isolated (*sensu* Mayr, 1970), had a high rate of queen loss, underwent the necessary initial genetic accidents for a high rate of worker thelytoky and possibly

had other, as yet undetermined, conditions that favoured worker thelytoky. Guy's (1976) analysis is thus important: the Cape peninsula (the centre of current *capensis* distribution: Ruttner, 1988) was islanded several times during the late Pleistocene, with probable predomination of very high winds (therefore frequent queen loss) and damp, cold winters. Similar, but less severe, weather conditions now occur at the peninsula, and *capensis* persists. The ability of *capensis* worker thelytoky to maintain itself in the face of hybridization with neighbouring *scutellata* has been documented (*see* above).

There is, however, the reciprocal question of why *capensis* worker thelytoky, with its manifest advantage of requeening from hopeless queenlessness, has not spread into the *scutellata* genotype. Moritz (1986b) modelled rate of queen loss and relative fitnesses of worker thelytoky and arrhenotoky in a Hardy-Weinberg equilibrium: worker thelytoky is advantageous if the rate of queen loss is high; arrhenotoky can be fixed in a population only if the fitness it confers is "considerably larger" than that conferred by thelytoky. This model assumed that "both reproductive strategies are under identical selective pressure within one gene pool." Moritz suggested that this is the present case in *capensis'* home range since there is now a high degree of hybridization throughout the Cape peninsula (Moritz and Kauhausen, 1984). However, the arrhenotokous systems of the vast majority of honeybee races are not confronted with hybridization with thelytokous neighbours. For these the Moritz model does not apply, because it does not take into account the primacy of arrhenotoky in the honeybee reproductive system (mentioned above), i.e. whatever the phylogeny of honeybee sociality, divergence between worker and queen will have been accompanied by arrhenotoky in both castes. If this evolution was accompanied by selection for the reproductive capacity of worker-produced drones during hopeless queenlessness, then the arrhenotokous laying worker

system will have provided an ongoing "inertia" against conversion to any other system such as worker thelytoky or, perhaps, the carrying of extra virgin queens during the mating period (Ruttner's alternative (ii) above). The case can be put quite simply. If honeybees have always had a successful system of propagating genes out of hopeless queenlessness, via worker arrhenotoky, why would they convert to an alternative system?

Some predictions about hopelessly queenless colonies that produce drones

The influence of natural selection on drone production by the hopelessly queenless colony gives a basis from which behaviour in the hopelessly queenless phase can be interpreted. Below, some of the more important points are listed as predictions (which can be tested), with relevant findings from the literature and from the present study. As noted above, it is expected that features which contribute to successful reproduction by the hopelessly queenless colony will be accentuated in races in which the incidence of this condition is high, so that such features will be differentiated between various races. The predictions below have been drawn mainly on the basis of colony-level processes in the hopelessly queenless colony and provide a foil to interpretations which emphasize the role of individual reproductive interests in this phase of honeybee life, such as that of Seeley (1985) who noted that

> "the best thing that [hopelessly queenless workers] can do . . . in order to maximize colony fitness is to produce one final crop of male reproductives . . . . But rather than rear these males as cooperatively and efficiently as posible, disharmony erupts . . . as workers compete to provide the eggs that will produce the males."

Seeley cited worker fighting and laying of many eggs to a cell as evidence of behaviour that promotes individual reproductive interests at

the expense of colony-level efficiency. In addition to worker fighting, Bourke (1988) interpreted emergence of false queens and production of queen substance by laying workers as "personal" reproductive behaviour. (Bourke did not discuss production of queen substance by workers with undeveloped ovaries - *see* chapter 3 - which might well be selfless contribution to colony stability.) Further, Evers and Seeley (1986) saw worker fighting in queenless colonies as primarily selfish by each attacker, even though there was a weakly significant preference for workers to attack half-sisters rather than full sisters, which indicated kin factions in the queenless colony. In chapter 4 of the present study it was shown that hopelessly queenless colonies do in fact produce "one final crop of male reproductives" despite the array of "disorderly" and "selfish" behaviours that have been enumerated. It is therefore submitted that consideration of natural selection and colony-level processes in reproduction by hopelessly queenless colonies will usefully broaden the debate. Some predictions from this standpoint are:

(i) Drones from hopelessly queenless colonies will join drone congregations and mate with queens. This has not yet been studied. It is the central prediction of the hypothesis drawn earlier.*

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* The hypothesis for the reproductive function of arrhenotokous laying workers was drawn on the "indirect evidence of complexity and constancy" (Williams, 1966, p.8) in processes in the hopelessly queenless colony, in the absence of direct evidence that drones of the hopelessly queenless colonies mate successfully. The hypothesis was thus arrived at through *teleonomy*, the practise of observing a distinct mechanism in an organism and then postulating that it must have a biological *(continued overleaf)*

(ii) Drones from hopelessly queenless colonies will be as efficient as other drones in ~~procuring~~ mating with queens. The general viability of worker-produced drones is reviewed above. There seems to be no reason why full-sized worker-produced drones should not be fully competitive. The capabilities of undersized drones are less certain: if they mate effectively, their production will be explained; if they do not mate, their production in high numbers in some hopelessly queenless colonies will require explanation. First, reproduction via males of the hopelessly queenless colony does not require a perfectly efficient system. Provided the normal-sized drones mate, then the undersized ones are not particularly important, and would be one of many "pointless" enterprises in the hopelessly queenless society, on a par, for example, with the unproductive construction of many queen cells (described in section 4.3.2.8). Second, Free and Williams (1974) found that queenless colonies preferred to rear drones in drone cells when they were offered them in Langstroth hives. Most observations on worker laying have been made on combs in Langstroth hives, which are designed to reduce the incidence of drone cells, so that rearing of drones in worker cells in these hives may be a consequence of the unavailability of drone cells. Observations on natural hives are required.

(*continued from page 119*) function (Mayr, 1974). The straightforward application of teleonomic reasoning in this manner would thus appear to be heuristic in the analysis of complex social processes, notwithstanding current polemic on the more abstract aspects of the concept (*see* e.g. Krimbas, 1984, pp.11, 37; Wallace, 1984).

(iii) To maximise the chances of mating, hopelessly queenless colonies will produce many drones. Four *scutellata* colonies produced 1000-4000 sealed drone cells (chapter 4); one European colony produced 6000 drones (Page and Metcalf, 1984).

(iv) To maximize chances of mating, hopelessly queenless colonies and their drones will survive for as long as they can. This modifies the need simply to produce as many drones as possible (iii above), to the need to maintain an optimum number of drones over a long period. A three- to four-fold increase in worker longevity, and thus lengthy survival of the hopelessly queenless colony, was found in *A. m. scutellata* (chapter 4).

(v) Oviposition by workers and rearing of their brood will commence as soon as possible after the onset of hopeless queenlessness. (*see* chapter 5).

(vi) Worker laying, and rearing of resultant brood, will be inhibited or restricted during queen replacement (swarming, supersedure and emergency replacement) so that the colony's resources are not dissipated during these critical phases. In some races workers lay during queen rearing (*see* chapter 5); in these cases, this activity will not prejudice the queen rearing process. During the queen rearing phases the colony is at high risk of hopeless queenlessness. The partial ovarian development by workers at these times (chapters 2 and 5) may simply be a consequence of diminishing inhibitory factors (queen substance and brood) - but there is the possibility that worker ovarian development in these phases is a preparative process that allows efficient, rapid onset of worker laying should the queen be lost, and so could be influenced by selection on the hopelessly queenless phase.

(vi) At onset of hopeless queenlessness all worker brood of the late

queen will be reared to adulthood, since these workers will be an asset in the survival and ultimate production of drones by the colony.

(vii) At onset of queenlessness, drones and drone brood of the late queen may be in the colony. If hopelessly queenless workers try to maximise transmission of their own genotypes, they could enhance their chances of doing so by ejecting queen drones once they themselves have started rearing their own progeny. This would concur with Hamilton's kin-selection hypothesis, which predicts that workers should favour their own sons over their brothers (Hamilton, 1972; West-Eberhard, 1975). Tolerance of queen-drones along with laying worker drones would indicate selection for transmission of the colony's genes, and would detract from Hamilton's prediction of selfish worker reproductives. Queen-drones are tolerated in newly queenless colonies (Butler, 1964, 1974) and in queen-rearing colonies (Free and Williams, 1975) but no observations have been made on the fate of these drones when worker drones are reared.

(viii) In races in which the absconding habit is developed, colonies will not abscond readily when hopelessly queenless (as determined by Martin, 1963). It will be reproductively advantageous for a hopelessly queenless colony to remain in an established nest and produce drones, rather than to abscond to start a new nest. There may be circumstances under which it will be in the interests of a hopelessly queenless colony to abscond, e.g. when all individuals are at risk from an ant attack.

(ix) A queenless free-flying swarm will occupy a nest site and will attempt to draw combs and rear drones in them. The occasional occurrence of such colonies was mentioned by Winston (1987). In

established colonies that become hopelessly queenless, comb is drawn and laid in (Darchen, 1957; Free, 1967; Taber and Owens, 1970).

(x) To achieve optimal production of drones, hopelessly queenless colonies will exhibit a special social organisation drawn from that of the queenright colony but with sources of control alternative to those mediated by the queen in the normal colony. Thus in the hopelessly queenless colony there will be:

(a) A source of queen pheromones, to maintain a basic social organisation. Production of these pheromones by queenless workers has been demonstrated (reviewed in chapter 3).

(b) Maintenance of activities vital to colony survival - housekeeping, foraging, colony defence, etc., as reviewed in chapter 2. *A. m. scutellata* colonies were maintained for three to four months after onset of queenlessness (chapter 4) so that basic activities must have been organised amongst the diminishing, ageing contingent of surviving workers.

(c) Maintenance of nurse bees over a long period, in the face of lack of input of young workers to take over nurse duties. This was inferred in the present study from the constant brood rearing in the hopelessly queenless *A. m. scutellata* colonies (chapter 4).

(d) Polyethistic specialisation of workers that lay eggs. In the present study, an age-polyethism in ovarian development was indicated (chapter 2). The development of false queens was determined by Sakagami (1958); Velthuis (1985) proposed that the younger workers that take up laying do so as an alternative to becoming foragers.

(xi) The genetic integrity of the normal queenright colony is based on

the nest-mate or kin recognition system (Holldobler and Michener, 1980; Breed and Bennett, 1987; Moritz and Southwick 1987a; Wilson, 1987). If the hopelessly queenless phase has been formed through natural selection of its reproductive capacity, then a nest-mate recognition system must be predicted for it. Mechanisms to eliminate the intrusion of foreign gene carriers into the hopelessly queenless colony will include:

(a) repulsion of strange queens (Sakagami, 1954), drones (*see* Rinderer *et al.*, 1985) and workers: this prediction is directly opposed to that of Weaver (1986) who said that "In any likely scenario . . . if the choice is between having no queen or accepting a strange queen, the workers should accept a joiner."

(b) resistance to invasion and takeover by other swarms (*see* section 7.5);

(c) no stealing of female eggs from other colonies for use in rearing replacement queens, as has been suggested to explain the advent of queens in hopelessly queenless colonies (Dimitrijevitsch, 1935; Fyg, 1950).

Many of the above predictions (or hypotheses) will undoubtedly be modified or invalidated in future research and replaced by more refined ones. However, thinking about hopeless queenlessness as a result of natural selection will be the only way in which this important phase of honeybee life will be properly understood.

PART 2

COLONY DEFENCE BEHAVIOUR IN *A. M. SCUTELLATA*: AN ETHOLOGICAL INVESTIGATION

CHAPTER 7

INTRODUCTION: COLONY DEFENCE BEHAVIOUR IN HONEYBEES

7.1 PREAMBLE

The experimental work of this study concerns the measurement of colony defence behaviour of honeybees against the human intruder, and against artificial stimuli designed to release components of this behaviour. Ever since the advent of the fierce Africanized bees in South America (Michener, 1975) this subject has attracted increasing attention. In particular, the responsiveness of different honeybee strains and races has often been measured, usually with a view to selecting colonies for the breeding of docility (*see* sections 8.4.5; 8.5.6). Alarm pheromones and associated behaviour have also been studied intensively (section 8.4.2). In this extensive body of work, ethological principles have received uneven attention and many fundamental aspects of the behaviour have not yet been adequately analysed. For example there was no attempt at a summarizing model for the behaviour until Collins *et al.* (1980) and subsequently there have been no attempts to critically assess, or improve, this model. As a step to address some fundamental issues, colony defence behaviour in the present study was approached as an ethological problem, with consequent emphasis on behaviour description, and demarcation of behaviour units and biological functions of the behaviour (see Hinde, 1970, pp.3-16; Aspey and Blankenship, 1977).

In the present study the term *colony defence behaviour* (CDB) in honeybees was restricted to the behaviour exhibited by worker bees when they defend their colony against natural enemies that either prey upon the bees themselves, or rob the stores or brood in the nest cavity. CDB may

also be released by certain artificial stimuli. The term aggression does not apply to CDB (Crewe, 1976, 1977; Koeniger, 1979) and should be restricted to intra-colonial fighting, as between newly emerged queens or between normal and ovary-developed workers (chapter 3), and to some inter-colonial interactions such as rejection of strange queens. These *aggressive* interactions are undoubtedly under selective forces distinct from those affecting colony defence against predators, even though some behaviours (e.g. stinging, biting) occur in both systems.

An unequivocal definition of CDB is difficult to derive. There are many kinds of natural enemies against which honeybees may "defend" themselves, and reviews of the topic usually lump all of them under *colony defence* e.g. as the

> "abilities of bees to protect themselves from other organisms, whether parasites or predators, diseases, casual intruders, or other bees (even of the same species) that may rob or take over the nest" (Michener, 1974, p.209; see also Wilson, 1971, p.533; Fletcher, 1978; Winston, 1987).

Seeley (1985) listed, in addition, nest construction in protective sites, reproduction by swarming, synchronous orientation flights, and the antibiotic properties of propolis and honey. Seeley's evolutionary analyses provide ample justification for this broad approach to colony defence. However, as argued below, when a specific kind of colony defence is analysed it is useful to focus on its function and thus on the selective forces that may have shaped it, to help in distinguishing it from other defensive behaviours which may have arisen in response to different selective forces. Thus Seeley *et al.* (1982) categorized the various forms of CDB in three Asian species of *Apis* on the basis of function at colony level by using field observations of responses to various kinds of predators. In the literature consulted for the present study no similar

attempt has been made for *A. mellifera*. A preliminary analysis is given in the remainder of this chapter. With a general perspective thus established, the factors that contribute to variation in intensity of CDB against mammalian stimuli, and methods that have been devised to measure them, are reviewed in chapter 8, as an introduction to the experimental work reported subsequently.

A tabulation of honeybee enemies in 11 categories based on the stimuli they present, against the kinds of responses they evoke, revealed six stimulus categories that evoke responses entailing either combat or mass avoidance against enemies near the nest (Table 7-1). These culminate in mass actions properly termed *colony* defence behaviour. The behaviour of the individual worker bee when disturbed at a distance from the nest is excluded from CDB. Responses to parasitoids, nest parasites and diseases (Table 7-1) form separate groups, none of which entail repulsion of enemies that pose an immediate, directly combative threat: apart from a brief assessment of defence against wax moths (section 7.5), they are considered no further in the present study.

The natural enemies that evoke CDB (Table 7-1) seem to fall into at least five categories: (1) predation or accidental disturbance by vertebrates, which elicits mass stinging attacks; (2) predation by wasps, which elicits colony "demoralization"; (3) intrusion by conspecific robbers; (4) takeover swarms; (5) predation by ants.

7.2 CDB AGAINST VERTEBRATES, PARTICULARLY MAMMALS

Among vertebrates in Africa, man and the honey badger, *Mellivora capensis* (Schreber), are predominant predators of the honeybee colony (reviewed by Smith, 1960; Fletcher, 1978; Seeley, 1985; Winston, 1987). Their predation typically entails opening of the nest cavity and removal and consumption of

TABLE 7-1. Natural enemies of honeybees: the stimuli they present and the responses they evoke.

Stimulus		Response				
Natural enemy	Action of enemy	Combative: fighting, stinging		Non-combative		
		mass attack (i)	mass defence-recruitment (ii)	colony 'demoralization' (iii)	absconding (iv)	other (specified) (v)
Man, honey badger, various primates, various reptiles (section 7.2)	(1) physical destruction of whole nest	+	+	(+)	(+)	–
'Casual' large intruders, eg. cattle, poultry	(2) disturbance near nest	+	+	?	?	–
Various birds, reptiles	(3) predation of individual bee near nest	+	+	?	?	–
Predatory wasps (section 7.3)	(4) predation at nest entrance	–	+	+	?	–
Predatory wasps, birds, reptiles, etc.	(5) predation of individuals away from nest	–	–	–	–	individual avoidance
Ants, eg. *Anomma* (section 7.5)	(6) predation of nest inmates, plundering of stores	–	+	–	–	–
Conspecific robber bees (section 7.4)	(7) enter colony, plunder stores	–	+	–	–	–
Conspecific swarms (section 7.5)	(8) enter colony, colony take-over	?	?	?	?	–
Parasitoids, eg. tachinids[a]	(9) brief contact with individual bee	–	–	–	–	individual avoidance
Nest parasites, eg. wax moth[b]	(10) contact with individual	–	–	–	(+)	eject individuals, colony-hygienic behaviour
Diseases[c]	(11) disease	–	–	–	(+)	colony-hygienic behaviour

References:

[a] Skaife (1926, 1979); [b] Milum (1972), Butler (1974), Eischen *et al.* (1986); [c] Rothenbuhler (1964 a, b).

the combs. Honeybees respond to mammalian nest robbers with one of their best known behaviours: stinging the intruder *en masse*.

The honey badger with its coarse hair and thick skin (Roberts, 1951; Smithers, 1971; Nowak and Paradiso, 1983) is well armoured against such attacks; the scent of its anal gland is said to repel bees intent on stinging (Mountain, 1972; Kingdon, 1977). Friedmann (1955, and see Queeney, 1952 and Friedmann and Kern, 1956) provided evidence supporting the legend that the honey badger follows the honey guide *Indicator indicator* (Sparrmann) to bees' nests although, of course, honey badgers find many nests without reference to these birds (Dorst and Dandelot, 1970; Rosevear, 1974), if only because much of their hunting is done at night. In a survey of 24 000 tribal beekeepers' hives in Tanzania, 2700 (i.e. 11%) were damaged by honey badgers in a year (Kingdon, 1977). Fifteen or more hives in an apiary may be badly damaged in a visit by one or two honey badgers, sometimes in a single night (Chorley, 1936; Guy, 1972). In Africa honey hunters and tribal beekeepers often remove all the combs of a colony, in depredations similar to those by honey badgers. Honey hunting occurs in most of the regions occupied by *A. m. scutellata* and is done intensively in some places (Fletcher, 1978; Seeley, 1985). It is clear that in these circumstances there is a strong selective pressure for vigorous defence by the honeybee colony against mammalian intruders. It is generally thought that the incidence of this sort of predation has been higher in savannah Africa than in Europe, so that a correspondingly higher defensive behaviour has evolved in *A. m. scutellata* (Seeley, 1985; Winston, 1987). Rinderer (1988) argues that man may have been the primary selective agent in these defensive differences because, in his view, man in Africa has always destroyed nests to extract honey (even in hived swarms) whereas in Europe the approach has always been gentler.

Although other African vertebrates are capable of destroying

colonies in honey badger or human fashion, for example baboons (*Papio* spp.) (Botha, 1970; Ambrose, 1978; Caron, 1978; Anderson *et al.*, 1983), most are casual or opportunistic predators, easily driven from a well defended nest but willing to consume a weak one, e.g. various rodents; monkeys (Caron, 1978), mongooses; civet cats (Smith, 1960). Others, such as toads and lizards, will prey on individual workers around the hive entrance (Smith, 1960; Morse, 1978a).

Many birds in Africa prey occasionally on honeybees (Fry, 1983) but some are known to prey heavily on them, especially alpine swifts (*Apus melba* (Linnaeus)) (Anderson *et al.*, 1983), bee-eaters (*Merops* spp.) (Botha, 1970; Ambrose, 1978; Fry, 1983, 1984), bush-shrikes (*Telophorus zeylonus* (Linnaeus)) (May, 1969), European swallows (*Hirundo rustica* Linnaeus) (Guy, 1972a), and drongoes (*Dicrurus adsimilis* (Bechstein)) (Botha, 1970; Buys, 1982). Birds may be attacked when they venture near honeybee nests (Hind, 1912; Fletcher, 1978). The rate of forager flights may be greatly reduced during attacks near the hive by bee-eaters (Fry, 1983). (Complete cessation of flights is the common response to wasp predation at the hive: *see* section 7.3.)

Certain vertebrate attributes, especially mammalian ones, have been identified as highly effective in releasing CDB, including physical disturbance to the nest (Michener, 1972, p.28; Fletcher, 1978), mammalian breath (Maschwitz, 1964a,b; Boch and Rothenbuhler, 1974), odour of sweat, rough or hairy surfaces, and rapid movement of dark objects or objects with colour contrasts (Free, 1961). Mammalian skin gives purchase to the barbs of the sting thus causing sting autotomy, a process that does not occur when honeybees sting the fragile intersegmental membranes of other arthropods (Butler, 1974; Eischen *et al.*, 1986): sting autotomy functions in the stinging of mammals (Hermann, 1971). Mammalian intruders thus present an array of stimuli that release CDB and honeybee defenders have

defensive responses and structures specific to them.

In CDB against vertebrates the workers that first detect the intruder may run into the nest and recruit defenders by releasing alarm pheromones through sting-fanning (Maschwitz, 1964a,b, 1966; section 8.4.2), or they may fly at the intruder and sting it, with sting autotomy and release of alarm pheromones (Ghent and Gary, 1962; Blum, 1969; Anderson *et al.*, 1983). Some workers may fly around the intruder with a characteristic jerky flight and high-pitched buzzing (Collins *et al.*, 1980; Rinderer, 1982). If the intruder persists there is rapid increase in the number of defenders. If the intruder retreats, defenders may follow over distances of a kilometer or more, particularly in the highly responsive races, notably *A. m. scutellata* and Africanized bees (Michener, 1972). If the attack is severe and prolonged, the intensity of the defence may diminish until the colony is "demoralized" (Farrar, 1968), or the colony may abscond (May, 1969, p.172; Fletcher, 1975).

Smith (1960) noted that African colonies in a state of extreme alertness produce a "menacing hiss" if an enemy comes near. In *A. cerana* Koeniger and Fuchs (1973) found a strong hissing response to stimuli associated with large predators: small predators such as wasps did not elicit it, so that it was distinct from the shimmering response by Asian honeybees to wasp predators, described in the next section.

CDB elicited by mammalian stimuli is highly variable in form and intensity (chapter 8). Nevertheless, it can be distinguished from other forms elicited by predatory wasps and by conspecific robber bees, described below.

7.3 CDB AGAINST APIVOROUS WASPS

Various species of wasp prey predominantly, some perhaps exclusively, on

honeybees. This predation takes at least four forms:

(i) Foragers are captured on flowers, e.g. by *Philanthus triangulum* (Fabricius) in Europe (De Jong, 1978) and by *Philanthus triangulum diadema* (Fabricius) in southern and central Africa (Mally, 1908; Attridge, 1909; Taylor, 1939; Skaife, 1953, 1979; May, 1969; Fletcher, 1978). These attacks may elicit combative or escape responses from the victim, but these responses do not fall within *colony* defence behaviour as defined in section 7.1.

(ii) Wasp predators pounce on bees at the nest entrance, e.g. *Palarus latifrons* Kohl in southern and central Africa (Mally, 1908; Attridge, 1909; Greathead, 1911; Taylor, 1939; Skaife, 1953, 1979; May, 1969; Fletcher, 1978).

(iii) In Africa, swarms clustered in the open may be attacked by "predatory wasps" (Hind, 1912).

(iv) In southeast Asia, workers on the open air nests of *Apis florea* Fabricius (and probably of *Apis dorsata* Fabricius) are picked off and killed by *Vespa tropica* Linnaeus wasps until the colony absconds, whereapon the wasps take the brood to their own nests (Seeley *et al.*, 1982). *Vespa orientalis* Linnaeus takes larvae, pupae and adults from *A. mellifera* (De Jong, 1978), as does *Vespa mandarinia* Smith from cavity-nesting *Apis cerana* Fabricius (and from imported *A. mellifera*) in the Far East (Matsuura and Sakagami, 1973).

In *A. florea* and *A. dorsata*, which do not nest in cavities, workers of the outer protective curtain of the nest cluster display the *shimmering* response to flying predators (Koeniger and Fuchs, 1975) such as *V. tropica*, and wasps that alight may be *balled* by several workers simultaneously. Shimmering may be elicited by the approach of a large intruder such as man

(Butler, 1974, p.151), but here the main defensive response is the mass stinging attack (Seeley *et al.*, 1982).

A. cerana may display shimmering behaviour (Butler, 1974; Koeniger and Fuchs, 1975) even though it usually nests in cavities into which workers retreat when accosted by wasps; if the wasps press their attack, they are balled in the confines of the nest entrance (Seeley *et al.*, 1982). *A. mellifera* colonies also ball wasps at the nest entrance, and cease flying under wasp attack (Ono *et al.*, 1987). The latter behaviour is termed *demoralization* by beekeepers, because the colony appears unwilling to come out and fight the wasps, and foraging time is lost. *Demoralization* appears to be a misnomer, since the behaviour is more likely a component of active defence (see Seeley *et al.*, 1982). For the present study the term *colony defensive retreat* is preferred. In *A. m. scutellata* this behaviour is common in in response to predation by *P. latifrons* (Anonymous, 1895, 1930, 1935; Mally, 1908; Greathead, 1911; Bates, 1930; Skaife, 1953, 1979; May, 1969; Mountain, 1983, 1969).

Shimmering does not occur in European races of *A. mellifera*, but it does in Cyprian *A. mellifera*, and may in the other Middle-Eastern and African races in regions where wasp predation is prevalent (Butler, 1974, p.14).

The fast zig-zag flight typical of *A. m. scutellata* may have evolved in response to wasp predation (Smith, 1958b; Butler, 1974).

It is clear that responses of honeybee colonies to wasp predation at the nest are essentially different from the CDB elicited by mammals such as man and honey badgers, as described in the previous section.

7.4 CDB AGAINST CONSPECIFIC ROBBER BEES

Another form of CDB, different from that displayed against vertebrates or

apivorous wasps, is the response of the colony to *conspecific robber bees*, which are workers that take honey from neighbouring colonies, often during nectar dearths (Butler, 1974; De Jong, 1978; Koeniger, 1982; Seeley, 1985) or, in the apiary, from combs exposed in beekeeping operations (Farrar, 1968; Root *et al.*, 1972; Cale *et al.*, 1975).

Butler and Free (1952) and Free (1955) described behavioural interactions at the hive entrance between robber bees and the guard bees of the defending colony, but did not mention colony defensive retreat ("demoralization") (section 7.3); this behaviour is not mentioned in this context in any other publication consulted for this study. On the contrary, robbing generally increased the number of guards and their readiness to defend. Butler and Free (1952) (see also Free, 1955) concluded that attack on robbers by guards was released by the characteristic swaying flight that robbers assume as they approach a colony. Alighting robbers were stung when they attempted to escape, whereas other workers that flew to the hive normally were examined and mauled if they presented a *submissive* posture but went unmolested if they presented a *dominant* posture. This interpretation was disputed by Ribbands (1954) who emphasized the role of the acquired odours of robbers in their recognition. Ribband's hypothesis was challenged in turn by e.g. Lecomte (1952) and Chauvin (1968a) in favour of a genetic origin of distinctive colony odour, an hypothesis which has recently been supported by Moritz and Southwick, (1987a). It is evident that defence against robbers constitutes a specialized intra-specific behaviour complex which stands apart from other kinds of CDB. Essentially, CDB against robbers is founded in a complex nest-mate recognition system (reviewed by Holldobler and Michener, 1980; Breed and Bennett, 1987). This element is absent from all other forms of CDB except, perhaps, colony takeover (section 7.5). The problem of whether robbing and/or colony takeover should be classified as

aggression (section 7.1) rather than as CDB is an open question, but is beyond the scope of the present study.

7.5 OTHER FORMS OF CDB

Invasion of an established colony by a swarm

No detailed description exists of the behavioural interactions in the takeover of one colony by another (Kigatiira, 1988). *A. m. scutellata* swarms fight (Pullinger, 1929; Hayter, 1947; Kigatiira, 1988) for hives (Mowbray, 1948) and have been seen to attack and oust strong imported European colonies (Simpson, 1906; Ntenga, 1964), particularly in times of dearth (Johnson, 1964; Fletcher, 1978). Africanized bees in South America also attack and take over European colonies (Kempff-Mercado, 1973; Michener, 1972, 1973, 1975), although they also achieve takeovers without fighting (Kempff-Mercado, 1973; Taylor, 1985; Rinderer, 1986a,b). Strong European *A. mellifera* colonies when robbing may kill weaker colonies of their own race, as well as *A. cerana* colonies (Koeniger, 1982). Defence against colony takeover would undoubtedly constitute a separate form of CDB, founded in the nest-mate recognition system and perhaps allied with defence against robbers (section 7.4).

CDB against ants

Many kinds of ants attack honeybee colonies, with varying degrees of severity (De Jong, 1978). Defending workers may kick at ants and fan them away from the hive (Spangler and Taber, 1970; see also Seeley *et al.*, 1982 on *A. cerana*). In tropical Africa the doryline army ants *Anomma* spp. and *Dorylus* spp. may consume the entire colony (stores, brood and adults) and

honeybees appear defenceless against these attacks (De Jong, 1978), although they may abscond (Chorley, 1936). No detailed description of responses to attacks by *Anomma* or *Dorylus* was found in the literature consulted, although their distinctive mode of attack (Wilson, 1971, p.72) and its high frequency in tropical Africa (Cheesman, 1948; Smith, 1960; Fletcher, 1978) could well be associated with a co-evolved response by *A. m. scutellata*.

Attacks on wax moths

In section 7.1 actions against wax moths (*Galleria mellonella* were excluded from CDB (as defined in the present study) on the grounds that the moths and larvae did not pose a combative, predatory threat. Wax moths are seen as ectosymbionts (Wilson, 1971, p.398) rather than as predators: their depredations are insidious, with a delayed effect following invasion of the colony. Nevertheless, *G. mellonella* larvae are stung and dragged from the hive, and moths may be attacked when they enter the hive (Nielsen and Brister, 1977, 1979). Eischen *et al.* (1982) compared intensities of attacks on *G. mellonella* moths by European and Africanized colonies and speculated that the more vigorous attacks by the latter were associated with the known higher defensiveness of these bees towards mammalian intruders. At present there is no evidence concerning this contention. Research on the problem would, however, be facilitated by reasoning about it from the broad perspective of the present review, in which CDB is surveyed with the view that different types of enemies may constitute separate selective forces, which may give rise to genetically and behaviourally discrete defensive behaviours.

7.6 DISCUSSION

In the foregoing review of the natural history of honeybee CDB four, possibly five, distinct forms of response to five different types of predation were evident. The differences between these responses consist in the behaviour *as it emerges at colony level*. Thus vertebrate intruders elicit guard recruitment and are flown at and stung *en masse* by colony defenders aroused and oriented in part by alarm pheromones, whereas wasp predators cause cessation of flying (colony defensive retreat), by which means combat is brought to the confines of the nest entrance (Seeley *et al.*, 1982). Both these forms differ from defence against conspecific robbers, which are mauled and stung at the nest entrance, and cause guard recruitment, but do not result in colony defensive retreat or flying attacks *en masse*. Even though various behaviours performed by individuals (stinging, biting, etc.) may occur in different forms of CDB, it seems likely that each form of CDB has evolved in response to a particular type of enemy.

The bald statement of the above conclusion may seem self evident, but it has important consequences for the study of CDB. In particular, the cues that release the different forms of CDB are important in the design of artificial stimuli in the experimental investigation of CDB. The effective use of artificial stimuli in the analysis of a defensive response was demonstrated by Koeniger and Fuchs (1975) who found that shimmering in *A. cerana* was not released by mechanical or chemical stimuli such as sound, vibration, flashing light, stationary objects, smoke, isopentyl acetate, and phenol: it was released only by the movement of a dark object against a light background. The intensity of shimmering induced by presenting an oscillating disc was affected by the size of the disc (which was optimal at 7 mm diameter, approximately the size of a wasp predator) as well as its

rate of movement and distance of presentation. These disc stimuli were thus interpreted to represent flying insect predators. In many investigations on CDB in *A. mellifera* small objects (pieces of leather, spheres of cotton, termed *targets* or *lures*) have been presented at the hive entrance, in the presence of the investigator, to elicit CDB which was then interpreted to represent colony responsiveness towards humans (*see* section 8.5.2). However, from the findings of Koeniger and Fuchs (1975) outlined above, it seems that the presentation of a wasp sized object by a human could constitute a simultaneous presentation of two very different stimuli, which can elicit two different forms of CDB: the response to the human is flying out and stinging, whereas the wasp-like object presented on its own might elicit a strong retreat response, particularly in regions in which predatory wasps are prevalent. Thus quantification of stings to a wasp sized bobbing object may (to some extent) be confounded as an index of colony responsiveness to humans. No matter whether or not lure tests are in fact confounded for this reason, the example shows that the more we know about the natural history of CDB, the better we will be able to devise ways of measuring it. The importance of referring artificially elicited behaviour to its natural context has been stressed in general ethology (e.g. Hinde, 1970, pp.57-145) but, as will be shown in the following reviews, it has not received sufficient attention in many studies of CDB intensity.

CHAPTER 8

REVIEW OF STUDIES ON CDB AGAINST HUMANS AND AGAINST ARTIFICIAL STIMULI

8.1 PREAMBLE

The great variation in CDB against the human intruder is a well-known characteristic of *A. m. scutellata* (Fletcher, 1978), as may be seen in successive bouts of CDB in a single colony, or when different colonies are compared (Collins *et al.*, 1980). Even though some races of honeybees are known to be generally more vigorous in their CDB than others (e.g. Africanized vs. European bees: Rinderer, 1982), the variation found within each race is usually great, so that Fletcher (1978) had to conclude that the contention that *A. m. scutellata* is a "vicious" race "is not valid in any absolute sense." The variation in the form of the behaviour arises from differences in the numbers of workers that participate in a bout of CDB (between one and several thousand), from the various defensive behaviours that each defender may perform (section 8.2), from the intensity with which these behaviours are performed, and from permutations of these factors.

The measurement and analysis of complex social behaviour such as CDB offers a special challenge in ethology, as aptly expressed by Wilson (1971, p.224):

> "The individual social insect . . . displays behavior patterns that are neither exceptionally ingenious nor exceptionally complex. The remarkable qualities of social life are mass phenomena that emerge from the meshing of these simple individual patterns by means of communication. In this principle lies the greatest challenge and

opportunity of insect sociology."

The requirements in such analyses are, first, description of the range of individual behaviours that comprise the social process; second, description of the mass action involved; third, derivation of methods for measuring the intensity, rate, or extent of the mass action; fourth, derivation of *correlated behaviour measurements*, especially for use in breeding (Rinderer, 1986d); and finally, the improvement of existing theory by critical analysis and experimentation. The basis of this approach for honeybee CDB against humans, and against certain artificial stimuli, is set out in this chapter, from which the experiments reported in chapter 9 were derived.

8.2 BEHAVIOUR OF THE INDIVIDUAL IN CDB

Collins *et al.* (1980) classified defensive responses of the individual honeybee to humans, and to various artificial stimuli, into four sequential steps, summarized below.

(1) *Alerting*. Initial response to danger stimulus may entail:

  (a) *Alert response*. Tense posture with cocked abdomen, wings extended or fanning, mandibles open and, occasionally, sting protracted;

  (b) *Recruiting response*. Worker runs into the hive with protracted sting and intermittent sting fanning, dispersing alarm-recruitment pheromones in the nest;

  (c) *Withdrawal response*. Workers retreat from the stimulus object.

(2) *Activating*. Alerted worker seeks stimulus object, walking or flying about the nest.

(3) *Attracting (or Orienting)*. Alerted worker orients to (or is attracted to) the stimulus object.

(4) *Culminating*. Mutually exclusive responses include:

(a) *Threat*:

(i) *Flying*. Worker flies rapidly round stimulus object (i.e. the "culminating stimulus"), with a characteristic high-pitched buzzing;

(ii) *Walking*. Worker walks, runs, towards stimulus object and makes body thrusts towards it, with antennal and prothoracic legs waving.

(b) *Retreat*: worker flees the stimulus object or the nest itself;

(c) *Contact-combative behaviour*:

(i) *Head-bumping* (in flight, up to 17 bumps may precede stinging in Africanized bees: Rinderer, 1982);

(ii) *Stinging* (plus release of alarm pheromones);

(iii) *Biting* (with possible release of alarm pheromones);

(iv) *Hairpulling* and *burrowing* into hair, clothes or fur.

While presumably all the listed responses are released by mammalian-type stimulus objects, certain of them may apply more in defence against conspecific robbers, i.e. walking-threat (4aii) (*see* section 7.4); or against predatory wasps, i.e. withdrawal (2c) and culminating-retreat (4b) (*see* section 7.3). Others, such as the contact-combat responses (4c) must be common to all three types of CDB.

Three-quarters of the categories entail release of alarm pheromones, viz. alerting (1a), recruiting (1b) and culminating (4aii), (4ci), (4cii), (4ciii). This reflects the broad basis of organisation *above the level of the individual* in honeybee CDB, as deduced from the natural history of the

behaviour (section 7.6), and as discussed below.

8.3 MASS ACTION IN CDB

Collins *et al.* (1980) based their model on the behaviour of the individual worker. This they took as the "basic unit of colony defence" because defensive behaviour by a single bee is commonly seen when colonies are manipulated, and because

> "collective defensive episodes can be viewed as an aggregate of individual responses with the activities and pheromone emissions of other members of the colony functioning as stimuli for the individual defending bees."

By contrast, in the present study the behaviour of the individual worker is considered a *component* of CDB, and the mass action against the intruder as the "basic unit" of the behaviour. This approach can be defended through the differences drawn in the previous chapter between several forms of CDB against different types of predators: these differences emerge in the behaviour at colony level, as mass actions, and are not distinguished on the basis of individual behaviour alone. Further, although a bout of CDB can be executed by a single worker it is usual, certainly in *A. m. scutellata*, that even slight interference with the nest will result in defensive action by several or many workers: the defensive response *en masse* is the normal expression of the behaviour. From this perspective the "collective defensive episodes" of Collins *et al.* (1980) are more than a straightforward aggregate of individual responses. Consider the case in which two workers "X" and "Y" at the nest entrance detect a danger stimulus in the form of a mammal close by. In response, bee X runs sting-fanning (Maschwitz, 1966) into the nest while bee Y flies up and stings the intruder, thereby labelling it with alarm pheromones (section 8.4.2) which

serve as an orienting and sting releasing stimulus to the many bees that have emerged from the nest in response to the alarm phermones emitted by bee X. The effect of these actions is of a higher order than the simple sum of the individual behaviours of bees X and Y. This exponentiation can be visualised by comparison with the lower order effect that results if, for instance, both bees respond "individually" either by retreating without sting fanning, or by flying about the intruder without releasing pheromones. Release of alarm pheromones is intrinsically a communicative social behaviour in which "insect colonies translate the numerous individual behavioural acts of its members into higher order effects." (Wilson, 1971, p.224). In fact, workers must be in a group (Moritz and Southwick, 1987b) of at least 20-40 before they register a typical response to alarm pheromones (Southwick and Moritz, 1985; Moritz and Burgin, 1987; Moritz *et al.*, 1987); Moritz (1988) showed that CDB must be measured as a group response in selecting colonies for breeding to reduce defensiveness. In the previous section it was noted that three-quarters of the categories of individual defensive behaviour of Collins *et al.* (1980) entail release of alarm pheromones. When it is considered that often hundreds or thousands of defenders participate in bouts of CDB, which generally take a recognizable form, the necessity of viewing the defensive mass action as the basic form of the behaviour is clear.

Bouts of CDB most often start at the hive entrance (Butler and Free, 1952; Gary, 1975; Seeley, 1985; Breed and Moore, 1988) (although this does not apply to colonies that do not nest in cavities - in these, the workers on the periphery of the cluster may react to intruders by releasing alarm pheromones: Morse, 1966). Maschwitz (1966) demonstrated the "typical course of alarm" in European bees by touching, with a pair of forceps, the bees at the nest entrance of a calm colony:

"The irritated bees . . . raise their abdomina . . . , protrude

their sting apparatuses [and] with wings whirring, run into the hive from which immediately emerges a score of excited bees, ready to attack any opponent."

The recruitment is a response to alarm pheromones released by the sting fanning of the irritated bees. The only other stimulus that elicits sting fanning is mammalian breath. No bees initially alarmed by pheromones respond by releasing their own pheromones. The latter observation, of Maschwitz, was confirmed by Shearer and Boch (1965), Boch *et al.*, (1970), Boch and Shearer (1971), and by Law and Regnier (1971) who also noted that release of alarm pheromone upon perception of such pheromones would lead to chain reactions in the colony, a process that does not occur.

Maschwitz (1966) distinguished between the effects of alarm pheromones released *within the nest*, which cause recruitment to the entrance, patrol running, flying near the nest, and readiness to attack; and release of them *outside the nest when intruders are stung*: these attract other defenders, thus localizing the attack. Alarm pheromones did not elicit attack when presented on "unattractive" objects such as plain white surfaces, but they did enhance stinging of an attractive object. Thus while alarm pheromones on a stimulus object attract and excite defender bees, attack is triggered by stimuli from the object itself. Phermonal tagging of mammal intruders is enhanced by sting autotomy (section 7.2).

Maschwitz did not refer to other work that has been done on *guard bees* (section 7.4). In undisturbed colonies "few if any bees guarded the hive entrances, but rapidly appeared if hives were physically disturbed by vigorous thumping" (Butler and Free, 1952). Sekiguchi and Sakagami (1966) (Japan; honeybee type not specified) found that 6-35% of workers in groups of the same age intermittently guarded the entrances of undisturbed colonies. The ages at which they did so varied widely but most were in

transition between house bee and field bee status; established foragers also guarded on occasion (*see* section 8.4.6). Schua (1952) in Germany found that the number of bees at the hive entrance, most of which he *assumed* were guards, was associated with the time of day (none at night, maximum at early afternoon) and with the weather (few in cold, more in hot, many before storms), and that colonies with many guards were the more ready to attack humans. The presence of conspecific robber bees causes recruitment of guards and prolongs their presence at the hive entrance (Butler and Free, 1952).

Not all workers at the hive entrance are guards. The nest entrance is a focal point for many other activities (Seeley and Morse, 1978) including Nasanov fanning (Free *et al.*, 1983); ventilation fanning (Gary, 1975); propolizing (Lovell and Root, 1972); "rocking movement" in polishing around the entrance (Gary, 1975); nest cleaning (Gary, 1975); and pausing before or after flight activities such as foraging, orientation, defaecation, nest cleaning, swarming, absconding. (The incidence of workers at the entrances of experimental colonies of *A. m. scutellata* is reported in chapter 9.)

Some workers responding to danger stimuli, or recruited by alarm pheromones in the nest, do not adopt the guard posture, but straight away take to the air (Blum, 1969). In *A. m. scutellata*,

> "When the state of alert is not reinforced they only intimidate the enemy by menacingly buzzing around and deliberately bumping into the enemy. When the attack is followed through, the smell of the stings implanted in the enemy serves as an added incitement and also as a guide to the target. Bees are then not easily dissuaded and may pursue the intruder for a considerable distance." (Anderson *et al.*, 1983).

Michener (1972) emphasized that Africanized bees (which are behaviourally similar to *A. m. scutellata*: Michener, 1975; Ruttner, 1986) in northern Brazil differ from other races in

> "their great sensitivity to colony disturbance, their ability to communicate alarm within and between colonies, and their capacity to respond quickly by massive attack on intruders . . . hundreds of bees become airborne and pursue and sting any animals or people within 100 m of the apiary."

A retreating observer in Michener's survey was followed for over a kilometre.

The capacity for vigorous mass attack has been well documented for *A. m. scutellata* (Fletcher, 1978) with many reports of livestock killed, from poultry to cattle (e.g. Edmunds, 1930). Murray (1964) described how a person survived an attack by submerging himself in a pool, where he remained for four and a half hours, being stung each time he emerged to take a breath: 2243 stings were later removed from his skin. The prevalence of mass attacks in *A. m. scutellata*, albeit usually of a lesser intensity than the extremes cited above, demands analysis of CDB at colony level. These are the effects experienced by the mammalian intruder, and this is the form of the behaviour that must ultimately be assessed in programmes aimed at breeding docile bees, even though correlated behaviour measurements (Rinderer, 1986d) may be preferred in the actual breeding programmes (*see* section 8.5.6).

Workers may respond to danger stimuli with behaviour not associated with attack. The engorging response to smoke in the colony (Free, 1968) is an emergency preparation for re-establishing the nest after a fire, or after fleeing from it (Newton, 1968); or for emergency actions, e.g. ventilation-fanning and water collection (Root *et al.*, 1972, p.647). Newton (1968) showed that low frequency vibrations in the hive, e.g. from

blows by falling weights, caused both "aggression" (i.e. CDB) and engorging, and so suggested that this engorging behaviour is a preparation in some of the bees for repairing damage to the nest after an attack. Some beekeepers recommend that a few sharp blows to the hive, after smoking, helps to calm fierce colonies before hive manipulation (Lownds, 1912; Martin, 1912a). If a colony is subjected to severe, prolonged attack, the defenders may cease defensive behaviour and may abscond (section 7.2).

8.4 FACTORS WHICH AFFECT VARIATION IN CDB

While the complex composition of CDB is the basis of its great variability, the factors that affect it are themselves complex and varied. Mention of them is scattered through many publications (see e.g. review by Goncalves and Stort, (1978). Fletcher (1978) classified these factors under the categories:

(i) *primary stimuli* (alert the bees, provoke attack);

(ii) *secondary stimuli* (whereby defenders orientate to the primary stimuli);

(iii) *recruitment stimuli* (alarm-recruitment pheromones);

(iv) *environmental factors* (lower the response-threshold to primary stimuli);

(v) *internal-colony factors* (lower the response-threshold to primary stimuli).

For the purposes of the present study, Fletcher's classification was modified as follows:

(1) *danger stimuli* (= "primary" and "secondary" stimuli);

(2) *alarm pheromones* (= "recruitment stimuli");

(3) *environmental factors* (= unchanged);

(4) *internal-colony factors* (= unchanged);

(5) *genetic factors* (= new category);

(6) *ontogenetic factors* (= new category).

Reasons for these changes are given in the reviews below. As in Fletcher's scheme, the categories overlap: the aim is simply to subdivide factors that affect CDB in order to facilitate comprehension of them.

In the revised classification categories (5) and (6) (genetic and ontogenetic factors) are not stimuli, whereas the others are. In general ethology, the term *stimulus* may refer to "a part or to a change in a part of the environment; or it may be confined to something which elicits an observable response." (Hinde, 1970, p.54). Categories (1) and (2) are the latter type of stimuli, while the former type includes categories (3) and (4), which in general usage are also termed *factors*.

8.4.1 Danger stimuli

Fletcher (1978) cited physical disturbance of the nest as the most effective "primary" stimulus that provokes stinging behaviour, as distinct from "secondary" stimuli such as movement and colour "whereby defenders orientate to the target." Fletcher noted, however, that movement could be regarded as either a primary or a secondary stimulus, as could odours of various animals, plants and minerals. Thus, distinguishing between "primary" and "secondary" stimuli does not provide any clear separation of the stimuli that elicit and mediate CDB. This problem can be circumvented by reference to the standard ethology of effective stimuli, from which we know that animals respond selectively to the many forms and changes in

physical energy to which they are constantly exposed, and that a response can be evoked through more than one physical modality - in every case, some features of the natural stimulus object have been shown to be of major importance in eliciting the response in question, while others have a lesser, or minor, effect (Hinde, 1970, p.57). Thus stimulus characters of major importance in releasing stinging of cloth balls by colony defenders were dark colour, sting venom odour, sweat, and movement, whilst "general bee odour" was of lesser importance (Free, 1961).

In cases in which stimulus objects evoke a specific response (e.g. stinging), the response is invariably elicited by relatively few stimulus characters, which together in the appropriate conformation are termed *sign stimuli*. In all Hinde's (1970) examples the natural stimulus object was taken as the point of reference and analysis was then made of *aspects* (or *attributes*) of the object. CDB has evolved to repulse specific objects (natural enemies) that intrude on the nest. Without an object to attack, a *full* sequence of CDB cannot occur. Thus the primary and secondary stimuli of Fletcher's classification are better regarded within a single category, the *effective danger stimuli*, abbreviated to *danger stimuli*. Physical disturbance to the nest, specified odours and movement are attributes that can release a full bout of CDB against the stimulus object. These, and other attributes of the stimulus object, are important in *orientation* of defenders, through the visual sense (movement, colour, colour contrasts) and through the olfactory sense (breath, sweat, alarm pheromones, certain inanimate odours). Combative responses such as biting or stinging are enhanced by certain tactile properties of the stimulus object, such as relatively high temperature, and furry surfaces (Maschwitz, 1966). With highly responsive *A. m. scutellata* colonies odour, or movement, alone may elicit stinging attacks (e.g. Lownds and Attridge, 1912; Edmunds, 1922; Crisp, 1939; Anonymous, 1943; Hayter, 1946, 1947; Fletcher, 1978) and even

inanimate objects may be stung (Papageorge, 1960) e.g. sacks of meal and bundles of clothing (Edmunds, 1930).

In general, natural stimulus objects present complex stimulus-composites, attributes of which may simultaneously elicit a variety of defence responses: the nature of the stimulus object is therefore associated with the variability of the defensive response.

In Table 8-1 some attributes of stimulus objects known release CDB are tabulated against the kinds of responses they elicit: the relative strengths of these responses are represented symbolically, for the general capacities of arousal, elicitation and orientation (Manning, 1972, p.38), and for specific responses. The relationships depicted are estimations derived from various analyses in the literature and are intended to give an overview of the differential effects on CDB of mammalian stimulus objects. They could of course be presented differently, depending on the evidence consulted but, no matter which interpretations are adopted, it is evident from the complexity displayed that none could give clearcut, mutually exclusive categories.

8.4.2 Alarm pheromones

Alarm pheromones are an intrinsic and major factor in CDB against humans (sections 7.2; 8.3). Fletcher (1978) discussed the contribution of IPA*

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* Iso-pentyl acetate, a major alarm releasing component of sting pheromone (Boch *et al.*, 1962; Boch and Shearer, 1971), which contains at least 23 other volatile compounds, many of which also elicit alarm (Blum *et al.*, 1978; Collins and Blum, 1982, 1983; Pickett *et al.*, 1982; Free *et al.*, 1983; Grandperrin, 1983; Blum and Fales, 1988; Whiffler *et al.*, 1988).

TABLE 8-1. Elements of colony defence behaviour evoked by aspects of mammalian danger stimuli, artificial stimuli, and alarm pheromones.

	Stimulus aspect	General response [a]			Specific response (in nest)				Specific response (to stimulus object)		
		arouse	orient to object	elicit a direct response	alert	move to entrance	sting fan	release mandibular pheromone	orient	sting	bite
Kinetic energy:	physical disturbance to nest [b c]	●●●	–	ooo	●●●	●●●	?	?	–	–	–
	physical irritation of worker [d]	–	–	●●●	–	–	●●●	?	–	ooo	o
Visual (stimulus object):	movement [d e f g h]	–	●●●	●●	–	–	–	–	●●●	●●	o
	light/dark contrast, with movement [e]	–	●●●	●	–	–	–	–	●●	●	o
Tactile (stimulus object):	mammalian skin, [d i] warmth, [d] fur, rough texture [e j]	–	–	●●●	–	–	–	–	–	●●●	●●●
Odour (stimulus object):	"general bee odour" [e]	–	o	o	–	–	–	–	o	●	o
	sweat [e]	–	o	o	–	–	–	–	o	●●	o
	inanimate, eg. paraffin [k l]	o	o	●●●	–	–	–	–	o	●●	o
	ex bee sting [e f g]	–	●	●●●	–	–	–	–	●	●●●	o
	ex bee mandibular gland (when biting) [d g m n]	–	o	●	–	–	–	–	o	●	o
Odour (free in nest):	mammalian breath [d o]	●●●	?	●●●	?	?	●●●	?	–	–	–
	ex bee sting [d]	●●●	–	●●●	●●●	●●●	–	?	–	–	–
	ex bee mandibular gland [d m p]	●	?	o	●	●	?	?	–	–	–

●●● = strong response
●● = response weaker than ●●● but stronger than ●
● = weak response
o = probably elicits response: no analysis in the literature
? = might (or might not) elicit response: no analysis in the literature
– = response not elicited, or situation does not apply

References: [a] Manning (1972); [b] Butler and Free (1952); [c] Fletcher (1978); [d] Maschwitz (1964b, 1966); [e] Free (1961); [f] Ghent and Gary (1962); [g] Free and Simpson (1968); [h] Collins and Kubasek (1982); [i] Hermann (1971); [j] Lecomte (1954, 1961); [k] Lownds and Attridge (1912); [l] Crisp (1939); [m] Boch and Shearer (1967); [n] Free (1987); [o] Seeley *et al* (1982); [p] Shearer and Boch (1965)

and 2HPT* to variation in CDB under "recruitment stimuli". However, in insect sociobiology recruitment is a loose descriptive term for communication that gathers nestmates where work is required, in food gathering, nest construction, nest defence, migration, etc. (Wilson, 1971, p.247). In the context of CDB the effect of sting and mandibular pheromones is better categorized under *alarm*, which subsumes recruitment as well as arousal, alerting, attracting, orienting, labelling and attack enhancement (Wilson, 1971, p.235; *and see* sections 8.2; 8.3; Table 8-1).

Alarm pheromones are social signals, the initial release of which is elicited by extraspecific sign stimuli (danger stimuli). When honeybee workers (near the hive) not involved in CDB perceive the pheromones they switch to defence; individuals already engaged in CDB may upon perception of the pheromones modify their behaviour (intensify it, or orient to and attack a pheromone-laden stimulus object) (Ghent and Gary, 1962; Maschwitz, 1964a,b, 1966; Blum, 1969; Shorey, 1973; Free, 1987). By their pervasive effects in CDB, alarm pheromones may be regarded as a physiological mediator of the behaviour analogous, say, to a rapidly acting mamalian hormone, e.g. adrenalin. In general, the more pheromones released (i.e. the higher the concentration and the wider the spread), the stronger the mass response (Blum, 1969; Collins *et al.*, 1987a), although artificially high concentrations may repel (Boch *et al.*, 1970; Boch and Shearer, 1971).

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* 2-heptanone, an alarm releasing substance in worker mandibular glands (Shearer and Boch, 1965), the only one known (Free *et al.*, 1983; Al Sa'ad *et al.*, 1985).

Alarm pheromones are associated with variation in CDB through the following factors:

(i) *The response threshold of the workers that first release alarm pheromones.*

(ii) *The response threshold of the workers that perceive alarm pheromones.* Understood in terms of the ratio of (i) the quantity of pheromone released to (ii) the concentration of molecules in the air required to elicit a response (Q/K ratio) (Bossert and Wilson, 1963; Wilson and Bossert, 1963; Crewe, 1977).

(iii) *The quantities of pheromones released*, which in a bout of CDB depends on the quantity released by individuals and on the number of individuals that release them, so that the pattern of release in space and time may differ from bout to bout. The latter factor has not been analysed.

The amount of IPA is nil in newly emerged workers and maximal in workers of guard age; 2HPT is absent in workers until they begin to guard or forage, and then increases steadily throughout the rest of their lives: the amount of alarm pheromone carried by a worker is loosely associated with its defence proclivities (Boch and Shearer, 1966, 1967; see also Whiffler *et al.*, 1988). Changes in the worker age structure in a colony will carry changes in quantities of alarm pheromones available for release and in this way may contribute to CDB variability.

Boch and Rothenbuhler (1974) found that workers from a strain (of European bees) known to be highly defensive had about a third more IPA than a docile strain although, within the fierce

strain, some highly responsive colonies had relatively little IPA in their workers, which indicated that efficiency in communicating alarm arose from a low response threshold in the perceivers of the pheromone rather than from high production of alarm pheromones. In fact, there were no correlations between amounts of IPA in workers and the responsiveness of their colonies to various measurements of CDB. Kerr *et al.* (1974) also reported no correlation between IPA levels and responsiveness to a moving lure, although there were significantly higher amounts of 2HPT in colonies highly responsive to the lure. However, these results, and those of Boch and Rothenbuhler (1974), should be taken as provisional since it can be argued that the behavioural measurements involved were confounded (*see* sections 8.5.2; 8.5.3; 8.5.6; and see Crewe, 1976 for criticism of pheromone analyses).

Amounts of IPA and 2HPT in *A. m. scutellata* workers of various ages (Crewe and Hastings, 1976; Whiffler *et al.*, 1988) were similar to those in European bees in Canada determined by Boch and Shearer (1966, 1967). Since *A. m. scutellata* is generally more highly responsive in defence than European honeybee races, it was concluded that inter-racial variation in this behaviour was founded on the workers' responsiveness to IPA and 2HPT and not on production of different quantities of the pheromones (Crewe and Hastings, 1976; Crewe, 1976, 1977). This was confirmed by Collins *et al.* (1988) in a comparison of IPA and 2HPT production and colony-defensiveness of Africanized and European bees - however, titres of nine other volatile sting components were significantly higher in Africanized bees and were well correlated with defensive levels: it was concluded that these pheromones would occur in higher concentrations around the intruder in defence by Africanized bees.

(iv) *The quality of pheromones released.* Crewe (1977) suggested that guard bees of *A. m. scutellata* produce characteristic quantities of IPA and 2HPT, and that workers may respond most highly to a specific blend of the two pheromones. However, other volatiles from the sting also release alarm so that blends could be complex and variable (Blum *et al.*, 1978; Collins and Blum, 1982, 1983; Blum and Fales, 1988) and thus contribute to CDB variability between colonies and between races. Collins *et al.* (1988) did not think it necessary to invoke synergies between pheromones to explain differences in defensive behaviour between European and Africanized bees.

(v) *Extrinsic factors.* Thresholds for alarm pheromone release and for responsiveness to them are likely to be associated with internal-colony factors such as age, state of engorgement, etc. (section 8.4.4) and with environmental factors such as ambient temperature, atmospheric electricity, and humidity (section 8.4.3), that generally influence intensity of CDB. Caged young workers responded more intensely to alarm pheromones under higher humidity (Collins, 1981). Workers adapted to alarm pheromones when they were artificially exposed to them for an hour or more, and so stung moving lures less than they did when they had not been exposed to the pheromones (Al-Sa'ad *et al.*, 1985. It is not known whether this effect on CDB intensity occurs under natural conditions, but it seems unlikely in view of the high volatility of the pheromones and the rapidity with which colonies return to normal after danger stimuli are removed (Free, 1987). Alarm pheromones are released as liquids that evaporate into the air, so that their concentration and persistence may be affected by ambient conditions such as wind (Southwick and Moritz, 1987), enclosed spaces, temperature,

humidity, and behaviour and physical nature of the objects stung.

(vii) *The context in which the pheromones are perceived during a bout of CDB.*

(1) *Within the nest*, alarm pheromones elicit arousal, alerting and recruitment to the nest entrance (section 8.3).

(2) *On the stimulus object*, alarm pheromones enhance attack: the object may be flown at, crawled on, bitten and stung. Alarm pheromone from the mandibular glands (2HPT) is released when a worker bites a stimulus object (Maschwitz, 1964a,b, 1966) and alerts other workers and labels the intruder for subsequent attack (Boch and Shearer, 1967). 2HPT has a weaker effect than equivalent concentrations of sting pheromone (Maschwitz, 1964a,b; Free and Simpson, 1968; Boch *et al.*, 1970). Sting pheromones in the nest do not cause pheromone release by the perceivers (section 8.3). In contrast, on a stimulus object they enhance further stinging and thus further pheromone release, with a "chain reaction" effect. When two objects are presented simultaneously, one with and one without alarm pheromones, the former will initially be stung the most (Free, 1961; Free and Simpson, 1968; Free *et al.*, 1983; Al-Sa'ad *et al.*, 1985). The advent of alarm pheromones on the stimulus object thus changes its stimulatory property, enhancing its efficacy as a danger stimulus. This effect has been overlooked in some methods of measuring CDB by presentation of leather or cotton "lures" at the hive entrance (*see* section 8.5.2; chapter 10).

(3) *Inter-colonial alarm communication.* In highly responsive

honeybee populations (e.g. Africanized bees of northern Brazil), disturbance to one colony may set off a "chain reaction that explodes within seconds; whole apiaries may go out of control." (Michener, 1975). This behaviour, which does not occur to the same degree in European races, may be facilitated by the release of large quantities of alarm pheromones, enhanced responsiveness to them, different behaviour in pheromone release (e.g. at hive entrances as well as on the stimulus object), and increased use of visual and auditory signals around the victim (Michener, 1972).

(4) *In contexts other than CDB against mammals.* Mandibular alarm pheromones (2HPT) released by defenders during mauling of conspecific robbers at the hive entrance may repel further invaders (Butler, 1966; Simpson, 1966). Sting and mandibular alarm pheromones are used as repellant labels on foreign queens near swarms (Morse, 1972, 1977; Boch and Morse, 1974). Thus alarm pheromones on non-mamalian stimulus objects (such as small lures: section 8.5.2) could sometimes have unforeseen repellent effects.

8.4.3 Environmental factors

Defensive responsiveness of honeybee colonies are strongly affected by weather and food supply.

A moving lure was attacked more in hot weather, and during approach of storms, than in cool weather (Schua, 1952; Southwick and Moritz, 1987). In a survey of CDB intensity in South America by means of a stinging bioassay ("moving lure test": *see* section 8.5.2), Michener (1972) suggested that the higher responsiveness of northern populations was associated with the higher temperatures of those regions. Two colonies measured at 20°C

and 26°C on the same day were more responsive at the higher temperature. At different temperatures over three days, the responsiveness of two colonies that had been selected for gentleness did not increase at higher temperatures whereas that of a "wild" Africanised colony did. Rothenbuhler (1974) supposed that the latter colony had a more "African" genotype, and so behaved more like African bees in tropical Africa.

Responsiveness to a stinging bioassay in two apiaries of Africanized bees 2500 km apart, with an average temperature - difference of 5°C, was 3-14 times higher at the warmer site (Brandeburgo *et al.*, 1977, 1982). Subsequent interchanging of 30 colonies produced higher responses at the warmer site, indicating a strong influence of climatic factors on the intensity of honeybee CDB. The association between defence responsiveness and temperature within each test site was equivocal: they were positively correlated at the warmer site, negatively at the cooler. (Similar tests by Stort, 1971, gave no correlation between temperature variation and defence responsiveness.) Relative humidity and defence responsiveness were positively correlated at the warmer site, but not at the cooler site. Brandeburgo *et al.* suggested that "other variables must be interacting with this behaviour." One possibility is unaccounted variability resulting from the measurement method that was used: *see* section 8.5.2. In European and Africanized colonies moved from a high-altitude (cool) site to a low-altitude (hot) site, Villa (1985) obtained significantly lower responses to a stinging bioassay (moving lure) at the high altitude site. He concluded that the temperature difference between the two sites (17°C and 34°C) was mainly responsible for the different responses, but could not discount other possible effects of altitude from factors such as atmospheric pressure, and ultraviolet radiation.

Responses of caged young workers to alarm pheromones were greater at higher temperatures; relative humidity affected only one aspect of the

responses measured (Collins, 1981). Oxygen consumption by winter bees in response to alarm pheromones were highest at 20°C and decreased above and below this temperature (Southwick and Moritz, 1985), whereas an increased response with higher temperatures was expected. The result may have been owing to the fact that guards of winter clusters are usually cooler than the cluster core.

Responses of colonies to a series of stimuli comprising alarm pheromones sprayed on the entrance, vibration to the hive and a moving lure test (section 8.5.2) were not correlated with ambient temperature, except for a negative correlation with the rate of recruitment to the pheromones (Collins and Kubasek, 1982). When a similar measurement method was applied repeatedly to six colonies (chosen randomly from a population), the intensity of the responses was highly variable, indicating the strong influence of "nonlinear environmental effects" - however, rankings in colony defensiveness over the measurement series were fairly constant (Moritz *et al.*, 1985). Thus, to reveal real differences in defensive responsiveness, sets of colonies should be measured at the same location, repeatedly over long periods (see also Moritz *et al.*, 1987). This approach was taken in the experiments reported in chapters 9 and 10. With an elaborate measurement routine designed to eliminate genetic factors, Southwick and Moritz (1987) obtained a significant correlation between defence responsiveness to moving lures and ambient temperature over a three month period. Although there were no significant correlations with four other meteorological factors (wind speed, solar radiation, relative humidity, barometric pressure), increasingly better predictions of defensive variance were obtained by successively adding them into a multiple regression. All five factors together accounted for 92% of the variance of the defensive response: stinging of moving lures was likely to be highest in hot, humid conditions with a bright sky and no wind.

Southwick and Moritz (1987) noted that their measurements were taken in a non-stormy temperate summer, and that weather effects might be different in the tropics and subtropics.

While the repeated moving-lure tests of Southwick and Moritz (1987) indicated a strong effect of temperature on CDB, Farrell (1977) did not find any such effect in six applications of moving-lure, "opening" and "cork" tests (section 8.5), even though the measurements were taken at various temperatures, ranging from 64 to 80°F, over a five-week period. Possible reasons for this were not given: design-flaws in each of the tests used by Farrell (*see* sections 8.5.2 and 8.5.3) could have been involved.

Fletcher (1978) thought that "since bees are poikilothermic", the claim that the irascibility of individual colonies is associated with high temperature is sound. However, the relationship is not that simple because "although bees are poikilothermic individually, they maintain a constant temperature in the brood nest between 34.5°C and 35.5°C, independent of outside temperature." (Lindauer, 1967) and so have highly homeothermic nests (Michener, 1974; see also Southwick and Mugaas, 1971; Southwick, 1982). While temperature in the nest core is fairly constant, the guards are usually found at the cooler periphery (Frisch, 1967) and so may be more directly influenced by ambient temperatures (Southwick and Moritz, 1985). Workers that stung an observer had a thoracic temperature of 36-38°C, independent of ambient temperatures between 7-23°C (Heinrich, 1979). In Kenyan bees propensity to attack was "partly correlated" with ambient temperature: at 26-29°C bees pursued the observer to 50 m from the hive, but at 8°C they pursued no more than 5 m. Heinrich postulated a minimum body temperature for attack which was *above* the minimum required for flight, so that the high body temperature of attacking bees was not a consequence of the attack behaviour, but was rather a partial "cause" of

it. Attack behaviour is not a simple function of body temperature, since highly responsive African bees had bodies no hotter than attacking bees of a more docile European race.

Besides direct effects on metabolism, temperature and humidity may affect the evaporation rates of alarm pheromones, and thus their efficacy in communicating alarm (section 8.4.2).

Changes in defensive responsiveness are partly associated with changes in electric fields in the environment and on the bees themselves Warnke (1976). "Irritability" of colonies increased in electrical fields under power lines (Anonymous, 1975).

In the literature there are many beekeeper's *opinions* about the relation between CDB variation and environmental conditions. They generally agree that bees in hot ambient conditions are often fiercer than in cooler conditions (Martin, 1912a; Smith, 1958a, 1960; Ntenga, 1969); that bees of warmer regions are often fiercer than those in cooler regions (Doidge, 1931; Edmunds, 1931; Hayter, 1948; Botha, 1966; Woyke, 1973); that transposition of colonies from cool to warm regions, or vice versa, have corresponding effects on temper (Guy, 1972; Mammo, 1976); and that colonies are often highly responsive in stormy or rapidly changing weather (Benson, 1921; Edmunds, 1930b).

Lowering of defence responsiveness during nectar flows in Europe was thought by Lecomte (1963) to be associated with a concomitant lowering of the number of guards with increased forager activity. In South Africa colonies become fiercer during nectar flows of *Aloe davyana* Schonland and *Eucalyptus grandis* Hill ex Maiden (Mountain, 1975; Doull, 1976). These flows occur in winter, so that hive heating cannot be important (Fletcher, 1978). The flows last for only part of the day (Mountain, 1975; Steinhobel, 1977), resulting in crowded hives.

"Defence is primarily a duty of the older bees, which are also

foragers . . . they cannot carry out both duties at the same time. When there is no forage these bees may remain at home as irritable guard bees . . . ." (Ribbands, 1954).

This is a translation of an environmental factor (flow termination) into an internal-colony factor (the number of old workers*) which may contribute to variability of CDB. Beekeepers commonly believe that CDB is reduced during periods of high forager flight-rate, since many potential defenders are then away in the field (Lundie, 1940; Kerr *et al.*, 1970). However, the possible connections between CDB and foraging rate are complicated by Lundie's (1940) finding that hives may be more congested during heavy flows than in dearths, since in dearths field bees spend long periods out foraging whereas in flows foraging time is reduced and field bees spend more time in the hive disgorging than they do foraging.

The factor of forager numbers in the hive may in some circumstances act against environmental influences that increase defensive responsiveness, such as high ambient temperature. For example, on hot days when high defensive responsiveness might be expected in every individual, colony responsiveness might be low if many potential defenders are out

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* Many authors refer to the number of workers in the eusocial insect colony as the "population" or "colony population", an often confusing usage since in general biology *population* usually means a *local* or *Mendelian* or *random-mating* population, i.e. a "community of potentially interbreeding individuals at a given locality" (Mayr, 1970). A colony of honeybees (or of any other eusocial insect) is not a population in the usual biological sense of the term (Rothenbuhler, 1960). "Colony population" should be used to refer to the number of colonies in a given locality, not to the number of workers in a colony.

foraging (in low flow conditions), whereas on cold days, with low individual responsiveness, colony responsiveness might be raised if foraging is reduced and many defenders remain in the colony (see Southwick and Moritz, 1987). Conversely, flight reducing effects of wasp predation (chapter 7) in hot conditions could set the conditions for extremely high CDB against mammalian predators.

Weather and nectar flow conditions affect the number of workers that engorge in response to vibration of the hive, or to smoke (Newton, 1968). Since engorging is connected with defence responsiveness (Newton, 1968), variations in it are a mechanism through which environmental factors may influence CDB.

The things against which honeybee colonies defend themselves must be considered under "environmental factors" that influence variation in CDB (even though they are also considered under "danger stimuli"). Ongoing, day to day actions of natural enemies must affect the state of alertness of a colony. Constant attacks by a variety of predators maintain high defence responsiveness in the honeybees of Tanzania (Smith, 1958a; Ntenga, 1969), and of South Africa (Fletcher, 1978). In addition, the attentions of conspecific robber bees elicit increased numbers of guards (section 7.4) which may thus increase propensity to attack other kinds of intruders (Martin, 1912b).

The density of colonies in an area may be an environmental factor in CDB variability (Collins *et al.*, 1980). Closely neighbouring colonies can communicate alarm pheromonally (section 8.4.2), and robbing may increase in high colony densities. Collins *et al.* (1980) classified environmental factors according to whether they operate *during* defensive behaviour (direct effects) or *before the onset* of a defensive behaviour (indirect effects). The resulting classification displays many factors that affect CDB (including internal-colony ones, separated in the present study under

section 8.4.4), and reflects that their sum effect on defence responsiveness must be transmitted via an intricate nexus of causes and effects.

8.4.4 Internal-colony factors

Internal-colony factors that may affect intensity of CDB were listed by Fletcher (1978) as colony size, swarming preparations, nectar flows, queenlessness, and food shortage. Although not defined in the literature, such factors in the honeybee colony are clearly analogous to the internal *intervening variables* postulated for changes in responsiveness to a constant stimulus in motivation, or drive, analyses of single animals (Hinde, 1959, 1970, p.194; Manning, 1972, p.59). Collins *et al.* (1980) listed several "indirect environmental conditions" that can be regarded as internal-colony factors in addition to those listed by Fletcher, viz: age distribution in colony; brood rearing; comb-building; crowding; diseases of adults or brood; nest destruction with no danger stimuli e.g. by moths, mice, etc; and nutritional levels, including water availability. Effects of internal-colony factors on CDB variability may conveniently be discussed under three broad categories (which are not mutually exclusive), as below.

(i) Polyethisms and CDB

Defense responses, alarm pheromones and venom do not occur in very young bees but reach optimal functional levels when guarding or foraging is first undertaken (section 8.4.6). CDB may thus be affected by the number of older (defensive) bees and of guards in the colony: e.g. an established colony, with a high proportion of old workers, might have a generally higher CDB than a newly established colony, which would have a relatively

high proportion of young workers. Whereas for some purposes it is convenient to regard the age-based polyethism of CDB as an internal-colony factor, the study of the development of the behaviour and its contribution to CDB variability falls naturally into a separate category, viz. *ontogenetic factors* (section 8.4.6).

(ii) Numbers of workers available to defend

Short term changes in the number of workers available to defend the colony are directly affected by environmental factors (section 8.4.3). Many beekeepers have associated colony strength with defence responsiveness: the larger the colony, the more workers available to defend (e.g. Smith, 1958b; Winston, 1987). Collins *et al.* (1982) found more rapid and extensive stinging of lures by larger colonies of both Africanized and European colonies. However, Boch and Rothenbuhler (1974) and Collins and Kubasek (1982) found no correlations, and some significantly negative correlations, between colony size and stinging of lures. Rothenbuhler (1974) also found a possible negative association between colony strength and responsiveness to Michener's (1972) moving lure test, and speculated that in the mixed Africanized/European population studied, the smaller (highly responsive) colonies may have been those with stronger African characteristics, since Africans swarm frequently and so may not build up large colonies as do the more docile, low-swarming Europeans.

(iii) Specific internal-colony factors and CDB

Colonies provided with 6 m^2 of empty comb responded faster to moving lures and stung them more often than colonies provided with 3 m^2 empty comb: volatiles from the comb may have acted as a primer pheromone for defensive

behaviour in some unknown function in which empty colonies benefit from heightened defensiveness (Collins and Rinderer, 1985).

The status quo of the colony changes markedly with preparations for supersedure, migratory absconding, or swarming, and this could affect CDB intensity. Many opinions on the possible effects of these factors may be found in the literature, but no properly constituted experiments.

In individual *A. m. scutellata* colonies, great variability in defensive responsiveness, over relatively short periods of days or weeks, has often been noted (Fletcher, 1978). Experimental analysis of the "intervening variables" that cause such variation will require the conceptual approach for the study of changes in responsiveness to a constant stimulus, as mentioned above. However, in current ethology of honeybee CDB, the constancy of any experimental danger stimulus has yet to be demonstrated (section 8.5.6) and, until one is available, rigorous analysis of intervening variables in CDB will be hampered.

8.4.5 Genetic factors

Because CDB comprises many behavioural and physiological entities (alarm pheromone production, responsiveness to alarm pheromones, participation by variable numbers of defenders who respond variably to complex stimuli, age polyethism) its inheritance must be complex and polygenic (Michener, 1975).

Genetic factors predominate in CDB variation between colonies kept in similar conditions, while environmental and internal-colony factors are important in CDB variation in the same colony at different times (Collins *et al.*, 1980). Changes in responsiveness in the same colony may, however, have a partly genetic basis, since the average queen may carry sperm from 7-17 drones (Moritz, 1983; Winston, 1987), so that a colony usually has sets of workers with different fathers: if the progeny of the various

fathers differ in defensive behaviour, then the responsiveness of the colony will vary depending on the proportions of the various genotypes in the colony at any particular time. (Transfer of brood from fierce to docile colonies increased the fierceness of the latter: Susaeta, 1974.)

Racial differences in defence responsiveness have long been known. For instance when African bees were first introduced to South America their "great aggressiveness" was to be eliminated by crossing with Italian bees, known to be gentle (Kerr, 1957). After the Africanized bees had established themselves around Sao Paulo, local beekeepers were supplied with 23 000 European queens in an attempt to reduce the defence responsiveness of the bees in that region (Goncalves *et al.*, 1972; Michener, 1975). When Kerr's African bees first escaped, many opinions on differences in temper of races, strains and colonies had been published (e.g. Adam, 1951, 1954) but there had been few attempts to measure these differences objectively. Since then several measurement methods have been developed, many in inheritance analyses of CDB in races and line-bred strains (section 8.5).

In manipulating unipaternate colonies of two strains Rothenbuhler (1964b; and see Rothenbuhler, 1960) was stung once by one strain and 143 times by the other. Stinging by backcrosses to the two lines indicated that more than one or two loci produced this behaviour difference (Rothenbuhler, 1967). With similarly bred European (gentle) and Africanized (fierce) bees, responsiveness to five "behaviour characters" of a moving lure test (Stort, 1971) indicated control by 8 genes (Kerr, 1974; Goncalves and Stort, 1978). The genetic systems that Stort inferred for his five behaviour characters (Stort, 1975a,b,c, 1976, 1980; and see reviews by Goncalves and Stort, 1978; Rinderer and Collins, 1986) were sometimes contrary to what would be predicted by common-sense reasoning. Responsiveness of F_1 colonies showed dominance of gentleness in (1) number

of stings in glove (2) number of stings in lure and (3) "observer persecution" (i.e. distance bees followed observer), whereas genes for fierceness dominated in (4) time to first sting and (5) time to become aggressive. Responsiveness of backcross colonies showed simple Mendelian segregations for characters (1) and (2) but complex polygenic inheritance for (3), (4) and (5), so that Stort (1974) had to contend that the "number of stings in the lure" are controlled by genes entirely different from those that control the "number of stings in the observer's glove". This is difficult to understand when it is considered that the two stimuli were in every test presented a metre from one another simultaneously at each hive entrance (*see* section 8.5.2). Common sense further dictates that the "behaviour characters" of a method to measure defensive intensity should be well correlated over repeated applications of the method (*see* sections 8.5.2; 9.1.1). However, Stort's (1974) genetically based glove/lure disparity was also reflected by the non-correlation between these two characters in a correlation matrix of the five characters in the Africanized backcrosses (Stort, 1978). Another unexpected non-correlation was obtained in this matrix, between the distance that bees followed the observer and the time to first sting in the lure. Further unexpected non-correlations were obtained in the matrix for the European backcrosses, between number of stings in gloves and time taken for colony to become "aggressive", and between number of stings in the lure and the distance that bees followed the observer. Rinderer and Collins (1986) suggested that the disparities in these results may have arisen through a highly polygenic regulation of some of the characters, or by the possible inclusive sampling by different measurement designs of behaviours controlled by different sets of genes (see also Collins, 1979). They proposed that "fine tuning of the processes of actual measurement could more clearly show the genotypes involved." In section 8.5.2 several

possibly serious confounding factors are identified for CDB measurement methods that rely on stinging of lures. It is suggested that these may have caused some of the disparate genetic and behavioural interpretations that have arisen from this test.

Many disparities, similar to those identified above for Stort's behaviour characters, may be found in the genetic interpretations for various CDB measurements in studies by e.g. Woyke (1969), Cosenza (1970, 1972), Boch and Rothenbuhler (1974), Kerr *et al.* (1974), Collins (1979, 1982), Rinderer (1982).

Despite current confusion about the details of the genetics of the differences in CDB intensity between races and strains there is, of course, no doubt that the differences are strongly influenced by genes (see e.g. Villa, 1985). The role of genes in CDB variability in colonies within panmictic populations is less clear than between races, owing perhaps to smaller differences in the behaviour between colonies from the same population. Michener (1972) found high CDB levels in northern Brazil, but much variability in the south (section 8.5.2), and could not say whether the cause was environmental or genetic. By reciprocal transfers of colonies between northern and southern Brazil, Brandeburgo *et al.* (1977, 1982; section 8.4.3) concluded that environmental factors predominated in the behaviour differences obtained, but Rothenbuhler (1979) suggested that the genetic factor of active selection for gentle colonies by beekeepers in the south was the basis of this difference. The measurement methods of Michener (1972) and of Brandeburgo *et al.* (1977, 1982) may have been partially confounded and could have contributed to the inconsistencies reported in those studies (section 8.5.2).

By laboratory measurement methods that reduced much of the variability from extrinsic factors that affect measurement of CDB in colonies (Collins and Rothenbuhler, 1978; Southwick and Moritz, 1985;

section 8.5.5), Collins (1980) and Moritz *et al.* (1985) found fairly consistent rankings in responsiveness between colonies from the same population, which were likely to have been genetically based. Subsequently Rinderer *et al.* (1983), Collins *et al.* (1984) and Moritz *et al.* (1985, 1987b) determined heritabilities of various defence responses which indicated that selective breeding within populations is possible but would be slow, so that crossing with docile European strains would be a more effective alternative (Collins and Rinderer, 1986; and see Rinderer, 1982). However, intra-population breeding is the only realistic option for bee breeding in Africa (Fletcher, 1978). Various selection strategies for general improvement of honeybees have been proposed (e.g. Anonymous, 1982; Moeller, 1976; Ruttner, 1972) and attempted e.g. by Szabo (1982) and Moeller (1976), who assessed defensive responsiveness of colonies through "expert opinion" (section 8.5.4). Szabo had little success, owing to variability from environmental factors.

There is a growing realization that genetic analyses and breeding programmes in honeybee defensive behaviour will benefit from improvements in measurement methods, particularly in the reduction of extrinsic effects on intensities recorded (e.g. Moritz *et al.*, 1985, 1987).

Genetic bases for some components of CDB have recently been established. Guard behaviour may influence CDB variability (section 8.4.6): Robinson and Page (1988) showed that differences between colonies in number and zealousness of guards is largely controlled by genetic factors. Collins *et al.* (1988) found higher quantities of nine alarm pheromones in Africanized (fierce) bees than in European (docile) bees. High heritabilities and genetic correlations were found among 12 alarm pheromone components, indicating a common genetic regulation (Collins *et al.*, 1987b).

8.4.6 Ontogenetic factors

(i) Pre-eclosional factors

Moritz *et al.* (1987b) interchanged eggs and young larvae between experimental colonies whose differing defensive temperaments were mostly genetically based. The responses of the resulting adults to a metabolic bioassay (*see* section 8.5.5) were well correlated with their genotypes and not with the genotype of the foster colony. Thus crossfostering of young larvae had no effect on alarm behaviour in the resulting adults.

(ii) Post-eclosional factors (especially age-based polyethisms)

Colony defence is one of many activities that a worker may perform during its life. Defense responsiveness and alarm pheromone and venom production in the individual changes with age, starting at low levels in newly emerged workers (Whiffler *et al.*, 1988) as does the olfactory capacity to sense alarm pheromones (Masson and Arnold, 1984; Winston, 1987, p.114). Possible effects of age-based production of alarm pheromones apon CDB variability are discussed in section 8.4.2. Age based sensitivity to alarm pheromones, and production of them, is hormonally controlled (Robinson, 1985, 1987). Workers perform a sequence of activities through their lifespan (Rosch, 1925, 1930, reviewed by Ribbands, 1952, 1953; Free, 1965; Wilson, 1971; Michener, 1974; Seeley, 1982, 1985, 1986; Winston, 1987), the first of which occur exclusively within the nest. Before they become guards or foragers, young bees do not normally participate in CDB (Ribbands, 1954), but may respond to alarm pheromones with wing flicks and increased locomotion (Collins and Rothenbuhler, 1978; Collins, 1980). Initial flights are undertaken at about 10-15 days of age (Ribbands, 1954), after

which the worker forages more frequently until in old age it does this almost exclusively.

Most workers commence foraging without having undertaken guarding (Butler and Free, 1952). The 5-15% that do become guards (Lindauer, 1953; Moore *et al.*, 1987) usually do so just before they start foraging (Rosch, 1925; Moore *et al.*, 1987; Breed and Moore, 1988), although foragers participate in CDB if the need arises (Butler and Free, 1952; Lindauer, 1953). Guards may vary in number and in responsiveness to danger stimuli (or "zealousness") (Lindauer, 1953; Sekiguchi and Sakagami, 1966; Breed and Moore, 1988). Since guards are usually the first to react in defence episodes, variability in their behaviour may contribute to variability in CDB, particularly in its rate of onset (Moore *et al.*, 1987; Breed and Moore, 1988; section 8.3). The role of guards in the variability of responsiveness to moving lure tests is discussed in section 8.5.2, and examined experimentally in chapter 10.

8.5 MEASUREMENT OF INTENSITY OF CDB

Given the great complexity of honeybee CDB against humans, the variety of stimuli that elicit it, the incremental effects caused by the defenders themselves, and the many extrinsic factors that influence responsiveness (reviewed in sections 8.3 and 8.4), it clearly will be difficult to obtain unconfounded measurements of the behaviour: much attention should be paid to the elimination of confounding factors from the measurement methods.

Current concern with improvement of CDB measurement methods is centred on the reduction of the effects of extrinsic factors in genetic analyses (section 8.4.5). Another way to improve measurement methods will be to identify defects in existing ones by *a priori* criticism using knowledge about the behaviour itself, and then to test the validity of

improved designs experimentally. This approach is attempted in the present study. The present review is a critique of existing methods of measuring CDB. In chapter 9 experiments are reported on an improved measurement method, designed in part from points drawn in the following review.

8.5.1 Responses to the observer: stinging and following

Rothenbuhler (1964a) counted stings received by observers during 98 standardized manipulations of experimental colonies, and found a distinct difference (1 sting vs. 143 stings) between two line-bred strains. He pointed out that sophisticated measurements are not necessary for a beekeeper to distinguish between honeybee strains that differ substantially in defence responsiveness. However, Szabo (1982) achieved less clear results with this method and noted the confounding influences of environmental conditions and of variability in manipulations by the beekeeper. A further disadvantage might accrue from the difficulty of counting all the stings received by the observer, e.g. on boots, back of veil, etc.

Beekeeper's clothing is designed to reduce stinging (smooth white material with as few colour contrasts as possible). Beekeepers are often mobbed by hundreds of angry bees but receive relatively few stings when properly dressed, whereas any suitable target in the vicinity, such as a moving piece of dark leather or an unprotected human, would be heavily stung (see Collins and Rinderer, 1986). Thus the defensive response to a beekeeper may be high in terms of numbers of flying defenders, but low as measured by numbers of stings received by him.

Michener (1972), Heinrich (1979) and Stort (1980) used the distance from the hive that attacking bees followed a human observer as a measurement of CDB intensity.

An advantage of these methods is that they elicit a response for which colonies are ultimately selected in breeding for gentleness, i.e. attacks to the beekeeper when he manipulates the colony.

8.5.2 Stinging bioassay: moving lures

By waving at the hive entrance a piece of black cloth at the end of a long stick, Schua (1952) found differences in responsiveness in various weather conditions, although the measurements were not rigorously quantified.

By presenting mechanically agitated woollen lures at the hive entrance and measuring time to first attack, Lecomte (1961) claimed to show that manipulations to a colony in an apiary raises the defence responsiveness of its immediate neighbours.

Stort (1970) devised a "moving lure test" to quantify the intensity of CDB. A cotton-filled leather ball of 2 cm diameter (Stort, 1975a) suspended on a thread was jiggled at the hive entrance and the following "behaviour characters" were recorded (Stort, 1974): time to first sting in lure; time until colony visibly irritated; number of stings in observer's glove after 60 s; number of stings in lure after 60 s; distance bees followed observer walking away after 60 s presentation (= the "observer persecution" of Stort, 1980). In addition, Stort (1970, 1971) measured time for colony to become calm after presentation of the lure (reviewed by Goncalves *et al.*, 1972; Goncalves, 1974; Goncalves and Stort, 1978).

Stort (1974, 1975a,b,c, 1976, 1978, 1980) applied this method to sets of colonies of known parentage (gentle European, fierce Africanized, and their hybrids and backcrosses). Each colony was subjected to the lure measurement five times at intervals of 10-20 min; the means were used as the indicators of responsiveness in each behaviour character. Stort was able to designate colonies "gentle" or "aggressive" and, according to their

segregation in crosses, identified genes for the behaviour characters and specified their dominances, epistatic interactions, and correlations with anatomic features. However, Stort obtained great variation in his results, between genotypes, between colonies, and between the behaviour measurements themselves, which he attributed either to the inherent variability of the behaviour or to control of the discrepant behaviour characters by different genes (Stort, 1974, 1975a,c, 1976, 1978; reviewed by Rinderer and Collins, 1986; *and in* section 8.4.5). Stort did not discuss the measurement method itself as a possible source of variation.

Several other studies used Stort's method to measure colony defensiveness, some with minor modifications in responses recorded or in lure design and presentation (e.g. Woyke, 1969; Cosenza, 1970, 1972; Michener, 1972; Kerr *et al.*, 1974; Farrell, 1977; Brandeburgo *et al.*, 1977, 1982; Stort and Chaud-Netto, 1978; Sugden and Furgula, 1982; Villa, 1985).

Kerr *et al.* (1974) obtained some interesting discrepancies in their behaviour measurements, but did not discuss them. For example, in the characters X_1 (time to first sting in ball) and X_3 (number of stings in ball 60 s after the first sting) they obtained:

in colony C6 : X_1 = 4.4 s X_3 = 54.6 stings

in colony C7 : X_1 = 19.6 s X_3 = 19.4 stings

which is understandable on the basis that the faster the onset of stinging, the more responsive the colony, thus the more stinging subsequently. However, in two other colonies they obtained:

in colony C2 : X_1 = 9.4 s X_3 = 63.0 stings

in colony C3 : X_1 = 19.2 s X_3 = 54.6 stings

in which the C3 ball took twice as long to be stung as the C2 one but subsequently received almost the same number of stings, which does not concur with the interpretation made above for colonies C6 and C7. Similar discrepancies are evident in the data of Michener (1972), whose Committee

incorporated Stort's test in an elaborate recording system (Michener's numbering):

(12) Rate of flight activity (number of workers entering the hive in 3 x 30 s periods);

(13) Number of stings and of bees pestering observer during count of (12);

(24) Time to first sting in a leather square (3 by 3 cm), jiggled 10 cm from entrance - at first sting, leather was moved to the entrance itself for 30 s, then observer walked away from hive (see (26) below);

(25) If no sting after 3 min at 10 cm from entrance (24 above), leather was moved to entrance and time to first sting there recorded - after 30 s, observer walked away (see (26) below);

(26) Distance bees followed observer and lure after either (24) or (25) above;

(27) Number of stings in leather after (26) above;

On completion of (26) the hive was smoked, opened and brood combs examined, whilst recording:

(18) Number of bees "pestering" an observer 17 m from hive;

(19) Number of bees pestering an observer 3 m from hive;

(20) Distance bees followed observer after hive was closed.

Eighty one colonies were tested in 21 apiaries along the length of the Brazilian coast. Most colonies were tested once only. The Committee (Michener, 1972, p.26) could not analyse this data rigorously because they were unable to recognize, measure or control many variables that may have caused variable responsiveness, e.g. physical environmental factors, colony size, flows, recent disturbances and differences among observers. The Committee was able to draw only the most general conclusions, namely that Africanized colonies were highly "aggressive" in northern Brazil but very

variable in this behaviour in the south: some Africanized colonies selected by beekeepers were "gentle" and pure Italian colonies were "very gentle" compared with Africanized colonies. The Committee (p.27) felt "that the tests of aggressiveness were meaningful", but did not discuss the relative merits of the components of their test.

In a "wild Africanized" colony measured thrice by Michener (1972), Rothenbuhler (1974) inferred a positive association between defence responses and the different temperatures on the three measurement days. In similar measurements on two other colonies that had been selected for gentleness there was no association between temperature and responsiveness. Rothenbuhler suggested that these differences were associated with the genetic characteristics of the colonies, but did not discuss the measurement method itself as a possible source of this somewhat puzzling variation.

With a moving leather lure, Farrell (1977) found faster time to first sting in backcrosses to a "fierce" line, from hybrids between two strains known to fierce and docile by expert opinion (section 8.5.4). However, the fierce backcrosses placed *fewer* stings in the lures than the supposedly docile backcrosses. Rinderer and Collins (1986) suggested that this discrepancy demonstrated the complex nature of CDB, and that it arose because some components of CDB "are inherited differently, while others have similar modes of inheritance and probably have some common genes." As discussed below, certain defects of the moving lure test could also provide an explanation for such discrepancies.

Brandeburgo *et al.* (1977, 1982) applied Stort's test twice to each of the 80 colonies in their two experimental apiaries, over 45 days. The great variation in the results led to the suggestion that "other variables must be interacting with this behaviour." Given the undoubted influence of day-to-day climatic conditions on CDB (section 8.4.3), some of this

variability could have arisen from variability of weather conditions over the 45-day test period, as well as from the test method itself, as discussed below.

Sugden and Furgula (1982) reduced Stort's method to a recording of the number of stings in the lure after a 60 s presentation, and in a comparison of six commercial lines in the USA found significant differences between some of them. This finding was challenged by a beekeeper as discussed in section 8.5.4.

For reasons unstated, Anderson (1977b, 1981) was unable to induce his bees (*A. m. scutellata*) to sting a lure somewhat larger than that of Stort (1974).

Finally, Al Sa'ad *et al.* (1985) measured stinging intensity by opening the hive and jiggling four lures together a few centimetres above the top bars for 60 s. In comparing the total number of stings delivered by two sets of 10 colonies, an expected result was obtained. Delaplane and Harbo (1987b) dragged a small leather square across the top-bars of a newly opened hive and recorded number of stings after 60 s. Variances for number of stings in five successive measurements were high but significant differences between queenless and queenright colonies were obtained. Potential problems associated with removing the hive lid while measuring CDB are discussed in section 8.5.3.

Stort (1974) noted that his time intervals of 10 min between tests on the same colony should be increased but did not say by how much, nor how an acceptable time could be determined. In Stort's approach, repeated tests spaced too closely could incur unnecessary variation in the intensities recorded if, as the tests progressed, some colonies became more responsive, so that later measurements would be higher than earlier ones: this incremental factor would be absent from other colonies with a more rapid calming time. Although it is clearly desirable to have lengthy

periods between tests, there is the converse risk that changes in ambient conditions during extended test periods could confound results. Stort (1970) found that Africanized bees took an average of 28 min to "calm down" after a moving lure test, so that experiments on this race with intervals of less than a half an hour would be at high risk from this incremental factor.

The advantages of the moving lure test appear to lie in its ease of execution and in the accuracy with which certain responses can be measured, such as time to first sting and number of stings deposited. However, in heavy attacks the number of stings left in the lure "may measure little more than the number of workers that the substrate can physically accommodate during the exposure period." (Gary, 1974; see also Collins *et al.*, 1982; Collins and Rinderer, 1986). This is an example of how the design of the response measurement may confound a bioassay of CDB intensity.

A further design flaw in the lure test is the confounding potential of the alarm pheromones released when the lure is first stung. In the measurement of CDB intensity it is axiomatic that the stimulus employed should be as constant as possible from test to test. Yet, in the presentation method of Stort (1974), the dangling lure may constitute two different stimulus forms during its 60 s presentation, neither of which are under the control of the investigator: first, the unstung lure and second, at an indeterminate time after the start of the presentation, the stung lure emitting alarm pheromones. Thus, for example, a lure stung 1 s after presentation is thereafter presented with alarm pheromones for 59 s, whereas in another test a lure stung 50 s after presentation is then presented with alarm pheromones for only 10 s. Given the incremental effects on the defensive response of alarm pheromones emanating from lures (Free, 1961; Free *et al.*, 1983; Pickett *et al.*, 1982; section 8.4.2) it is

clear that in the two cases cited, two distinctly different stimuli will have been given over the 60 s presentation period. This problem is not mentioned in the literature on moving lure measurements of CDB intensity, although a similar one was mentioned by Gary (1974) when bees released alarm pheromones during alarm pheromone bioassays with lures which could be stung. Also, in tests of defensive responses of bees stimulated by alarm pheromones in small cages, very young bees are preferred as subjects because they cannot release pheromones of their own, which would interfere with the artificial pheromonal stimuli (Collins and Rothenbuhler, 1978; Collins and Blum, 1982, 1983; Moritz *et al.*, 1985). In tests of older caged bees, Southwick and Moritz (1985) designed their method to nullify uncontrolled pheromone emissions by the subjects.

The confounding potential of alarm pheromones in the lure test may be exacerbated by the fact that the first sting on the lure is often the act of a single individual. The first individual to sting may in some cases be exceptionally responsive, as in the case of a "zealous guard" (Butler and Free, 1952; Sekiguchi and Sakagami, 1966); or of a bee that has had an annoying experience just prior to the presentation of the lure; or even of a bee accidentally bumped by the lure itself (e.g. see photographs in Stort, 1974). Thus, in studies endeavouring to measure the level of CDB of the "colony as a whole" a zealous first stinger of a lure may give an aberrantly rapid score for the "time to first sting." In such cases the rest of the colony might take relatively longer to become aroused than it would if the responsiveness of the first stinger was more in line with the responsiveness of the majority of the defenders of the colony. This factor could for example, explain the large discrepancies obtained by Michener (1972) and Kerr *et al.* (1974, enumerated above) between "time to first sting in lure" (individual response) and "number of stings in lure" (colony response).

The emphasis that a lure test may place on the behaviour of an individual is inimical to the requirement that a test of CDB intensity should gauge the defensive proclivity of the colony as a whole, such as that evoked by a danger stimulus perceived by all the hive inmates simultaneously, e.g. a vibration to the hive, or a mammalian odour blown into the hive.

Another factor that might affect the constancy of the moving lure stimulus is the presence of the observer who, while presenting the lure stimulus, himself constitutes a different danger stimulus for honeybees (section 7.2). The recorded discrepancies between defensive responses to simultaneous presentations of lures and humans (e.g. the difference between stings in glove and stings in lure found by Stort: section 8.4.5) raise the question of which measurement more closely reflects the defensive responsiveness of the colony against the human intruder (see also Southwick and Moritz, 1987). Villa (1985) experienced this problem when a colony that had responded mildly to a lure nevertheless responded to a hive manipulation with a "most intense defensive episode", which led him to note that his lure test "measured only initial reaction time and initial strength of reaction and therefore only quantified reactions to minor disturbances." At an extreme it is not difficult to visualize an investigator receiving a full mass attack from a fierce colony whilst jiggling a lure largely ignored by the defenders flying out to attack him. Or, perhaps in less responsive colonies, lures might be avoided owing to their similarity to apivorous wasps, which evoke "colony defensive retreat" (section 7.6). If the differences in defensive responsiveness to simultaneous presentations of the human and the small moving lure do arise partly from the wasp-like characteristics presented by the latter stimulus, then these differences will have a partly genetic basis, although not of the kind originally envisaged by Stort (1974) (section 8.4.5).

From these *a priori* criticisms it is evident that some of the variability reported for responses to the moving lure test could have arisen from confounding effects of the test method itself, although in many cases such variability has been dismissed as owing to the inherent variability of the behaviour, or to control of different characters by different genes (section 8.4.5).

The moving lure methods of Schua (1952) and Stort (1974) were incorporated into a *composite stimulus* by Collins and Kubasek (1982) and Collins *et al.* (1982, 1984, 1988) entailing presentation of: (i) large amounts of artificial alarm pheromone at the hive entrance (ii) a physical jolt to the colony and (iii) mechanically agitated leather squares (5 by 5 cm) at the entrance and 45 cm away from it. Moritz *et al.* (1985) devised a similar method but omitted the physical jolt. Moritz *et al.* (1987a,b, 1988) and Southwick and Moritz (1987) (i) removed the hive lid (ii) placed a filter paper soaked in alarm pheromone on the top bars and (iii) waved a leather square 5 cm above the top bars for 15 s. The main difference between these composite tests and the straightforward lure presentation was in the presentation of large amounts of alarm pheromone before revealing the lure. The presence of these alarm pheromones in the vicinity of the lures might diminish confounding effects of pheromone releases by the bees themselves, although Free *et al.*, (1983) thought that high concentrations of alarm pheromones on one lure may have incited stinging of another presented simultaneously 40 cm away.

The above tests with composite stimuli were applied in well-designed experiments which took cognizance of environmental effects by presenting the test to all the experimental colonies more or less simultaneously, and repeating it at widely spaced intervals, in some cases intermittantly over periods of several months. The tests gave satisfactory results in a variety of investigations, e.g. elements of the composite response recorded

by Collins and Kubasek (1982) were well correlated; Africanized colonies stung lures significantly more than European colonies (Collins *et al.*, 1982; Rinderer, 1982); inter-colony rankings by the field test of Moritz *et al.* (1987) were similar to the rankings indicated by a laboratory test (section 8.5.5); and weather factors accounted for 92% of the response variance in the test of Southwick and Moritz (1987). Nevertheless, all these studies recorded substantial variation, as reflected in various inconsistencies, e.g. unexpected non-correlations between some of the measurement categories of Collins and Kubasek (1982) and Collins *et al.* (1984); discrepant rankings in responsiveness of a set of colonies (Moritz *et al.*, 1985); and high variability of the realized heritability of defensive behaviour of honeybee colonies (Moritz *et al.*, 1987). This variability was assigned mainly to environmental effects (Moritz *et al.*, 1987; Southwick and Moritz, 1987). However, the previously mentioned criticisms of lure tests must apply also to the use of lures in composite tests, and may have contributed to the variability recorded. In addition, Collins and Kubasek (1982) and Collins *et al.* (1982) noted that in highly responsive colonies counts of guards recruited to the entrance board were underestimates since, in response to the danger stimuli, many defenders immediately flew from the entrance: it was thought that counts of flying defenders near the hive would provide better measurement of defender recruitment (Collins and Rinderer, 1986).

The validity of the moving lure test as used by Michener (1972) and Stort (1974) was tested experimentally in chapter 10.

8.5.3 "Opening", "cork" and "breath" tests

Boch and Rothenbuhler (1974) devised three bioassays of CDB:

1. *The "cork" test.* A cork with IPA evaporating from it was placed at the hive entrance. The response was measured as the increase in number of bees at the entrance after a 3 min presentation. Differences were shown between two strains known by expert opinion (section 8.5.4) to differ in defensive responsiveness. Responses of hybrids and backcrosses indicated dominance of gentleness. Advantages of this test are its repeatability and simplicity, although the information it yields about CDB is meaningful only in connection with other tests more representative of the mammalian intruder. The problem of using guard recruitment as an index of honeybee defensive responsiveness is discussed below.

2. *The "opening" test.* The hive covers were removed "gently". The response was measured on a scale of 0 (no apparent response) to 4 (highly irritated) during a 3 min exposure. A significant difference was obtained between colonies of two strains of different defensive responsiveness, whereas responses of hybrids were "intermediate, indicating a lack of dominance" even though cork and breath tests indicated dominance of gentleness. Thus the response variability was explained in terms of genetic factors. The advantages of this test are its easy execution and the fact that it constitutes a stimulus directly applicable to the human intruder. However, the stimulus delivered is likely to be inconstant from test to test: consider for instance the different stimuli produced in lifting a clean hive top and a heavily propolized one. Honey supers over the brood chamber in some hives, but not others, could further confound this test. Farrell (1977) could not use this test at temperatures below 60°F for fear of killing the brood by chilling. Some pilot observations on this test are reported in section 9.1.2.

3. *The "breath" test.* The observer blew three puffs of breath into the

hive entrance and measured the change in the number of bees at the entrance 3 min after the stimulus. Responses to this test were similar to those evoked by the cork test, i.e. there was a significant difference between docile and highly responsive strains, and a gentle response in the hybrids. Advantages of the test are its simplicity of execution and its direct applicability to the mammalian intruder (only mammalian breath and mechanical disturbance to workers are known to elicit sting fanning: section 8.4.1). Disadvantages may include inconstancy in stimulus form and strength, e.g. different vigour of puffs; different types of breath. A more serious problem is the measurement of the response as the change in the number of workers at the entrance board after the stimulus. Bees at the entrance of an undisturbed colony are not necessarily guards or defenders (six other activities are listed in section 8.3) and so may not respond defensively when disturbed, e.g. they might fly off to forage, or retreat into the hive. Further, Collins and Kubasek (1982) and Collins *et al.* (1982) noted that in highly responsive colonies guards were often depleted by workers flying from the hive entrance to attack various stimulus objects. In guard recruitment, therefore, we have a quantity expected to be high in high responses, but which in some cases may be *low* in otherwise highly vigorous responses. Indeed, Boch and Rothenbuhler (1974) obtained a negative value in one of their breath tests.

In hybrids between two strains known to be fierce and docile by expert opinion (section 8.5.4), Farrell (1977) found dominance of fierceness in backcrosses to the fierce line in responses to Boch and Rothenbuhler's cork and opening tests. This differed from Boch and Rothenbuhler's findings (above) in hybrids between Brown (fierce) and van Scoy (gentle) lines.

An assessment of the validity of guard recruitment as a measurement of CDB is given in chapter 9.

8.5.4 The expert opinion

A common method of assessing of CDB intensity has been through the opinions of experts: beekeepers and bee researchers, e.g. Gilbert (1938), Adam (1951, 1954, 1961, 1983), Moeller (1976), Ruttner (1977c), Collins and Rothenbuhler (1978), Anonymous (1982), Taber (1985), Eischen *et al.* (1986), Moore *et al.* (1987). For a variety of expert opinions on the responsiveness of Africanized bees see Kerr *et al.* (1970, 1982), McGregor (1970), Cornejo *et al.*, (1973), Cantwell (1974), Taylor and Williams (1975), Morse (1976), Laidlaw (1977), Taber (1977), Blum *et al.* (1978). The expert opinion cannot be dismissed as meaningless owing, say, to the complexity of the behaviour and the lack of control of confounding factors, since the reliability of the expert's opinion has never been rigorously tested, just as the reliability of most of the CDB measurement methods reviewed above has escaped critical analysis and experimental testing.

The response of the colony to the beekeeper is in principle acceptable as a basis for measurement of CDB since the human is a major natural predator of the honeybee colony and the response evoked has evolved against this form of predation (section 7.2). Thus Rothenbuhler's (1964b) and Szabo's (1982) counts of stings to an observer during a manipulation on the colony (section 8.4.1) were little more than a formal quantification of something that would be assessed intuitively by the experienced beekeeper.

The expert's opinion may differ from the results of more formal tests, as in the case of the opposition of Mraz (1982) to the finding of Sugden and Furgula's (1982) lure test (section 8.5.2) that there "is no reason for a beekeeper to tolerate this degree of aggressiveness in [Mraz-line] honeybees." Mraz said that the bees were acceptable since he often worked shirtless and veil-less in large apiaries of these bees. Who is correct? Given the potential confounding factors of the moving lure test

(section 8.5.2) and the fact that the conclusion of Sugden and Furgula was based on only three tests of two colonies per strain, it would be understandable should a potential user of the bees choose on the basis of Mraz's opinion.

But this is not to denigrate any effort to quantify CDB by specific measurements. The need for an accurate method could not be more emphatically demonstrated than by the history of the selection of the African colonies that were initially taken to Brazil by Kerr in 1956, of which 26 escaped to found the Africanized race that occupies much of South and Central America today (Michener, 1975; Rinderer, 1986a). In Africa Kerr was at pains to select queens from gentle colonies for his importation, by taking queens recommended by beekeepers and researchers and by his own assessment (i.e. by expert opinion), as when in Tanzania he rejected colonies that were "extremely wild" (Kerr, 1957) and as when in Angola he selected queens from "relatively gentle" colonies (Kerr, 1967). From the first African colonies established in Brazil, before the escapes, many were eliminated because of *bad temper* or low productivity (Kerr, 1957; Woyke, 1969; Portugal Araujo, 1971). This selection was to continue in a programme of breeding to combine the desirable traits of African and European races, including gentleness (Kerr, 1957; Michener, 1972; Goncalves, 1975). No test method to quantify CDB for these selections was mentioned in the literature about this programme. With hindsight, of course, it is clear that the programme should not have been attempted without a scientifically sound method for measuring CDB.

8.5.5 "Defensive" responses of caged workers

Lecomte (1961) measured attacks on woollen lures in small cages containing 150 workers and claimed that the different responsiveness of samples from

colonies of different races reflected the different responsiveness of the races as assessed by beekeepers (the data were not rigorously analysed). Lecomte (1961, 1968) claimed also that this method showed consistent differences between colonies of a single population.

Behavioural and metabolic (oxygen consumption) responses to alarm pheromones of small groups of caged workers were developed by Collins and Rothenbuhler (1978) and Southwick and Moritz (1985). These methods reduced the influence of environmental and internal-colony factors problematic in field tests, and were heuristic in investigations of several aspects of defensive behaviour (see Collins, 1979, 1980, 1981, 1982; Collins and Blum, 1982, 1983; Collins *et al.*, 1984; Moritz and Burgin, 1987; Rinderer *et al.*, 1983; Whiffler *et al.*, 1988). An essential problem is to know the extent to which the responsiveness of a worker sample in the laboratory correlates with responsiveness to the colony in the field. The metabolic test of Southwick and Moritz (1985), which measured a single variable, showed very low within-colony variance and it ranked the responsiveness of a set of five colonies in the same way as did a composite field test (which measured number of stings in a moving lure: section 8.5.2), even though the results of the field test were highly variable over the 13 recordings, which were taken weekly (Moritz *et al.*, 1985). Thus this laboratory test may provide shortcuts in breeding programmes, because field tests must be extensively repeated to distinguish genetic differences from environmentally induced ones.

The cage test of Boch and Rothenbuhler (1978) reflected differences in defensiveness of strains as assessed in the field by expert opinion. However, Collins *et al.* (1984) obtained less clear-cut correlations between cage measurements and "composite stimuli" measurements (section 8.5.2) of a set of fierce (Africanized) and docile (European) colonies and their backcrosses. The cage measurement (time to react to IPA) had *low*

phenotypic correlations with the "composite" measurements. The "composite" measurements themselves showed a wide range of "phenotypic correlations", ranging from .12 to .99, in which just under half were above .5 (no significance levels were given). As stated in previous discussion, such differences would be caused by (i) different genetic systems controlling the defensive behaviour of European and Africanized bees; (ii) environmental effects (likely to be strong since each colony in the field was measured twice only); (iii) confounding factors in the design of the field test (i.e. lure test: section 8.5.2).

8.5.6 Discussion

From the reviews in the present chapter it seems fair to say that there has been insufficient attention to the design of CDB measurement methods for whole colonies. In fact, there have been no experiments to compare various artificial danger stimuli and CDB responses to see if some are more consistent, or of better quality, than others. This may owe largely to the fact that many of the CDB experiments did not need measurement methods designed with full ethological rigour, for three main reasons.

First, in many investigations the subjects included sets of colonies known through expert opinion to differ in defence responsiveness, so that when the fierce strain or race gave responses significantly higher than the docile, nothing more was required (even though it is a commonplace that with distinctly fierce and docile colonies a difference will be obtained with almost any sort of measurement method: Rothenbuhler, 1964b).

Second, even though many results were confusing in some finer details, on the broad level most studies yielded the expected results, particularly when large sets of colonies under different treatments (or of differing genetic composition) were compared. Even the study of

Brandeburgo *et al.* (1977, 1982), which was undoubtedly severely confounded in several ways (*see* sections 8.4.3; 8.5.2), showed that two sets of colonies were much more responsive to Stort's test when kept in a warmer region of Brazil.

Third, in studies of the inheritance of *metric characters* (Falconer, 1981, p.96) in selected crosses (e.g. Cosenza, 1970; Boch and Rothenbuhler, 1974; Kerr *et al.*, 1974; Stort, 1975a,b,c, 1976, 1980; Collins, 1979, 1982; Collins *et al.*, 1984; Rinderer *et al.*, 1983; Moritz *et al.*, 1986b, 1987), some sort of interpretation in genetic terms will always be possible, even if the measurement method for the character is partially confounded. This is the case even in the determination of specific heritabilities (h^2) (Falconer, 1981, p.148; Collins, 1986) (unless negative variance components arise: see Collins *et al.*, 1984). Thus in some of these studies, particularly those undertaken before Collins and Rothenbuhler (1978), Collins and Kubasek (1982) and Moritz *et al.* (1985), there was perhaps less concern with confounding factors than there would have been in more ethologically oriented work. The greatest reservations about the meaning of a CDB measurement method were expressed in the one study (Michener, 1972) in which many unbred colonies of a single race were measured.

In breeding bees the geneticist is, of course, careful to select meaningful measurements and to take them accurately, in order to determine relative breeding values (Collins, 1986; Rinderer, 1986d). However, in their requirements for measurement accuracy, geneticists emphasize elimination of environmental and internal-colony effects. Very often, easily executed *correlated measurements* are preferred and large efforts for small gains in precision in actual behaviour measurements are generally regarded as unproductive. If by these means geneticists can get clear breeding values (or heritabilities) for behaviour characters, they are satisfied (Rinderer, 1986b). Nevertheless, geneticists acknowledge that

improvement in behaviour measurements may in certain instances improve genetic analyses, as when, in reviewing the differing conclusions that have been made about the genetics of CDB, Rinderer and Collins (1986) said that "it is possible that fine tuning of the processes of actual measurement could more clearly show the genotypes involved." The reviews in the present chapter indicate that in some tests, more than "fine tuning" is required: even though the moving lure test has yielded some clear inheritance patterns in CDB (section 8.4.5), it is likely that geneticists would prefer a less confounded measurement method should one be available. Improved methods have in fact been devised by geneticists (e.g. Collins and Kubasek, 1982; Moritz *et al.*, 1985), although the reasons for such developments were not based on critiques of previously existing tests. The requirements of the geneticist for simple, meaningful CDB measurements, and the criteria for such measurements set by ethological investigations of the behaviour, should be compatible. Further, geneticists need ethologically sound bioassay methods for CDB as a basis for judging the usefulness of their correlated measurements (Collins *et al.*, 1984; Moritz *et al.*, 1985).

With hindsight, it is instructive to consider what sort of CDB bioassay would today be deemed adequate for the importation of African honeybees to the Americas: the critical question is whether any of the CDB measurement methods reviewed above would suffice to identify indisputably docile colonies in Africa for such importation. This question is, perhaps, a realistic yardstick by which the adequacy of a CDB measurement method may be judged. Indeed, this criterion will in the future become increasingly important, especially when it is realized that an effective method of improving the Africanized bees will be to introduce desirable genotypes chosen from the vast variety available in Africa (see Taylor, 1985; Rinderer, 1986b; Fletcher, 1988) (this would be in addition to the selection from the Africanized gene pool and cross-breeding with European

strains, advocated e.g. by Michener, 1973; Cantwell, 1974; Levin, 1977; Taylor, 1985; Collins and Rinderer, 1986).

While a great deal has been learned from the studies reviewed, they have in various ways ignored the need to study the CDB response as a *complex mass action*. The investigations reported in the next chapter are an attempt to devise measurement methods for the behaviour as a mass behaviour.

CHAPTER 9

DEVELOPMENT AND APPLICATION OF A METHOD TO MEASURE CDB IN HONEYBEES

9.1 DEVELOPMENT OF A METHOD TO MEASURE CDB

9.1.1 Introduction

From the critiques of chapters 7 and 8, several basic requirements for a method to measure CDB may be drawn:

(i) the type of CDB to which the results are referred must be specified (CDB against apivorous wasps, robbers, colony takeover, humans, etc);

(ii) the aspects of the natural stimulus that are represented by an artificial stimulus should be specified (section 8.4.7);

(iii) the experimental stimulus employed should be of constant or controlled strength and form (section 8.4.1);

(iv) the aspects of the response that are abstracted as measurements must be considered in relation to the CDB type under study;

(v) the accuracy of the response measurements should be specified (section 8.5);

(vi) the effects of environmental, internal-colony, and genetic factors must be accommodated (section 8.4);

(vii) the entire method should be assessed critically for possible confounding effects (section 8.5);

(viii) if results indicate intrusion of confounding effects, these should be isolated and the method modified to exclude them.

The development of an unconfounded CDB measurement method is thus unlikely

to prove simple. However, from the reviews of chapters 7 and 8 a potentially heuristic approach, hitherto not addressed seriously in the literature, may be drawn. In section 8.5.2 the relationships between two moving lure measurements, X_1 (time to first sting) and X_2 (number of stings in lure), were assessed critically on the basis that a relatively fierce response in one measurement in a colony should be accompanied by a similarly fierce response in the other measurement, while in another colony a docile response in one measurement should be accompanied by a docile response in the other measurement. These relationships should hold in all types of measurement of colony defence intensity, in any particular colony, at all intensities of colony response. Repeated applications of a good CDB measurement on a set of colonies should rank them consistently according to their intrinsic levels of responsiveness. If serious discrepancies arise, an explanation must be sought, e.g. in environmental, internal-colony, or genetic effects. If no explanation can be made, the measurement method should be re-examined for confounding effects. Because "good", "sound", "true", "meaningful", "reliable", etc. behaviour measurements will accurately reflect the intensity of every defensive bout measured, they should be *highly correlated* with one another over repeated tests on the same colony, and between measurements (taken simultaneously) on different colonies. Conversely, "bad", "unsound", "meaningless", i.e. confounded measurements, which will not accurately reflect the intensity of each bout, should be uncorrelated both with one another and with good measurements. This criterion implicitly assumes (or predicts) that any extrinsic factor that has a major effect on CDB intensity will be highly correlated with variation in CDB intensity.

The criteria drawn above seem to be the best in the light of present knowledge about honeybee CDB. They are of course by no means the only ones possible (e.g. genetically based ones could be drawn), and they could be

found to be inadequate (e.g. potential defenders out foraging on a hot day could negate a predicted incremental effect of the heat; repetition of measurement routines might affect responsiveness). In this sense the criteria are hypotheses. However, their validity could only be tested by a CDB measurement known to be good, of which none are available. This element of circularity in the analysis of the soundness of CDB measurements must at present simply be acknowledged as requiring attention in the study of CDB. Advances will be possible only through assessment of CDB data against criteria for good CDB measurements. If among several different CDB measurements some are found to fit the criteria and some not, then the validity of the criteria will be strengthened somewhat. On the other hand, if none of a variety of well designed measurements is found to fit a particular criterion, this would indicate the need to reappraise that criterion. By assessing both criteria and behaviour variables *pari passu*, the circularity in the problem should eventually be broken.

9.1.2 Preliminary observations

From the various CDB test methods reviewed in section 8.5, three were chosen for study: the "moving lure" method, because of its wide use; and the "opening" and "breath" tests, because they seemed to deliver stimuli directly applicable to CDB against the human intruder, the main point of interest of the study. An advantage of these tests was that they could be executed by a single observer.

Before any data were recorded the tests were performed many times (on colonies established as described in section 9.2.2) and the general features of the responses were noted. In the *moving lure tests* several of the confounding factors outlined in section 8.5.2 were apparent, particularly crowding of lures, accidental bumping of flying workers by the

lure, and attacks on the observer while the lure was ignored. Lengthy initial attacks on lures by lone individuals were seen sometimes, but on other occasions the first attack was soon followed by a mass attack. This discrepancy indicated the existence of the confounding factor of the individual initial attacker and its release of alarm pheromones, as outlined in section 8.5.2. A further drawback was seen in highly responsive colonies, which would occasionally launch massive, really dangerous attacks, forcing the observer with the lure to retreat before completion of the test (as experienced also by Michener, 1972). These high responses mostly occurred after the continuous presentation of a lure beyond 15-30 s. For the present study, where the intention was to test colonies repeatedly from day to day, such high responses were undesirable since it was evident that the loss of many defenders through sting autotomies could affect subsequent responses. This test was later analysed more rigorously (chapter 10).

The *opening test* (section 8.5.3) constituted a highly variable danger stimulus. For example, the propolis under the lids of some hives would snap and crack when the lids were removed on cool days when the propolis was brittle, causing more vibration than on warm days. There was always the danger of accidentally bumping a hive or crushing workers who would then release alarm pheromones. Some hives with empty supers appeared to give delayed responses, since fewer defenders were immediately exposed than they were in full hives. As with the lure test, colonies would occasionally reach a dangerous level of response before completion of the stimulus, which was exacerbated by the replacement of the hive lid. In other cases apparently unresponsive colonies would suddenly attack when hive covers were replaced.

In the *breath test* it became evident that there was more to the response than just the guard recruitment measured by Boch and Rothenbuhler

(1974). In highly responsive colonies workers sometimes flew and attacked the observer as he arrived at the colony, although more often the first attacks were elicited during the breath stimulus. To administer this stimulus the observer knelt to one side of the hive and blew three strong, one-second puffs at one-second intervals directly into the hive entrance, from a distance of about 60 mm (after Boch and Rothenbuhler, 1974). Care was taken not to touch any bees stationed on the entrance board. The administration of this stimulus generally produced an immediate, characteristic response comprising a loud buzzing "roar" from within the hive and recruitment of defenders, some rushing about the flight board and the others flying about in characteristic jerky flight with a high-pitched whine and occasional head-bumps (as described by Collins and Kubasek, 1982; Rinderer, 1982; section 8.2), called *pestering* by Michener (1972). If the observer remained near the hive the pestering would in some colonies be maintained for a while and then diminish, whereas in others it would build up to a mass attack, sometimes forcing the observer to retreat. If after giving the breath stimulus the observer walked off behind the hive and stood at a point 10 m away, a relatively modest group would follow and continue to pester without any buildup to mass attack. When the observer returned to the hive 3 min later to count the guards, highly responsive colonies would renew their attack at relatively low levels, whereas docile colonies would remain calm. Finally, when the observation was completed, the observer was able to rid himself of persistent pesterers by walking into a dense papaw (papaya) plantation 12 m behind the row of hives, and remaining there for a few minutes.

Of the three measurement methods, the breath test seemed to have the best potential to produce unconfounded measurements of CDB intensity, and a measurement routine was devised as described in the next section.

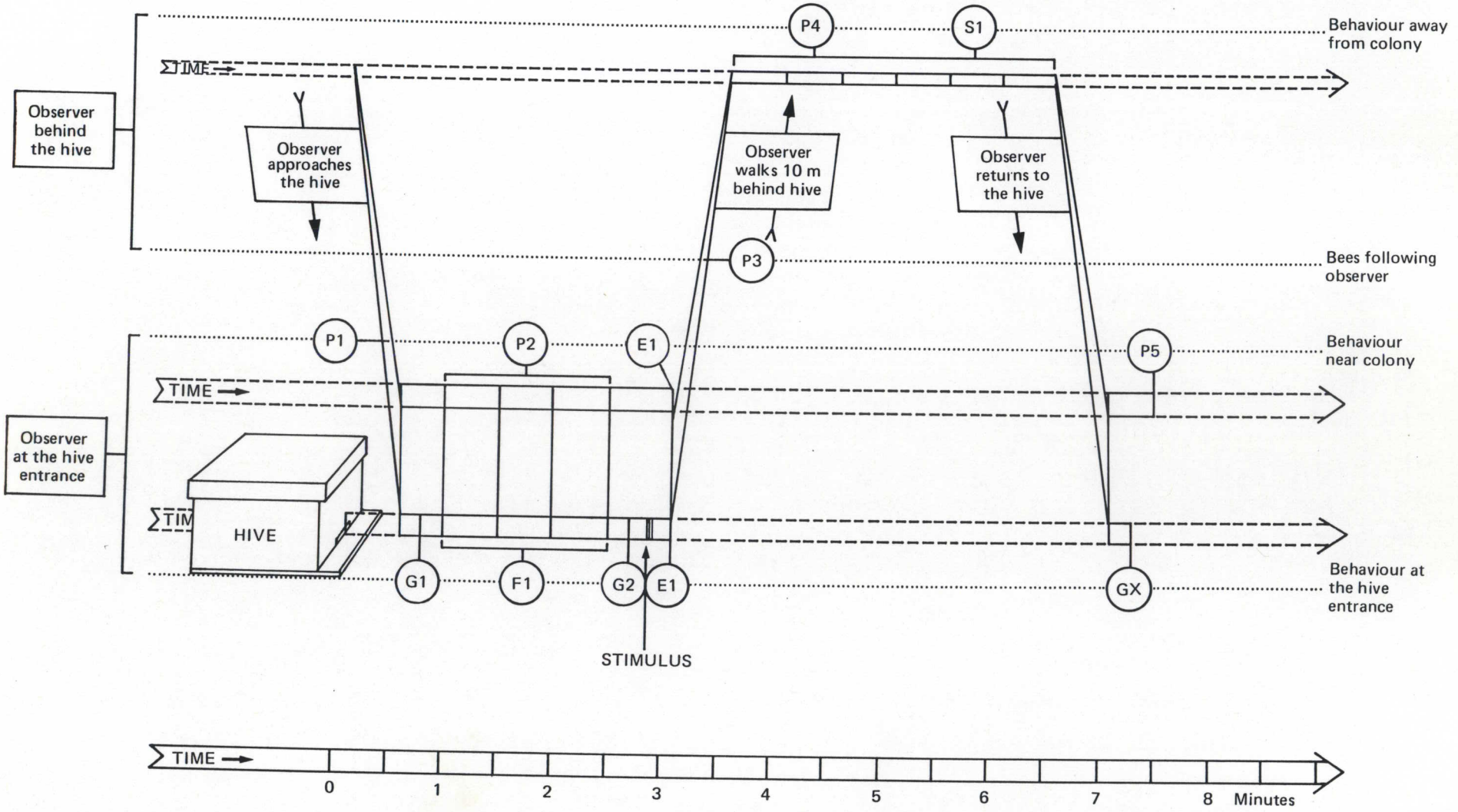

FIGURE 9-1. Plan of the movements of the observer relative to timing of measurements during the breath test measurement routine. Variables are defined in the text.

9.1.3 A CDB measurement routine

A measurement routine was imposed on the sequence of responses to the breath test described above (Fig. 9-1). Its timing was fitted around the 3 min interval between the breath stimulus and the counting of guards used by Boch and Rothenbuhler (1974). Five kinds of variable were recorded:

"P" = pestering;

"F" = forager flight;

"G" = guards at hive entrance;

"E" = response to stimulus at hive entrance;

"S" = stings to observer.

Some variable types were recorded more than once, as indicated by the numbers attached to them in the listing below, which describes the variables in the approximate order in which they were recorded in the field:

P1 Number of workers that flew up at the observer on his arrival at the hive.

F1 Number of workers that flew into the hive per 30 s (mainly returning foragers): average of 3 counts of 30 s each.

P2 Number of workers that attacked the observer per 30 s during the count of F1: average of 3 counts of 30 s each.

G1 Number of workers on the flight-board ("guards") when the observer first arrived at the hive.

G2 Number of workers on the flight-board after the 90 s count of F1.

G3 Number of workers on the flight-board 3 min after administration of the breath stimulus (column GX, Appendix Table 9-1) *minus* G1, i.e. increase or decrease in the number of workers on the flight-board 3 min after administration of breath stimulus (roughly

equivalent to "number responding to breath stimulus" of Boch and Rothenbuhler, 1974).

E1 Initial response to 3 x 1 s puffs of human breath blown into the hive entrance: i.e. observer's assessment of the initial "buzzing roar", defender recruitment and flying attack, noted on a scale of 0 = no response to 5 = full, mass attack.

P3 Number of workers that followed the observer as he walked to a point 10 m behind the hive after administering the breath stimulus.

P4 Number of workers that pestered the observer standing 10 m behind the hive after administering the breath stimulus: average of 6 counts of pesterers at 30 s intervals after administration of the breath stimulus.

S1 Number of stings received by the observer during P4.

P5 Number of bees that pestered the observer upon his return to the hive to count G3.

After recording P5 the observer walked into dense foliage 12 m behind the hives to rid himself of pesterers. After 2 min the next colony was tested. The recording of a breath test took 5 min; tests on successive colonies were thus taken at 7 min intervals; a recording of five colonies took 35 min. If a colony due to be tested had an orientation flight in progress it was left for another and was then tested at the end of the measurement session. Successive testing of adjacent colonies was avoided whenever possible.

The five experimental colonies were labelled A-E. *A variable name prefixed by one of these letters refers to the variable as measured in that particular colony*, e.g. DG3 = "G3 in colony D", i.e. change in number of workers on the flight-board 3 min after administration of the breath

stimulus, in colony D.

Most of the measurements employed in this routine were counts or estimations of groups of bees, flying into the hive or around the observer, or which guarded the hive entrance, or responded during the breath stimulus, during set time intervals. Whilst flights into the hive can be accurately recorded with a click-counter, it is difficult to make accurate estimations of guards and flying bees, and the experience of the observer is an important factor. Indeed, at present the only attempts to count flying defenders have been by Michener (1972) who simply estimated them directly, and by Collins and Rinderer (1986) who attempted to photograph airborne bees but have not reported the results in detail. Collins and Kubasek (1982) said that the lack of a method to account for flying bees was the greatest inadequacy of their field test. In the present study direct counts of flying defenders were made since it was felt, after many pilot observations, that consistent estimations could be obtained. When the flying bees numbered about 15 or below they were counted directly. Larger groups, up to about 50, were estimated on the basis of multiples of 10, above which they were estimated in multiples of 50 or 100 (the larger the group, the greater the inaccuracy of the count). Guards at the hive entrance were estimated in much the same way as the flying defenders. Similarly, the initial response to the breath test (response E1) was made on a scale of 0 (no response) to 5 (immediate full mass attack), with gradations of 0.25 between each unit. Thus an element of the investigation was to see whether CDB can be studied effectively through direct estimates by an experienced observer. Partly for this reason, many repetitions (n = 59) of measurements were made on the experimental colonies.

The measurement routine was designed to elicit *moderate* defence responses, by:

(i) the use of a mild danger stimulus, i.e. the breath stimulus described above;

(ii) the departure of the observer from the hive immediately after administration of the stimulus;

(iii) the prevention of inter-colony alarm communication by siting the hives far apart, with screens of vegetation between them;

(iv) the use of fairly docile colonies.

9.2 ASSOCIATIONS BETWEEN THE BREATH TEST VARIABLES

9.2.1 Introduction

The criterion (drawn in section 9.1.1) that good CDB variables in honeybees should be well correlated over repeated measurements, both within and between colonies, was examined in the present study.

9.2.2 Methods and materials

Five normal (section 3.2.1) colonies (designated A-E) of the local *A. m. scutellata* bees were aligned at 10-20 m intervals in a windbreak of trees with abundant low foliage, so that each colony was screened from its neighbours. Standard Langstroth hives were used, with entrances 120 mm wide and 12 mm high, opening onto flight-boards extending 50 mm from the hive body. The colonies were measured by the breath test (section 9.1.3) on 59 days, mostly in daily succession, although three days were missed over the total observation period of 62 days. The order in which the colonies were tested was changed from day to day. Measurement sessions started at midday.

The extent to which pairs of the various CDB measurements varied

together over the observation series was estimated by correlation analysis (Sokal and Rohlf, 1981, p.561). Pearson's *r* was used when the variables concerned had approximately normal distributions (Sokal and Rohlf, 1969, p.498). Distributions were assessed by scrutinizing histograms and by the */X/* ratio (Bliss, 1967, p.145) between the skewness and kurtosis values (Nie *et al.*, 1975; Anonymous, 1983, 1986) for each variable. Distributions ranged from approximately normal through increasing positive skewness (Spiegel, 1972) to extremes in which mostly zeros were scored. Transformations for non-normal variables were determined by the application of Taylor's Power Law (Taylor, 1961, 1970; Southwood, 1978). Variables in which only zeros were scored throughout the observation period, in one or more of the experimental colonies, were dropped from the initial analyses (i.e. variables P1, P2 and S1). In variables with non-normal distributions which could not be transformed to normality, correlations were estimated by Kendall's *tau* (Hays, 1973, p.788; Blalock, 1979, p.434) which, because of its superior capacity to handle numerous ties in data (Nie *et al.*, 1975, p.289; Blalock, 1979, p.439), was preferred over Spearman's *rho*, the other commonly used distribution-free method for estimating associations between pairs of variables.

In the initial analyses the correlation matrices, between seven behaviour variables, comprised 21 correlation coefficients (Table 9-1, p.206). Relationships amongst these coefficients were determined by cluster analysis, which was chosen because it makes fewer assumptions than most other multivariate techniques for reducing matrices (such as factor analysis, principal components analysis, and discriminant analysis), and allows a diversity of data representations (Aspey and Blankenship, 1977; De Ghett, 1978). Cluster analysis of a matrix of "individuals" (which in the present study were bivariate correlation coefficients between CDB variables) results in several mutually exclusive subgroups (clusters)

within which individuals are relatively similar and between which individuals are relatively different (Aspey and Blankenship, 1977).

Several clustering methods are available for similarity matrices (such as correlation matrices), namely single linkage, complete linkage, and average linkage (UPGMA, WPGMA, UPGMC and WPGMC) (Sneath and Sokal, 1973; Anonymous, 1986). In trial runs of these methods on correlation matrices from the data, WPGMA consistently gave the most readily interpretable clusters in relation to visual appraisal of the matrices and so was chosen for the analysis. On the same matrix, group-average methods (of which WPGMA is one) generally produce more distinct clusters than the other common SAHN (Sequential, Agglomerative, Hierarchic, Nonoverlapping clustering methods) methods, viz. single and complete linkage (Gower, 1967; Lance and Williams, 1966; Sneath and Sokal, 1973, p.228). Although this method of choosing a clustering algorithm might be questioned by the biometric purist, it is defended on the grounds that the aim in using the technique was simply as a preliminary aid to visualize the groupings of the variables as afforded by their correlation coefficients. Green (1979, p.15) and Crovello (1970) provide support for this approach in choosing descriptive biometric methods.

9.2.3 Results and discussion

The CDB variables in the present study, with $n = 59$ in each colony, were different from most CDB variables in the literature (reviewed in section 8.5) which consisted either of several recordings on a colony on one day (e.g. Stort, 1974) or of once- or twice-daily recordings made at intervals of weeks or months (e.g. Brandeburgo *et al.*, 1977, 1982; Collins and Kubasek, 1982). The latter studies analysed differences in responsiveness of sets of colonies in different environments, or given different stimuli,

whereas the present study examined consistency of relationships among CDB variables in a few colonies measured repeatedly over a long period, as also done by Moritz *et al.* (1985) and Southwick and Moritz (1987) in their field measurements of CDB.

Restricting comment first to the matrix of 21 Pearson correlation coefficients between the seven CDB measurements of colony A (Table 9-1), G1-G2 was the most highly correlated pair of variables with r = .922, followed by P3-P4 (r = .888), P4-P5 (r = .803) and P3-P5 (r = .734). These high correlations can be explained partly by the fact that each pair of variables measured a similar type of behaviour. G1 was the number of bees at the entrance initially; G2, the number at the entrance after the observer had stood near the colony for 90 s. Thus the presence of the observer during the count of F1 was not associated with variable changes in the number of guards in the 59 observations made on colony A. Variables P3, P4 and P5 were all counts of the bees pestering the observer after the breath stimulus, which increased or decreased in number according to the responsiveness of the colony and to the progression of the stimulus routine. Over the 59 observation days the intensities of P3, P4 and P5 in colony A had a fairly low range, from a maximum of a group of 36 pesterers over the 3 min of P4, to no response at all (Appendix Table 9-2). The high correlations between these variables indicates that the pestering habits of the defenders of colony A were, during 3 min following the breath stimulus, *consistent* over a range of intensities over the 59 observation days. It is understandable that P3-P5 were somewhat less well correlated than P3-P4 and P4-P5 since there was a greater time interval between P3 and P5, as well as additional danger stimuli.

Variables G1 and G2 had significant negative correlations with G3, at r = -.333, which means that G3 was a different kind of measurement from G1 and G2. This deduction was strengthened by the correlations of the

TABLE 9-1. Correlation matrices of seven CDB variables in colonies A–E (Pearson coefficients; $n = 59$). Variables are defined in the text.

Colony	Behaviour variable	G1	G2	G3	E1	P3	P4
A	G1						
	G2	.922**					
	#G3	−.452**	−.333**				
	E1	.514**	.636**	.039			
	P3	.469**	.520**	.022	.772**		
	P4	.535**	.568**	−.038	.782**	.888**	
	#P5	.483**	.531**	.119	.622**	.734**	.803**
B	G1						
	G2	.970**					
	G3	−.283	−.258				
	E1	.557**	.550**	−.015			
	#P3	.385*	.386*	−.268	.422**		
	P4	.619**	.599**	−.341*	.649**	.722**	
	#P5	.136	.136	−.028	.240	.341*	.351*
C	G1						
	G2	.934**					
	#G3	−.260	−.165				
	E1	.638**	.714**	.160			
	P3	.759**	.790**	−.047	.774*		
	P4	.816**	.851**	.013	.820**	.891**	
	P5	.753**	.787**	−.080	.809**	.849**	.907**
D	G1						
	G2	.759**					
	#G3	−.234	−.202				
	E1	.608**	.484**	−.308			
	#P3	.364*	.409*	−.231	.673**		
	P4	.450**	.474**	−.278	.755**	.857**	
	#P5	.180	.176	−.130	.301	.602**	.643**
E	G1						
	G2	.819**					
	#G3	−.489**	−.393*				
	E1	.520**	.543**	−.397*			
	#P3	.573**	.480**	−.258	.594**		
	#P4	.442**	.497**	−.195	.635**	.551**	
	#P5	.035	.022	.284	.220	.121	.438**

The coefficients are not significantly different from zero unless marked *($P < 0.01$) or **($P < 0.001$).

best transformation has been applied but variable remains non-normal.

other variables of the analysis, E1, P3, P4 and P5, with G1, G2 and G3. With G3 they were without exception non-significant, whereas with G1 and G2 they were significant, and remarkably even, at levels between .469 and .636. It is thus possible to assign G3 the status of "odd man out" in the array of seven CDB measurements made on colony A, whereas G1 and G2 appear to be on the periphery of the domain of E1, P3, P4 and P5.

Although the relationships between G1 (or G2) and the other variables of the analysis were fairly clear, this was not the case with other relationships (outside the highly correlated pairings P3-P4 and P4-P5, discussed above). For instance, r between P3-P5 = .734; P3-E1 = .722; P4-E1 = .782; P5-E1 = .622. The similar magnitudes in this array make it difficult to visualize the relationships between the variables: they are clarified by the dendrogram in (Fig 9-2), which also corroborates the relationships drawn above by inspection of the raw coefficients. G1-G2 as the primary cluster was well isolated from the neighbouring cluster of P3-P4-E1-P5, and G3 was separated entirely from these two clusters. Within the central cluster E1 was nearer to the pairing P3-P4 than was P5.

Thus, over the 59 observation days in colony A, three different kinds of variable were determined:

(i) G1-G2

(ii) G3

(iii) E1-P3-P4-P5

The dendrogram produced from the Kendall coefficients between the seven variables of colony A (Fig. 9-2) was similar to that for the Pearson coefficients in every respect except in the placings of E1 and P5, which were interchanged in position. This indicates that the non-normal distributions of G3 and P5 did not seriously affect Pearson's r as an indicator of the relationships between the variables, and the interpretations made above from the dendrogram based on Pearson

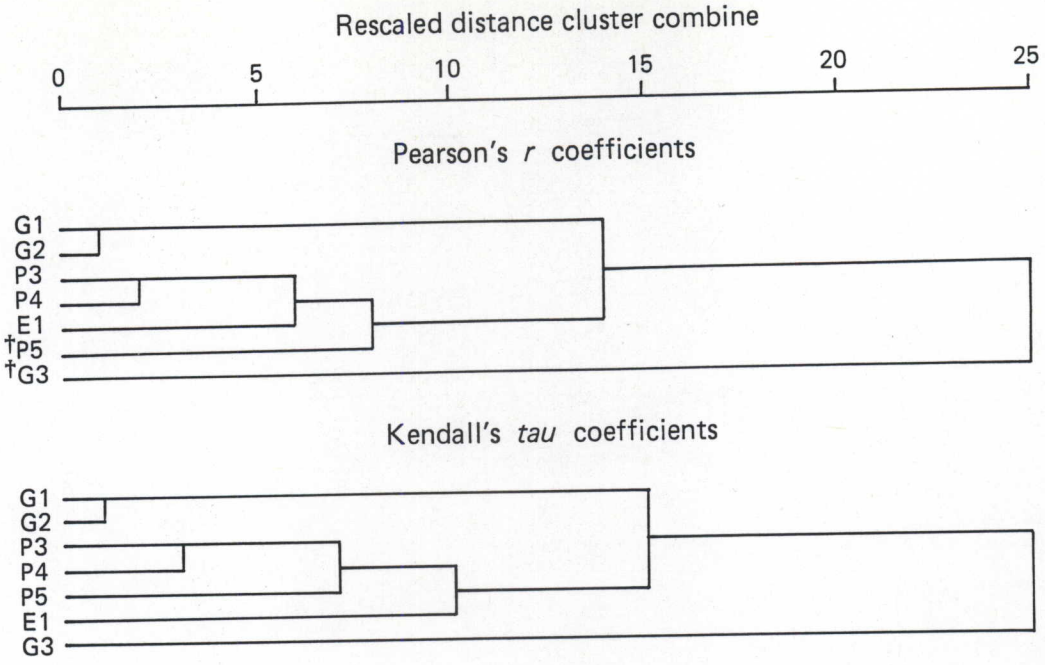

FIGURE 9-2. Dendrograms from cluster analyses of associations between seven CDB variables in colony A. Pearson coefficients are given in Table 9-1. Variables are defined in the text.
† best transformation has been applied but variable remains non-normal.

TABLE 9-2. Correlated *t*-tests between means of G1 and G2 in colonies A–E (n = 59; DF = 58). Variables are defined in the text.

Variable pairs	Mean of log-transformation	Standard deviation of log	t	P†
AG1	0.961	0.671	−0.33	.741
AG2	0.973	0.691		
BG1	0.744	0.417	−0.95	.344
BG2	0.757	0.406		
CG1	1.126	0.445	4.64	.000**
CG2	1.029	0.432		
DG1	0.190	0.224	1.01	.317
DG2	0.170	0.212		
EG1	0.521	0.235	−0.39	.697
EG2	0.528	0.259		

† Two tailed test of significance.
**Significant difference at $P < 0.001$.

coefficients would be similar for the Kendall's *tau*-based dendrogram.

The highly correlated variables G1 and G2 in colony A had very similar means (Table 9-2), as they did in colonies B, D and E. However, in colony C the mean of G2 (= 16.3) was substantially *lower* than that of G1 (= 21.5), as confirmed in a correlated *t*-test (Sokal and Rholf, 1969; Nie *et al.*, 1975) between G1 and G2 from each colony (Table 9-2), which showed no significant differences in colonies A, B, D and E but a highly significant difference in colony C where G2 was less than G1. This reduction in the number of guards in colony C in response to the 90 s presence of the observer standing to one side of the entrance must have been the result either of some guards retreating into the hive, or of some guards flying from the entrance to attack the observer, as noted by Collins and Kubasek (1982) and Collins *et al.* (1982) (section 8.5.2). Evidence in favour of the latter conjecture exists in the relative magnitudes of P1 and P2 (the numbers of workers that flew at the observer on his arrival and during his initial stay of 90 s at the hive entrance). P1 was zero in colonies A, B, D and E, but in colony C its mean was 4.7 (range 0-30) (Appendix Table 9-2). Similarly, P2 was zero in colony D, very low in colonies A, B and E, but high in colony C (mean = 11.8; range = 0-83). A simple explanation is that in the undisturbed state colony C usually (over the 59 measurement days) had many more guards at its entrance than the other colonies (substantiated in section 9.2), many of which flew to attack and pester the observer when he arrived at the hive. Indeed, colony C was in general the most highly responsive of the five experimental colonies (section 9.2), so that the reduction in the number of guards at its entrance over the observation time of 90 s was contrary to the notion of Boch and Rothenbuhler (1974) that the change in the number of guards at the entrance is a simple index of colony defensiveness. Because the difference between G1 and G2 constituted no response in four colonies, and was

negative in colony C, G2 was dropped from further analyses of the CDB variables. There was a qualitative difference between G1 and the other CDB variables: G1 was not a *response*, either to the initial presence of the observer (as were P1, P2, G2), or to the breath stimulus (as were G3, E1, P3, P4, P5). Rather it represented a *state* in the undisturbed colony, with the potential to reflect the defensive responsiveness, or "alertness" of the colony. However, it also had the potential to reflect other factors, since the workers at the undisturbed hive entrance may have been engaged in a variety of non-defensive behaviours (section 8.3).

With the dropping of G2, the primary cluster of G1-G2 in colony A (in Fig. 9-2) was replaced in the new six-variable cluster for colony A by P3-P4 (Fig. 9-3), and G1 was relegated to the position of the last-admitted variable to the cluster of P and E variables, flanked on the other side by G3, still the most dissimilar variable of the matrix. Within the P-E cluster, E1 retained the same relative position that it occupied in the seven-variable cluster, between P4 and P5.

In dendrograms for the six behaviour variables G1, G3, E1, P3, P4 and P5 in the other four colonies of the analysis, B, C, D and E (Fig. 9-3), G3 was the most dissimilar variable in every case. The primary cluster was P3-P4 in colonies B and D whereas in colony C it was P4-P5 with P3 clustered closely. In colony E the primary cluster was E1-P4, with P3 clustered closely. Thus P3 and P4 were highly correlated in all five colonies, while the other flight-attack variable, P5, ranged from a close association with P3-P4 in colony C, to the second-most dissimilar variable in colonies B and E, a position it relinquished to G1 in colonies A and D. In all five colonies, P3, P4 and E1 constituted the most similar trio of variables, their proximity to one another being broken only once, by P5 in colony C.

Several variables in the above analysis could not be normalized, and

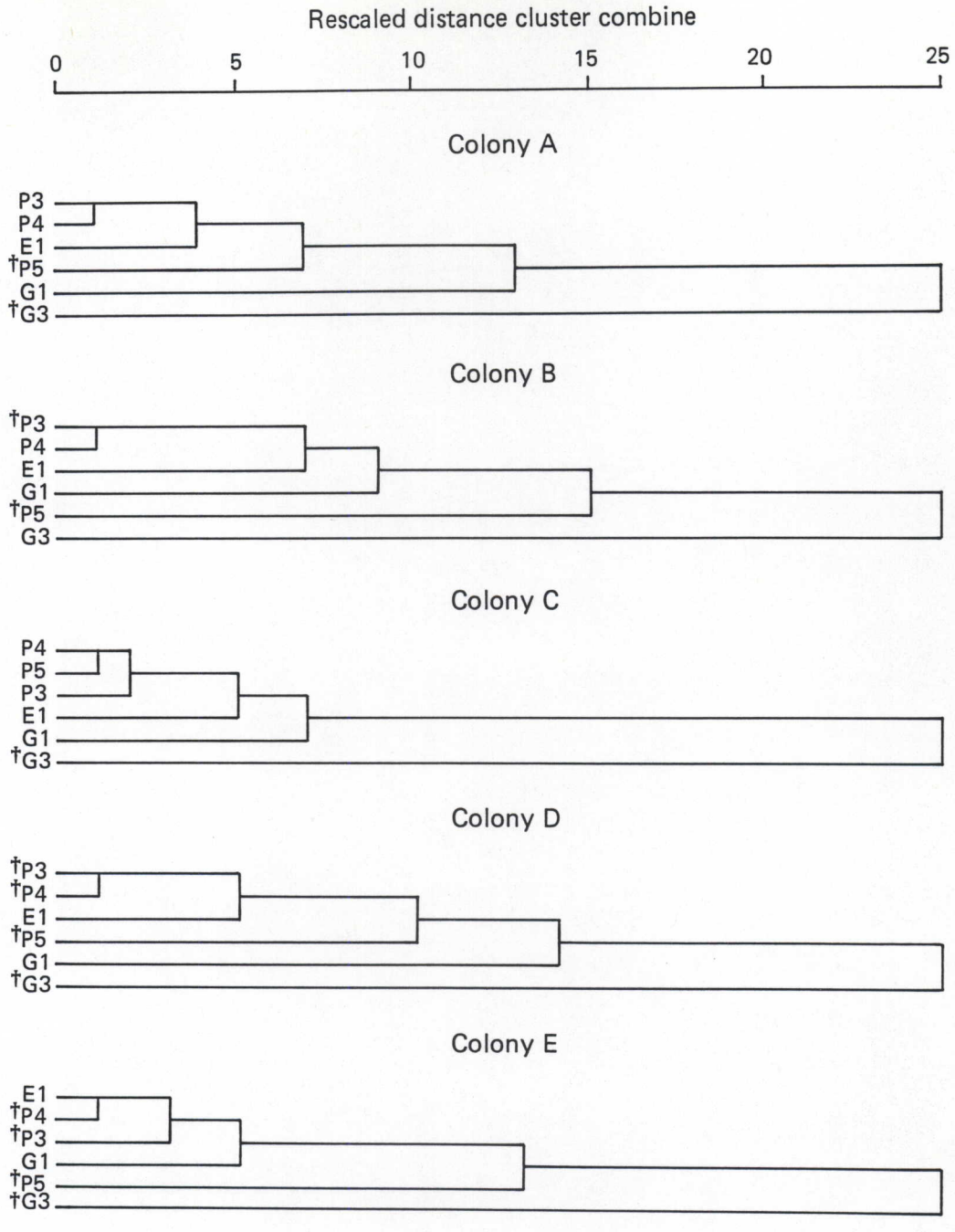

FIGURE 9-3. Dendrograms from cluster analyses of Pearson's *r* between six CDB variables in colonies A–E. Variables are defined in the text.

† best transformation has been applied but variable remains non-normal.

they may have affected the relationships depicted by Pearson's *r*. As a check, dendrograms were produced from the distribution-free Kendall's *tau* for the same sets of variables (Fig. 9-4): they yielded much the same information as the Pearson-based ones, but placed G1 more closely with the E1-P3-P4 grouping.

At this point, four distinct measurement types (as opposed to three in the analysis of colony A alone) have been identified on the basis of their associations revealed by cluster analysis:

(i) G1

(ii) G3

(iii) E1-P3-P4

(iv) P5

P1, P2 and S1 were too low in four of the five experimental colonies to be used in the general analysis.

In terms of the criteria for good CDB measurements stated at the outset, the consistent close clustering between the two different response types E1 and P3-P4 in all five colonies indicates (weakly) that these variables may have provided a better measure of CDB than the more labile G1, G3 and P5. The consistencies obtained in the relationships between the variables indicates that the measurements, which were estimates and counts by the observer (*see* section 9.1.2) were accurate enough for the purposes of this study. The further consistencies reported in the remainder of the study serve to support this contention.

The "composite" dendrogram from the correlation matrix of six variables for all five colonies together (Fig. 9-5) showed a remarkably consistent series of clusterings of E1-P3-P4 in colonies A, B, C and D. G1 was included in this clustering only in colony C, and was attached more distantly in colony D. P5 entered this clustering in colonies A and C. Otherwise, G1 and P5 were scattered through the dendrogram, as were all the

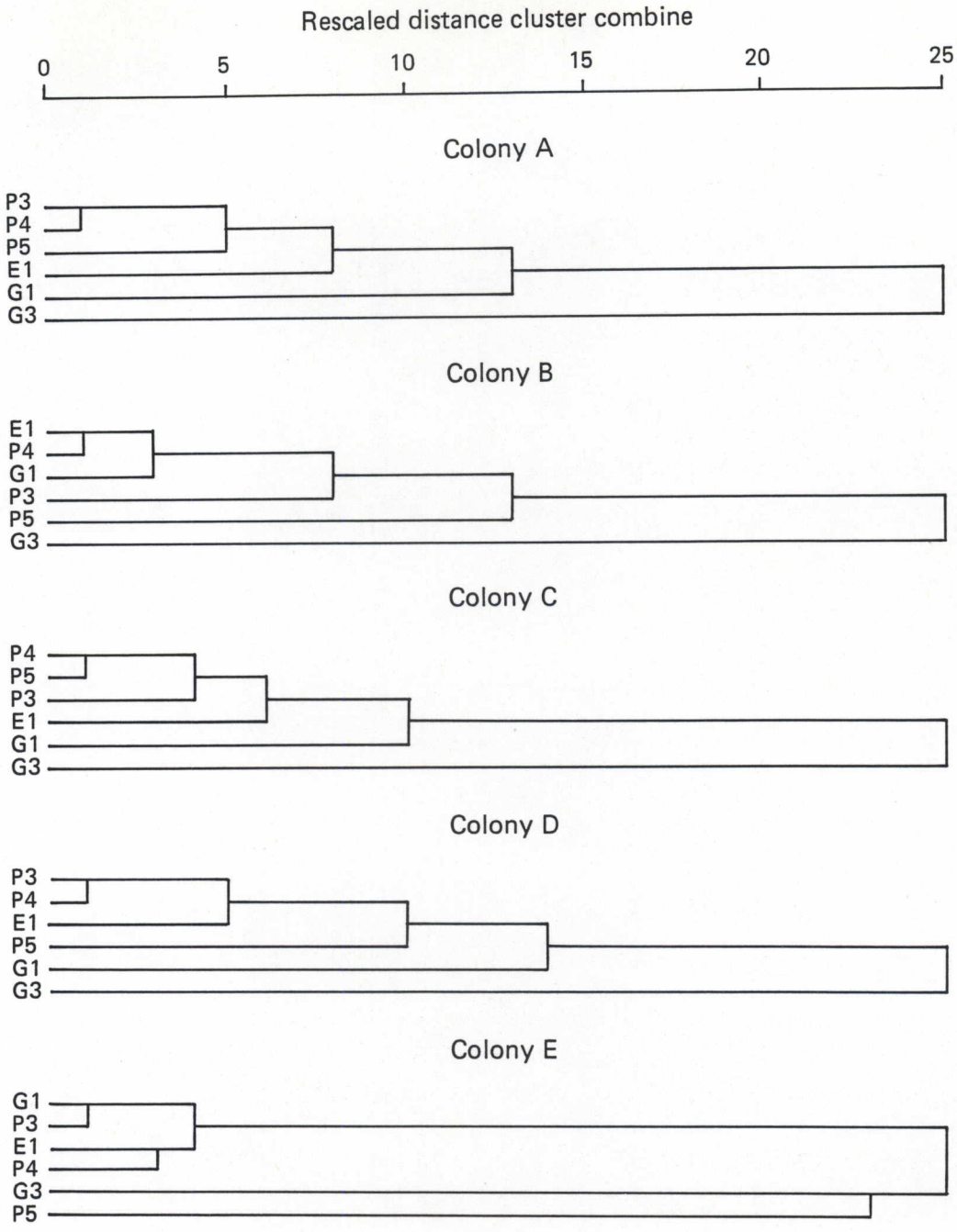

FIGURE 9-4. Dendrograms from cluster analyses of Kendall's *tau* between six CDB variables in colonies A–E. Variables are defined in the text.

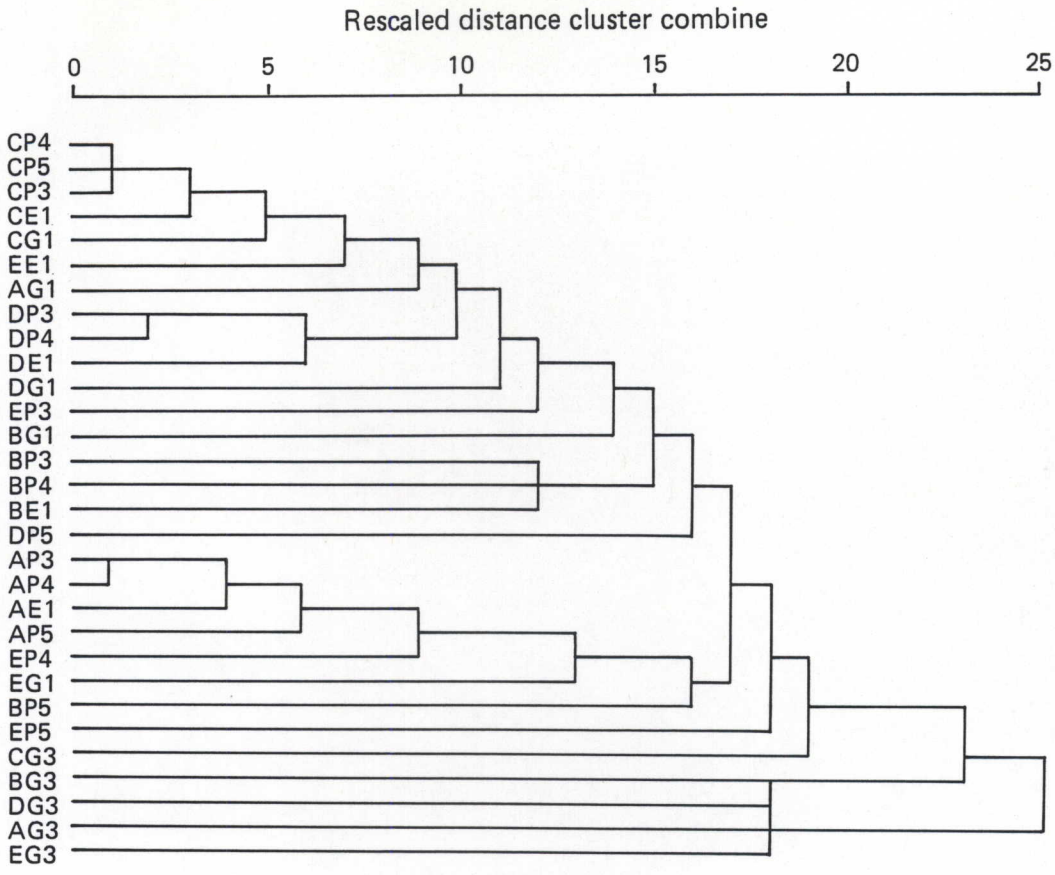

FIGURE 9-5. Dendrogram from cluster analysis of Pearson's *r* between six CDB variables in colonies A–E together. Variables are defined in the text.

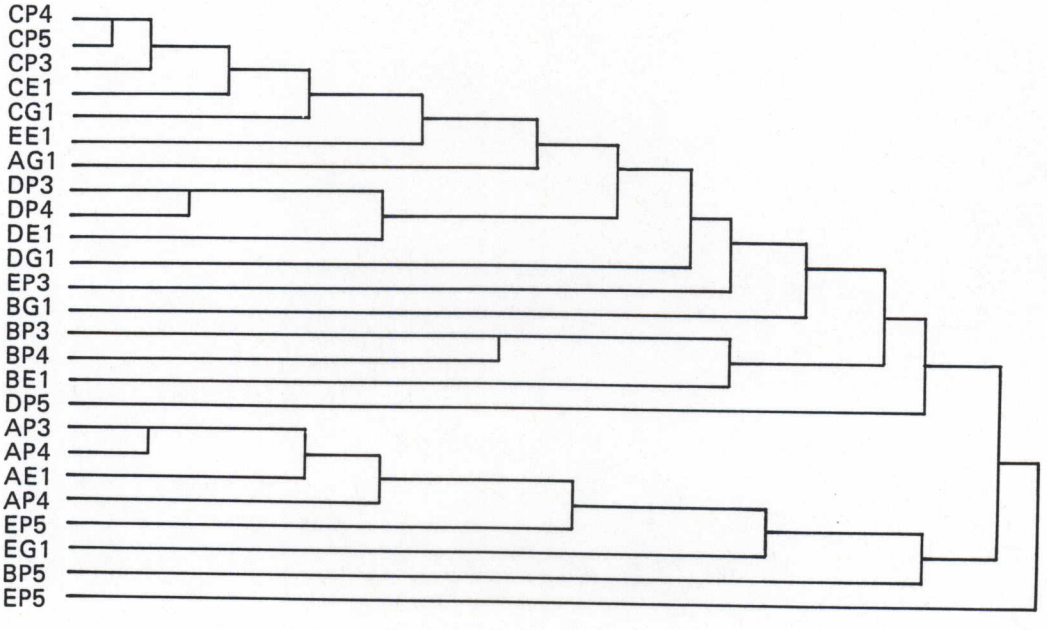

FIGURE 9-6. Dendrogram from cluster analysis of Pearson's *r* between five CDB variables in colonies A–E together (ie. variable G3 was dropped from the analysis shown in Fig. 9-5). Variables are defined in the text.

variables of colony E*. The G3's were so unlike the other variables that the relationships between the latter were unaffected in the dendrogram produced when G3 was dropped from the analysis (Fig. 9-6). The relationships depicted by these composite dendrograms were essentially equivalent to those obtained above from the clusters of separate colonies, with the important additional information that, with the exception of colony E, variables E1, P3 and P4 were more alike one another *within* each colony than each variable-type was to its equivalents in other colonies. The latter case would have been indicated if clusters of variables-of-a-kind were obtained. These relationships were shown more clearly when all variables of colony E, and all G3, were dropped from the analysis (Fig. 9-8), which produced the groupings:

Colony A: AE1-AP3-AP4-AP5

Colony B: BE1-BP3-BP4

Colony C: CG1-CE1-CP3-CP4-CP5

Colony D: DE1-DP3-DP4

Scattered amongst these were the dissimilar variables AG1, BG1, DG1, BP5 and DP5.

Even though the composite dendrograms showed that correlations

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* The all-colony dendrogram based on Kendall's *tau* indices (Fig. 9-7) yielded the same basic intra-colony clusterings as those from *r* coefficients (Fig. 9-5), with stronger displacement of P5 of colonies B, D and E to the group of most dissimilar variables, which included G3 of all colonies. As with the *tau*- and *r*- based dendrograms for separate colonies (discussed above), the great similarities between the *tau*- and *r*- based composite dendrograms serve to strengthen the interpretations made on the basis of the *r* dendrograms alone.

between E1, P3 and P4 were strongest *within* the colonies A, B, C and D, each of these variables was also highly correlated with its counterpart in the other experimental colonies (Table 9-3). Further, there were high correlations in pairings between E1-P3-P4 from different colonies (*see* Table 9-4 which, together with Table 9-3, comprises the coefficients from which the composite dendrogram of Fig. 9-5 was produced): between E1-P4 there was only 1 non-significant pair in 20 pairings; there were 2 non-significant pairings between E1-P3 and between P3-P4; between P4-P5 there were 7 non-significant pairings, 4 of them in colony E. By contrast, G3 had no significant positive correlations with E1, P3 or P4 in any pairing, but in fact had some significantly negative correlations with these variables (Table 9-4). Even amongst G3 inter-colony pairings (Table 9-3) there were only 2 significant correlations out of a possible 10. G3 was thus *very* different from the other variables of the analysis. In terms of the criteria for good CDB measurements stated at the outset, this result indicates (weakly) that E1-P3-P4 were better measurements of CDB than G3.

The affinities of P5 between colonies, and with other variables, appeared to place it on the periphery of the array E1-P3-P4. There were 5 out of 10 significant pairings between colonies in P5 (Table 9-3). P5 of colony C was significantly correlated with E1 in all colonies; P5 in colony A was significantly correlated with AE1, CE1 and DE1 (Table 9-4). Otherwise, there were no significant inter-colony pairings between E1 and P5. This pattern of correlations seems to have been associated with the responsiveness of each colony in P5: the means of this variable were very low (below 1) in colonies B, D and E, higher in colony A (= 2.3) and very much higher in colony C (= 65.8): in the low-responding colonies many observations registered as zero (Appendix Table 9-1). It thus seems that when responses to P5 were sufficiently high, this variable was very similar to E1. In comparison with P5-E1, there was a greater number of significant

TABLE 9-3. Correlation matrices of each of six CDB variables in colonies A–E (Pearson coefficients, n = 59). Variables are defined in the text.

Behaviour variable/ Colony		Colony			
		A	B	C	D
G1	B	.428**			
	C	.574**	.599**		
	D	.477**	.359**	.472**	
	E	.396*	.267	.417*	.466**
G3	B	.100			
	C	.107	−.026		
	D	.227	.438**	.022	
	E	.461**	.227	.017	.242
E1	B	.484**			
	C	.761**	.501**		
	D	.587**	.610**	.635**	
	E	.675**	.595**	.688**	.676**
P3	B	.492**			
	C	.604**	.488**		
	D	.485**	.543**	.604**	
	E	.417*	.353*	.481**	.440**
P4	B	.471**			
	C	.683**	.592**		
	D	.697**	.623**	.743**	
	E	.550**	.289	.561**	.600**
P5	B	.483**			
	C	.454**	.267		
	D	.362*	.469**	.349*	
	E	.058	−.015	.136	.068

The coefficients are not significantly different from zero unless marked *(P <0.01) or **(P <0.001).

TABLE 9-4. Correlation matrices of pairs of CDB variables in colonies A–E (Pearson coefficients; $n = 59$). Variables are defined in the text. Coefficients between variables *within* a colony are underlined.

Behaviour variable/ Colony		Colony				
		A	B	C	D	E
				Variable G3		
G1	A	−.452**	−.387*	−.019	−.250	−.508**
	B	−.387*	−.283	−.008	−.109	−.513**
	C	−.351*	−.242	−.260	−.135	−.560**
	D	−.319	−.305	−.184	−.234	−.327
	E	−.201	−.040	−.007	−.250	−.489**
				Variable E1		
G1	A	.514**	.330	.508**	.614**	.609**
	B	.254	.557**	.361*	.508**	.381*
	C	.580**	.547**	.638**	.729**	.623**
	D	.496**	.462**	.470**	.608**	.517**
	E	.458**	.365*	.385*	.484**	.520**
				Variable P3		
G1	A	.470**	.329	.616**	.446**	.372*
	B	.195	.385*	.378*	.240	.148
	C	.475**	.388*	.759**	.491**	.370*
	D	.432**	.338*	.581**	.364*	.477**
	E	.459**	.396*	.438**	.366*	.573**
				Variable P4		
G1	A	.535**	.490**	.568**	.535**	.388*
	B	.215	.619**	.482**	.362*	.100
	C	.517**	.588**	.816**	.629**	.490**
	D	.418**	.373*	.488**	.450**	.390*
	E	.448**	.309	.450**	.394*	.442**
				Variable P5		
G1	A	.483**	.244	.465**	.324	−.031
	B	.103	.136	.333*	.174	−.035
	C	.374*	.216	.753**	.325	.128
	D	.379*	.218	.501**	.180	.076
	E	.218	.100	.382*	.093	.035
				Variable E1		
G3	A	.039	−.282	−.101	−.439**	−.358*
	B	−.318	−.015	−.248	−.209	−.159
	C	.094	−.103	.160	−.113	.087
	D	−.126	−.175	−.202	−.308	−.277
	E	−.313	−.353*	−.444**	−.543**	−.397*
				Variable P3		
G3	A	.022	−.078	−.281	−.191	−.250
	B	−.287	−.268	−.329	−.251	−.075
	C	.154	.140	−.047	.104	−.046
	D	−.041	−.178	−.122	−.231	−.216
	E	−.363*	−.339*	−.507**	−.360*	−.258

TABLE 9-4. *Continued*

Behaviour variable/ Colony		Colony				
		A	B	C	D	E
				Variable P4		
G3	A	-.038	-.224	-.264	-.192	-.107
	B	-.300	-.341	-.252	-.355*	-.072
	C	.173	-.025	.013	.071	.202
	D	-.093	-.223	-.190	-.278	-.198
	E	-.435**	-.439**	-.508	-.459**	-.195
				Variable P5		
G3	A	.119	.034	-.204	-.102	.156
	B	-.278	-.028	-.332	-.344*	.074
	C	.131	.108	-.080	.066	.231
	D	-.030	-.071	-.229	-.130	.060
	E	-.239	-.197	-.360*	-.251	.284
				Variable P3		
E1	A	.772**	.407*	.641**	.399*	.435**
	B	.354*	.422**	.435**	.311	.370*
	C	.688**	.501**	.774**	.561**	.436**
	D	.494**	.410*	.650**	.673**	.605**
	E	.488**	.299	.625**	.479**	.594**
				Variable P4		
E1	A	.782**	.468**	.663**	.598**	.611**
	B	.311	.649**	.563**	.443**	.359*
	C	.703**	.515**	.820**	.729**	.574**
	D	.529**	.600**	.669**	.755**	.539**
	E	.528**	.504**	.660**	.618**	.635**
				Variable P5		
E1	A	.622**	.293	.682**	.239	.225
	B	.122	.240	.448**	.126	.225
	C	.485**	.332	.809**	.316	.051
	D	.410*	.316	.584**	.301	.095
	E	.293	.238	.570**	.312	.220
				Variable P4		
P3	A	.888**	.394*	.639**	.654**	.597**
	B	.554**	.722**	.553**	.543**	.322
	C	.665**	.509**	.891**	.722**	.491**
	D	.500**	.541**	.575**	.857**	.557**
	E	.379*	.267	.405*	.467**	.551**
				Variable P5		
P3	A	.734**	.355*	.643**	.279	.092
	B	.383*	.341*	.523**	.335*	.086
	C	.481**	.287	.849**	.381*	.180
	D	.406*	.317	.550**	.602**	.199
	E	.234	.152	.399*	.063	.121
				Variable P5		
P4	A	.803**	.483**	.681**	.362*	.006
	B	.361*	.351*	.484**	.496**	.092
	C	.469**	.315	.907**	.366*	.177
	D	.555**	.358*	.706**	.643**	.187
	E	.421**	.123	.486**	.209	.438**

The coefficients are not significantly different from zero unless marked * (P <0.01) or ** (P <0.001).

inter-colony correlations between P5 and P3, and even more between P5 and P4. In the measurement routine, P5 was recorded directly after P4, and was a measurement of the same kind of behaviour. The high inter-colony correlations involving colonies that responded minimally in P5 indicate that this variable was in these cases very similar to P4. The fewer high inter-colony correlations between P5-P3 were indicative of a lessening of similarity between P5-P3 over P5-P4, with even less similarity between P5 and E1. Thus when colony responsiveness in P5 was sufficiently high, this variable was very similar to the group E1-P3-P4 but, in the less responsive colonies, a decline in affinities between P5 and P4-P3-E1 was apparent. This situation was reflected in the dendrogram for these correlations (for all variables of all colonies together) (Fig. 9-5) in which AP5 and CP5 clustered closely with E1-P3-P4 of their respective colonies, but BP5, DP5 and EP5 were not included in E1-P3-P4 of their colonies.

The high correlations of G1 with E1, P3 and P4 in almost every inter-colonial pairing (Table 9-4) indicated a high degree of similarity between these variables.

Associations between P1, P2, S1 and other CDB variables in colony C

Substantial responses in P1, P2 and S1 were recorded only in colony C, the most highly responsive colony in the experiment (section 9.2). The close clustering in colony C of P2 with E1, P3, P4 and P5 (Fig. 9-9) indicated P2 as very similar to these measurements, whereas P1 was on the periphery of this clustering and S1 well removed from it. P1 was in fact highly correlated with all the variables in colony C except S1 and G3 (Table 9-5). S1 was significantly correlated with E1, P4 and P5 at $P < .001$, and with P2, G1 and P3 at $P < .01$ (Table 9-5). These correlations were, however, without exception substantially lower than those between P1 and E1-P3-P4-

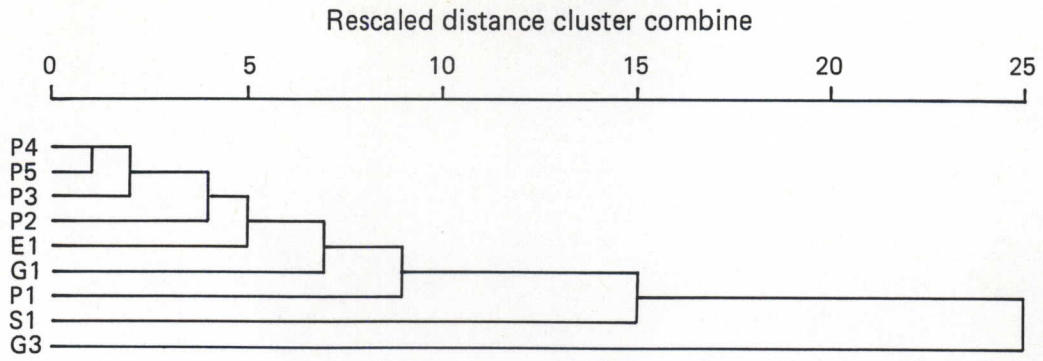


FIGURE 9-9. Dendrogram from cluster analysis of Pearson's *r* between nine CDB variables in colony C, including P1, P2 and S1. Variables are defined in the text.

TABLE 9-5. Correlation matrix of nine CDB variables in colony C, including P1, P2 and S1 (Pearson coefficients; $n = 59$). Variables are defined in the text.

	P1	P2	G1	G3	E1	P3	P4	S1
P2	.814**							
G1	.638**	.763**						
G3	−.086	−.136	−.260					
E1	.678**	.796**	.638**	.160				
P3	.664**	.818**	.759**	−.047	.774**			
P4	.663**	.808**	.816**	.013	.820**	.891**		
S1	.296	.360*	.380*	.302	.463**	.397*	.512**	
P5	.705**	.856**	.753**	−.080	.809**	.849**	.907**	.459**

The coefficients are not significantly different from zero unless marked *($P < 0.01$) or **($P < 0.001$).

P5, and P2 and E1-P3-P4-P5, so that in general S1 appeared to be on the borderline as a reliable CDB measurement in colony C (*assuming* that E1-P3-P4 are generally reliable measurements (and that P5 was a reliable measurement in colony C), as concluded in section 9.5).

Associations between forager flight rate and CDB variables

The flight rate in the undisturbed colony (i.e. variable F1: section 9.1.3) was the only behaviour variable recorded that was known not to be a colony defence response.

Dendrograms between the CDB variables G1, G3, E1, P3, P4, P5, and F1 (Figs. 9-10; 9-11), showed that F1 was not closely associated with the main clustering of "good" CDB variables E1-P3-P4, except in colony B. Nevertheless, in each colony F1 was significantly correlated with all the "good" CDB variables (Table 9-6), except in one instance, between F1 and P5 in colony E. F1 was uncorrelated with G3 in colonies A-D, and significantly negatively with it in colony E (Table 9-6), which again emphasizes the difference of G3 among the variables investigated, and points to G3 as likely to be a confounded or meaningless measurement of CDB. Thus, although the "good" CDB measurements were more highly correlated amongst themselves, flight rate was also well correlated with them, so that it is likely that the intensities of the two behaviours were influenced by largely the same factors (*see* section 9.5).

9.2.4 Conclusion

Given the inherent variability of CDB, the many factors that may affect it (chapter 8), and the difficulty in quantifying mass actions of honeybees, the fact that high correlations were obtained between behaviour variables

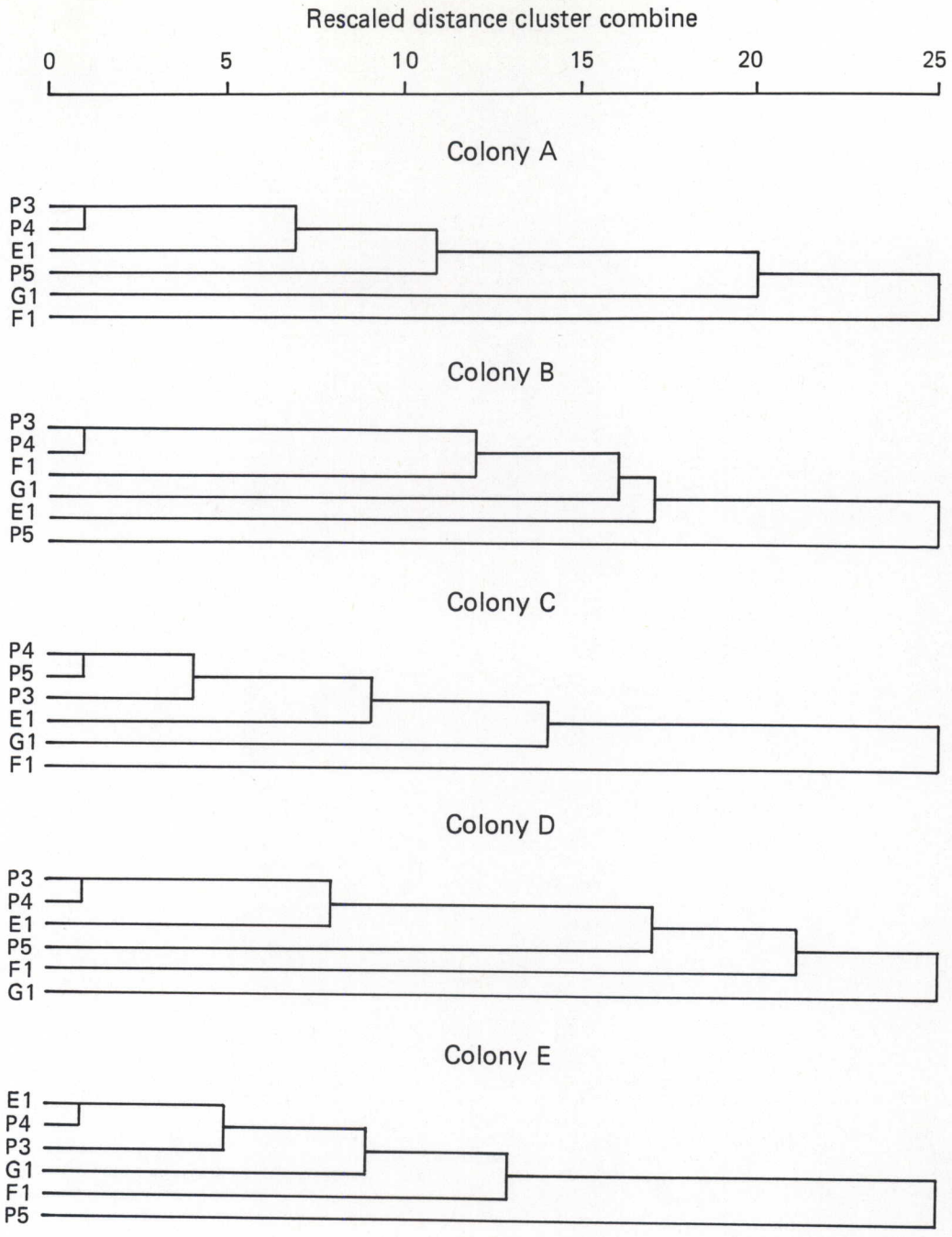


FIGURE 9-10. Dendrogram from cluster analysis of Pearson's *r* between flight rate (F1) and five CDB variables in colonies A–E. Variables are defined in the text.

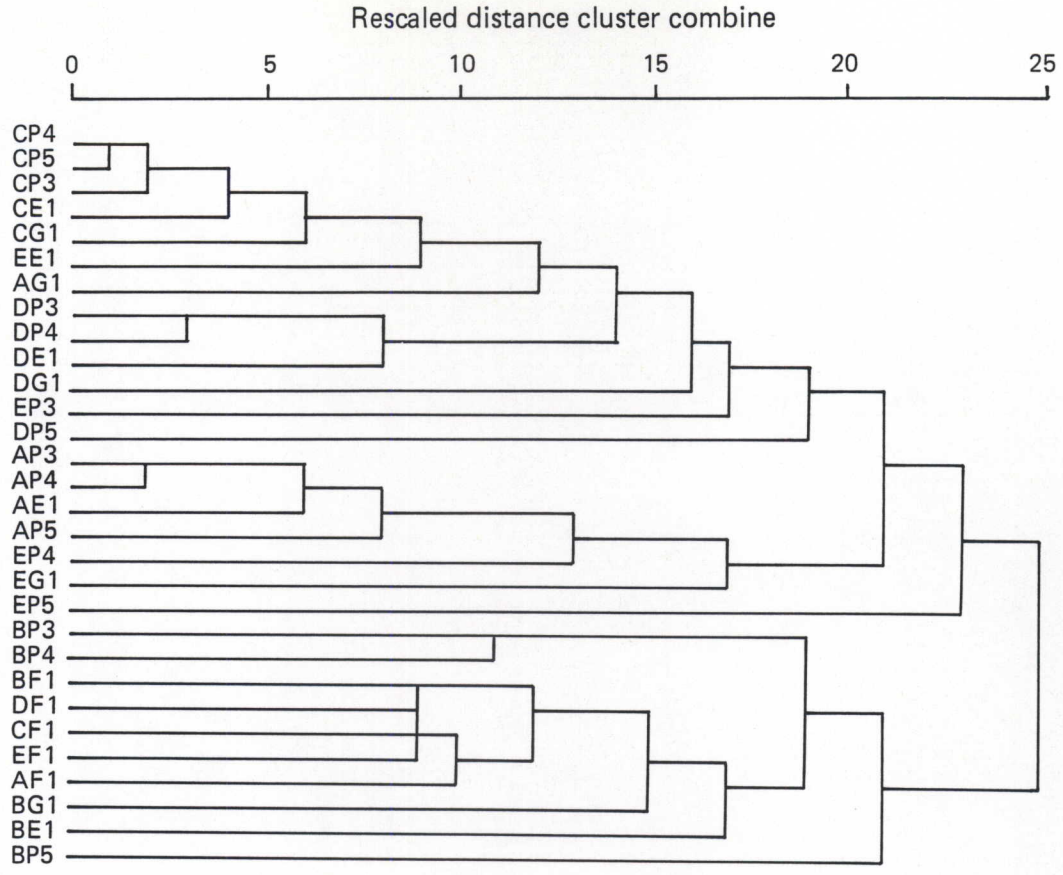


FIGURE 9-11. Dendrogram from cluster analysis of Pearson's r between flight rate (F1) and five CDB variables in colonies A–E. Variables are defined in the text.

TABLE 9-6. Correlation matrix of flight rate (F1) and six CDB variables in colonies A--E (Pearson coefficients, $n = 59$). Variables are defined in the text.

Flight rate/ Colony		Behaviour variables					
		G1	G3	E1	P3	P4	P5
F1	A	.496**	−.256	.457**	.475**	.506**	.437**
	B	.582**	−.218	.469**	.475**	.602**	.372*
	C	.622**	−.131	.494**	.483**	.518**	.438*
	D	.411*	−.072	.520**	.387*	.548**	.448*
	E	.443**	−.606**	.629**	.378*	.375*	.028

The coefficients are not significantly different from zero unless marked * ($P < 0.01$) or ** ($P < 0.001$).

of the breath test in colonies measured daily over a two-month period is in itself a useful advance, and provides a heuristic alternative to the other CDB analysis methods reviewed in section 8.5. Although the associations between the CDB variables of the five experimental colonies were by no means all clear-cut, the present approach as an initial exploratory technique was encouraging, enabling as it did the identification of:

(i) the variables E1, P3 and P4 as likely to be good measurements of colony defence responsiveness in comparison to the other variables of the analysis;

(ii) P1, P2 and P5 as likely to be good measurements of CDB, but with a higher response threshold than E1, P3 and P4;

(iii) G2 as an unequivocally useless CDB measurement;

(iv) G3 as very different from the other variables, and likely to be a poor measurement of colony defence responsiveness;

(v) G1 as a potentially useful indicator of the "state of defence responsiveness" of each colony but somewhat different from E1-P3-P4, so that its relation to CDB requires further investigation.

(vi) S1 as likely to be an unreliable CDB measurement.

In terms of the criterion that good CDB measurements should be well correlated over repeated measurements (section 9.1.1), the consistent close association between the two different measurement types E1 and P3-P4 indicates these as likely to be the best measurements of CDB in the array of measurements taken. However, at this stage the possibility is not altogether discounted that another, distinctly different, measurement (G3 or possibly G1) could be the good measurement of CDB, and all the others meaningless. Thus evidence from examination of other, allied hypotheses must be assessed in conjunction with the above analysis (*see* below).

9.3 RANKING OF COLONY RESPONSIVENESS TO THE BREATH TEST

9.3.1 Introduction

If the defensive responsiveness of a set of normal (section 3.2.1) colonies is measured more or less simultaneously, and distinct differences are obtained between some of them, it is hypothesized that a good CDB measurement method will reflect these differences when it is applied again (provided that the colonies have been given sufficient time to settle down); consistent inter-colonial rankings in defensive responsiveness will not be obtained by poor CDB measurement methods (section 9.1.1; Moritz *et al.*, 1985).

9.3.2 Methods and materials

The data from 59 daily breath tests on five colonies (A-E) (sections 9.1.3; 9.2.2) were analysed by comparing the average intensity (arithmetic mean) of the six variables G1, G2, E1, P3, P4 and P5 by anova (oneway analysis of variance, or Model I anova: Snedecor and Cochran, 1967), and by seven multiple comparison procedures (MCP) (Norusis, 1983; see also Bliss, 1967; Snedecor and Cochran, 1967; Sokal and Rohlf, 1981). To accommodate the assumptions of anova (Sokal and Rohlf, 1981, p.400) each variable was analyzed in its nearest to normal distribution. Transformations that bring normality generally also improve additivity and reduce variance heterogeneity (Southwood, 1978, p.13; Sokal and Rohlf, 1981, p.418), as shown by three tests for homogeneity of variance (Table 9-7): no variable had significantly homogeneous variance in all three tests, although there was near-homogeneity in G1, E1, P3 and P4, as reflected by the max/min variance ratio test. The problem of variance heterogeneity is lessened in

TABLE 9-7. Three tests for homogeneity of variance in six CDB variables in colonies A–E. Variables are defined in the text.

Variable	Test		
	Cochran's C	Bartlett-Box F	Max/Min Variance
G1	.810‡	199.2‡	1 521.7‡
G1§	.347†	10.5†	3.9†
G3	.970‡	167.4‡	157.6‡
G3§	.940‡	142.8‡	96.2‡
E1	.246†	3.2†	2.4†
P3	.995‡	280.1‡	1 359.7‡
P3§	.527‡	17.0†	5.3†
P4	.997‡	351.5‡	12 625.4‡
P4§	.461†	20.6†	7.5†
P5	.993‡	334.0‡	17 307.8‡
P5§	.470†	34.0†	17.3†

‡Variable highly heterogeneous. †Variable slightly or moderately heterogeneous. §Variables in logarithmic transformation.

In Max/Min variance ratio $F_{max\ 0.05}$ (5.58) = 2.04, above which variances are significantly heterogeneous (Sokal and Rohlf, 1981, p. 405). In Cochran's C and Bartlett-Box F tests, the larger the value, the higher the heterogeneity (Norusis, 1983, p. 113).

anovas with equal sized samples in all variables (Glass *et al.*, 1972; Hays, 1973; Nie *et al.*, 1976) so that the above-named variables were considered admissible to anova. The more deviant variables G3 and P5 were also analysed by anova and, as a check, by Kruskal-Wallis "anova", a distribution-free method (Sokal and Rohlf, 1981, p.429).

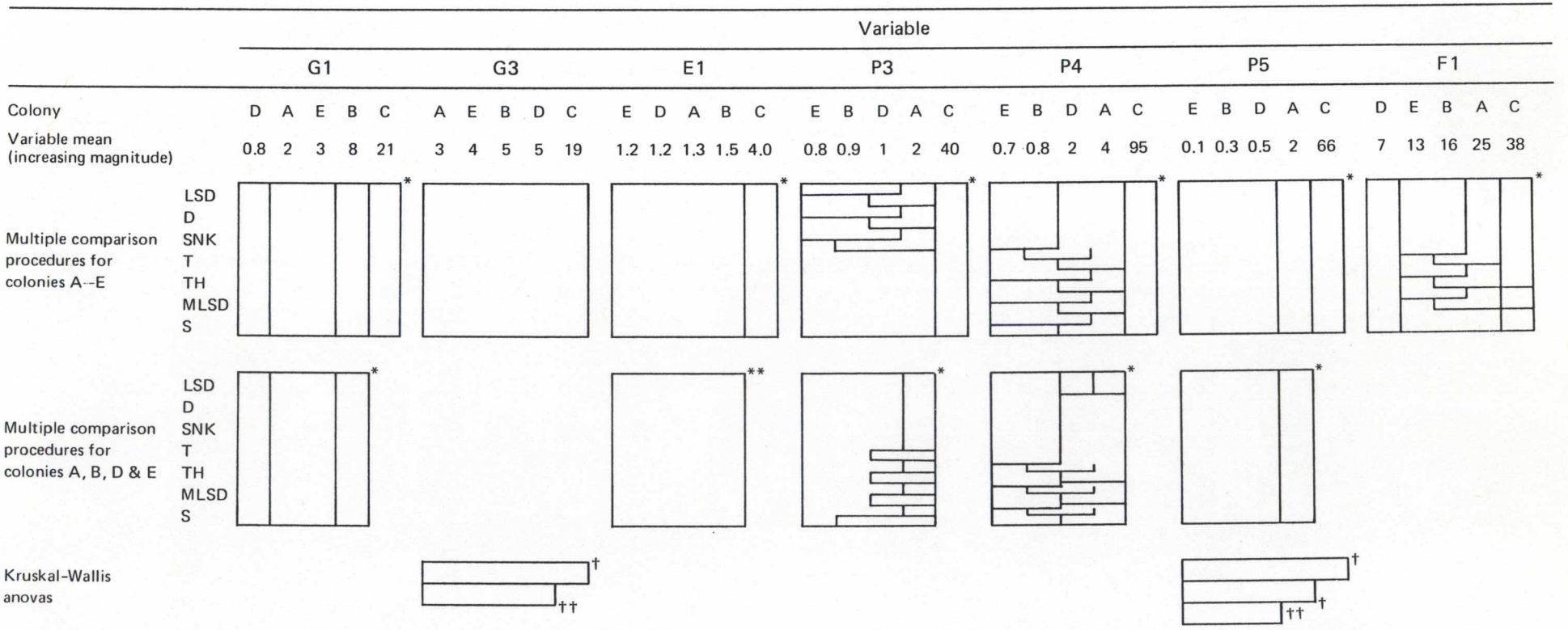
At the end of the series of 59 CDB measurements the following internal-colony factors were measured in each experimental colony: number of framesides covered by bees; number of cells with honey, pollen or brood.

9.3.3 Results and discussion

F-tests indicated significant differences between the means of the five experimental colonies in all variables except G3 (Table 9-8). The magnitudes of the natural means of the other five CDB variables showed that colony C was much more responsive than the other four colonies, which were similar in all variables, except colony D in G1. This was confirmed in every variable except G3 by the application of seven kinds of MCP to the set of five colonies (Table 9-8). However, the Kruskal-Wallis analysis for G3 also indicated that colony C was significantly different (Table 9-8). Because G3 had fairly severe heterogeneity of variance, the non-parametric Kruskal-Wallis test was more likely to be valid.

G1 was the only variable in which all MCP's indicated a colony (colony D) with a mean significantly *smaller* than the means of the other four colonies, which rendered G1 the most different variable in the comparison-of-means analysis. The means of colonies A, B, D and E, as reflected by variables E1, P3, P4 and P5, were of roughly similar magnitudes, which explains why each variable ranked the colonies somewhat differently, both when the influential colony C was included or was dropped from the anovas (Table 9-8).

TABLE 9-8. Anovas and multiple comparisons of means of CDB and flight-rate variables in colonies A–E (n = 59). Variables are defined in the text.



* F test indicates significant difference between means ($F_{0.01}$ 5,58).

† Kruskal-Wallis anova indicates significant difference between means.
†† Kruskal-Wallis anova indicates no significant difference between means.

Vertical lines in the multiple comparison blocks represent discontinuities between subsets of groups identified, whose highest and lowest means do not differ by more than the shortest significant range for a subset of that size. Half-space vertical lines indicate overlapping subsets. The multiple comparison procedures are listed in order of decreasing power (Nie *et al.*, 1975), from LSD which requires the least difference between means to show significant differences, to Scheffe's test, the most conservative, which requires the largest difference between means to show a significant difference (Norusis, 1983). LSD = Least Significant Difference method; D = Duncan's multiple range test; SNK = Student-Neuman-Keuls test; T = Tukey's alternate procedure; TH = Tukey's honestly significant difference; MLSD = Modified LSD; S = Scheffe's test (Nie *et al.*, 1975).

Thus, from a set of randomly chosen colonies from a single population of honeybees, 59 daily applications of the breath test identified four colonies of similar (mild) temperament, with no substantial differences between them in variables G3, E1, P3 and P5, and one (colony C) of significantly fiercer temperament. This arrangement in colony temperaments was not particularly suitable for the examination of the criterion for good CDB measurements stated at the outset. More informative would have been a set of colonies of several *chosen* grades of temperament, or a set comprising strains of known disposition, mild and fierce, such as used by Rothenbuhler (1964b; *and see* section 8.5.1).

In the experimental colonies, differences in CDB intensity were likely to have been associated with internal-colony and/or genetic factors, assuming that (i) differences from environmental effects were negated by having the colonies in one area, and by measuring them all within 35 min at the same time each day; (ii) that the breath stimulus used was a constant and relevant danger stimulus; and (iii) that the behaviour measurements taken were sound reflections of the CDB intensity of the colonies. The five colonies used were too few to allow decisive analysis of the effect of the internal-colony factors that were measured, but limited insight may be gained by comparing them with the ranking of CDB intensity in the colonies (Table 9-9). Colony C, the only highly responsive colony, had the most bees, honey stores and brood. But the possibility that the first of these factors was unequivocally connected with high defensive responsiveness was reduced by the almost equally high number of bees in colony B, a low responding colony. The amount of honey stored in colony C was not much higher than in some other colonies, and colony B had more pollen stores. The quantity of brood in colony C was, however, much greater than in the other colonies. Thus the results indicate that the number of bees and amount of stores were unlikely to have been directly

TABLE 9-9. Defensive responsiveness[¶] of colonies A–E as indicated by five variables; foraging rates (F1)[¶]; quantities of bees, brood and stores in each colony. Variables are defined in the text.

	Colony				
	A	B	C	D	E
Variable					
E1, P3	–	–	+++	–	–
P4	+	–	+++	–	–
P5	+	–	+++	+	–
G1	+	++	+++	–	+
F1	++	+	+++	–	+
Number of framesides covered by bees	13 ++	19 +++	19 +++	5 +	8 +
Number of cells with brood (x 100)	6 –	18 +	123 ++++	13 +	48 ++
Number of framesides with honey	2 +	9 ++	14 +++	3 +	7 ++
Number of framesides with pollen	0.4 #	1.9 #	1.5 #	0.7 #	0.4 #

– very low
\+ low
++ medium
+++ high
++++ very high
\# equivalent

[¶] Relative defensive responsiveness and foraging rates abstracted from Table 9-8.

connected with CDB intensity, whereas amount of brood might have been more directly associated with it.

Comparison of the means of F1 (flight rate over 59 observation days) in the five experimental colonies by anova (Fig. 9-8) showed that colony C had the significantly highest mean, as it had in the CDB variables E1, P3, P4 and P5, and in G1 and G3. Colony A had the next highest mean, significantly lower than that of colony C, but somewhat higher than the others, as also in AP3 and AP4. F1 was similar to G1 in that the mean for colony D was significantly *smaller* than the means for the other four colonies. Thus the colonies with the highest and lowest foraging rates over the 59 observation days also had, on average, the highest and lowest number of guards and it may be that the number of bees at the entrance was highly dependent on the flight rate rather than on the defensive proclivities of the colonies. Flight rate was not associated with the number of bees in the colonies measured since it was significantly lower in colony B than in colony C, which had roughly equal numbers of bees (Table 9-9).

9.4 ASSOCIATIONS BETWEEN SOME WEATHER MEASUREMENTS AND COLONY RESPONSIVENESS TO THE BREATH TEST

9.4.1 Introduction

Honeybee colonies are generally fiercer in hotter climatic conditions (section 8.4.3). Southwick and Moritz (1987) (*see* section 8.4.3) demonstrated that variation in defensive intensity in colonies measured intermittently over a three month period was strongly associated with variation in ambient temperature. Other experiments have, however, produced uneven results for the effect of weather on CDB: the use of

confounded measurement methods may have contributed to this (section 8.4.3). If temperature has a significant effect on CDB intensity it should be highly correlated with good CDB measurements administered repeatedly, whereas poor CDB measurements will be poorly correlated. This relationship should obtain with any other weather factors that affect CDB (*see* section 9.1.1).

The assessment of the effects of weather on CDB variability is a complex problem, with two main aspects: (i) to separate the contribution of weather factors from amongst the many other factors that affect CDB variability, reviewed in chapter 8; (ii) to separate the effects of various weather components apon CDB, both from one another and from the effects of the weather as a whole.

Weather variables may have complicated relationships with one another, or are often well correlated, so that measurements under a variety of conditions will be necessary to isolate the effective variables. For example, in the case of humidity, cold air can hold proportionately less water vapour than warmer air, so that the relationship between temperature and humidity (in terms of saturated vapour pressure) is exponential (Kaye and Laby, 1959; Ahrens, 1985). Humidity measurements are thus much affected by temperature: e.g. at 10°C and 19.7% relative humidity the saturation deficit is 7.4 mm, whereas at 20°C, 7.4 mm saturation deficit is reached at 57.7% relative humidity (Waterhouse and Amos, 1968, p.36). In an insect that detects humidity by sensing evaporation rate through changes in temperature, chemical composition, osmotic pressure, or mechanical stresses in the receptors during evaporation, saturation deficit would be more appropriate as a measurement of the evaporating power of the air than relative humidity. On the other hand, relative humidity would be a more appropriate measurement in an animal with humidity receptors that function in the same way as olfactory receptors (Pielou, 1940; Dethier, 1963). The

responses of many insects to humidity are better explained by saturation deficit than by relative humidity (Lees, 1943) although in some the opposite has been found (Pielou, 1940). Little appears to be known about the honeybee worker's sense of atmospheric humidity other than that, inside the hive, it is acute enough to detect small changes (of as low as 5%) in relative humidity (Kiechle, 1961). However, there appears to be little control of humidity within the hive, although high humidities may influence fanning (Simpson, 1961). Foragers smell liquid water with their antennae (Ribbands, 1955; Kuwabara and Takeda, 1956), but this need not be the sense by which they register atmospheric humidity. In recent studies of nest homeostasis, including thermoregulation and honey ripening in which worker's humidity sense must be important, humidity is scarcely mentioned (see e.g. Keinrich, 1981, 1985; Seeley and Heinrich, 1981; Kronenberg and Heller, 1982; Seeley, 1985; Winston, 1987; Southwick, 1988). The effects of humidity on CDB variation is by no means as clear as that of temperature. Humidity may affect the evaporation rate of alarm pheromones (section 8.4.2) or, in conjunction with temperature, it could affect the responsiveness of the defenders themselves (section 8.4.3). Where weather and defensive responsiveness were rigorously measured, humidity was not important in response variation (Collins, 1981; Southwick and Moritz, 1987). In South America, less rigorous measurements, and beekeepers opinions, have claimed higher CDB in hot, humid conditions (Brandenburgo *et al.*, 1977, 1982; Michener, 1975).

The present study examines associations between responsiveness to the breath test for CDB, and ambient temperature, humidity, wind strength and wind direction.

9.4.2 Methods and materials

The data from 45 of the 59 daily breath tests on five colonies (A-E) (sections 9.1.3; 9.2.2) were compared by correlation analyses with the ambient weather variables measured at the time of each test (humidity data were lost for 14 of the 59 observation days). The distributions of the variables were scrutinized and transformed where necessary, as described in section 9.2.2. Kendall coefficients were calculated between variables that violated the requirements for use in Pearson correlations. The weather factors recorded were:

T — *Atmospheric temperature* in shade (°C) (measured directly).

RH — *Relative humidity* (%) (measured directly by aspirated psychrometer).

VP — *Vapour pressure*, calculated from RH and T by the relationship

$$VP = \frac{RH}{100} \times SVP \text{ at } T$$

where SVP (*saturated vapour pressure*) is a constant at a particular T (Weast, 1978; Pearce and Smith, 1983; Ahrens, 1985; Houghton, 1985).

SD — *Saturation deficit*, calculated from VP and T by the relationship SD = SVP - VP at T

where SVP is a constant at a particular T (Houghton, 1985).

HT — *"Humiture"*, a "comfort index" for humans representing the combined effects of temperature and humidity, calculated from the relationship

HT = T + VP - 10

where VP is in hectopascals and T is in °F (this expression pers. comm. O.S. McGee, Dept. Geography, University of Natal; see also Ahrens, 1985; Houghton, 1985).

W *Prevailing wind strength*

1 = none

2 = light

3 = moderate

4 = high

5 = gusty

D *Wind direction*

1 = none

2 = North

3 = NE

4 = East

5 = SE

6 = South

7 = SW

8 = West

9 = NW

10 = Variable

9.4.3 Results and discussion

Bearing in mind that the data were the product of 45 midday measurement sessions taken over a period of about 60 days, during which there was a considerable variation in weather conditions (range of temperature = 9-30°C; range of relative humidity = 9-93%: *see* Appendix Table 9-1), there were many surprisingly high correlations between weather and behaviour variables, particularly with temperature, humiture, saturation deficit and relative humidity (Table 9-10). However, temperature, relative humidity and saturation deficit were highly correlated with one another (Table 9-11), which raised the problem (mentioned in section 9.4.2) of the extent to

TABLE 9-10. Correlation matrices of six CDB variables and seven weather variables in colonies A–E ($n = 45$). Variables are defined in the text.

Behaviour variable/ Colony		Weather variable						
		T†	HT†	SD†	RH†	VP†	W‡	D‡
G1	A	.595**	.482**	.561**	−.502**	−.164	−.187	−.069
	B	.683**	.749**	.417*	−.321	.245	−.028	.112
	C	.713**	.678**	.519**	−.476**	.025	−.006	.044
	D	.518**	.428*	.503**	−.371	−.126	−.005	−.050
	E	.440*	.254	.526**	−.545**	−.346	.128	−.141
G3	A	−.542**	−.478**	−.453*	.439*	.062	.134	.055
	B	−.202	−.088	−.303	.257	.223	−.058	−.145
	C	−.079	−.077	−.040	.050	−.006	−.076	.016
	D	−.158	.029	−.370	.347	.389*	.033	.183
	E	−.675**	−.550**	−.603**	.609**	.177	.049	−.085
E1	A	.438*	.344	.434*	−.342	−.146	−.096	−.026
	B	.744**	.734**	.571**	−.392*	.085	−.105	−.122
	C	.624**	.525**	.584**	−.437*	−.129	−.055	−.041
	D	.776**	.702**	.663**	−.485**	−.051	.036	.095
	E	.674**	.553**	.635**	−.515**	−.166	−.089	−.204
P3	A	.431*	.294	.470*	−.406*	−.241	−.079	.232
	B	.527**	.503**	.450*	−.328	.023	−.197	−.044
	C	.688**	.556**	.638**	−.570**	−.192	−.125	−.017
	D	.550**	.460*	.522**	−.384*	−.119	.043	−.022
	E	.456*	.366	.466*	−.326	−.132	.032	−.072
P4	A	.444*	.324	.454*	−.396*	−.201	−.217	.150
	B	.717**	.720**	.556**	−.372	.111	−.101	−.011
	C	.786**	.664**	.696**	−.627**	−.156	−.094	−.005
	D	.646**	.497**	.651**	−.523**	−.236	−.002	.114
	E	.434*	.328	.444*	−.346	−.172	.051	−.019
P5	A	.238	.115	.301	−.294	−.237	−.100	.265
	B	.160	.221	.058	.026	.156	−.349	−.305
	C	.616**	.501**	.570**	−.505**	−.165	−.112	−.046
	D	.223	.112	.278	−.276	−.212	−.048	−.211
	E	−.183	−.121	−.215	.197	.109	.052	−.036

† Pearson coefficients

‡ Kendall coefficients

The coefficients are not significantly different from zero unless marked * ($P < 0.01$) or ** ($P < 0.001$).

TABLE 9-11. Correlation matrices of weather variables (n = 45). Variables are defined in the text.

Weather variable	Weather variable					
	T	HT	RH	SD	VP	W
			Pearson coefficients			
HT	.892**					
RH	−.692**	−.310				
SD	.863**	.549**	−.911**			
VP	−.093	.367	.744**	−.567**		
			Kendall coefficients			
HT	.728**					
RH	−.509**	−.200				
SD	.728**	.415**	−.790**			
VP	−.171	.128	.680**	−.458**		
W	.095	.006	−.186	.176	−.186	
D	.027	.010	−.124	.096	−.094	.536**

The coefficients are not significantly different from zero unless marked * (P <0.01) or ** (P <0.001).

which temperature and humidity *each* contributed to the variation in the behaviour variables. When the effects of temperature were held constant in a partial correlation analysis (Sokal and Rohlf, 1981, p.656), it was evident that temperature could account for most of the high correlations between the weather variables and the behaviour variables (Table 9-12). Conversely, of course, the partial correlations produced when saturation deficit was held constant indicated no role for temperature in the variation in the CDB variables (analysis not tabulated). There is no set statistical method that will indicate unequivocally the direction of causation in situations such as this, and common sense or experiential knowledge about the relationships must be used (see e.g. Nie *et al.*, 1975, p.384; Sokal and Rohlf, 1981, p.592f). Comparison of the zero order (bivariate) correlation coefficients of Table 9-10 with the first order (partial) correlation coefficients of Table 9-12 shows that the low associations between vapour pressure and the behaviour variables were largely unaffected when temperature was controlled. Vapour pressure was *not* correlated with temperature (Table 9-11), whereas relative humidity and saturation deficit were highly correlated with it. However, vapour pressure, relative humidity and saturation deficit were significantly correlated with one another (Table 9-11) (as expected from their arithmetic relationships with one another: section 9.4.2). If humidity itself had anything to do with the high correlations between the CDB variables and relative humidity and saturation deficit, then higher correlations would be expected between the behaviour and vapour pressure. As it is, the high correlations of the behaviour with relative humidity and with saturation deficit are better explained as owing to the significant influence of temperature on these humidity expressions. This argument is clarified in the path diagram (see e.g. Sokal and Rohlf, 1981, p.645) of these relationships as causative pathways (Fig. 9-12) which shows that if a

TABLE 9-12. Matrices of first order partial correlation coefficients between weather variables and six CDB variables in five colonies, controlling for temperature (n = 45). Variables are defined in the text.

Behaviour variable/ Colony		Weather variable					
		HT	SD	RH	VP	W	D
G1	A	-.135	.116	-.155	-.135	-.326	-.170
	B	.424*	-.467*	.288	.424*	-.155	.050
	C	.131	-.273	.034	.131	-.134	-.052
	D	-.091	.129	-.020	-.091	-.081	-.127
	E	-.341	.322	-.370	-.341	.082	-.213
G3	A	.014	.034	.106	.014	.240	.138
	B	.209	-.259	.166	.209	-.034	-.126
	C	-.014	.056	-.007	-.014	-.067	.025
	D	.381	-.468*	.333	.381	.053	.205
	E	.156	-.057	.267	.156	.180	-.014
E1	A	-.117	.122	-.059	-.117	-.168	-.084
	B	.232	-.212	.255	.232	-.297	-.310
	C	-.091	.115	-.009	-.091	-.170	-.143
	D	.034	-.021	.114	.034	-.096	.014
	E	-.141	.141	-.090	-.141	-.234	-.380
P3	A	-.223	.214	-.164	-.223	-.147	.205
	B	.085	-.013	.061	.085	-.311	-.122
	C	-.177	.121	-.179	-.177	-.292	-.130
	D	-.081	.112	-.006	-.081	-.029	-.101
	E	-.102	.160	-.016	-.102	-.027	-.139
P4	A	-.179	.157	-.137	-.179	-.305	.112
	B	.256	-.178	.248	.256	-.273	-.131
	C	-.135	.055	-.186	-.135	-.311	-.151
	D	-.231	.243	-.137	-.231	-.107	.056
	E	-.146	.153	-.070	-.146	-.003	-.075
P5	A	-.222	.195	-.184	-.222	-.134	.247
	B	.174	-.162	.192	.174	-.377	-.329
	C	-.137	.096	-.138	-.137	-.240	-.147
	D	-.197	.172	-.173	-.197	-.077	-.244
	E	.094	-.115	.099	.094	.076	-.016

The coefficients are not significantly different from zero unless marked * (P <0.01).

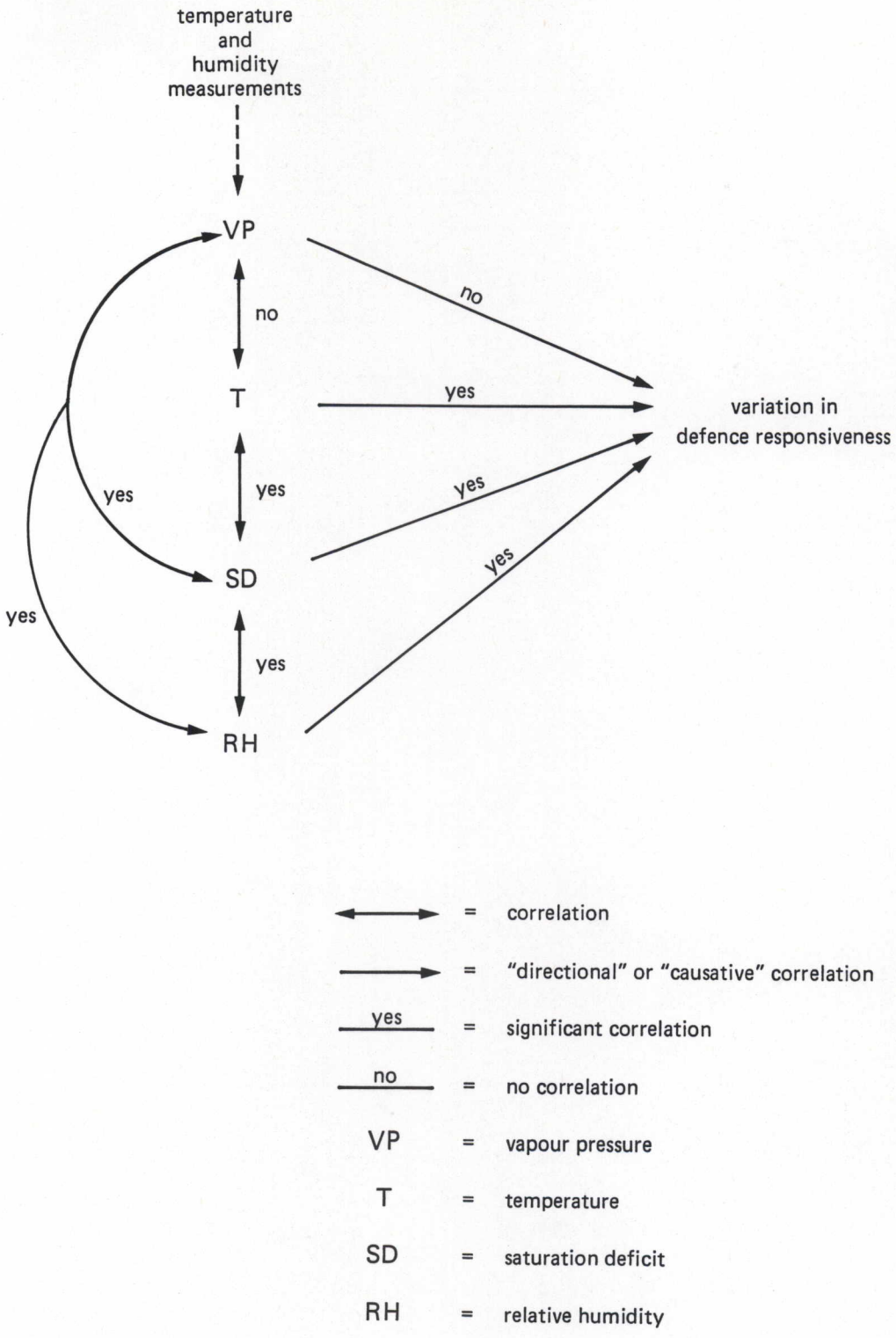


FIGURE 9-12. Path diagram of the significant and non-significant first order correlations between three humidity measurements and temperature, and their "causative" correlations with variation in defence responsiveness.

behaviour variable *was* correlated, independently of temperature, with any one humidity measurement, the cross-correlations between the three humidity measurements would have ensured high correlations between behaviour variables and the other two humidity measurements, independently of temperature.

The lack of influence of humidity in the present series of observations is also reflected in the way in which most of the high zero order correlations between humiture and behaviour (Table 9-10) were strongly reduced in the partial coefficients when temperature was controlled (Table 9-12). It is emphasized that the conclusion for a minimal influence of humidity on CDB must be restricted to the weather regime that obtained during the observations, in which humidity was closely and inversely correlated with temperature.

Wind direction and wind strength were measured on an ordinal scale so that their use in Pearson correlation coefficients of association with behaviour variables (Table 9-10) was not entirely appropriate (Labowitz, 1972; Nie *et al.*, 1975); Kendall's *tau* coefficients of the bivariate relations between these variables and the behaviour variables were calculated (Table 9-10) as a check (section 9.2.2). Both sets of coefficients indicated no significant correlations between wind direction or wind strength with any behaviour variable other than P5 (negative correlations indicated by *tau*). It is concluded that the direction of the wind and its strength did not have any effect on the variation of CDB over the observation period.

Thus temperature was the only weather variable among those recorded that had a significant effect on the variation of the behaviour variables. Temperature was significantly and positively correlated with the behaviour variables G1, E1, P3 and P4 in every colony, whereas in P5 there was only one significant correlation, in colony C (Table 9-10). In G3 there were no

significant correlations with temperature in colonies B, C and D. The significant correlations with temperature in colonies A and E were *negative*, meaning that, on the 45 observation days (over a 60-day period), fewer guards were recruited 3 min after the breath stimulus on warmer days than on cooler, whereas the opposite was indicated by the four other measurement types G1, E1, P3 and P4.

In terms of the criteria drawn in the introduction (9.4.1) for the association between CDB variation and effective weather factors, the CDB variables G1, E1, P3 and P4 stand as "good" variables while G3, with its inconstant nill and negative associations with temperature, stands revealed as an unreliable, or meaningless, CDB measurement. P5, with its inconstant association with temperature, stands as a generally unreliable indicator of CDB intensity: it did, however, provide a good measurement in colony C, the most highly responsive of the colonies (section 9.3).

The significant partial coefficients between BG1 and humiture, saturation deficit and vapour pressure, and between EG1 and relative humidity (Table 9-12), indicate that humidity did have a significant, positive effect on the number of guards at the entrance of the undisturbed colony B, whereas it may have had a somewhat negative effect on this behaviour in colony E. Although these results are equivocal, they may at a push be taken as a hint that G1 as a measurement was slightly different from the other three sound CDB measurements, E1, P3 and P4, which together had no significant first order association in any colony with any humidity measurement.

The coefficients of determination, (r^2) between temperature and the behaviour variables (Table 9-13) represent the percentage proportion in each CDB variable (the dependent variable) that is explained by temperature (the independent variable) in the linear relationship between each pairing (Nie *et al.*, 1975, p.327; Sokal and Rohlf, 1981, p.571). The highest

TABLE 9-13. Coefficients of determination (r^2) between temperature and CDB variables in colonies A–E (n = 45). Variables are defined in the text.

Behaviour variable/ Colony		r^2	Behaviour variable/ Colony		r^2
	A	.35**		A	.19*
	B	.47**		B	.28**
G1	C	.51**	P3	C	.47**
	D	.27**		D	.30**
	E	.19*		E	.21*
	A	−.29**		A	.20*
	B	−.04		B	.51**
G3	C	−.01	P4	C	.62**
	D	−.02		D	.42**
	E	−.45**		E	.19*
	A	.19*		A	.06
	B	.55**		B	.03
E1	C	.39**	P5	C	.34**
	D	.60**		D	.05
	E	.45**		E	−.03

The coefficients are not significantly different from zero unless marked * (P <0.01) or ** (P <0.001).

associations, in which more than 50% of the behaviour variation was explained by temperature variation, were r^2 = .62 in CP4, = .60 in DE1, = .55 in BE1, = .51 in CG1 and = .51 in CP4, so that in these instances temperature was a *very* important factor controlling the intensity of the defensive responses. From the general levels of association (Table 9-10), it is concluded that temperature was a highly significant factor in the average intensity of CDB, and the results roughly concur with that of r = .539 between temperature and defence responsiveness obtained by Southwick and Moritz (1987) from pooled measurements on 36 European colonies taken at weekly intervals over three months (they also found no correlation between relative humidity and defensiveness).

Associations between P1, P2, S1 and weather variables

Substantial responses in P1, P2 and S1 were recorded in colony C, the only highly responsive colony in the experiment (section 9.2).

Bivariate Pearson *r* coefficients (and Kendall *tau* coefficients, as a check) showed no relation between CS1 and any weather variable (Table 9-14), which detracts from its already dubious status as a CDB measurement, drawn in section 9.2. In contrast, CP1 and CP2 had significant correlations with temperature, relative humidity, and saturation deficit, but not with vapour pressure. In the first order partial correlation coefficients, controlling for temperature (Table 9-14), there were no significant correlations between CP1, CP2 and vapour pressure. Following the reasoning about the relationships between behaviour, temperature and humidity employed above for the other CDB variables of the analysis, it is clear that temperature was the only weather variable measured that had a significant effect on the intensity of CP1 and CP2 in the experiment. These significant correlations with temperature indicate that P1 and P2

TABLE 9-14. Correlation matrices of CDB variables CP1, CP2 and CS1 with weather variables (n = 45). Variables are defined in the text.

Behaviour variable	Weather variable						
	T	HT	SD	RH	VP	W	D
				Pearson coefficients			
CP1	.601**	.436*	.639**	−.529**	−.278	.014	.078
CP2	.669**	.554**	.620**	−.492**	−.158	.002	.048
CS1	.246	.222	.214	−.201	−.017	−.054	−.089
				Kendall coefficients			
CP1	.479**	.391**	.504**	−.404**	−.194	−.023	−.016
CP2	.464**	.401**	.426**	−.330*	−.116	−.038	−.036
CS1	.146	.050	.161	−.155	−.120	−.069	−.087
				First order partial correlation coefficients, controlling for T			
CP1		−.279	.297	−.195	−.279	−.075	.014
CP2		−.129	.112	−.053	−.129	−.109	−.036
CS1		.006	.004	−.044	.006	−.087	−.121

The coefficients are not significantly different from zero unless marked * (P <0.01) or ** (P <0.001).

were good measurements of CDB in the highly responsive colony C, but had a higher response threshold than variables such as E1, P2 or P3. P1 and P2 were thus similar to P5 (see section 9.2.3).

Associations between forager flight rate and weather variables

The flight rate in the undisturbed colony (i.e. variable F1: *see* section 9.1.3) was the only behaviour variable recorded that was known not to be a colony defence response.

F1 was in every colony significantly correlated with temperature, relative humidity, and saturation deficit, but not with vapour pressure (Table 9-15). In the first order partial correlation coefficients, controlling for temperature (Table 9-15), there were no significant correlations with vapour pressure. Following the reasoning about the relationships between behaviour and temperature and humidity employed above for the other CDB variables of the analysis, it is concluded that temperature was the ony weather variable measured that had a significant affect on the intensity of F1.

Inter-colony correlations for F1 were without exception high (Table 9-16), indicating the strong influence of common factors, an important one of which was identified above as ambient temperature. The influence of temperature on both F1 and the CDB variables (derived in section 9.2.3) provides a parsimonious explanation for the significant correlations that existed between them, the only other possible explanation in the present results being that F1 was somehow behaviourally connected with CDB. The distinction between F1 and the CDB variables as functionally unconnected behaviour types is intuitively reasonable.

TABLE 9-15. Correlation matrices of flight rate (F1) and weather variables in colonies A–E (n = 45). Variables are defined in the text.

Flight variable	Weather variables						
	T	HT	SD	RH	VP	W	D
			Pearson coefficients				
AF1	.503**	.361	.504**	−.533**	−.240	.005	−.024
BF1	.630**	.624**	.481**	−.357	.077	−.130	−.020
CF1	.691**	.696**	491**	−.418*	.109	−.193	−.114
DF1	.522**	.503**	.382*	−.329	.033	−.087	.038
EF1	.632**	.558**	519**	−.494**	−.073	−.236	−.158
			First order partial correlation coefficients, controlling T				
AF1		−.224	.160	−.296	−.224	−.067	−.093
BF1		.176	−.159	.142	.176	−.270	−.117
CF1		.241	−.288	.117	.241	−.388*	−.266
DF1		.097	−.159	.053	.097	−.178	−.024
EF1		−.018	−.067	−.101	−.018	−.408*	−.297

The coefficients are not significantly different from zero unless marked * (P <0,01) or ** (P <0.001).

TABLE 9-16. Correlation matrix of flight rate in colonies A–E (Pearson coefficients; n = 59).

	A	B	C	D
B	.641**			
C	.672**	.695**		
D	.641**	.757**	.664**	
E	.742**	.726**	.757**	.714**

** Coefficient significantly different from zero at P <0.01.

9.5 DISCUSSION: THE BREATH TEST AS A METHOD TO MEASURE CDB

The encouraging fact that high correlations were obtained between behaviour variables, both within and between colonies, over a two-month observation period, was discussed in section 9.2. Similarly encouraging were the high correlations between behaviour variables and temperature (section 9.4) and the regularity in differences in response intensity between colonies (section 9.3). Although each of these three analytical methods was itself limited in the information it could produce, combined evidence from the three methods (summarized in Table 9-17) allows stronger inferences about the relative worth of the measurements and about the behavioural bases of their interrelationships.

First, E1, P3 and P4 fitted all the criteria for good CDB measurements (section 9.1.1) to such a high degree that not only were they the best of the CDB measurements studied, but also appeared likely to be reliable CDB measurements in their own right. From a broader perspective, because the present study has *directly assessed* the empirical validity of CDB measurements, E1, P3 and P4 are among the few reasonably reliable indicators of intensity of honeybee colony defence behaviour at the present time (*see* critique of other CDB measurements in section 8.5).

Among the four variable types (G1, G3, P5, and E1-P3-P4) indicated by the cluster analyses of section 9.1, G3 was identified as the most different variable, in that it was not associated with the consistent clustering E1-P3-P4. The fact that G3 was not only different, but also very likely to be worthless as a CDB measurement, was reinforced by its non-significant correlations with ambient temperature (section 9.4). An important confounding factor in G3, similar to that identified by Collins *et al.* (1982) and Collins and Kubasek (1982) for guard recruitment in their CDB measurement routine, was the tendency for the entrance guards to fly

TABLE 9-17. Quality of CDB variables as indicated by three analysis methods in colonies A–E.

Variable	Method of analysis			
	Association between variables¶	Ranking of colonies†	Association with temperature‡	Conclusion
P1	Low responses in ABDE Good in C	Low responses in A–E, no analysis possible	Low responses in ABDE Good in C	High response threshold but probably Good
P2	Low responses in ABDE Good in C	Low responses in A–E, no analysis possible	Low responses in ABDE Good in C	High response threshold but probably Good
G1	Good	Different from E1 – P1 – P4	Good	Not a defense *response*
G2	Equivalent to G1 in ABDE, negatively correlated in C, ie. poor in C	Dropped from analysis	Dropped from analysis	Useless
G3	Most different variable	Similar to E1 – P3 – P4	Poor	Poor
E1	Highly correlated in all colonies. Good	Similar ranking of colonies. Good	Good	Good
P3			Good	Good
P4			Good	Good
S1	Low responses in ABDE Poor in C	Low responses in A–E, no analysis possible	Low responses in A–E, no analysis possible	Poor/useless
P5	Low responses in ABDE Good in C	Good	Low responses in ABDE Good in C	High response threshold but probably Good

¶ section 9.2 † section 9.3 ‡ section 9.4

when encountering a danger stimulus such as the breath test, which in some cases even resulted in *negative* correlations with ambient temperature when a variety of other CDB measurements (particularly E1-P3-P4) indicated a positive association.

Although G3 was clearly worthless as a measurement of CDB intensity, it nevertheless indicated differences in CDB levels (rankings) between colonies in the same way as the good variables (section 9.2). Perhaps the greater values for G3 in colony C were associated simply with the greater number of guards (G1) in that colony which, as argued below, need not be directly associated with CDB intensity. If this were the case, then, in studies of defensive differences between colonies (e.g. as by Boch and Rothenbuhler, 1974; Moritz *et al.*, 1985: *see also* 8.5.3; 8.5.6), measurements as useless as G3 might well register differences and so seem to be a valid indicator of CDB, whereas in fact such differences would be ancillary manifestations of a more general phenomenon associated with guard bees (and possibly other bees) at the hive entrance. Rankings of colony responsiveness are, on their own, inadequate to assess the worth of CDB measurements.

It is difficult to assess the status of G1 as a measurement of CDB, since it was not a defensive *response* to a direct danger stimulus, but was rather a "state" in the undisturbed colony (section 9.2.3). Evidence in favour of G1 as a reliable indicator of CDB include, first, the fact that in the undisturbed colony the guards are behaviourally concerned with CDB, and are likely to initiate bouts of it, particularly when a danger stimulus is presented at the hive entrance (Eischen *et al.*, 1986; Moore *et al.*, 1987; section 8.3). Second, G1 was well correlated with the good CDB variables E1-P3-P4 (section 9.2.3), and all four variables were well correlated with temperature (section 9.4). All this might well indicate G1 as a good CDB measurement. However, an alternative explanation is that the

high correlations between G1 and E1-P3-P4 may have arisen through a common dependence on ambient temperature, as was likely in the high correlations between F1 and the CDB variables (section 9.4). A different behavioural basis, but common dependence on temperature (and perhaps also on other, unidentified factors) would explain the frequent, slight differences between G1 and the good CDB measurements throughout the study, as in the dendrograms of section 9.2.3, in the anovas of section 9.3, and in the few, but exceptional, correlations between G1 and humidity (section 9.4). These results can, however, do no more than indicate weakly that G1 may have been different from E1-P3-P4. Collins and associates have also obtained equivocal results concerning the defensive proclivities of the bees at the entrance of the undisturbed colony. Undisturbed Africanized colonies had significantly more guards than more docile European colonies (Collins *et al.*, 1982). Collins *et al.* (1988) found that quantities of certain alarm pheromones in workers were significantly correlated with the defensiveness of their colonies, but were not correlated with numbers of guards in undisturbed colonies, an expected result, "since stimuli for defensive behaviour have not been presented at this time." Bees at the entrance of the undisturbed colony may include non-guards (section 8.3) and, in a bout of CDB, many workers besides the guards may participate. The initial number of guards therefore does not necessarily reflect the defensive responsiveness of a colony in the same way as do attacks on the observer. In practical terms, few beekeepers would forgo protective clothing to manipulate an African colony designated docile solely because few bees guarded the entrance. This conclusion, made on general grounds, disagrees with that of Moore *et al.* (1987) who proposed that the number of guards is associated with colony defensiveness (although this defensiveness was assessed only by expert opinion). The phenomenon requires more stringent analysis.

From the present analyses, it was not possible to identify any one of the good measurements, E1, P3 or P4 as better than the others as a CDB measurement: a variable that seemed best in one analysis would be usurped by another in a different analysis. However, the strengths and weaknesses of E1, P3 and P4 may be assessed in terms of their general design. E1 was not a direct count but a judgement of the intensity of a composite response - its accuracy was therefore strongly dependent on the experience of the observer. However, it would be difficult to improve the present design of E1 without resorting to sophisticated measuring devices. In P3 and P4, workers flying about the observer could be counted until they numbered above about 15, when counts gave way to estimates of unknown accuracy. These measurements were thus more accurate in the lower responses and the design of the measurement method, to keep responses moderate (section 9.1.2) was of undoubted value in this respect.

The accuracy of the counting technique for flying bees could be improved in many ways, e.g. use of a second observer to count bees flying around the stimulus-observer; use of a walk-in room or tent with flight-exit traps to facilitate counting of pesterers; photography of responses (see e.g. Collins and Kubasek, 1982).

The timing of P3 and P4 in relation to the breath stimulus was devised pragmatically, from pilot observations, as likely to provide optimal CDB measurements. While this timing was found to be adequate for the colonies tested, it could probably be improved by experimentation. P5, taken three min after the breath stimulus, consistently registered above zero only in colony C, the most highly responsive colony, in which it appeared to be equivalent to P3 (section 9.2.3). Thus, although potentially a good CDB measurement, P5 was recorded too long after the stimulus and so its response threshold was too high for four out of the five colonies of the experiment.

P1 and P2 were elicited by the stimulus of the observer standing near the otherwise undisturbed colony, whereas E1 was elicited by the breath stimulus and P3, P4 and P5 were elicited by the breath stimulus plus the subsequent presence of the observer (section 9.1.3). The high correlations between the responses to these various stimuli (P1, P2 and P5 in colony C only) (section 9.2.3) indicate their general validity as sign stimuli for CDB. Although the many meaningful results obtained in the study indicated that the breath stimulus as applied by the observer must have been adequately consistent, its consistency could doubtlessly be improved by a mechanical applicator delivering standardized puffs of moist air enriched with carbon dioxide.

Important in the design of measurement methods for CDB is the elimination of confounding factors of the kind specified for moving lures in section 8.5.2, where the first sting in the lure changed the nature and potency of the stimulus in an entirely uncontrolled manner. In a measurement sequence such as that of the present study, stings to the observer could have a similar effect: the method whereby the observer retreated from the hive immediately after administering the danger stimulus is thought to have largely circumvented this problem; longer periods between testing of colonies would eliminate it. Dousing of the hive entrance (or the observer himself) with artificial alarm pheromones (Collins and Kubasek, 1982; Moritz *et al.*, 1985, 1987) might serve to reduce this factor in studies in which several colonies are measured in quick succession.

Stung or unstung, the observer when near the experimental colony may unwittingly constitute a confounding factor. In the measurement sequence of the present study, colonies A, B, D and E scarcely responded to the initial 90 s presence of the observer, as shown by their nil or negligible responses in P1, P2 and G2. However, in the highly responsive colony C,

the number of guards at the entrance after the initial 90 s (G2) was substantially *lower* than the initial number (G1), many of them having flown and attacked the observer (section 9.2.3). Thus when colony C received the breath test, some of its guards had already responded to the previous danger stimulus of the observer standing near the hive. The initial forager count of 90 s was thus to some extent a confounding factor in the comparison of the responses of colony C with the other four colonies. It was, however, not especially important in the present study since the responses of colony C to the breath test were very well correlated with the responses of the other four colonies. Nevertheless, the measurement sequence of the present study would be improved by the elimination of any presence of the observer at the colony before the administration of the breath stimulus.

It is concluded that the breath test and the measurement routine devised for it in the present study offers a meaningful and relatively unconfounded method of measuring CDB against humans in that:

(i) the breath stimulus is a known attribute of the human intruder, the only one known to elicit sting fanning besides mechanical disturbance to guard bees (section 8.3);

(ii) the breath stimulus is of consistent form and strength and it is not affected by responses of the bees themselves, as in the case of e.g. lure stimuli (section 8.5.2; chapter 10) - the stimulus reaches many hive inmates simultaneously;

(iii) the aspects of the response abstracted as measurements (E1, P3 and P4) were responses either to the stimulus itself or to the observer - they were thus relevant to CDB against humans.

The major disadvantage of the method was its heavy reliance apon the experience of the observer, which limits its use as a general technique in honeybee studies. However, the method would be readily mastered by persons

sufficiently motivated to take an intelligent interest in the work.

The stinging of stimulus objects in CDB measurement routines causes two major problems, mentioned previously: the uncontrolled advent of alarm pheromones during a measurement sequence; and, in repeated observations on a colony, the loss of defenders in mass stinging attacks can confound results. For these reasons the present CDB measurement method was designed to reduce stinging. In any CDB measurement method, the stimulus object most suitable to register stings, the observer, is invariably dressed in clothing designed to reduce stinging as much as possible. The one measurement of stinging in the present study (S1) produced equivocal results. However, the ideal CDB measurement method will include a sound method of measuring stinging. Or, if stinging is avoided, the relation of the non-stinging responses with colony stinging behaviour must be determined. A great deal of imaginative design and testing of measurement methods for stinging will have to be undertaken before either of these goals is attained.

CHAPTER 10

ASSESSMENT OF A STINGING BIOASSAY AS A MEASUREMENT OF CDB

10.1 INTRODUCTION

In section 8.5.2 several factors with the potential to confound the moving lure test were identified. However, no evaluation of this test as a measurement of CDB was found in the literature. In chapter 9 responses to the breath stimulus of Boch and Rothenbuhler (1974) were evaluated by experimentally addressing the criteria that good measurements of CDB intensity should be highly correlated with one another and with factors that affect their intensity (section 9.1.1). In that study several "good" measurements of CDB were identified, and temperature exerted a significant effect on the intensity of these variables. In the present study the method of evaluation for breath test responses of chapter 9 was applied to a lure test derived from Michener (1972: *see* section 8.5.2). The results were compared with two "good" breath-test measurements, taken in the same observation periods.

10.2 METHODS AND MATERIALS

On 20 observation days during a series of 59 daily breath tests (section 9.2.2) a set of five colonies (A-E) was submitted to a moving lure test directly after the breath test routine. The lure tests were administered to the colonies in the same order as the breath tests and, because both tests were of 7 min duration, the hiatus between the breath test and the lure test was the same in each colony (35 min). The lure was a 30 by 30 mm

square of soft black leather, 1-2 mm thick, suspended on a metre-long thread. A new lure was used for every test. The observer stood to one side of the hive and jiggled the lure in the air, 10 cm in front of the hive entrance, in the following measurement routine:

LG Number of workers on the flight-board when the observer first arrived at the hive.

LF Number of workers that flew into the hive per 30 s (mainly returning foragers); average of 3 counts of 30 s each (approximately equivalent to measurement 12 of Michener, 1972, *see* section 8.5.2).

LT Time at which lure was first stung in a presentation starting at the termination of LF above (approximately equivalent to measurement 24 of Michener, 1972, *see* section 8.5.2).

LS Number of stings in the lure after a 30 s presentation directly at the hive entrance, which commenced at the first sting in LT (approximately equivalent to measurement 27 of Michener, 1972, *see* section 8.5.2).

LS2 If the lure was unstung after a 3 min presentation in LT, it was moved to the entrance for 30 s and the number of stings recorded (approximately equivalent to measurement 27 of Michener, 1972, *see* section 8.5.2).

LT2 If the lure was stung during LS2, LT2 = number of seconds to first sting (approximately equivalent to measurement 25 of Michener, 1972, *see* section 8.5.2).

LP Number of workers that pestered the observer at the end of LS or LS2 (based on measurements by Michener, 1972, of bees pestering observer).

The duration of the lure presentation could vary between 2 min, if the lure was stung immediately it was presented (i.e. LF = 90 s + LS = 30 s), up to 5 min, if the lure was not stung, (i.e. LF = 90 s + LT = 180 s + LS = 30 s). In the maximum duration, a further 2 min was taken before moving on to the next colony, during which the observer retired into thick foliage in an adjacent papaw (papaya) plantation to rid himself of any bees that flew about him (*see* section 9.1.3). When the lure was stung in less than 3 min the observer retired to the foliage after the 30 s presentation at the entrance (i.e. after LS2) until 7 min had elapsed since the start of the test. Thus each lure test lasted 7 min, the same as each breath test. On completion of measurement LS, or LS2, all bees clinging to the lure were flicked off it as the observer walked to the foliage. This measurement routine thus followed that of Michener (1972, p.76) up until Michener's departure from the hive after presenting the lure. The main differences in the present method were, first, the lure was removed from the bees abruptly at the end of the LS measurements, and second, each observation was terminated by the retreat of the observer into adjacent foliage. These changes omitted Michener's measurement (26) of the distance that the bees followed the observer away from the hive; consequently, the count of the number of stings in the lure (LS) was not equivalent to Michener's number of stings in lure, i.e. his measurement 27 in which the lure was left exposed to attack while the observer walked away from the hive.

In several colonies, LT and LS were not normally distributed; application of Taylor's power law (section 9.2.2) showed no transformation that would bring normality. The distribution-free Kendall's *tau* was therefore used to assess the associations between the variables of the lure test, the ambient temperature at the time of each test, and the following measurements from the breath test (section 9.1.3), recorded over the 20-day period of the lure tests:

F1 - forager flight rate;

G1 - initial number of workers at hive entrance;

E1 - initial response to breath stimulus;

P4 - number of workers that pestered the observer after the breath stimulus.

In pilot observations (section 9.1.2) there were occasions when the observer was "pestered" by flying bees but the lure was not stung; sometimes bees clung to the lure but did not sting, or the lure was completely covered by workers so that stinging could not be observed and potential stingers could not get to the lure. The incidence of these responses in the present series of lure tests was recorded.

10.3 RESULTS AND DISCUSSION

In 52 out of 100 presentations in five colonies the lure was not stung during LT (Table 10-1). These cases had to be dropped from the analysis since they could not be given a value: assignation of a zero score would indicate an instantaneous stinging of the lure; the assignation of some maximum value, such as 180 s, would imply that the lure had been stung at that point, when in fact it was not stung at all. No sting in LT therefore constituted a *null* measurement. The dropping of null measurements from an analysis constitutes a form of data selection, which when frequent will give biased results.

One way to eliminate the defect of null measurements in LT would be to continue presenting the lure until it is stung, as done by Cosenza (1970), Kerr *et al.* (1974) and Villa (1985). In the first two studies, maxima of 10 min and 19.4 min to first sting were recorded in European colonies. Such lengthy periods would, however, not be tolerable in the likes of the present investigation in which the duration of each test

TABLE 10-1. Scores of "lure" and "breath" bioassays in colonies A-E. Variables are defined in the text.

Day	F1	LF	G1	LG	E1	P4	LT	LS	LS2	LT2	LP	*a*	*b*	*c*	T
							Colony A								
17	35	23	4	10	2.00	15.0	-1	-1	2	20	0				20.0
18	48	40	3	5	2.00	9.2	15	2	-1	-1	0				24.5
19	32	30	3	5	1.50	11.0	-1	-1	0	-1	0				22.0
20	29	30	4	4	2.50	13.0	130	5	-1	-1	0				26.0
21	5	16	2	7	1.75	5.8	179	1	-1	-1	3				19.0
22	6	38	2	10	2.75	7.3	15	3	-1	-1	0				26.0
23	38	48	4	5	1.75	6.8	-1	-1	0	-1	5			+	26.0
24	0	11	0	0	0.50	0.0	-1	-1	0	-1	0				12.0
25	No observation														
26	36	24	4	5	2.00	7.5	-1	-1	0	-1	0				25.0
27	33	22	2	3	1.00	0.7	-1	-1	0	-1	2				26.0
28	29	28	15	10	2.00	1.0	-1	-1	0	-1	0			+	27.0
29	5	2	1	1	0.50	1.2	-1	-1	0	-1	0				17.0
30	19	7	0	0	0.25	0.5	-1	-1	0	-1	0				18.0
31	20	8	1	3	1.25	2.0	-1	-1	0	-1	5				19.5
32	26	7	7	5	1.50	3.5	-1	-1	0	-1	2				17.5
33	24	22	2	2	2.00	5.2	-1	-1	0	-1	3				22.5
34	37	18	3	5	0.75	0.5	-1	-1	0	-1	5				25.0
35	15	16	2	3	0.25	0.0	-1	-1	0	-1	3				24.0
36	0	0	0	0	0.00	0.0	-1	-1	0	-1	0	+			9.5
37	8	1	1	1	2.50	0.5	-1	-1	0	-1	0	+			12.5
							Colony B								
17	21	20	5	5	1.00	3.3	10	12	-1	-1	0				20.0
18	27	15	10	5	1.50	2.5	35	9	-1	-1	0				24.5
19	9	10	10	5	1.25	1.2	22	8	-1	-1	0				22.0
20	13	13	10	7	2.00	1.0	45	2	-1	-1	0				26.0
21	1	5	2	3	0.50	0.0	25	1	-1	-1	0	+			19.0
22	18	40	10	15	2.50	1.2	10	2	-1	-1	0	+			26.0
23	21	34	10	10	2.00	1.0	-1	-1	0	-1	0			+	26.0
24	0	0	0	0	0.50	0.0	-1	-1	0	-1	0				12.0
25	No observation														
26	22	15	5	5	1.75	0.3	-1	-1	0	-1	5			+	25.0
27	20	12	5	3	1.00	0.5	70	3	-1	-1	1	+			26.0
28	13	16	7	10	2.50	1.3	65	2	-1	-1	0				27.0
29	3	9	0	2	0.50	0.0	-1	-1	0	-1	1				17.0
30	11	14	4	3	1.50	0.2	-1	-1	0	-1	0				18.0
31	10	5	2	5	0.50	0.0	-1	-1	0	-1	0			+	19.5
32	4	5	2	4	0.25	0.0	-1	-1	0	-1	0				17.5
33	11	13	4	5	0.75	0.5	-1	-1	1	25	0				22.5
34	13	10	4	5	1.50	0.7	-1	-1	0	-1	0				25.0
35	12	6	3	6	1.00	0.3	75	5	-1	-1	0				24.0
36	0	0	0	0	0.00	0.0	-1	-1	0	-1	0				9.5
37	1	0	0	0	1.00	0.0	-1	-1	0	-1	0				12.5

TABLE 10-1. *Continued.*

Day	F1	LF	G1	LG	E1	P4	LT	LS	LS2	LT2	LP	a	b	c	T
							Colony C								
17	37	33	20	20	4.00	97.0	115	24	-1	-1	30				20.0
18	43	40	45	25	4.75	400.0	25	13	-1	-1	20				24.5
19	35	35	25	20	4.00	76.0	20	13	-1	-1	100	+			22.0
20	53	55	30	30	4.75	167.0	5	1	-1	-1	50	+			26.0
21	27	27	25	25	4.00	62.0	18	3	-1	-1	10	+			19.0
22	34	36	30	35	4.75	225.0	28	5	-1	-1	50	+			26.0
23	29	47	20	20	4.50	121.0	12	4	-1	-1	50	+			26.0
24	61	17	3	1	3.25	12.0	-1	0	-1	-1	5				12.0
25	No observation														
26	32	32	15	25	4.00	121.0	25	3	-1	-1	20	+			25.0
27	30	45	10	25	4.00	49.0	-1	0	-1	-1	20	+		+	26.0
28	57	60	25	50	4.25	62.0	30	5	-1	-1	20				27.0
29	16	14	10	15	3.50	37.0	27	2	-1	-1	5				17.0
30	26	30	3	20	3.75	48.0	12	12	-1	-1	20				18.0
31	13	14	15	20	3.50	33.0	25	8	-1	-1	20	+			19.5
32	17	22	10	20	3.50	38.0	175	2	-1	-1	10				17.5
33	31	35	30	30	4.00	88.0	15	2	25	25	10				22.5
34	42	35	25	25	4.00	108.0	5	1	-1	-1	30				25.0
35	32	24	15	15	4.00	62.0	23	2	-1	-1	15				24.0
36	4	0	0	1	3.50	0.5	71	3	-1	-1	1	+			9.5
37	13	3	1	4	3.75	6.3	15	10	-1	-1	2				12.5
							Colony D								
17	6	5	0	4	1.50	2.7	-1	0	-1	-1	0				20.0
18	13	9	2	4	1.50	4.0	20	14	-1	-1	0				24.5
19	6	6	3	4	2.00	4.2	55	1	-1	-1	0	+			22.0
20	6	4	2	4	2.50	6.7	-1	-1	4	15	0				26.0
21	2	2	1	4	1.25	1.3	-1	0	-1	-1	0				19.0
22	6	13	2	4	2.75	7.3	120	9	-1	-1	0				26.0
23	9	12	2	4	1.75	5.2	-1	0	-1	-1	2			+	26.0
24	1	1	0	4	0.00	0.0	-1	0	-1	-1	0				12.0
25	No observation														
26	8	2	1	4	2.00	1.8	60	6	-1	-1	0	+			25.0
27	5	3	2	4	1.50	3.0	18	1	-1	-1	0				26.0
28	3	6	3	4	2.50	0.3	-1	0	-1	-1	1				27.0
29	2	2	0	4	0.50	0.0	-1	0	-1	-1	0				17.0
30	2	1	0	4	0.00	0.0	-1	0	-1	-1	0				18.0
31	3	1	0	4	0.75	0.0	-1	0	-1	-1	0				19.5
32	1	1	2	4	1.50	0.5	-1	0	-1	-1	0				17.5
33	3	1	0	4	2.00	2.0	-1	0	25	25	0				22.5
34	4	4	1	4	2.00	6.3	-1	0	-1	-1	0				25.0
35	1	1	0	4	2.50	2.2	-1	0	-1	-1	0				24.0
36	0	0	0	4	0.00	0.0	-1	0	-1	-1	0				9.5
37	0	0	0	4	0.00	0.0	-1	0	-1	-1	0				12.5

TABLE 10-1. *Continued.*

Day	F1	LF	G1	LG	E1	P4	LT	LS	LS2	LT2	LP	*a*	*b*	*c*	T
							Colony E								
17	8	18	2	8	1.50	0.0	167	1	-1	-1	0				20.0
18	13	6	3	1	1.00	0.0	-1	-1	4	10	0				24.5
19	20	9	5	2	1.25	0.7	30	4	-1	-1	5				22.0
20	15	20	7	4	1.75	1.7	-1	-1	2	27	0				26.0
21	5	17	5	10	1.50	0.8	-1	-1	1	28	0	+			19.0
22	16	10	4	10	2.50	2.5	150	1	-1	-1	5				26.0
23	14	15	4	5	1.50	0.7	-1	-1	7	10	5	+			26.0
24	1	1	0	1	0.25	0.0	-1	0	-1	-1	0				12.0
25	No observation														
26	12	14	3	5	1.75	0.5	20	3	-1	-1	0	+			25.0
27	17	16	5	5	1.75	1.2	110	1	-1	-1	2	+			26.0
28	13	9	5	5	3.50	1.3	112	1	-1	-1	0				27.0
29	7	8	4	5	0.75	0.2	143	1	-1	-1	2				17.0
30	5	4	2	4	0.00	0.3	15	3	-1	-1	0				18.0
31	8	5	2	7	0.50	0.5	10	7	-1	-1	0	+			19.5
32	9	4	6	3	1.00	1.0	-1	-1	3	10	3				17.5
33	6	5	1	3	0.50	0.2	72	1	-1	-1	2				22.5
34	13	8	3	5	1.50	0.0	100	2	-1	-1	0				25.0
35	9	7	5	5	0.75	0.2	-1	-1	1	15	0				24.0
36	0	0	0	0	0.00	0.0	-1	0	-1	-1	0			+	9.5
37	13	9	2	1	0.50	0.0	3	4	-1	-1	0				12.5

Column *a*: lure covered by 20-40 clinging workers

Column *b*: lure attacked but not stung

Column *c*: lure not stung but pestered by 5-15 flying workers

A blank in column *a, b or c* means no response observed

-1 = no response or, in LT and LT2, a *null measurement* as defined in the text

(breath and lure) was specified. Another remedy for a null LT would be to strengthen the intensity of the stimulus after a certain period of non-response to the initial presentation, so generating "secondary" responses that would constitute subordinate extensions on the scale of the "primary" measurements. This is in fact what LT2 constituted in the present study (derived from Michener, 1972, where measurement 25 was taken if there was no sting in measurement 24: *see* section 8.5.2). However, this palliative was itself defective. First, the value of LT2 could also be null, which did not solve the problem of the preceding null measurement in LT. Second, responses in LT2 could range from 0-30; anything in this range would be numerically equivalent to fast responses in LT. To make LT2 subordinate to LT, a transformation would have to be applied to LT2 to make it greater than the maximum value of LT, i.e. greater than 180 s, e.g. by increasing each value of LT2 by 180, thus making it a direct continuation of LT. However, if this were done, values above 180 s would be qualitatively different from lower values since at 180 s the intensity of the stimulus was changed by the experimenter, so that it would not be easy to make a meaningful connection between LT and LT2.

In the 52 null LT responses of the present study, 43 of the subsequent 52 LT2 measurements were null (Table 10-1), i.e. there were 9 LT2 measurements in the entire study. This result indicates that, in the colonies measured, if LT was null then it was highly likely that the lure not would be stung in LT2 and thus, in the present study, LT2 served no useful purpose.

The designs of LS and LS2, which were linked to those of LT and LT1, were also problematic because LS and LS2 were qualitatively different from one another. LS was a response to a freshly stung lure emitting alarm pheromones for 30 s, whereas LS2 was a response initially to an unstung lure, then, if the lure was stung, to a stung lure for a proportion of the

remaining 30 s presentation time (incremental effects of alarm pheromones on lures are outlined in section 8.5.2). In addition, both LS and LS2 were confounded by the uncontrolled advent of the first sting, as described for Stort's lure test in section 8.5.2.

The dropping of the null measurements in LT did not leave much to analyse, since there were only 4 responses in colony A, 5 in colony D, and 9, 18 and 12 in colonies B, C and E respectively (Table 10-1).

Over the days when responses for LT occurred in colonies B, C and E, Kendall coefficients between E1, P4, LT and LS in each colony indicated significant associations only between E1 and P4 in colony C, and LT and LS in colony E (Table 10-2) (correlations with LP are discussed at the end of this section). The low correlations between E1 and P4 in colonies B and E are explained by the data selection enforced by the dropping of the null results for LT, since over the entire 20 days of the lure tests, E1 and P4 in colonies B and E were significantly correlated (Table 10-3). This provides empirical evidence of the strong effects that data selection through null LT recordings may have upon comparisons between CDB measurements. There appears to be no way to remedy such effects.

The high negative correlation between LT and LS in colony E is the result expected when faster onset of stinging and higher numbers of subsequent stings both occur in fiercer responses (*see* sections 8.5.2 and 9.1.1). However, this result does not necessarily mean that ELT and ELS were good CDB measurements in this case, since they were not correlated with EE1 and EP4 (Table 10-2), the two measurements of known good quality in the study. Further, ELT and ELS were not correlated with temperature, whereas EE1 and EP4 were well correlated with it over the same observation days (Table 10-4, p.270) as expected of good CDB measurements (*see* below). In colony C responses for LT and LS were registered on almost all 20 observation days (n = 18), and here there were no correlations between LT

TABLE 10-2. Correlation matrices of CDB variables E1, P4, LT, LS and LP in colonies B C and E (Kendall coefficients). Variables are defined in the text.

Colony/ Behaviour variable		Behaviour variable			
		LT	LS	LP	E1
B†	LS	−.059			
	LP	.359	.000		
	E1	−.030	−.123	−.250	
	P4	−.400	.471	−.239	.388
C‡	LS	.167			
	LP	−.177	.181		
	E1	−.210	.084	.543*	
	P4	−.182	.007	.576*	.764**
E§	LS	−.721*			
	LP	.288	−.290		
	E1	.457	−.402	.160	
	P4	.173	−.092	.380	.410

The coefficients are not significantly different from zero unless marked * (P <0.01) or ** (P <0.001).

† n = 9
‡ n = 18
§ n = 12

} = observation days on which LT-responses occurred, as recorded in Table 10-1, and see text.

TABLE 10-3. Correlation coefficients between behaviour variables F1 and LF, G1 and LG, E1 and P4, E1 and LP, P4 and LP in colonies A–E (Kendall coefficients; $n = 20$). Variables are defined in the text.

Behaviour variable/ Colony		Behaviour variable	Behaviour variable/ Colony		Behaviour variable
		LF			LG
	A	.499*		A	.673**
	B	.687**		B	.667**
F1	C	.477*	G1	C	.657**
	D	.706**		D	.—
	E	.471*		E	.224
		P4			LP
	A	.563**		A	−.116
	B	.598**		B	−.140
E1	C	.767**	P4	C	.607**
	D	.628**		D	.087
	E	.504**		E	.373
		LP			
	A	−.182			
	B	−.010			
E1	C	.571*			
	D	.215			
	E	.198			

The coefficients are not significantly different from zero unless marked * ($P < 0.01$) or ** ($P < 0.001$).

.— = coefficient cannot be calculated.

and LS (Table 10-2); neither LT nor LS were correlated with E1 or P4, whereas the latter two variables were well correlated. In terms of the critera for associations between good CDB variables (*see* sections 9.1.1; 10.1) these results indicate that LT and LS were poor measurements of CDB intensity.

Relations between CDB variables from two different tests (breath test, followed 35 min later by lure test) could have been confounded by disturbance to the colony in the first test, or by changes in ambient conditions between tests. That the status quo of the colonies was not much changed between tests was indicated by (i) the similar rates of foraging in each colony at the start of each test (significant correlations between F1 and LF, and (ii) similar numbers of guards at the start of each test in colonies A, B and C (significant correlations between G1 and LG) (Table 10-3).

Over the days when responses for LT occurred in colonies B, C and E comparison of the Kendall coefficients between temperature and E1, P4, LT and LS (Table 10-4) showed that E1 was the best correlated with temperature in all three colonies, significantly so in colonies C and E, and nearly so in colony B ($P = .012$). P4 was not correlated with temperature in colony B, but was significantly correlated with it in colony C, and nearly so in colony E ($P = .021$). By contrast, LT and LS were in every case not correlated with temperature. These results, even though based on relatively few measurements, indicate the superiority of E1 and P4 over LT and LS as measurements of CDB intensity. If the strength of association between temperature and CDB is taken to be a critical criterion of the quality of a CDB measurement, then the results indicate that LT and LS were worthless measurements in the present experiments.

Over the entire 20 days of the lure tests, E1 in colonies B, C and E was significantly correlated with temperature (Table 10-4). P4, poorly

TABLE 10-4. Correlations between ambient temperature and CDB variables E1, P4, LT, LS and LP in colonies A–E (Kendall coefficients). Variables are defined in the text.

Colony	Behaviour variables							
	Colony B: $n = 9$; C: $n = 18$; E: $n = 12$†					$n = 20$		
	E1	P4	LT	LS	LP	E1	P4	LP
A	—	—	—	—	—	.369	.311	.157
B	.708(*)	.118	.294	−.333	.246	.703**	.570**	.091
C	.712**	.664**	−.128	−.062	.652**	.714**	.623**	.623**
D	—	—	—	—	—	.688**	.604**	.390
E	.720*	.528(*)	.400	−.393	.156	.699**	.425	.165

The coefficients are not significantly different from zero unless marked (*) ($P < 0.05$) or * ($P < 0.01$) or ** ($P < 0.001$).

† Measurements were made on observation days on which LT-responses occurred, as recorded in Table10-1, and see text.

— = coefficient not calculated.

correlated with temperature over the nine observation days dictated by LT nulls in colony B, was highly correlated with temperature over the full 20-day observation period, whereas EP4 remained uncorrelated and CP4 remained highly correlated. An effect of data selection through dropping of null LT measurements thus appears to have intruded only in BP4.

The fact that E1 was in every instance better correlated with temperature than was P4 indicated that, in the circumstances of the observations, E1 was a better measurement of CDB than was P4.

The reduction of the data available for analysis occasioned by the null measurements in LT precluded analysis of the ranking of response intensity of the colonies by anova, as done for variables from the breath test, in section 9.2.

The lure was attacked but not stung on only 3 of the 100 presentations to the five colonies (Table 10-1). However, it was often

difficult to see when the lure was first stung. A sting was unequivocal when a worker grasped the lure, applied its abdomen to the leather and then moved away, leaving its sting apparatus visible on the black surface. But the first worker on the lure would at other times grasp and perhaps bite at it, but clearly did not sting it immediately. Such actions could entail release of mandibular alarm pheromones before stinging (incremental effects of alarm pheromones on CDB are outlined in section 8.4.2). Other workers would lower the abdomen to the leather for extended periods, so that the observer had to judge when a sting commenced: in these cases it could not be directly observed whether a first sting had been executed because the method required the lure to remain in position at the hive entrance; also, once a first sting had been decided upon, although perhaps not actually seen, the lure was moved to the entrance, where often it was mounted by other bees. These difficulties constitute confounding factors in the measurement of "time to first sting" in the estimation of CDB intensity with small-lure stimuli.

Collins *et al.* (1982) thought that in highly responsive colonies, the number of stings in the lure after a 30 s presentation was an underestimation

> "because the targets were completely covered by bees within a few seconds of being presented. Since the bees remained on the targets . . . other bees could not reach the surface with their stings."

In the present study this problem occurred on all 19 occasions when the lure was completely covered by bees (20-40 in number), since in these cases the maximum number of stings in the leather was only 13 (on day 77, colony C: Table 10-1) and the mean number of stings per lure was 3.8.

Occasionally the lure was pursued by a group of 5-15 flying workers, but was not stung (Table 10-1). More often, particularly in the highly

responsive colony C, the observer was pestered by flying bees that paid no attention to the lure, in response LP (Table 10-1). Over the observation days when LT was not null LP was not correlated with LT or LS; it was significantly correlated with E1 and P4 in colony C, but not in colonies B and E (Table 10-2). When n = 20 there were significant correlations between LP and E1, and LP and P4, only in colony C (Table 10-3). Only LP in colony C was significantly correlated with temperature, when n = 20, and when null LT's were dropped (Table 10-4). These significant correlations in colony C were associated with the singularly high responsiveness of colony C, in the experimental set A-E (*see* section 9.2). The significant correlations of LP with E1, P4 and temperature in colony C (in contrast to no correlations between LT, LS and E1, P4 and temperature) indicate that LP was a response to the observer, similar to P4, rather than a direct response to the lure stimulus. Thus in highly responsive colonies (colony C in the present study) the presence of the observer holding the lure may provide a stimulus more relevant to CDB than does the lure stimulus itself - this may be a useful aspect for future analysis by those interested in using small lures as CDB sign stimuli.

10.4 CONCLUSION

The capacity for null measurements is a feature that should be eliminated from CDB measurement designs since, whenever nulls occur, they force data selection into the analysis and so may cripple it. A null measurement as a result of no response may occur in any measurement of the time to a response within a specified period. Such measurements are not problematic when a response always occurs. Among the CDB measurement methods reviewed in chapter 8, several with the capacity for null measurements exist, viz. time to first sting in a 60 s lure presentation (Stort, 1974, 1975a;

Brandeburgo *et al.*, 1977, 1982); time to first sting in a 180 s lure presentation (Michener, 1972; Farrell, 1977); time for colony to become "aggressive" in a 60 s lure presentation (Stort, 1974; 1976); time until bees emerged after alarm pheromone was sprayed at the entrance, and time for first bee to land on a lure, in the 90 s test sequence of Collins *et al.* (1982), also used by Collins *et al.* (1984) and Collins and Rinderer (1985); time to response in a 60 s presentation of alarm pheromones (Collins and Rothenbuhler, 1978; Collins, 1979, 1980, 1981, 1982; Rinderer *et al.*, 1983; Collins *et al.*, 1984); time to attack a wax moth in a 10 min presentation (Eischen *et al.*, 1986). Methods of accommodating null measurements in analyses are not mentioned in most of these publications, although Collins and Rothenbuhler (1978) said that "if the bees did not respond, 'no reaction' was recorded rather than a time" - a policy followed in subsequent work by Collins (1979, 1981). Eischen *et al.* (1986) recorded 600 s if there was no response at 10 min. In Michener's (1972) raw data, response 24 (time to first sting) was frequently recorded as " - ", but also as " 0 " in apparently strong responses in which a zero would mean an instantaneous stinging of the lure (e.g. NB3, NA1, NA2, p.89) and also in weak responses in which it is unlikely that the lure would have been stung instantaneously (eg. SH1, p.90): therefore, the problem of null measurements in response 24 does not appear to have been addressed in Michener's study.

The difficulties that arose from the design of LT, LS, LT2 and LS2 are evidence (additional to that drawn in section 8.5.2) that lure tests may at first sight appear to provide reasonable methods to assess CDB intensity, but on closer examination may prove complex and confounded: an *a priori* critical analysis followed by experimental assessment should be applied to all CDB measurement methods before they are applied in the investigation of CDB.

The foregoing analysis has demonstrated that the initial experimental assessment of a CDB measurement method need not be particularly elaborate or extensive to reveal critical weaknesses *provided the unknown measurements can be compared against CDB measurements of known good quality* (e.g. E1, P3 and P4: section 9.5). The heuristic power of analysing sets of repeated CDB measurements is also evident, in that the method allowed the application of powerful statistical methods such as correlation analysis, and so revealed shortcomings in the design of measurements which were overlooked in *a priori* assessment (section 8.5.2) and by the designers themselves, e.g. the generation of null measurements by LT. These features of the approach (outlined fully in section 9.1.1) allow it to yield more critical information on the quality of CDB measurements than does the alternative approach of the application of one or a few tests per colony for the analysis of singular values in arrays of colonies, as has been the predominant method in CDB studies to date (reviewed in chapter 8).

Thus from the present results it is possible to conclude that (i) the design of the measurement sequence of Michener (1972) was flawed in that LT allowed null measurements when bees failed to sting; (ii) when LT was not null, LT and LS were likely to be poor measurements of CDB; and (iii) if adequate CDB measurements are possible with a lure stimulus, then they could be found by improving designs through application of the methods of assessment of the present study.

CHAPTER 11

DISCUSSION - ETHOLOGY AND THE MEASUREMENT OF HONEYBEE CDB

The advent of the Africanized bees in South America, and their impending invasion of the southern USA, has spurred numerous studies of honeybee CDB. It is generally acknowledged that the readiness with which Africanized bees attack humans (and livestock) constitutes an intermittent public health hazard in tropical South America, and there is little doubt that when these bees colonize the southern USA there will be adverse public reaction. The massive USA bee industry, involving honey and wax production, the package bee industry, and the pollination of billions of dollars' worth of crops, could be severely disrupted (Taylor, 1985; Rinderer, 1986a,b; Needham *et al.*, 1988). Consequently, the main thrust of the work on honeybee CDB has been towards the breeding of gentler strains (section 8.4.5). The primary requirement in such work is an adequate method to quantify the behaviour. It is somewhat surprising, therefore, that a principal measurement method of CDB (the moving lure test) can be shown by *a priori* analysis to contain several potentially serious flaws (section 8.5.2), and that in an experimental comparison with measurements known to be of reasonably sound design, the moving lure test showed up badly on several counts (chapter 10). A possible reason why partially flawed tests have survived unnoticed in the study of CDB was drawn in section 8.5.6: even gross measurement methods, such as the number of stings on a person performing a standard manipulation on a hive, will reveal expected differences between colonies that differ greatly in their defensive behaviour (Rothenbuhler, 1964b; section 8.5.1); so too, of course, will more refined but nevertheless flawed methods such as the moving lure test. Practically all of the numerous studies on the genetics of CDB (reviewed in sections 8.4.5; 8.5.6)

were done on sets of colonies comprising two parental strains known to differ distinctly in their defensive responsiveness, with hybrids between the two and backcrosses to the parental strains. Since the measurement methods used (predominantly the moving lure test) invariably yielded the expected differences between the parental strains, nothing more was required. However, among the backcrosses, some of whose levels of response were less distinct than those of the parental strains, anomalous results were often obtained, in the form of unexpectedly different intensities yielded by different components of particular tests. These anomalies were invariably explained in terms of the complexities of multi-gene inheritance and control of the behaviour (even though many of the anomalies can also be explained by flaws in the test methods themselves) (sections 8.4.5; 8.5). Further, in determinations of heritabilities, a genetic interpretation will always be possible, no matter what the flaws in the test method employed (section 8.5.6). The point is that, in the genetic studies on CDB published so far, highly refined measurement methods have not been necessary for meaningful results to be obtained (section 8.5.6). It should perhaps be noted here that, first, this explanation for the persistence of flawed measurement methods in CDB studies is not intended to denigrate the substantial advances that have been made in the genetic studies that have employed these methods. Second, the "composite" tests initiated by Collins and Kubasek (1982) and subsequently used by Collins and co-workers, Moritz (1985, 1987a, b, 1988) and Southwick and Moritz (1987), have yielded results which are much less open to the criticisms that apply to the work in which simple lure tests were used (section 8.5.2). Nevertheless, a recommendation arising from the present study is that all CDB measurement methods should be experimentally tested for confounding factors in their design (e.g. as done for the breath test in chapter 9 and the lure test in chapter 10).

Another broad point that emerges from the present study is the need to specify the relationship between the behaviour evoked by a measurement method and its meaning for defensiveness against the human intruder. This problem is more complex than it may first appear, and is founded in the great complexity of CDB itself, in the plethora of factors that affect the expression of CDB, and in the wide variety of natural enemies that assail the honeybee colony (reviewed in chapters 7 and 8). In any attempt to breed docile honeybees the ultimate aim must be to produce colonies tolerant of the human intruder, in the form of beekeepers and casual or accidental disturbers of the colony, such as agricultural personnel and their machines and livestock. Beekeepers and bee-researchers can usually designate a colony fierce, docile or intermediate simply by opening it and manipulating a few frames - this has in fact been a predominant, and useful, method of assessing colony defensiveness (section 8.5.4). However, in beekeeping manipulations, intensity of CDB is greatly affected by vibrations to the hive, human (mammalian) odours, movement, and alarm pheromones from workers accidentally crushed (section 8.4), none of which can be controlled sufficiently to provide a constant stimulus. For rigorous analysis of CDB it is thus necessary to devise *artificial* danger stimuli of more constant strength and form than those presented by beekeepers' manipulations, i.e. it is necessary to abstract aspects of the full mammalian stimulus to suit the requirements of repeatability. Each artificial danger stimulus will, of course, have a particular relationship with the full mammalian stimulus, which may be difficult to assess accurately - some are obviously fairly close to it, e.g. breath and opening tests (section 8.5.3; chapter 9) and others are very abstracted from it, e.g. moving lure and cork (alarm pheromone) tests, and "defensive" responses of caged workers (sections 8.5.2; 8.5.3; 8.5.5). In the literature, the associations between responsiveness of caged workers and

responsiveness of their colonies in field tests have been debated to good effect (section 8.5.5); it is also generally recognized that the presentation to colonies of alarm pheromones on inanimate objects (cork tests) constitutes a highly restricted stimulus-response situation (sections 8.4.1; 8.4.2; 8.5.3). In contrast, results of lure tests have often been taken to represent colony responsiveness to the human intruder. However, in the reviews of the present study it is argued from several viewpoints that the lure test is unlikely to fulfil this requirement - besides its flaws as a stimulus *per se* (mentioned above *and see* section 8.5.2), there is the possibility that a small lure bobbing at the hive entrance represents a predatory wasp rather than a mammalian stimulus. If this is the case, then the lure test would be highly inappropriate as a means to determine responsiveness of colonies to humans.

Clearly, what is needed is a measurement method that adequately reflects colony responsiveness to the human intruder, as a benchmark against which other tests, desirable perhaps for their simplicity of execution, can be compared. There is at present no such test available in the study of CDB, and there has been no specific attempt to derive one. In the present study (chapter 9) a move towards this goal was attempted in the testing of an array of responses to the breath stimulus and to presence of the observer at the hive before and after administration of the stimulus. The observer himself constituted a particular type of "full mammalian stimulus", a relatively weak one, since his dress and behaviour during the test were designed to minimise stinging. The breath stimulus is a relatively unconfounded abstraction of an important component of the full mammalian danger stimulus - in the complex mass response to breath blown into the hive, some workers respond to mammalian breath with a specifically evolved response, sting-fanning (section 8.5.3). In the analysis of the relationships between the array of nine kinds of response to the breath

stimulus it was found that three (initial response to breath stimulus and two counts of defenders flying about the observer after the stimulus) were better measurements of CDB than the other six, on the grounds that they were consistently well correlated with one another, both within each colony tested, and between different colonies. The good correlations of the three superior responses with ambient temperature indicated that they were probably good CDB measurements in their own right. It has thus been shown that it is in general possible to assess the relative merits of components of CDB measurement methods, and specifically that it is possible to make good measurements of colony defenders flying about a human observer. Further work in this direction would undoubtedly advance our understanding of CDB against humans.

Finally, it is submitted that with the emphasis that has been placed on the genetic analysis of CDB, consideration of the ethology and of the natural history of the behaviour has been somewhat neglected. There is urgent need for behavioural-ecological studies similar to that of Seeley *et al.* (1982) to be conducted on CDB of feral honeybee populations in Africa (*see* chapter 7), as well as for further purely ethological analyses. Ethologists have formulated powerful methodologies for unravelling meaningful behaviour units from complex behaviour, the full meaning of which can then be extracted by analysis of the biological functions, and the behaviour genetics, of the units. The pursuit of such studies will facilitate the current quest for a method to breed genuinely docile bees.

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APPENDIX TABLE 3-1. Colonies I, II, III and IV. Data for marked groups: age, numbers introduced and recovered, and proportions recovered. The data are displayed in Figs. 3-1, 3-5 and 3-6.

State of colony when marked groups were introduced	Group *a*	Number of days: old when sampled (total age) *b*	Number of days: old at dequeening *c*	Number of days: spent in queenless colony *d*	Number of days: colony queenless when group introduced *e*	Number of workers: in group placed in colony *f*	Number of workers: sampled at dequeening for dissection *g*	Number of workers: adjusted after sample at dequeening *h*	Number of workers: recovered at final sampling *i*	Number of workers: house bees recovered *j*	Number of workers: field bees recovered *k*	% workers recovered: at final sampling* *l*	% workers recovered: house bees† *m*	% workers recovered: field bees‡ *n*	% ratio house bees: field bees§ *o*
COLONY I															
Queenless	K	6	NA	7	6	200	NA	200	125	125	0	63	100	0	63:0
	J	10	NA	10	3	200	NA	200	134	134	0	67	100	0	67:0
Age of dequeening	I	13	0	13	NA	200	NA	200	136	133	3	68	97	3	66:2
Queenright	H	16	3	13	NA	250	10	240	144	143	1	60	99	1	59:1
	G	19	6	13	NA	250	10	240	134	130	4	56	97	3	54:2
	F	23	10	13	NA	248	10	238	119	115	4	50	97	3	49:1
	E	26	13	13	NA	250	10	240	150	140	10	63	93	7	59:4
	D	33	20	13	NA	250	10	240	132	118	14	55	89	11	49:6
	C	39	26	13	NA	300	10	290	68	57	11	23	84	16	19:4
	B	47	34	13	NA	300	10	290	30	27	3	10	90	10	9:1
	A	53	40	13	NA	300	10	290	0	0	0	0	NA	NA	NA
COLONY II															
Queenless	K	3	NA	3	7	200	NA	200	173	173	0	86	100	0	86:0
	J	7	NA	7	3	200	NA	200	141	136	5	71	96	4	68:3
Day of dequeening	I	10	0	10	NA	200	NA	200	129	116	13	65	90	10	58:7
Queenright	H	13	3	10	NA	250	10	240	169	145	17	68	90	10	61:7
	G	16	6	10	NA	200	10	190	119	93	26	63	78	22	49:14
	F	20	10	10	NA	243	10	233	102	79	23	44	81	19	36:8
	E	23	13	10	NA	250	10	240	121	85	36	50	70	30	35:15
	D	30	20	10	NA	250	10	240	61	28	33	25	46	54	11:14
	C	38	28	10	NA	300	10	290	28	9	19	10	32	68	3:9
	B	46	36	10	NA	300	10	290	3	1	2	1	NA	NA	NA
	A	52	42	10	NA	300	10	290	1	1	0	0,3	NA	NA	NA
COLONY III															
Queenless	K	6	NA	7	6	200	NA	200	168	148	20	84	88	12	74:10
	J	10	NA	10	3	200	NA	200	158	110	48	79	70	30	55:24
Day of dequeening	I	13	0	13	NA	200	NA	200	98	65	33	49	66	34	32:17
Queenright	H	16	3	13	NA	250	10	240	68	24	44	28	35	65	10:18
	G	19	6	13	NA	250	10	240	72	29	43	30	40	60	12:18
	F	22	9	13	NA	250	10	240	119	28	91	50	24	76	12:38
	E	26	13	13	NA	300	10	290	114	5	99	39	4	96	1,6:37
	D	33	20	13	NA	300	10	290	57	0	57	20	0	100	0:20
	C	40	27	13	NA	306	10	296	18	1	17	6	6	94	0,4:5,6
	B	47	34	13	NA	300	10	290	0	0	0	0	0	0	NA
	A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
COLONY IV															
Queenless	K	6	NA	7	6	200	NA	200	173	171	2	86	99	1	85:1
	J	10	NA	10	3	200	NA	200	172	172	0	86	100	0	86:0
Day of dequeening	I	13	0	13	NA	200	NA	200	122	112	10	61	85	15	52:9
Queenright	H	16	3	13	NA	250	10	240	146	118	28	61	81	19	49:12
	G	19	6	13	NA	250	10	240	120	82	38	50	68	32	34:16
	F	23	10	13	NA	250	10	240	104	45	59	43	43	57	18:25
	E	26	13	13	NA	300	10	290	148	83	65	51	56	44	29:22
	D	33	20	13	NA	300	10	290	96	19	75	33	20	60	7:26
	C	40	27	13	NA	300	10	290	40	5	35	14	14	86	2:12
	B	47	34	13	NA	300	10	290	2	0	2	0,7	NA	NA	NA
	A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

NA = not applicable; * (column i ÷ column h x 100); † (column j ÷ column i x 100); ‡ (column k ÷ column i x 100); § (column l x column m x 100 ÷ column l x column n x 100)

APPENDIX TABLE 3-2. Colonies I, II, III and IV. Ovary development in the marked age groups. The data are presented graphically in Figs. 3-7, 3-8, 3-10 and 3-11.

State of colony when marked groups were introduced	Group	Number of days: old when sampled (total age)	old at dequeening	spent in queenless colony	colony queenless when group introduced	Ovarian development : numbers dissected: Field bees: total	0	1a	1b	2*	House bees: total	0	1a	1b	2	Ovarian development : % of total dissected†: Field bees: 0	1a	1b	2	1a+1b+2	House bees: 0	1a	1b	2	1a+1b+2
COLONY I																									
Queenless	K	7	NA	7	6	0					25	21	3	1							84	12	4		16
	J	10	NA	10	3	0					25	21	3	1							84	12	4		16
Day of dequeening	I	13	0	13	NA	3	1	1	1		25	11	11	2	1						44	44	8	4	56
Queenright	H	16	3	13	NA	1				1	25	4	14	5	2						16	56	20	8	84
	G	19	6	13	NA	4	2			2	25	6	8	10	1						24	32	40	4	76
	F	23	10	13	NA	4	1	3			25	15	8	1	1						60	32	4	4	40
	E	26	13	13	NA	9	6	3			25	16	6	3		67	33		33		64	24	12		36
	D	33	20	13	NA	14	12	2			25	21	4			86	14		14		84	16			16
	C	39	26	13	NA	11	11				25	24	1			100	0		0		96	4			4
	B	47	34	13	NA	3	3				25	24	1								96	4			4
	A	53	40	13	NA	0					0														
						34	29	5	0	0	200	121	53	21	5‡										
							85	15	0	0		60	27	10	3§										
COLONY II																									
Queenless	K	3	NA	3	7	0					25	25									100				
	J	7	NA	7	3	5	4	1			25	22	3								100				
Day of dequeening	I	10	0	10	NA	13	10	3			25	21	4			77	23		23		84	16			16
Queenright	H	13	3	10	NA	17	6	9	2		25	13	11	1		35	53	12	65		52	44	4		48
	G	16	6	10	NA	25	10	14	1		25	15	10			40	56	4	60		60	40			40
	F	20	10	10	NA	23	19	4			25	21	4			83	17		17		84	16			16
	E	23	13	10	NA	25	24	1			25	21	4			96	4		4		84	16			16
	D	30	20	10	NA	25	25				25	25				100			0		100				0
	C	38	28	10	NA	19	19				9	9				100			0		100				0
	B	46	36	10	NA	2	2				1	1													
	A	52	42	10	NA	0					1	1													
						147	113	31	3	0	159	125	33		1 0‡										
							77	21	2	0		78	21		1 0§										
COLONY III																									
Queenless	K	7	NA	7	6	20	16	2	1		25	25				80	10	5	5	20	100				
	J	10	NA	10	3	25	22	2	1		25	24	1			88	8	4		12	96	4			
Day of dequeening	I	13	0	13	NA	25	13	9	2	1	25	19	6			52	36	8	4	48	76	24			24
Queenright	H	16	3	13	NA	~~25~~ 24	~~4~~ 11	~~15~~ 10	~~1~~		~~24~~ 25	~~14~~ 9	~~10~~ 15	~~1~~		~~36~~ 58	~~60~~ 42	~~4~~		~~64~~ 42	~~58~~ 36	~~42~~ 60	~~4~~		~~42~~ 64
	G	19	6	13	NA	25	21	4			25	17	8			84	16			16	68	32			32
	F	22	9	13	NA	25	21	4			25	23	2			84	16			16	92	8			8
	E	26	13	13	NA	25	25				5	5				100				0					
	D	33	20	13	NA	25	25				0					100				0					
	C	40	27	13	NA	17	17				1	1				100				0					
	B	47	34	13	NA	0					0														
	A	not placed																							
						166	136	27	2	1	100	68	31		1 0‡										
							82	16	1	1		68	31		1 0§										
COLONY IV																									
Queenless	K	7	NA	7	6	2	1	1			25	23	2								92	8			8
	J	10	NA	10	3	0					25	21	4								84	16			16
Day of dequeening	I	13	0	13	NA	10	7	2	1		25	22	3			70	20	10		30	88	12			12
Queenright	H	16	3	13	NA	25	9	15	1		25	6	15	3	1	36	60	4		64	24	60	12	4	76
	G	19	6	13	NA	25	6	19			25	11	12		2	24	76			76	44	48		8	56
	F	23	10	13	NA	25	12	13			25	19	6			48	52			52	76	24			24
	E	26	13	13	NA	25	14	11			25	20	5			56	44			44	80	20			20
	D	33	20	13	NA	25	24	1			19	19				96	4			4	100				0
	C	40	27	13	NA	25	25				5	5				100				0					
	B	47	34	13	NA	2	2				0														
	A	not placed																							
						160	97	61	2	0	144	97	41		3 3‡										
							61	38	1	0		67	29		2 2§										

NA = not applicable. A blank in a column for ovary development means 'zero'. * '0 1a 1b 2' are symbols for stages of ovary development: see Fig. 3-4. † The states of ovarian development in each group are expressed as % of the total number dissected in the group, provided that 9 or more individuals were dissected. ‡ Sums of stages of ovarian development in marked groups A to I. § The sums of stages of ovarian development expressed as % of the total number of marked individuals dissected in the colony.

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APPENDIX TABLE 3-3. Colonies V and VI. Age data for marked groups, and ovary development in samples of 50 taken from each group after the colonies had been queenless for 27 days. The data are presented graphically in Figs. 3-9 and 3-12.

State of colony when marked groups were introduced	Group	Number of days: old when sampled (total age)	old at dequeening	spent in queenless colony	colony was queenless when group introduced	Ovarian development: Individuals dissected: 0	1a	1b	2*	% of total number dissected: 0	1a	1b	2
COLONY V													
Queenless	S	15	NA	15	12	42	7	1		84	14	2	
	R	18	NA	18	9	44	5	1		88	10	2	
	Q	21	NA	21	6	46	3	1		92	6	2	
	P	24	NA	24	3	42	6	1	1	84	12	2	2
Day of dequeening	O	27	0	27	NA	47	3			94	6		
Queenright	N	30	3	27	NA	32	11	5	2	64	22	10	4
	M	33	6	27	NA	22	19	7	2	44	38	14	4
	L	36	9	27	NA	27	17	2	4	54	34	4	8
COLONY VI													
Queenless	S	15	NA	15	12	48	2			96	4		
	R	18	NA	18	9	42	6	2		84	12	4	
	Q	21	NA	21	6	49	1			98	2		
	P	24	NA	24	3	45	5			90	10		
Day of dequeening	O	27	0	27	NA	46	3	1		92	6	2	
Queenright	N	30	3	27	NA	44	5		1	88	10		2
	M	33	6	27	NA	31	14	2	3	62	28	4	6
	L	36	9	27	NA	42	8			84	16		

NA = not applicable. A blank in a column for ovary development means 'zero'. * '0 1a 1b 2' are symbols for stages of ovary development: see Fig. 3-4.

APPENDIX TABLE 4-1. Pilot observations, colonies 2 and 3. Queen cells drawn (and their contents), and comb cells with laying worker brood, on observation days after dequeening. (Data are condensed from Appendix Table 4-2, and presented graphically in Figs. 4-4 and 4-5.)

Number of days after dequeening	Comb cells			Queen cells				Totals	
	eggs	larvae	brood	empty	eggs	larvae	sealed	queen cells drawn	comb cells occupied
COLONY 2									
1									
2				11				11	
3				6				6	
4				3				3	
5	30								30
6	800				6			6	800
7	2 200				6			6	2 200
8	2 800	larvae present,			4			4	2 800
9	3 000	but not			6			6	3 000
10	3 000	recorded		6	3			9	3 000
13	2 500	4 250	31	2	5	3		10	6 781
15	1 490	2 900	40	2	7			9	4 430
17	680	2 030	1 620		8	4		12	4 330
COLONY 3									
1									
2				6				6	
3				3				3	
4				1				1	
5									
6					4			4	
7	50			1	2			3	50
8	400			1	1			2	400
9	2 000			1	3			4	2 000
10	3 500				2			2	3 500
13	1 500	100			8	7		15	1 600
15	900	140		3	6	6		15	1 040
17	600	650	5		9	3		12	1 255

A blank in a column means 'zero'.

APPENDIX TABLE 4-2. Pilot observations, colonies 2 and 3. Comb cells with laying worker brood, frame by frame, on observation days after dequeening. (Data are presented graphically in Fig. 4-2.)

Number of days after dequeening	Frame number	Comb cells (worker and drone)		
		eggs	unsealed larvae	sealed brood
COLONY 2				
5	2	30		
6	2	700		
	3	100		
7	2	700		
	3	600		
	4	600		
	5	300		
8	2	833		
	3	733		
	4	733		
	5	300		
	6	200		
9	2	800		
	3	800		
	4	600		
	5	600		
	6	200		
10	2	800		
	3	800		
	4	600		
	5	600		
	6	50		
13	2	300	400	
	3	900	750	21
	4	300	2 100	10
	5	1 000	1 000	
15	2	600	100	
	3	800	400	30
	4	50	1 800	
	5	40	600	10
17	2	300	30	70
	3	200	200	400
	4	100	1 000	800
	5	80	800	350
COLONY 3				
8	5	400		
9	4	1 000		
	5	1 000		
10	4	1 050		
	5	2 000		
	6		450	
13	4	1 300	100	
	5	200		
15	4	700	40	
	5	200	100	
17	5	600	50	5
	6		600	

A blank in a column means 'zero'.

APPENDIX TABLE 4-3. Colonies A, B, C and D. Total numbers of drone cells on each frame side. Data are presented graphically in Fig. 4-7.

Frame number		Number of drone cells in colonies			
		A	B	C	D
1	i	36	152	0	0
	ii	25	122	0	0
2	i	0	0	305	0
	ii	0	0	305	0
3	i	0	153	946	61
	ii	0	214	946	61
4	i	214	3	336	66
	ii	214	2	336	61
5	i	275	30	260	16
	ii	244	20	260	15
6	i	92	122	10	61
	ii	92	92	10	61
7	i	25	122	0	10
	ii	25	61	0	10
8	i	0	0	15	20
	ii	0	0	15	23
9	i	61	61	0	10
	ii	61	61	0	10
Total drone cells in each colony		1 364	1 215	3 744	485

i, ii = two sides of Langstroth frame.

APPENDIX TABLE 4-4. Colonies V, VI, A, B, C and D. Queen cells drawn (and their contents), and comb cells with laying worker brood, on observation days after dequeening. (Data are condensed from Appendix Table 4-5, and presented graphically in Figs. 4-4, 4-7, 4-8, 4-9 and 4-10.)

Number of days after dequeening	Worker cells						Drone cells							Queen cells				
	eggs	young larvae	old larvae	unsealed larvae	sealed brood	all brood stages	eggs	young larvae	old larvae	unsealed larvae	sealed brood	all brood stages	% drone cells occupied*	empty	eggs	larvae	sealed	all queen cells
									COLONY V									
4																		
5													ND	4	1			5
6							3					3		3				3
8	1 750					1 750	83					83			3			3
19	940	2 480	1 750	4 230	1 750	6 965	800					800			4			4
27	3 090	1 420	440	1 860	960	5 910	260	250	100	350	211	821		4	3	9	2	15
							290	20	15	35	170	495		10	1	3	2	16
									COLONY VI									
4																		
5													ND	2				2
7	780					780								3				3
19	410	110	301	411	307	1 128	1 700					1 700		2				2
									90	90	1 500	1 590		8		1	4	13
									COLONY A									
4																		
5														12				15
7	432					432	29					29	2	14	2			8
12	1 956					1 956	1 151					1 151	84		13			13
17	1 110	2 352	775	3 127	37	4 274	213					213	16	3	8	1		11
23	30	390	740	1 130	800	1 960	845	27	45	72	4	921	67	5	3			8
30	70	485	535	1 020	1 060	2 150							0	2	1			3
37	107	180	448	628	865	1 600							0		8	4		12
44	35	170	175	345	385	765	45					45	3		3	6		9
51		50	97	147	185	332	60		5	5	5	70	5		6	3		9
58	46	84	93	177	126	349	20	20	20	90	30	90	7		7			7
65	40	205	213	418	80	538	2			2		2	0,4	4	4			8
72	15	25	30	55	46	116									2			2
79	600	40	20	60		660												
89																		

APPENDIX TABLE 4-4. *Continued.*

Number of days after dequeening	Worker cells						Drone cells							Queen cells				
	eggs	young larvae	old larvae	unsealed larvae	sealed brood	all brood stages	eggs	young larvae	old larvae	unsealed larvae	sealed brood	all brood stages	% drone cells occupied*	empty	eggs	larvae	sealed	all queen cells
COLONY B																		
4														1				2
7							10							3				3
12	455					455	150							1	6			7
17	1 615	500	1 000	1 500		3 115	120	20	50	70		190	16		4	4		8
23	20	1 000	1 250	2 250	1 500	3 770		20	30	50	55	105	9			4		6
30	530	450	785	1 235	1 805	3 570	45	25		25	45	115	9	1	4			8
37	60	255	210	465	1 010	1 535	55		10	10	40	105	9		6	5		12
44	38	45	117	162	260	460	100	1	5	6	35	141	12	2			1	8
51	40	60	90	150	122	312	63	10	15	25	10	98	8	1	5	1		7
58	35	55	43	98	102	180	80	10	10	20	15	115	9		4			4
65	40	55	110	165	55	260	177		10	10	35	222	18		1			1
72	10	10	5	15	10	35	140		2	2	9	151	12		1			1
79	35	20	60	80	3	118	184				3	187	15	1	1			3
91	130	20	25	45	14	189	16				3	19	2		2			2
98	30	25	30	55		85	5					5	0,4					
105																		
COLONY C																		
6							1 405					1 405	38		1			1
12	2 930	6 330	870	7 200		10 130	555	270	985	1 255		1 810	48		6			5
20	440	2 320	2 550	4 870	4 120	9 430	15	110	280	390	1 150	1 555	42		3	1		4
27	195	605	1 085	1 690	2 070	3 955	1 110		20	20	650	1 780	47		6	1		10
35	160	1 170	425	1 595	724	2 479	1 119	125	45	170	140	1 429	38	1		1		2
42	290	475	835	1 310	270	1 870	1 300	20	25	45	33	1 378	37	1				1
48	140	205	305	510	100	750	1 105				10	1 115	30					
55	150	460	430	890	40	1 080	780				3	783	21					
69							70					70	2					
76																		

APPENDIX TABLE 4-4. *Continued.*

Number of days after dequeening	Worker cells						Drone cells							Queen cells				
	eggs	young larvae	old larvae	unsealed larvae	sealed brood	all brood stages	eggs	young larvae	old larvae	unsealed larvae	sealed brood	all brood stages	% drone cells occupied*	empty	eggs	larvae	sealed	all queen cells
COLONY D																		
6							2					2	0,4	3	5			8
12	503	20		20		523	4					4	0,8		4	1		5
20	260	1 000	1 450	2 450	1 090	3 800			40	40	90	130	27	13	2	1		16
27	340	110	270	380	2 276	2 986	30		25	25	157	212	44	1	1	4	1	7
35	190	1 360	840	2 200	398	2 788	30	8	8	16	65	111	23		1	2		3
42	75	245	1 300	1 545		1 620	10		15	15		25	5			2		2
48	335	70	85	155		490	30	10	10	20		50	10					
55	30	185	120	305	2	337	10				1	11	2					
69	30	70	120	190	26	246												
76	85	240	65	305	3	393												
83	40	60	70	130	5	179												
89	45	45	65	69	6	139												
93	70†	37†	32†															

A blank in a column means 'zero'. * Total number of drone cells in colonies A, B, C and D are given in Appendix Table 4-3. ND = not determined.
† Brood was dead (blackened, shrivelled) when examined.

APPENDIX TABLE 4-5. Colonies V, VI, A, B, C and D. Queen cells drawn (and their contents), comb cells with laying worker brood (frame by frame), and numbers of adults on observation days after dequeening. (Data are summarized in Appendix Table 4-4, and presented graphically in Figs. 4-3, 4-11, 4-13 and 4-14.

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
COLONY V																
4	4 ii									1.1(j)	1(lj)			ND	ND	ND
	6 i									2						
5	3 ii									1						
	5 ii									1						
	6 i					1(1)										
6	3 ii					20(1-3)										
	4 ii															
	5 i										1(5)					
	ii					30(3-10)					2(5)					
	9 i					30(3-10)										
	ii					1(1)										
8	3 i					2(1)										
	ii					200(2-10)										
	4 i	300(1-6)				250(2-15)										
	ii	150(1-6)									1(15)					
	5 i	300(1-10)									1(15)					
	ii	200(1-10)				200(10-15)										
	6 i	500(1-10)				150(10-25)										
	ii	300(1-5)									2(10)					
19	3 i				10	60(10)	100	50	20							
	ii				15	80(10)	150	50	20	1		4(yj)	2(oj)			
	4 i	100(1-3)	100	100	300											
	ii	100(1-3)	100	100	300											
	5 i		300	100	200	20(10)			20	1						
	ii		300	100	200	100(10-15)			30							
	6 i	10(1-3)	10	50	200				60	1	2(25)	2(yj)				
	ii	10(1-3)	20	50	200				50							
	7 i	20(1-3)	500	500	300				5	1	1(yj)					
	ii	700(1-10)	600	600												
	8 i		50	50	50											
	ii		500	100	20				6							

APPENDIX TABLE 4-5. *Continued.* (Colony V, Colony VI)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
27	3 i					90(5-15)	10	10								
	ii					90(5-15)	10	5	80	2(j)			1(oj)			
	4 i	100(1-3)	100	20	50											
	ii	200(1-3)	200	20	100											
	5 i	240(1-3)	150	100	60				10							
	ii	500(1-3)	90	80	70	100(10-20)			20	1		1(yj)				
	6 i	400(1-5)	100	50	140	10(10-20)			20		1(10)					
	ii	400	200	60	200				30	1(j)						
	7 i	400	200	40	100					4		1(yj)				
	ii	500	300	30	100											
	8 i	50	30	20	80					2		1(yj)	1(oj)			
	ii	300	50	20	60				10							
										COLONY VI						
4	6 i									1				ND	ND	ND
	8 i									1						
5	4 ii									2						
	8 i									1(j)						
7	4 i	50(1-3)				600(1-6)				2						
	ii	200(1-3)				700(1-6)										
	5 i	100(1-3)				200(1-6)										
	ii	400(1-3)				200(1-6)										
	6 i	30(1-3)														
19	3 i				16											
	ii				6					3(j)						
	4 i		10	20	50			50	700	2						
	ii			10	20			40	700			1(yj)				
	5 i		10	30	10				100	3			2(yj)			
	ii		30	50	60											
	6 i	100	20	30	60											
	ii	300	30	100	50											
	7 i			6	1											
	ii			30	20								2(oj)			

APPENDIX TABLE 4-5. *Continued.* (Colony VI, Colony A)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
	8 i	5	5	20	7											
	ii	5	5	5	7											
27	3 i		7	8	3											
	ii	20	20	10	20											
	4 i					5	45	15	490							
	ii					10	80	20	700							
	5 i	40	80	190	250											
	ii	20	50	60	360											
	6 i	120	380	110	180											
	ii	200	400	120	150											
	7 i	150	90	60	33											
	ii	110	110	25	21											
	8 i	10	50	40	8											
	ii	50	20	20	17											
										3		1(y);1(yj)	1(oj)			
										COLONY A						
4	5 i									3						
	ii									1(½)				12.9		
	7 i									1;13(½)						
	ii									4						
	9 i									6						
5	5 i					12(1)				1						
	ii					10(1)								ND		
	7 i					3(1)				8(½)	2(1)					
	ii					4(1)				4						
	9 i									1						
7	3 i	200(1-10)									1(2)					
	ii	100(1-3)												ND		
	4 i	2(1)				214(1-10)										
	ii	10(5)				214(1-10)										
	5 i	10(1)				275(5)					1(5)					
	ii	10(3)				244(5)					2(5;3)					

APPENDIX TABLE 4-5. *Continued.* (Colony A)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
	6 i					92(5-10)										
	ii	100(1-5)				92(5-10)										
	7 i										1(3);3(2);2(1)					
	ii					20(5)					1(5);1(3);1(2)					
12	3 i	50(1-3)								1				8		
	ii	10(1-3)								2	1(3)					
	4 i	300(1-3)				6(1-3)										
	ii	6(1)				3(1)										
	5 i					20(1-5)					1(3);1(8)	1(yj)				
	ii	40(1-3)				10(1-3)										
	6 i	300(1-5)				92(1-5)										
	ii	50(1-5)				50(1-5)										
	7 i	500(1-3)														
	ii	200(1-3)				20(1-15)					1(1);1(3);1(5);1(7)					
	8 i	200(1-3)														
	ii	200(1-3)														
	9 i	100(1-3)				10(1-3)										
17	3 i	5														
	ii	5	2											6		
	4 i	300	100	200		200(1-10)				2						
	ii	50				200(1-10)										
	5 i	100	100	40	4	200(1-8)	10	5	2		1(5)					
	ii	200	50	100	10	200(1-8)	2			2	1(10)					
	6 i	50	100			10	10	20								
	ii	100	100	5	3	5	5	20								
	7 i	50	200	100		10										
	ii	100	300	300	20	20			2	1	1(1)					
	8 i	100	900	10												
	ii	50	500	20												
23	4 i	10	50	10	30					1(j)				ND		
	ii	10	10	10												
	5 i			150	150											
	ii		10	150	150											

APPENDIX TABLE 4-5. *Continued.* (Colony A)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
	6 i			10	10											
	ii		10	100	200											
	7 i		100	100	100											
	ii		100	100	100					1(j)	1(4)					
	8 i	10	10	10	10											
	ii		100	100	50											
30	4 i	5	10	10	10											
	ii		5	5	10									4.5		
	5 i	10	50	50	200						2(2)					
	ii	5	30	50	100						2(1)	1(oj)				
	6 i		10	50	50						1(1)	1(y)				
	ii	10	20	150	200						1(1)					
	7 i	20	100	100	300											
	ii	20	200	100	150						2(2)	1(2y);1(o)				
	8 i		10	10	10											
	ii		50	10	30											
37	4 i			2	10											
	ii			1	5									4		
	5 i	20	20	50	150						1(3)					
	ii	7	10	70	100						1(1)					
	6 i	10	50	70	100	25(5-10)						2(yj)				
	ii			5.5	30	10(5-10)										
	7 i	10	10	100	200						1(5)					
	ii	20	50	100	200	10(5)						3(yj);1(oj)				
	8 i	20	20	20	20											
	ii	20	20	30	50											
44	4 i				10											
	ii				40									3		
	5 i			10	50											
	ii			10	50		5	5			1(1);1(2)					
	6 i		10	20	50	40(10)					1(1)					
	ii			5	20	20(15)										
	7 i	5	20	30	50						1(2)	1(oj)				
	ii	10	20	30	50						1					
											1(5)	1(yj);1(oj)				

APPENDIX TABLE 4-5. *Continued.* (Colony A)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
	8 i	10	100	30	30											
	ii	10	20	50	35											
51	5 i			5	30						1(1)			1.5		
	ii			10	30						2(1)					
	6 i		10	10	35	20	20	20	30		1(3)					
	ii			2	10											
	7 i		30	40	40											
	ii		10	30	40						1(3);1(5)	1(2j)				
58	5 i			3	15						1(1)			1.5		
	ii				15											
	6 i	1	4	10	25											
	ii				6					3(j)	1(5)					
	7 i		5	20	10	2(3)				1						
	ii	15	15	20	25						1(2);1(5)					
	8 i	20	50	30	15											
	ii	10	10	10	15											
65	5 i				10									1.5		
	ii				10											
	6 i				10											
	ii				5											
	7 i			3	5											
	ii		5	10	20											
	8 i	20	100	100	20						1(3)					
	ii	20	100	100												
72	5 i				10						1(5)					
	ii				7									1		
	6 i				9											
	ii				5											
	7 i				3											
	ii				6											
	8 i	5(1-3)	5	10	6											
	ii	10	10	20												
79	8 i	300	30	10												
	ii	300	10	10										0.5		

APPENDIX TABLE 4-5. *Continued.* (Colony A, Colony B)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number		eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
89																	
91															0.5		20
93																150	10
96																150	10
98																120	10
103																32	10
																5	
										COLONY B							
4	6	i									1						
7	5	ii									1				18.5		
	6	i									2				ND		
		ii					10(15)										
12	3	ii									1						
	5	i													ND		
		ii	5(1)														
	6	i	100(1-3)				50(3-10)					1(2)					
		ii	300(1-5)				50(3-10)					2(3);1(4)					
	7	i	30(1-3)				30(3-10)					1(4)					
		ii	20(1-3)				20(1-5)										
17	3	i					50(1-5)					1(3)					
		ii					50(1-5)								9		
	4	i	600(1-3)														
		ii	400(1-3)														
	5	i	300(1-3)	200									1(yj)				
		ii	100(1-3)										1(yj)				
	6	i	100	200	400		10	10	20			1(1);1(2)	1(yj)				
		ii	100	100	600		5	5	20			1(3)	1(yj)				
	7	i	10					5	10								
		ii					5					1(3)					
23	4	i	10	400	200												
		ii		200	300										ND		
	5	i		100	200	200							1(o)	25(½)			
		ii			100	100							1(yj)				
	6	i			100	400			10	20	1		2(y)				
		ii			100	500			10	20	1						

APPENDIX TABLE 4-5. *Continued.* (Colony B)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
	7 i				100		10	5	10							
	ii			50	200		10	5	5							
	8 ii	10	200	200												
30	4 i	10	50	50	150									ND		
	ii	10	150	200	200							1(yj)				
	5 i	5	20	50	200						1(1)					
	ii	5	10	30	150											
	6 i	10	50	150	300	20	10		20			1(oj)				
	ii	50	100	200	500	20	10		20							
	7 i	20	50	20	100	5	5		5			1(o);2(y)				
	ii	20	10	50	100											
	8 i			5	5											
	ii		10	30	100											
37	4 i	10	10	30	150									ND		
	ii	10	10	30	150											
	5 i			20	100						1(3)	1(o)				
	ii			5	60						1(3)	2(yj)				
	6 i		100	50	200	20(10)		5	20							
	ii		50	30	200	20(10)		5	15		5(3)	2(o)				
	7 i	10(1-3)	50	30	70	10(5-10)			5							
	ii	10(1-3)			20	5(5)										
	8 i	10(1)	5	5	10											
	ii	10(1)	30	10	50											
44	4 i	10	10	10	50											
	ii	10	10	10	50									6		
	5 i			10	20	10(5-10)		5	10	1						
	ii			5	10				5	1	1(2)		1			
	6 i	3	5	20	40	30(5-10)			10		3(1);1(3)					
	ii	5	10	30	30	30(5-10)	1		10							
	7 i			5	25	20(1-5)										
	ii			2	5	10(1-5)										
	8 i	5	5	20	10											
	ii	5	5	5	20											

APPENDIX TABLE 4-5. *Continued.* (Colony B)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
51	4 i				20											
	ii				20											
	5 i				15	3(2-5)				1						
	ii				5						2(1)	1(oj)				
	6 i	5(1)	5	10	10	20(5-10)					3(2)					
	ii	5(1)	30	20	20	20(5-10)										
	7 i	5	5	5	10	20										
	ii	5			2											
	8 i	10	10	10	10											
	ii	10	10	10	10											
58	4 i				10											
	ii				10									2.5		
	5 i			3	8											
	ii			2	7						1(2)					
	6 i	5(1)	5	5	30	30(1-5)	5	5	5		1(2);1(5)					
	ii	30(1)	30	30	30	30	5	5	10							
	7 i															
	ii					20(1-5)					1(3)					
	8 i			3	2											
	ii				5											
65	3 i				5											
	ii				5									2		
	4 i				5											
	ii				5											
	5 i	10			5											
	ii	10			5				5							
	6 i															
	ii	20	50	100	20	77(1-10)		5	10		1(2)					
	7 i					80(1-10)		5	20							
	ii		5	10	5	20(1-5)										
72	6 i					70(5-10)			4		1(2)					
	ii	5(1)	10	5	10	60(3-10)		2	5					ND		
	7 i					10(1-5)										
	ii	5(1)														

APPENDIX TABLE 4-5. *Continued.* (Colony B, Colony C)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number		eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
79	6	i					95(5-10)				1						
		ii	5	5	10		89(5-10)			3		1(5)		1 (d.emerging)	1.5		
	7	i	10	5	20												
		ii	10	10	30	3											
91	6	i					1(1)										
		ii					15(1-2)								1.75		30
	7	i	100(1)	10	15	4						1(2)					
		ii	30	10	10	10				3		1(4)					
98	6	i					5(1)										
		ii	5	5	15											1 530	30
	7	i	20	20	15												
		ii	5														
105																1 400	20
111																1 000	20
115																	
								COLONY C									
6	3	i					650(1-3)					1(2)			16		
		ii					715(1-3)										
	4	i					10(1)										
		ii					10(1)										
	5	i					10(1)										
		ii					10(1)										
12	2	i	20	400			4(3)					1(2j);1(4)			9		
		ii	100	20			1(2)										
	3	i	200	900			195(10)	65	455			3(5j)					
		ii	300	1 000			65(5-10)	65	390								
	4	i	300	800	200		20(10)	5	5								
		ii	200	1 000	100		10(10)	5	5								
	5	i	400	800	5		130	65	65								
		ii	200	400	20		130	65	65								
	6	i	300	300								1(3j)					
		ii	300	700	500												
	7	i	100														
		ii	100	10													

APPENDIX TABLE 4-5. *Continued.* (Colony C)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
	8 i	10														
	ii	400														
20	2 i	20	100	250	450									7		
	ii		50	200	300						1(2j)	1(oj)				
	3 i	20	50	50	200	5	20	30	500							
	ii	20	50	50	200	10	10	20	500							
	4 i	20	50	150	450											
	ii		20	100	400											
	5 i	50	100	200	300		50	100	100							
	ii	50	100	100	250		30	130	50							
	6 i	50	200	300	500											
	ii	50	200	400	400						1(2)					
	7 i	20	300	100	100											
	ii	20	400	300	350											
	8 i	20	300	300	200						1(2)					
	ii	100	400	50	20											
27	2 i	50	50	100	250									ND		
	ii	5	5	5	100						2(5j)					
	3 i	10		20	100	150		10	325							
	ii			10	100	700(5–10)		10	130							
	4 i	20	20	30	200											
	ii	20	20	20	200											
	5 i	10	10	40	10	130(10)			130							
	ii	50	20	10	10	130(10)			65							
	6 i	10	10	10	150											
	ii				200						1(3)	1(oj)				
	7 i	20	30	10	100						2(2);3(3);1(2j)					
	ii		40	30	250											
	8 i				300											
	ii		400	800	100											
35	2 i	5	20	30	50											
	ii	5	20	40	40									6		
	3 i	10(1)	10	10	20	200(5–10)	60	10	50							
	ii			5	20	500(5–10)	50	30	20	1(j)						

APPENDIX TABLE 4-5. *Continued.* (Colony C)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
	5 i	20	30	60	40	150(5)	5		20							
	ii	10	40	50	4	150(5)	5		20							
	6 i	5	10	10	20											
	ii	10	20	20	20											
	7 i	20	400	30	100							1(oj)				
	ii	20	500	50	120											
	8 i	40	40	10	100	5(1-5)			10							
	ii	10	70	50	130	4(1-5)			10							
42	2 i				20											
	ii		10		15									5		
	3 i	20	10	20	10	390(1-5)	20	20	20		1(1)					
	ii	10	40	10	10	585(1-5)		5	3							
	4 i	50	50	30	20											
	ii	40	200	30	20											
	5 i	50	100	400	20	130(1-5)			5							
	ii	100	20	300	50	195(1-5)			5							
	6 i	10	40	10	10											
	ii	10	5	20	10											
	7 i			5	5											
	ii			10	20											
	8 i				10											
	ii				50											
48	3 i				10	520(1-10)			10							
	ii	10	5	5		585(1-10)								2		
	4 i	10	10	40	10											
	ii	20	20	50	10											
	5 i	50	100	130	30											
	ii	50	70	80	40											
55	3 i					390(3-10)										
	ii					390(3-10)			3					2		
	4 i	50(1)	100	100	20											
	ii	50	60	80												
	5 i	30	200	150	20											
	ii	20	100	100												

APPENDIX TABLE 4-5. *Continued.* (Colony C, Colony D)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
69	3 i					40(1-5)									1 160	30
	ii					30(1-5)										
76															450	20
83																
									COLONY D							
6	3 i					1(1)								8		
	ii					1(1)										
12	3 ii	100(1)								3	5(1)					
	4 i	3(1)				4(5)					2(2);2(3)			5		
	ii	200(1)														
	5 i	200(1)										1(yj)				
	ii	100(1-3)														
20	3 i	20	150	250				20		12(j)				5		
	ii		100	200	10			20	10							
	4 i	20	100	200	350											
	ii	30	100	300	250											
	5 i	20	250	100	70											
	ii	20	150	100	60						1(2)	1(oj)				
	6 i	100	100	100	150				30	1(j)	1(2)					
	ii	50	50	200	200				50							
27	3 i	10	10	20	20	5(10)		5	20	1(j)				2		
	ii	5	50	5	100	5(10)		5	15		1(3)	1(yj)	1(o)			
	4 i	20	5	10	500	5(10)		5	51							
	ii			20	500	5(10)		5	56			1(yj)				
	5 i	5	5	10	300	5(10)		5	5							
	ii			30	300	5(10)			10			1(oj)				
	6 i		20	100	350							1(oj)				
	ii		20	50	200											
	7 i	200		20	1											
	ii	100		5	5											
35	3 i	10	300	20	20	5(5)	5	5	20		1(2)			1		
	ii	20	150	30	30	5(5)			20							

APPENDIX TABLE 4-5. *Continued.* (Colony D)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
	4 i	30	300	50	90	5(5-10)			5							
	ii	20	100	20	60	5(5-10)			5			1(yj);1(o)				
	5 i	20	80	40	50	5(5)	3	3	5							
	ii	20	50	20	50	5(5)			10							
	6 i	50	150	70	50											
	ii	10	30	40	40											
	7 i	5	100	300	3											
	ii	5	100	250	5											
42	3 i	5	5	15		5(5)		5								
	ii	5	5	5		5		5						2.5		
	4 i	10	10	10				5								
	ii	10	10	10								2(oj)				
	5 i	5	5	10												
	ii	10	10	10												
	6 i	20	100	50												
	ii	10	100	20												
48	3 i					10(1-5)	5	5								
	ii					29(1-5)	5	5						3		
	4 i	150	20	20												
	ii	150	20	20												
	5 i	5	5	10												
	ii	10(1-3)	5	5												
	6 i	20	20	20												
	ii			10												
55	3 i	10	50	20		10(3-10)										
	ii	5	100	50	2									2.5		
	5 i		10	10												
	ii	5	5	10												
	6 i	5	10	10												
	ii	5	10	20												
69	4 i	10	20	20	3											
	ii	10	10	30	10									1.5	2 010	20
	5 i	10	20	20	3											
	ii	20	20	50												

APPENDIX TABLE 4-5. *Continued.* (Colony D)

Number of days after dequeening	Worker cells					Drone cells				Queen cells				Number of adults		
	frame number	eggs	young larvae	old larvae	sealed brood	eggs	young larvae	old larvae	sealed brood	empty	eggs	larvae	sealed	framesides	individual workers	individual drones
76	4 i	30	30	20	3											
	ii	30	150	20										2	1 430	20
	5 i	5	10	5												
	ii	20	50	20												
83	4 i	20(1)	30	50												
	ii	20	30	20	5										1 820	
89	3 i	10	10	20	3											
	ii	20	20	30											1 037	
	4 i	10	10	10												
	ii	5	5	5	3											
93																

A blank in a column means 'zero'. i, ii = two sides of Langstroth frame. ND = not determined.

( ) designates cell contents:

(y) = young larvae
(o) = old larvae
(j) = royal jelly
(½) = queen cell half-drawn over a drone cell
(*numeral*) = approximate number of eggs per cell, eg. 5(1–3) = five cells with 1 to 3 eggs each.

APPENDIX TABLE 9-1. Raw data for breath tests and weather measurements in colonies A-E. Variables are defined in sections 9.1.3 and 9.4.2. Blank spaces in the data-field indicate missing data.

Day	P1	F1	P2	G1	G2	GX	G3	E1	P3	P4	S1	P5	T	RH	VP	SD	PWDSC
								Colony A									
1	0	37	0	3	2	1	-2	0.00	0	0.0	0	0	17.0	26	0.503	1.432	00010
2	0	56	0	5	4	5	0	0.50	2	3.0	0	2	22.0	27	0.713	1.927	01510
3	0	39	0	0	0	5	5	0.50	0	1.0	0	0	24.0				03710
4	0	4	0	0	0	5	5	0.00	0	0.0	0	0	18.0	45	0.927	1.133	01310
5	0	43	0	3	6	8	5	2.00	4	5.0	1	3	25.0	20	0.633	2.531	02710
6	0	0	0	0	0	10	5	1.00	0	0.0	0	0	15.5	63	1.108	0.651	32403
7	0	37	0	1	3	5	4	2.50	2	2.3	0	2	19.0	53	1.163	1.031	00010
8	0	11	0	2	1	5	3	0.50	0	0.0	0	0	17.0	55	1.064	0.871	01110
9	0	34	0	5	3	2	-3	1.50	3	5.7	0	3	22.0	68	1.795	0.845	00010
10	0	23	0	2	2	5	3	1.50	3	2.0	0	0	21.0				00010
11	0	38	0	1	2	3	2	2.00	1	1.7	0	5	24.0				01710
12	0	12	0	1	1	10	9	2.00	2	4.7	0	2	15.0	78	1.328	0.375	01311
13	0	44	1	3	5	10	7	2.00	5	5.8	0	5	20.0	25	0.584	1.751	00010
14	0	37	0	3	5	5	2	2.00	2	6.5	1	0	22.0	32	0.655	0.985	00010
15	0	31	0	2	2	5	3	2.00	5	8.5	0	5	22.0	29	0.766	1.874	01910
16	0	45	0	5	3	10	5	2.50	10	36.0	0	30	26.0	17	0.571	2.786	00010
17	0	35	0	4	4	15	11	2.00	2	15.0	0	15	20.0				01710
18	0	48	1	3	5	5	2	2.00	2	9.2	0	5	24.5				01510
19	0	32	3	3	3	5	2	1.50	5	11.0	0	10	22.0	36	0.950	1.690	01910
20	0	29	0	4	5	10	6	2.50	10	13.0	0	10	26.0	17	0.571	2.786	02810
21	0	5	0	2	3	10	8	1.75	1	5.8	1	5	19.0	54	1.185	1.009	01201
22	0	6	0	2	2	2	0	2.75	2	7.3	0	0	26.0	30	1.007	2.350	01310
23	0	38	0	4	5	4	0	1.75	5	6.8	0	3	26.0	13	0.436	2.921	01910
24	0	0	0	0	0	4	4	0.50	0	0.0	0	0	12.0				00003
25	No observation																
26	0	36	0	4	4	3	-1	2.00	5	7.5	0	10	25.0	17	0.538	2.626	01710
27	0	33	0	2	1	2	0	1.00	1	0.7	0	0	26.0	10	0.336	3.021	04102
28	0	29	0	15	15	5	-10	2.00	0	1.0	0	1	27.0	12	0.427	3.133	01112
29	0	5	0	1	0	2	1	0.50	1	1.2	0	0	17.0	37	0.716	1.219	01310
30	0	19	0	0	0	3	3	0.25	0	0.5	0	1	18.0	24	0.495	1.566	01110
31	0	20	1	1	1	5	4	1.25	2	2.0	0	1	19.5				03310
32	0	26	0	7	5	5	-2	1.50	1	3.5	0	1	17.5	30	0.599	1.398	00010
33	0	24	0	2	2	5	3	2.00	2	5.2	0	3	22.5	20	0.545	2.178	01910
34	0	37	0	3	2	0	-3	0.75	0	0.5	0	0	25.0	16	0.506	2.657	01110
35	0	15	0	2	2	2	0	0.25	0	0.0	0	0	24.0	9	0.268	2.712	02310
36	0	0	0	0	0	25	25	0.00	0	0.0	0	0	9.5	93	1.103	0.083	32403
37	0	8	0	1	2	30	29	2.50	1	0.5	0	2	12.5	65	0.941	0.506	00011
38	0	36	0	1	2	5	4	2.00	2	3.3	2	0	17.0				01910
39	0	15	0	3	2	12	9	1.75	2	3.7	0	3	22.0				03111
40	0	45	0	3	2	2	-1	2.00	1	5.3	0	1	22.5	31	0.844	1.879	00010
41	0	42	0	5	5	5	0	2.50	3	3.0	0	3	28.0	21	0.793	2.982	03110
42	0	20	0	2	1	2	0	1.00	2	1.8	0	0	20.0	54	1.261	1.074	00010
43	0	29	0	1	1	1	0	1.50	1	3.3	0	0	25.0	38	1.202	1.961	00011
44	0	22	0	1	1	5	4	1.50	3	4.2	0	0	30.0	22	0.932	3.306	00012
45	0	53	0	3	3	5	2	3.00	5	3.8	0	2	34.0				01712

APPENDIX TABLE 9-1. *Continued.*

Day	P1	F1	P2	G1	G2	GX	G3	E1	P3	P4	S1	P5	T	RH	VP	SD	PWDSC
46	0	4	0	0	0	5	5	0.00	0	0.0	0	0	15.0				02203
47	0	0	0	0	0	0	0	0.00	0	0.0	0	0	9.0	87	0.997	0.149	00003
48	0	0	0	0	0	3	3	0.00	0	0.0	0	0	12.0	54	0.168	1.232	01501
49	0	59	0	0	0	10	10	0.50	0	0.0	0	0	17.0	39	0.754	1.180	03311
50	0	15	0	1	2	5	4	1.75	2	0.8	0	0	23.0	20	0.561	2.244	02311
51	0	14	0	2	1	5	3	0.75	0	0.0	0	0	22.0	45	1.188	1.452	01110
52	No observation																
53	No observation																
54	0	8	0	0	0	5	5	0.00	0	0.0	0	0	18.0	73	1.504	0.556	01102
55	0	16	0	0	0	2	2	1.50	1	1.3	0	0	23.0	42	1.178	1.627	02511
56	0	23	0	0	0	5	5	0.00	0	0.0	0	0	20.0	54	1.261	1.074	01902
57	0	32	0	5	5	2	-3	1.00	0	0.2	0	0	27.0	73	2.510	0.961	00012
58	0	17	0	2	2	2	0	0.00	0	0.0	0	0	25.0	45	1.424	1.740	01510
59	0	18	0	5	5	7	2	1.00	0	0.2	0	0	24.0				01910
60	0	50	0	1	2	5	4	2.00	1	2.8	0	1	31.0				00010
61	0	6	0	0	0	3	3	0.50	0	0.0	0	0	21.0				02111
62	0	10	0	2	3	1	-1	0.25	0	0.0	0	0	22.0	42	1.109	1.531	01910
								Colony B									
1	0	20	0	2	2	15	13	1.00	0	0.0	0	0	17.0	26	0.503	1.432	00010
2	0	49	0	20	27	15	5	1.00	2	1.3	1	0	22.0	27	0.713	1.927	01510
3	0	38	1	5	7	15	10	1.00	1	0.3	0	0	24.0				03710
4	0	1	0	2	1	10	8	1.00	0	0.0	0	0	18.0	45	0.927	1.133	01310
5	0	25	0	3	3	5	2	2.50	2	1.2	0	0	25.0	20	0.633	2.531	02710
6	0	0	0	0	0	15	15	1.00	0	0.0	0	0	15.5	63	1.108	0.651	32403
7	0	15	0	15	15	20	5	2.75	1	0.8	2	1	19.0	53	1.163	1.031	00010
8	0	9	0	5	5	10	5	0.50	0	0.0	0	0	17.0	55	1.064	0.871	01110
9	0	35	0	5	8	15	10	2.00	0	0.0	0	5	22.0	68	1.795	0.845	00010
10	0	30	0	3	2	8	5	1.00	0	0.0	0	0	21.0				00010
11	0	24	0	5	4	8	3	0.50	0	0.7	0	0	24.0				01710
12	0	8	0	1	1	10	9	0.50	0	0.0	0	0	15.0	78	1.328	0.375	01311
13	0	15	0	4	4	10	6	1.00	1	0.7	0	1	20.0	25	0.584	1.751	00010
14	0	34	0	4	5	5	1	2.00	1	2.0	0	0	22.0	32	0.655	0.985	00010
15	0	29	0	7	5	10	3	1.50	1	1.0	0	0	22.0	29	0.766	1.874	01910
16	0	30	0	5	3	10	5	2.75	5	4.0	3	3	26.0	17	0.571	2.786	00010
17	0	21	0	5	5	10	5	1.00	4	3.3	0	3	20.0				01710
18	0	27	1	10	10	2	-8	1.50	2	2.5	0	2	24.5				01510
19	0	9	0	10	10	15	5	1.25	2	1.2	0	0	22.0	36	0.950	1.690	01910
20	0	13	0	10	10	15	5	2.00	2	1.0	1	0	26.0	17	0.571	2.786	02810
21	0	1	0	2	2	10	8	0.50	0	0.0	0	0	19.0	54	1.185	1.009	01201
22	0	18	0	10	10	10	0	2.50	2	1.2	2	0	26.0	30	1.007	2.350	01310
23	0	21	0	10	10	10	0	2.00	1	1.0	0	0	26.0	13	0.436	2.921	01910
24	0	0	0	0	0	0	0	0.50	0	0.0	0	0	12.0				00003
25	No observation																
26	0	22	0	5	5	5	0	1.75	0	0.3	0	0	25.0	17	0.538	2.626	01710
27	0	20	0	5	5	5	0	1.00	0	0.5	0	0	26.0	10	0.336	3.021	04102
28	0	13	0	7	10	10	3	2.50	0	1.3	0	0	27.0	12	0.427	3.133	01112
29	0	3	0	0	1	5	5	0.50	1	0.0	0	0	17.0	37	0.716	1.219	01310

APPENDIX TABLE 9-1. *Continued.*

Day	P1	F1	P2	G1	G2	GX	G3	E1	P3	P4	S1	P5	T	RH	VP	SD	PWDSC
30	0	11	0	4	4	10	6	1.50	1	0.2	0	0	18.0	24	0.495	1.566	01110
31	0	10	0	2	2	5	3	0.50	0	0.0	0	0	19.5				03310
32	0	4	0	2	2	10	8	0.25	0	0.0	0	0	17.5	30	0.599	1.398	00010
33	0	11	0	4	3	3	-1	0.75	0	0.5	0	0	22.5	20	0.545	2.178	01910
34	0	13	0	4	3	10	6	1.50	0	0.7	2	0	25.0	16	0.506	2.657	01110
35	0	12	0	3	4	10	7	1.00	2	0.3	0	0	24.0	9	0.268	2.712	02310
36	0	0	0	0	0	00	0	0.00	0	0.0	0	0	9.5	93	1.103	0.083	32403
37	0	1	0	0	0	4	4	1.00	0	0.0	0	0	12.5	65	0.941	0.506	00011
38	0	11	0	15	15	15	0	1.00	1	0.0	0	0	17.0				01910
39	0	2	0	4	5	10	6	2.00	0	0.3	0	0	22.0				03111
40	0	10	0	5	3	7	2	2.00	1	0.3	1	0	22.5	31	0.844	1.879	00010
41	0	21	0	3	5	5	2	2.50	1	1.7	0	0	28.0	21	0.793	2.982	03110
42	0	19	0	5	5	10	5	2.00	0	1.0	0	0	20.0	54	1.261	1.074	00010
43	0	17	0	5	5	20	15	2.75	2	1.7	1	0	25.0	38	1.202	1.961	00011
44	0	44	0	10	10	20	10	3.00	5	2.2	3	1	30.0	22	0.932	3.306	00012
45	0	24	0	10	10	10	0	3.25	5	4.7	2	0	34.0				01712
46	0	1	0	0	2	10	10	0.50	0	0.0	0	0	15.0				02203
47	0	1	0	0	0	5	5	0.00	0	0.0	0	0	9.0	87	0.997	0.149	00003
48	0	7	0	0	0	5	5	0.50	0	0.0	0	0	12.0	54	0.168	1.232	01501
49	0	15	0	15	15	25	10	1.00	0	0.7	0	0	17.0	39	0.754	1.180	03311
50	0	18	0	10	10	20	10	2.50	0	0.2	0	0	23.0	20	0.561	2.244	02311
51	0	19	0	5	5	10	5	2.50	0	1.0	0	0	22.0	45	1.188	1.452	01110
52	No observation																
53	No observation																
54	0	13	0	2	3	20	18	1.75	0	0.0	0	0	18.0	73	1.504	0.556	01102
55	0	14	0	5	5	20	15	2.00	0	0.7	0	0	23.0	42	1.178	1.627	02511
56	0	8	0	2	2	20	18	1.25	0	0.0	0	0	20.0	54	1.261	1.074	01902
57	0	29	0	100	100	100	0	2.00	5	4.0	0	0	27.0	73	2.510	0.961	00012
58	0	24	1	25	20	30	5	1.50	0	1.0	0	0	25.0	45	1.424	1.740	01510
59	0	17	0	25	25	30	5	2.00	0	1.0	0	0	24.0				01910
60	0	28	0	15	15	20	5	3.00	0	2.0	2	1	31.0				00010
61	0	7	0	15	15	15	0	2.25	0	0.3	0	0	21.0				02111
62	0	20	0	15	15	20	5	1.50	0	1.3	0	0	22.0	42	1.109	1.531	01910
Colony C																	
1	0	58	0	3	1	5	2	3.00	5	8.3	0	3	17.0	26	0.503	1.432	00010
2	0	67	0	10	7	30	20	3.0	15	38.0	5	30	22.0	27	0.713	1.927	01510
3	0	42	6	10	10	20	10	4.00	10	35.0	5	30	24.0				03710
4	0	28	2	10	5	20	10	3.00	5	29.0	2	30	18.0	45	0.927	1.133	01310
5	0	52	10	15	15	30	15	4.50	20	140.0	15	50	25.0	20	0.633	2.531	02710
6	0	6	0	1	3	20	19	3.00	10	9.0	2	15	15.5	63	1.108	0.651	32403
7	0	39	5	15	12	35	20	4.00	30	53.0	5	50	19.0	53	1.163	1.031	00010
8	0	18	10	10	10	50	40	4.50	20	92.0	5	100	17.0	55	1.064	0.871	01110
9	5	66	17	30	20	35	5	4.50	30	65.0	0	50	22.0	68	1.795	0.845	00010
10	0	52	8	10	10	25	15	4.50	50	65.0	0	50	21.0				00010
11	10	53	20	20	20	15	5	4.75	50	100.0	10	100	24.0				01710
12	0	19	7	15	5	20	5	4.00	15	52.0	0	50	15.0	78	1.328	0.375	01311
13	10	61	20	20	20	25	5	4.50	50	90.0	5	80	20.0	25	0.584	1.751	00010

APPENDIX TABLE 9-1. *Continued.*

Day	P1	F1	P2	G1	G2	GX	G3	E1	P3	P4	S1	P5	T	RH	VP	SD	PWDSC
14	5	50	20	15	15	35	20	4.50	100	108.0	1	100	22.0	32	0.655	0.985	00010
15	20	60	30	30	30	50	20	4.75	100	108.0	5	100	22.0	29	0.766	1.874	01910
16	10	61	15	20	20	100	80	4.75	50	400.0	20	200	26.0	17	0.571	2.786	00010
17	5	37	12	20	20	30	10	4.00	50	97.0	0	50	20.0				01710
18	20	43	67	45	50	100	55	4.75	300	400.0	20	200	24.5				01510
19	10	35	18	25	10	25	0	4.00	50	76.0	0	75	22.0	36	0.950	1.690	01910
20	20	53	50	30	25	30	0	4.75	100	167.0	0	100	26.0	17	0.571	2.786	02810
21	0	27	8	25	20	25	0	4.00	50	62.0	0	50	19.0	54	1.185	1.009	01201
22	20	34	40	30	20	30	0	4.75	100	225.0	5	200	26.0	30	1.007	2.350	01310
23	5	29	18	20	10	15	5	4.50	100	121.0	0	100	26.0	13	0.436	2.921	01910
24	1	61	5	3	1	10	7	3.25	10	12.0	2	20	12.0				00003
25	No observation																
26	3	32	18	15	15	10	5	4.00	50	121.0	0	100	25.0	17	0.538	2.626	01710
27	5	30	10	10	5	10	0	4.00	50	49.0	0	30	26.0	10	0.336	3.021	04102
28	10	57	12	25	25	25	0	4.25	50	62.0	2	40	27.0	12	0.427	3.133	01112
29	0	16	1	10	7	30	20	3.50	10	37.0	0	50	17.0	37	0.716	1.219	01310
30	0	26	1	3	5	20	17	3.75	10	48.0	0	40	18.0	24	0.495	1.566	01110
31	2	13	2	15	15	20	5	3.50	15	33.0	0	30	19.5				03310
32	0	17	0	10	10	20	10	3.50	30	38.0	2	30	17.5	30	0.599	1.398	00010
33	10	31	18	30	20	30	0	4.00	50	88.0	2	100	22.5	20	0.545	2.178	01910
34	10	42	23	25	15	15	-10	4.00	50	108.0	0	100	25.0	16	0.506	2.657	01110
35	2	32	6	15	15	15	0	4.00	30	62.0	0	50	24.0	9	0.268	2.712	02310
36	0	4	0	0	0	25	25	3.50	0	0.5	0	4	9.5	93	1.103	0.083	32403
37	0	13	0	1	2	20	19	3.75	2	6.3	0	10	12.5	65	0.941	0.506	00011
38	3	30	2	10	10	50	40	4.00	10	85.0	0	100	17.0				01910
39	5	22	10	20	20	500	480	4.50	80	500.0	30	100	22.0				03111
40	10	58	20	25	20	30	5	4.25	20	39.0	0	100	22.5	31	0.844	1.879	00010
41	5	53	10	15	5	20	5	4.50	20	63.0	0	75	28.0	21	0.793	2.982	03110
42	5	38	10	15	10	25	10	4.00	20	77.0	0	80	20.0	54	1.261	1.074	00010
43	2	43	7	15	15	25	10	4.50	30	105.0	0	50	25.0	38	1.202	1.961	00011
44	15	50	30	10	10	30	20	4.75	150	500.0	10	200	30.0	22	0.932	3.306	00012
45	30	70	83	300	200	300	0	4.75	200	500.0	20	300	34.0				01712
46	0	4	0	3	1	25	22	3.25	5	12.0	0	5	15.0				02203
47	0	1	0	0	0	25	25	3.00	0	0.5	0	5	9.0	87	0.997	0.149	00003
48	0	1	0	0	0	25	25	3.00	0	2.7	0	10	12.0	54	0.168	1.232	01501
49	2	38	4	20	5	20	0	3.50	2	18.0	10	20	17.0	39	0.754	1.180	03311
50	0	29	5	20	20	40	20	4.25	20	71.0	10	75	23.0	20	0.561	2.244	02311
51	5	52	20	100	50	30	-70	3.50	50	125.0	0	150	22.0	45	1.188	1.452	01110
52	No observation																
53	No observation																
54	0	16	1	5	3	15	10	3.25	5	8.3	0	10	18.0	73	1.504	0.556	01102
55	5	34	1	10	10	20	10	3.75	0	18.3	0	10	23.0	42	1.178	1.627	02511
56	0	18	0	5	5	20	15	3.75	10	19.0	0	20	20.0	54	1.261	1.074	01902
57	0	78	3	30	25	100	70	4.00	30	79.0	10	50	27.0	73	2.510	0.961	00012
58	0	51	3	15	5	10	-5	3.25	5	15.0	0	10	25.0	45	1.424	1.740	01510
59	0	38	0	20	20	24	4	4.00	15	40.0	0	10	24.0				01910
60	10	78	5	32	25	50	18	4.50	30	100.0	20	75	31.0				00010
61	0	23	0	5	3	15	10	3.00	5	7.5	0	5	21.0				02111
62	0	42	1	5	5	5	0	3.00	1	14.0	0	5	22.0	42	1.109	1.531	01910

APPENDIX TABLE 9-1. *Continued.*

Day	P1	F1	P2	G1	G2	GX	G3	E1	P3	P4	S1	P5	T	RH	VP	SD	PWDSC
								Colony D									
1	0	16	0	0	0	5	5	0.00	0	0.0	0	0	17.0	26	0.503	1.432	00010
2	0	20	0	0	1	8	8	1.00	2	5.3	0	5	22.0	27	0.713	1.927	01510
3	0	19	0	0	0	1	1	0.50	0	0.2	0	0	24.0				03710
4	0	1	0	0	0	4	4	0.50	0	0.0	0	0	18.0	45	0.927	1.133	01310
5	0	13	0	1	1	6	5	1.50	0	1.7	0	0	25.0	20	0.633	2.531	02710
6	0	6	0	0	0	25	25	0.00	0	0.0	0	0	15.5	63	1.108	0.651	32403
7	0	19	0	4	3	4	0	2.00	0	0.5	0	0	19.0	53	1.163	1.031	00010
8	0	6	0	1	1	10	9	0.50	0	1.0	0	0	17.0	55	1.064	0.871	01110
9	0	38	0	1	2	5	4	3.00	5	4.7	3	1	22.0	68	1.795	0.845	00010
10	0	10	0	1	0	10	9	1.00	0	0.2	0	0	21.0				00010
11	0	11	0	1	0	5	4	2.00	3	5.2	0	2	24.0				01710
12	0	3	0	0	0	10	10	0.50	0	0.3	0	0	15.0	78	1.328	0.375	01311
13	0	4	0	0	0	10	10	0.50	0	1.5	1	1	20.0	25	0.584	1.751	00010
14	0	5	0	0	0	5	5	1.00	3	3.8	1	2	22.0	32	0.655	0.985	00010
15	0	9	0	1	2	10	9	1.50	1	2.3	0	0	22.0	29	0.766	1.874	01910
16	0	28	0	3	2	5	2	2.00	5	9.2	1	5	26.0	17	0.571	2.786	00010
17	0	6	0	0	0	10	10	1.50	2	2.7	0	2	20.0				01710
18	0	13	0	2	1	5	3	1.50	1	4.0	0	3	24.5				01510
19	0	6	0	3	3	10	7	2.00	2	4.2	0	0	22.0	36	0.950	1.690	01910
20	0	6	0	2	2	10	8	2.50	2	6.7	0	0	26.0	17	0.571	2.786	02810
21	0	2	0	1	0	20	19	1.25	1	1.3	0	0	19.0	54	1.185	1.009	01201
22	0	6	0	2	2	2	0	2.75	2	7.3	0	0	26.0	30	1.007	2.350	01310
23	0	9	0	2	2	5	3	1.75	3	5.2	0	0	26.0	13	0.436	2.921	01910
24	0	1	0	0	0	8	8	0.00	0	0.0	0	0	12.0				00003
25	No observation																
26	0	8	0	1	1	2	1	2.00	0	1.8	0	0	25.0	17	0.538	2.626	01710
27	0	5	0	2	2	3	1	1.50	3	3.0	0	2	26.0	10	0.336	3.021	04102
28	0	3	0	3	0	1	-2	2.50	0	0.3	0	0	27.0	12	0.427	3.133	01112
29	0	2	0	0	0	1	1	0.50	0	0.0	0	0	17.0	37	0.716	1.219	01310
30	0	2	0	0	0	1	1	0.00	0	0.0	0	0	18.0	24	0.495	1.566	01110
31	0	3	0	0	0	13	13	0.75	0	0.0	0	0	19.5				03310
32	0	1	0	2	0	5	3	1.50	0	0.5	0	0	17.5	30	0.599	1.398	00010
33	0	3	0	0	0	0	0	2.00	1	2.0	0	0	22.5	20	0.545	2.178	01910
34	0	4	0	1	1	0	-1	2.00	3	6.3	0	5	25.0	16	0.506	2.657	01110
35	0	1	0	0	0	0	0	2.50	5	2.2	0	0	24.0	9	0.268	2.712	02310
36	0	0	0	0	0	0	0	0.00	0	0.0	0	0	9.5	93	1.103	0.083	32403
37	0	0	0	0	0	5	5	0.00	0	0.0	0	0	12.5	65	0.941	0.506	00011
38	0	2	0	0	0	4	4	0.25	0	0.8	0	0	17.0				01910
39	0	3	0	0	1	0	0	1.25	2	4.8	0	0	22.0				03111
40	0	2	0	1	1	0	-1	0.75	0	0.8	0	0	22.5	31	0.844	1.879	00010
41	0	5	0	1	1	0	-1	2.00	2	2.3	0	0	28.0	21	0.793	2.982	03110
42	0	2	0	0	0	8	8	1.00	1	0.7	0	0	20.0	54	1.261	1.074	00010
43	0	4	0	0	0	5	5	2.00	1	2.0	0	0	25.0	38	1.202	1.961	00011
44	0	6	0	2	1	2	0	2.00	2	3.7	0	0	30.0	22	0.932	3.306	00012
45	0	13	0	2	1	1	1	3.00	10	16.0	3	3	34.0				01712
46	0	1	0	0	0	5	5	0.00	0	0.0	0	0	15.0				02203

APPENDIX TABLE 9-1. *Continued.*

Day	P1	F1	P2	G1	G2	GX	G3	E1	P3	P4	S1	P5	T	RH	VP	SD	PWDSC
47	0	1	0	0	0	1	1	0.00	0	0.0	0	0	9.0	87	0.997	0.149	00003
48	0	0	0	0	0	0	0	0.00	0	0.0	0	0	12.0	54	0.168	1.232	01501
49	0	3	0	0	1	0	0	0.50	0	0.0	0	0	17.0	39	0.754	1.180	03311
50	0	5	0	1	1	15	14	0.75	0	0.0	0	0	23.0	20	0.561	2.244	02311
51	0	10	0	2	2	5	3	1.50	0	0.7	0	0	22.0	45	1.188	1.452	01110
52	No observation																
53	No observation																
54	0	1	0	0	0	20	20	0.50	0	0.0	0	0	18.0	73	1.504	0.556	01102
55	0	4	0	0	0	15	15	1.75	0	0.8	0	0	23.0	42	1.178	1.627	02511
56	0	1	0	0	0	20	20	0.25	0	0.0	0	0	20.0	54	1.261	1.074	01902
57	0	6	0	1	2	10	9	1.50	1	0.3	0	0	27.0	73	2.510	0.961	00012
58	0	4	0	0	0	5	5	1.25	0	0.7	0	0	25.0	45	1.424	1.740	01510
59	0	5	0	0	0	5	5	1.50	0	0.6	0	0	24.0				01910
60	0	15	0	0	0	2	2	2.00	0	4.0	0	1	31.0				00010
61	0	1	0	2	3	10	8	1.00	0	0.0	0	0	21.0				02111
62	0	6	0	0	0	5	5	1.00	0	0.0	0	0	22.0	42	1.109	1.531	01910
								Colony E									
1	0	12	0	4	3	5	1	1.00	0	0.0	0	0	17.0	26	0.503	1.432	00010
2	0	35	0	2	1	7	5	0.50	0	0.0	0	0	22.0	27	0.713	1.927	01510
3	0	25	0	1	3	5	4	0.50	0	0.3	0	0	24.0				03710
4	0	5	0	1	1	12	11	0.50	0	0.0	0	0	18.0	45	0.927	1.133	01310
5	0	19	0	4	5	5	1	1.50	0	0.7	0	0	25.0	20	0.633	2.531	02710
6	0	1	0	1	0	25	24	0.50	0	0.0	0	2	15.5	63	1.108	0.651	32403
7	0	32	1	5	5	8	3	1.50	1	0.3	1	1	19.0	53	1.163	1.031	00010
8	0	4	0	0	0	10	10	1.00	0	0.0	0	0	17.0	55	1.064	0.871	01110
9	0	33	1	3	3	5	2	2.00	3	1.8	0	0	22.0	68	1.795	0.845	00010
10	0	22	0	3	2	6	3	2.00	0	0.7	0	0	21.0				00010
11	0	13	0	0	0	2	2	2.00	0	0.7	0	0	24.0				01710
12	0	7	0	2	4	15	13	0.50	0	1.5	0	0	15.0	78	1.328	0.375	01311
13	0	32	0	3	3	3	0	1.50	2	1.0	0	0	20.0	25	0.584	1.751	00010
14	0	39	0	5	5	5	0	2.50	1	0.5	0	0	22.0	32	0.655	0.985	00010
15	0	21	0	2	5	5	3	1.00	0	0.8	0	0	22.0	29	0.766	1.874	01910
16	0	25	0	4	5	5	1	2.00	0	2.7	0	0	26.0	17	0.571	2.786	00010
17	0	8	0	2	3	5	3	1.50	0	0.0	0	0	20.0				01710
18	0	13	0	3	1	0	-3	1.00	0	0.0	0	0	24.5				01510
19	0	20	0	5	5	5	0	1.25	2	0.7	0	0	22.0	36	0.950	1.690	01910
20	0	15	0	7	5	5	-2	1.75	5	1.7	1	0	26.0	17	0.571	2.786	02810
21	0	5	0	5	5	10	5	1.50	2	0.8	2	0	19.0	54	1.185	1.009	01201
22	0	16	0	4	4	4	0	2.50	5	2.5	0	0	26.0	30	1.007	2.350	01310
23	0	14	0	4	5	5	1	1.50	0	0.7	0	0	26.0	13	0.436	2.921	01910
24	0	1	0	0	0	10	10	0.25	0	0.0	0	0	12.0				00003
25	No observation																
26	0	12	0	3	3	5	2	1.75	3	0.5	0	0	25.0	17	0.538	2.626	01710
27	0	17	0	5	5	5	0	1.75	2	1.2	0	0	26.0	10	0.336	3.021	04102
28	0	13	0	5	8	12	7	3.50	5	1.3	0	0	27.0	12	0.427	3.133	01112
29	0	7	0	4	4	5	1	0.75	1	0.2	0	0	17.0	37	0.716	1.219	01310
30	0	5	0	2	2	10	8	0.00	0	0.3	0	0	18.0	24	0.495	1.566	01110

APPENDIX TABLE 9-1. *Continued.*

Day	P1	F1	P2	G1	G2	GX	G3	E1	P3	P4	S1	P5	T	RH	VP	SD	PWDSC
31	0	8	0	2	3	6	4	0.50	0	0.5	0	0	19.5				03310
32	0	9	0	6	4	6	0	1.00	0	1.0	0	0	17.5	30	0.599	1.398	00010
33	0	6	0	1	3	3	2	0.50	0	0.2	0	0	22.5	20	0.545	2.178	01910
34	0	13	0	3	3	3	0	1.50	0	0.0	0	0	25.0	16	0.506	2.657	01110
35	0	9	0	5	5	5	0	0.75	1	0.2	0	0	24.0	9	0.268	2.712	02310
36	0	0	0	0	0	20	20	0.00	0	0.0	0	0	9.5	93	1.103	0.083	32403
37	0	13	0	2	1	15	13	0.50	0	0.0	0	0	12.5	65	0.941	0.506	00011
38	0	4	0	5	5	10	5	1.25	0	0.5	0	0	17.0				01910
39	0	6	0	2	3	10	8	2.25	0	4.8	0	2	22.0				03111
40	0	30	0	2	1	1	-1	2.25	0	0.2	0	0	22.5	31	0.844	1.879	00010
41	0	20	0	4	2	0	-4	1.25	1	1.0	0	0	28.0	21	0.793	2.982	03110
42	0	11	0	3	5	6	3	1.25	0	0.0	0	0	20.0	54	1.261	1.074	00010
43	0	16	0	5	5	5	0	1.50	1	1.2	0	0	25.0	38	1.202	1.961	00011
44	0	21	0	5	3	10	5	2.00	5	0.3	0	0	30.0	22	0.932	3.306	00012
45	0	32	0	5	5	10	5	3.00	5	8.2	0	2	34.0				01712
46	0	2	0	2	2	8	6	1.00	0	0.2	0	0	15.0				02203
47	0	0	0	0	0	15	15	0.00	0	0.0	0	0	9.0	87	0.997	0.149	00003
48	0	0	0	1	1	20	19	0.00	0	0.0	0	0	12.0	54	0.168	1.232	01501
49	0	7	0	7	7	10	3	1.00	0	0.2	0	0	17.0	39	0.754	1.180	03311
50	0	11	0	2	3	15	13	1.50	0	0.2	0	0	23.0	20	0.561	2.244	02311
51	0	12	0	2	2	10	8	0.75	0	0.0	0	0	22.0	45	1.188	1.452	01110
52	No observation																
53	No observation																
54	0	2	0	0	0	10	10	0.25	0	0.0	0	0	18.0	73	1.504	0.556	01102
55	0	9	0	3	2	10	7	1.50	0	0.5	0	0	23.0	42	1.178	1.627	02511
56	0	4	0	1	1	0	-1	0.00	0	0.0	0	0	20.0	54	1.261	1.074	01902
57	0	16	0	2	2	5	3	1.00	0	0.0	0	0	27.0	73	2.510	0.961	00012
58	0	8	0	1	2	3	2	1.00	0	0.0	0	0	25.0	45	1.424	1.740	01510
59	0	12	0	1	2	1	0	1.00	0	0.0	0	0	24.0				01910
60	0	32	0	3	3	3	0	2.00	0	0.0	0	0	31.0				00010
61	0	4	0	3	1	2	-1	0.50	0	0.0	0	0	21.0				02111
62	0	10	0	0	1	1	1	1.00	0	0.2	0	0	22.0	42	1.109	1.531	01910

APPENDIX TABLE 9-2. Descriptive statistics for breath test variables of colonies A-E. Variables are defined in section 9.1.3.

Behaviour variable/ Colony		Mean	Standard deviation	Minimum	Maximum
	A	0.00	0.00	0.00	0.00
	B	0.00	0.00	0.00	0.00
P1	C	4.75	6.67	0.00	30.00
	D	0.00	0.00	0.00	0.00
	E	0.00	0.00	0.00	0.00
	A	24.92	16.10	0.00	59.00
	B	16.29	11.36	0.00	49.00
F1	C	37.81	19.52	1.00	78.00
	D	6.59	7.12	0.00	38.00
	E	13.53	10.05	0.00	39.00
	A	0.10	0.44	0.00	3.00
	B	0.05	0.22	0.00	1.00
P2	C	11.76	15.97	0.00	83.00
	D	0.00	0.00	0.00	0.00
	E	0.03	0.18	0.00	1.00
	A	2.25	2.41	0.00	15.00
	B	7.97	13.54	0.00	100.00
G1	C	21.54	39.72	0.00	300.00
	D	0.78	1.02	0.00	4.00
	E	2.83	1.85	0.00	2.00
	A	2.32	2.45	0.00	15.00
	B	8.10	13.58	0.00	100.00
G2	C	16.27	26.48	0.00	200.00
	D	0.68	0.92	0.00	3.00
	E	2.92	1.91	0.00	8.00
	A	73.39	5.79	60.00	99.00
	B	75.20	5.11	62.00	88.00
G3	C	89.54	64.16	0.00	550.00
	D	75.39	5.88	68.00	95.00
	E	74.40	5.80	66.00	94.00
	A	1.28	0.88	0.00	3.00
	B	1.50	0.84	0.00	3.25
E1	C	3.96	0.57	3.00	4.75
	D	1.22	0.85	0.00	3.00
	E	1.21	0.77	0.00	3.50

APPENDIX TABLE 9-2. *Continued.*

Behaviour variable/ Colony		Mean	Standard deviation	Minimum	Maximum
	A	1.73	2.23	0.00	10.00
	B	0.86	1.41	0.00	5.00
P3	C	40.25	51.90	0.00	300.00
	D	1.07	1.82	0.00	10.00
	E	0.76	1.50	0.00	5.00
	A	3.50	5.54	0.00	36.00
	B	0.85	1.08	0.00	4.70
P4	C	94.99	121.58	0.50	500.00
	D	2.06	2.90	0.00	16.00
	E	0.68	1.30	0.00	8.20
	A	0.09	0.34	0.00	2.00
	B	0.34	0.78	0.00	3.00
S1	C	3.78	6.67	0.00	30.00
	D	0.15	0.58	0.00	3.00
	E	0.07	0.31	0.00	2.00
	A	2.27	4.78	0.00	30.00
	B	0.29	0.89	0.00	5.00
P5	C	65.80	60.26	3.00	300.00
	D	0.54	1.28	0.00	5.00
	E	0.12	0.46	0.00	2.00