

**CONSEQUENCES OF HABITAT FRAGMENTATION FOR THE
POLLINATION OF WILDFLOWERS IN MOIST UPLAND
GRASSLANDS OF KWAZULU-NATAL**

By

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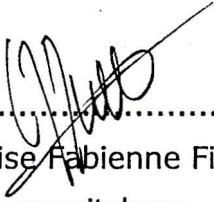
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Preface

These studies, carried out under the supervision of Dr Steven D. Johnson in the School of Botany and Zoology, University of Natal, Pietermaritzburg, represent original work on my part and have not been submitted in any form for any degree or diploma to any other University. Where use has been made of the work of others it is duly acknowledged in the text.



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To my boyfriend, John Harris, thank you for your understanding, love and support during some very hard times apart.

To Mom and Dad, thank you for your endless emotional support, encouragement and unconditional love, and for giving me the opportunities to come this far.

Frontispiece

The author and Ronny Alexandersson hand-pollinating *Gladiolus longicollis* flowers at site M1, Gilboa Estate.



Abstract

Large areas of moist upland grassland in KwaZulu-Natal are severely fragmented due to large scale farming of exotic trees. The aims of this thesis were to determine whether habitat fragmentation of these grasslands has a detrimental effect on plant-pollinator interactions and hence the reproductive output of the wildflower species occurring there and whether the magnitude of this effect can be predicted by breeding and pollination system characteristics.

The 24 wildflower species included in this study appear to support a rich and diverse pollinator community, including long-tongued solitary bees, long-tongued flies, hawkmoths and sunbirds. Two thirds of the wildflower species appear relatively specialised in terms of pollination, with six species entirely dependent either on a single species or a specific functional type of pollinator for pollination. The majority of wildflower species (90%) were found to be incapable of autonomous self-pollination and thus dependent on pollinators for fruit and seed set. At least six species are obligately xenogamous. Little evidence was found for pollen limitation in undisturbed moist upland grassland, suggesting that these grasslands are characterised by high levels of pollinator activity. Greater levels of pollen limitation of reproductive output in habitat fragments was evident in two species, suggesting that depressed reproductive output in habitat fragments may be the consequence of a decrease in the quantity and/or quality of pollinator services.

Significant detrimental effects of habitat fragmentation on reproductive output were evident in two thirds of the wildflower species, with 94% of the species exhibiting overall declines in seed set per flower from the continuous grassland site to the habitat fragments. The median decline in seed set per flower for the wildflower species was found to be 33.0%. Significantly more species experienced overall declines in reproductive output than would have been expected by chance alone. Only specificity of the pollination interaction in terms of number of pollinator taxa involved was found to be significantly related to percentage change in seed set from continuous to fragment habitats. This effect was diminished when other factors were included in a multiple regression. Results support Bond's (1994) hypothesis that degree of specificity in pollination systems is important in determining extinction risk of a given plant species. Declines in reproductive output of a range of wildflower species in grassland habitat fragments may affect the local persistence of these populations, particularly if recruitment is seed-limited.

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'What escapes the eye, however, is a much more insidious kind of extinction: the extinction of ecological interactions. Many of the remaining participants of these interactions will probably hold on for many years, but they constitute little more than a haphazard, semi-self-sustaining zoo and botanical garden.' Janzen (1974).

CHAPTER 1

INTRODUCTION

HABITAT FRAGMENTATION AND PLANT-POLLINATOR MUTUALISMS

There is a consensus in the scientific community that 'the current massive degradation of habitat and extinction of many of the Earth's biota is unprecedented and is taking place on a catastrophically short timescale' (Novacek & Cleland 2001). There is an urgent need to understand the processes by which these extinctions are taking place in order to make any useful attempts at mitigation. One of the more important drivers of environmental change is the increased anthropogenic use of land (Sala *et al.* 2000). Under the pressures of an exploding human population and the large scale expansion of agriculture, industrial development and housing, large expanses of natural vegetation are being destroyed, leaving only isolated remnants of the indigenous flora and fauna surrounded by a transformed matrix of alien vegetation or development. This fragmentation of natural habitat is considered to be one of the greatest threats to terrestrial biodiversity worldwide (Jennersten 1988; Rathcke & Jules 1993; Turner 1996).

A more insidious threat resulting from the fragmentation of habitat is its potential to disrupt vital ecological interactions (Janzen 1974). Individual species do not exist in isolation but are always part of a complex ecological web in which the different organisms interact with one another as predators, prey, competitors or mutualists (Didham *et al.* 1996; Memmott 1999). Disruptions to these intricate relationships, such as those between a plant species and its pollinators, may often go unnoticed because of the longevity of the surviving partners. Yet the repercussions of the disruption of plant-pollinator mutualisms are potentially serious; pollination is important as the first stage in the sexual reproduction of angiosperms and as such is essential to primary productivity and the preservation of biodiversity in terrestrial ecosystems (Kevan 1999). By some estimates, over 90% of modern angiosperms are pollinated by animals (Buchman

& Nabhan 1996). The substantial connectance of plant-pollinator mutualisms in this web of interactions means the loss of component species may elicit additional losses, perhaps even cascades of extinction (Rathcke & Jules 1993; Olesen & Jain 1994; Memmott 1999).

Despite the vital role played by pollination interactions in the maintenance of biodiversity, they are increasingly under threat from humans through habitat fragmentation, agriculture and grazing, pesticide and herbicide use, the introduction of non-native species and the prospect of global climatic change (Kearns *et al.* 1998). Claims of the disruption of pollination systems as a result of human activities have been made on every continent except Antarctica (Allen-Wardell *et al.* 1998; Kearns *et al.* 1998). Widespread declines have been detected among central European bee faunas (Westrich 1996). Worldwide nearly 200 species of wild vertebrate pollinators may be on the verge of extinction (Nabhan 1996) and there are numerous documented examples of plant-pollinator mutualisms in which one or more of the partners have actually gone extinct (Weller 1994; Ladley & Kelly 1995; Mawdsley *et al.* 1998; Robertson *et al.* 1999; Elmqvist 2000).

Many of the claims of pollinator declines are based on short term pollinator surveys, or on evidence of pollination deficits in plants, both of which may simply reflect the substantial natural or short term variation inherent in plant-pollinator systems rather than genuine trends in abundance and diversity (Roubik 2001; Thomson 2001; Williams *et al.* 2001). An extensively replicated, long-term study of orchid bees (*Euglossini*) in the tropical moist forests in Panama for example showed that no aggregate trend in abundance occurred from 1979 to 2000 (Roubik 2001). Similarly, resampling of the bee fauna of Carlinville, Illinois, USA, nearly a century after the first survey was carried out revealed it to be remarkably persistent, despite the continued dominance of crop-farming and use of insect control tools in that region (Marlin & LaBerge 2001). Other studies carried out on bees and butterflies in cities and other highly anthropogenic environments also exemplify the resilience of some pollinator species to habitat disturbance (Edwards 1996; Klemm 1996).

The extent of native pollinator declines around the world is thus still barely understood and generalisations may be premature. The lack of strong evidence however is no cause for complacency and there is an urgent need for further investigation of the effects of anthropogenic landscape changes, such as habitat fragmentation, on plant-pollinator mutualisms.

POTENTIAL CONSEQUENCES FOR THE POLLINATOR COMMUNITY

Habitat fragmentation has the potential to disrupt the interactions between a plant and its pollinators. Plant species may experience lower and inferior pollinator visitation and depressed reproductive output in remnant habitat fragments as a result of changes in the pollinator community (Rathcke & Jules 1993). Such changes may include a decline in pollinator abundance and diversity, changes in the species composition of the pollinator fauna and changes in pollinator behaviour.

Changes in pollinator abundance and diversity

Habitat fragments are expected to support fewer pollinators than continuous habitats because of the limited availability of pollinator resources. The reduced sizes of plant populations in habitat fragments may limit the carrying capacity for pollinators in terms of nectar and/or pollen availability (Kearns and Inouye, 1997; Rathcke and Jules, 1993; Kremen and Ricketts, 2000). In sub-alpine meadows of northeastern Utah for example, the floral resources of a fragment have been found to be important in determining the number and diversity of colonizing *Bombus* queens in early summer (Bowers, 1985). Fragmentation may also result in the reduced availability of non-food resources such as rendezvous plants and nesting sites (Spears 1987; Rathcke & Jules 1993; Kremen & Ricketts 2000; Cane 2001). Specialised pollinator nesting requirements for example include specific soil attributes, spaces under rocks, old rodent burrows or holes formed in dead wood by wood-boring insects, as well as nearby mud, resins, pebbles or plant hairs which may be used to line, partition and plug nests (Cane 1991; O'Toole & Raw 1991; Kearns & Inouye 1997). A decrease in the availability of these resources may thus further limit the abundance and diversity of pollinators that a given habitat fragment can support. Because larger habitat fragments are likely to contain a greater diversity of habitat types and hence a

greater variety of pollinator resources than smaller ones, fragment size may determine the number of pollinator species able to persist within a given fragment (Rolstad 1991; Saunders *et al.* 1991; Baz & Garcia-Boyero 1995).

The holometabolous life cycles of many insect pollinators add to the complexity of supporting healthy pollinator communities within an isolated habitat fragment (Fischer 1998). A given fragment must meet the needs of all life cycle stages whose mobility and habitat requirements differ substantially (Samways 1993; Thomas 1996; Cane & Tepedino 2001). Often different arthropod life stages require resources from different habitats, all within flight range of each other (Janzen 1987; Olesen & Jain 1994; Baz & Garcia-Boyero 1995; Fischer 1998; Cane 2001). For example, the hawkmoth that feeds on the nectar from flowers of the orchid *Cynorkis uniflora* which grows on mountain sides in Madagascar is also dependent on nearby threatened forest areas in which their larval host plants occur (Nilsson *et al.* 1992). This cross habitat dependency makes a system more vulnerable to human habitat destruction; the elimination of a given habitat type from a habitat mosaic will lead not only to a loss of species breeding in those habitats, but also to a reduction in the species richness of other remaining habitat types (Janzen 1987; Olesen & Jain 1994). Unfortunately the multiple habitat needs of different groups of pollinators are seldom congruent (Fischer 1998).

The removal of natural vegetation and its replacement with cultivation during the process of fragmentation may significantly alter the fluxes of radiation, wind, water and nutrients across the landscape (Saunders *et al.* 1991). Such changes may directly affect the abundance and distribution of pollinator species, depending on the physiological tolerances of the species (Murcia 1995, 1996).

Because pollinators visit flowers while foraging for resources, a change in the size, density and isolation of plant populations may also influence the degree to which pollinators are attracted and the subsequent visitation rates (Fritz & Nilsson 1994; Ågren 1996; Groom 1998). Pollinator visitation may be lower in small populations than in large ones, leading to increased levels of pollen limitation of reproductive output and reduced population viability (the Allee effect, see also Lamont *et al.* 1993; Ågren 1996; Groom 1998). Large patches of

catnip, *Nepeta cataria* (Lamiaceae), for example not only attracted more visitors, but were also less likely to be rejected if approached (Sih & Baltus 1987). At the other end of the scale however, pollinator satiation may occur in very large populations of flowering plants, leading to a decrease in per flower visitation rates (Conner & Neumeier 1995). In *Nepeta cataria* patch size was found to be a good predictor of pollinator abundance, explaining 63% of the variation in pollinator limitation of fruit set (Sih & Baltus 1987). Increased pollen limitation of reproductive success with a decrease in population size was found in *Primula veris* and *Gentiana lutea* (rare, self-incompatible perennials) (Kéry *et al.* 2000), the tristylous herb *Lythrum salicaria* (Lythraceae) (Ågren 1996), the rare, self-incompatible herb *Rutidosia leptorrhynchoidea* (Asteraceae) (Morgan 1999), three non-rewarding orchids, *Orchis spitzelii*, *Orchis palustris* and *Anacamptis pyramidalis* (Fritz & Nilsson 1994), the almost entirely outcrossing *Dombeya acutangula* (Sterculiaceae), a native tree on La Réunion (Gigord *et al.* 1999), American ginseng, *Panax quinquefolius* (Araliaceae) (Hackney & McGraw 2001), *Banksia goodii* (Proteaceae) (Lamont *et al.* 1993) and the self-compatible herb *Clarkia concinna* (Onagraceae) (Groom 1998). In the latter two species the decline in reproductive output was especially severe with total reproductive failure experienced by five of the nine smallest populations of *B. goodii* (Lamont *et al.* 1993) and nine of the 12 tiny isolated patches of *C. concinna* (Groom 1998). A decline in reproductive success with decreasing population size is not ubiquitous however and Bigger (1999) found degree of pollen limitation to be unrelated to flowering patch size in the rare endemic *Aster curtus* (Asteraceae) in relic prairies of Pacific Northwest. Seed production and germinability were similarly found to be relatively unaffected by population size in the self-compatible *Leucochrysum albicans* subsp. *albicans* var. *tricolor* (Asteraceae) (Costin *et al.* 2001).

Although plant population density is not directly affected by the reduction in habitat area associated with fragmentation, the population may decline over the long term, leading to a lower density in a given habitat fragment. The foraging efficiency of a pollinator is related to the density of available food items (Ingvarsson & Lundberg 1995) and such changes may affect the degree to which pollinators are attracted to the flowers, independently of population size. Significant declines in per flower visitation rates and reproductive success at low

density were evident for the self-incompatible *Brassica kaber* (Brassicaceae) (Kunin 1993), *Lesquerella fendleri* (Cruciferae) (Roll *et al.* 1997) and *Delphinium nuttallianum* (Ranunculaceae) (Bosch & Waser 1999).

The degree of isolation of a given flowering plant population may influence the number and diversity of pollinators attracted to the population (Ågren 1996; Sih & Baltus 1997; Groom 1998). The quantity and quality of pollinator visitation may thus decline with increasing isolation of the habitat fragment, especially if the degree of isolation is greater than the foraging range of the pollinators. Plants in less isolated populations of the naturally patchy, self-incompatible *Calystegia collina* (Convolvulaceae) for example were more likely to receive compatible pollen during pollination than were plants in more remote populations (Wolf & Harrison 2001). Isolated patches of catnip, *Nepeta cataria*, tended to receive fewer visits and tended to show higher than expected degree of pollinator limitation (Sih & Baltus 1987). The combined effects of isolation and patch size on the naturally patchily distributed, self-compatible *Clarkia concinna* (Onagraceae) resulted in an apparent threshold for isolation, such that beyond a certain distance, pollen receipt and seed production dropped sharply, unless plants are in sufficiently large patches which attract pollinators regardless of their degree of isolation (Groom 1998). Despite criticisms by Cane (2001) that the study does not modify the pollinator habitat between the established 'islands' of potted populations of *Sinapis arvensis* (Cruciferae) and *Raphanus sativus* (Cruciferae), Steffan-Dewenter and Tscharntke (1999) found that increasing isolation of potted populations from each other and from adjacent species rich grasslands resulted both in decreased abundance and diversity of flower-visiting bees and significantly lower seed set. In a similar study however, Schulke and Waser (2001) found that the hummingbird and bumblebee pollinators of *Delphinium nuttallianum* readily flew the 400 m separating potted populations, resulting in substantial pollination in these populations. Degree of isolation from mainland or from other conspecific populations was also not found to have a significant effect on seed set on the tristylous *Lythrum salicaria* (Ågren 1996). Similarly, degree of pollen limitation of populations of the rare endemic *Aster curtus* (Asteraceae) in relic prairies of Pacific Northwest was found to be unrelated to flowering patch size or isolation (Bigger 1999).

Changes in pollinator species composition

Because certain pollinator species cope better with the changes associated with fragmentation than others, shifts in the species composition of remnant pollinator fauna may occur in addition to changes in abundance and diversity (Rathcke & Jules, 1993; Aizen & Feinsinger, 1994; Olesen & Jain, 1994). In some studies habitat fragments were even invaded by new pollinating species (Aizen & Feinsinger 1994; Elmqvist 2000). Larger populations of *Brassica nigra* (Brassicaceae) were visited by significantly greater numbers of honey bees and syrphid flies and significantly fewer small bees than small populations on a per plant basis (Conner & Neumeier 1995). These changes in species composition may cause a decrease in the efficiency with which flowers are pollinated (Groom 1998). Even relatively minor differences in pollinator composition and abundance may have a measurable demographic impact on plant populations because different species of pollinator, even within the same guild or order, are known to differ in the quantity and quality of pollen they transport to flowers of the same plant species (Herrera 2000; Thomson 2001). The efficiency of a given pollinator in effecting fertilisation of a flower is determined by a number of factors, including its morphology (feeding apparatus, hairiness, overall body size), its behaviour when visiting a flower (with respect to the probability of contacting anthers or stigmatic surfaces) and its movement patterns between flowers (the degree to which pollinators will be flower constant and the level of outcrossing achieved) (Lindsey 1984; Kunin 1997; Herrera 2000). The Mediterranean shrub *Lavandula latifolia* (Lamiaceae) is pollinated by nearly 80 species of bees, butterflies and flies (Herrera 2000). In a study carried out by Herrera (2000) flowers pollinated predominantly by butterflies and small bees were found to set proportionally more fruits, each fruit containing more seeds (which had a greater probability of producing a seedling in the field) than those pollinated by large bees and flies. This greater reproductive success was most likely the result of the greater distances flown by butterflies and small bees between consecutive flower visits, resulting in more between-plant interfloral flights and a greater deposition of outcross pollen (Herrera 2000).

Changes in pollinator behaviour

Fragmentation of habitat may bring about changes in the behaviour of remnant pollinator species. Pollinator foraging tactics change in response to changes in food availability because they behave so as to maximise their food intake per unit time spent foraging (Pyke *et al.* 1977). Pollinators may adjust flight distance between flowers in response to the volume of recently received nectar rewards (Bronstein 1995). Waddington (1983), for example, found that bumblebees leaving inflorescences with high rewards flew short distances, whereas nearby flowers were passed and relatively long flights were made after visits to nectar-poor inflorescences. Lower visitation rates in fragments may lead to an accumulation of nectar in the flowers and thus an increased likelihood that visitors encountering the patch would probe more flowers per plant, leading to higher levels of geitonogamous crosses (Kwak 1987; Ågren 1996; Bosch & Waser 1999; Cane & Tepedino 2001). Germination success of *Silene regia* (Caryophyllaceae) seeds from smaller populations was found to be low and highly variable because of reduced hummingbird visitation and reduced interplant movements in small populations (Menges 1991).

Flight behaviour of pollinators may also change in response to the boundary of the fragment itself (Aizen & Feinsinger 1994; Groom 1998). Long-distance foragers such as large robust birds, moths or bats (which may have foraging radii of 8-25 km) are important for pollen transfer between habitat fragments provided pollen is viable for long enough and sufficient pollen carryover takes place (Motten 1986; Fritz & Nilsson 1994; Berge *et al.* 1998; Corbet 2000; Murren 2002). Large bees can also travel large distances, although it is unlikely that they ever travel more than a moderate fraction of their potential flight distance (Rathcke & Jules 1993; Bronstein 1995). Smaller birds, beetles, wasps, butterflies and noctuid moths travel relatively short distances and for many species inter-fragment foraging is precluded by distances from 50 m to 1 km between fragments, thus restricting pollen flow to within the fragment (Feinsinger *et al.* 1987; Rathcke & Jules 1993; Murcia 1996; Debinski & Holt 2000). For example, the Fender's Blue butterfly was found to significantly modify its behaviour within 10-22 m from the habitat boundary (Schultz & Crone 2001). Behavioural responses to the matrix habitat differ even among closely related species (Ricketts 2001). Even pollinators that have no difficulty crossing a gap

between patches may remain within local patches and preferentially visit nearby plants within the patch (Rasmussen & Brødsgaard 1992). An increase in within-fragment pollen transfer is evident in insular populations of mustard and radish (Steffan-Dewenter & Tscharntke 1999) and in the tropical forest tree species *Shorea siamensis* (Dipterocarpaceae) and *Anacardium excelsum* (Anacardiaceae) in logged and fragmented habitats in Thailand and Costa Rica (Ghazoul & McLeish 2001).

The low availability of floral rewards facing pollinators restricted to habitat fragments may force usually specialist, constant species to feed from many more plant species than normal in each foraging bout, potentially resulting in increased pollen wastage (Kunin 1993; Bronstein 1995; Murcia 1996). Greater proportions of heterospecific flowers within a fragment may further reduce visitor constancy and hence the quality of pollinations for a given plant species (Kunin 1997).

POTENTIAL CONSEQUENCES FOR PLANT POPULATIONS

Depressed reproductive output

As mentioned above, changes in the pollinator community may result in a decline in visitation rate to flowers in remnant habitat fragments. Such inferior pollinator visitation may lead to pollen-limitation of plant reproductive output and depressed levels of seed set. Declines in seed production may in turn limit recruitment in a fragment plant population, potentially limiting population growth and the persistence of the population through time (Rathcke & Jules 1993). The abiotic changes associated with habitat fragmentation also have the potential to affect reproductive output by changing the microclimate and availability of resources in the remnant fragment (McCall & Primack 1985; Saunders et al 1991; Cunningham 2000b).

Depressed seed set or viability may also result from a decline in the quality of pollen deposited on the stigma. Changes in pollinator type or movement may change the degree of relatedness between pollen donors and receptors, and potentially decrease genetic variability and fitness via inbreeding or outbreeding depression (Menges 1991; Waser & Price 1991; Oostermeijer *et al.* 1994; Young & Merriam 1994; Fischer & Matthies 1997). The situation could be made worse should plant populations exhibit spatial genetic structure as a result of limited

dispersal of both pollen and seeds, resulting in a greater incidence of biparental inbreeding, ie pollinations between related individuals (Ellstrand & Elam 1993; Lamont *et al.* 1993; Bosch & Waser 1999). Smaller populations may suffer greater inbreeding depression than larger ones because of a reduced effectiveness of selection relative to genetic drift; deleterious recessives could become fixed by chance instead of being eliminated by selection (Ellstrand & Elam 1993). In plant populations that naturally experience high levels of inbreeding the frequency of deleterious recessive alleles may decline as they become homozygous and are purged by selection (Ellstrand & Elam 1993; Young *et al.* 1996; Van Treuren *et al.* 1994). Naturally inbreeding populations should thus be less vulnerable to inbreeding depression than typically outbreeding populations (but see Barrett & Kohn 1991).

Increased extinction risk

A decrease in the population sizes of plants either with the initial clearing of vegetation or as a result of changes in pollinator visitation or abiotic conditions may increase the short term extinction risk of the population from the effects of environmental and demographic stochasticity (Barrett & Kohn 1991). The magnitude of reduction in population size with fragmentation may be affected by the size of the remnant habitat fragment; the larger the fragment, the more likely it is that populations will be large and able to withstand the risks of local extinction (MacArthur and Wilson, 1963; 1967; Noss & Csuti 1997). Smaller fragments are thus expected to retain fewer plant and pollinator species than larger ones. Cane (unpublished data in Kremen and Ricketts, 2000) found that small habitat fragments in the Sonoran desert tended to lose members of the specialist pollinator guild. The relationship between fragment size and species abundance and diversity has been found to hold true for a number of investigations involving both plant and pollinator species (Robinson and Quinn, 1988; Aizen & Feinsinger 1994b; Summerville & Crist 2001; Collinge 2000; but see Debinski & Holt 2000).

Severe reductions in population size can create genetic bottlenecks because remaining individuals contain only a small sample of the original gene pool (Barrett & Kohn 1991; Young *et al.* 1996). Empirical data generally confirm the positive associations between population size and genetic variation (Ellstrand &

Elam 1993). Remnant populations that stay small and isolated for several generations may continue to lose genetic diversity through random genetic drift (Young *et al.* 1996). Isolation of small fragmented populations appears to play a role in reduced genetic diversity of the tropical tree *Pithecellobium elegans* (Hall *et al.* 1996). A decline in variability can affect the fitness levels of a species, as evident in *Gentiana lutea* (Gentianaceae) and *Primula veris* (Primulaceae) (Kéry *et al.* 2000) and *Ipomopsis aggregata* (Polemoniaceae) (Heschel & Paige 1995), and limits the capacity of a species to adapt to changing environmental conditions, thus increasing the long term risk of population extinction (Barrett & Kohn 1991; Heschel & Paige 1995; Kéry *et al.* 2000).

According to the theory of island biogeography (MacArthur & Wilson 1963, 1967) more isolated habitat fragments may experience higher rates of extinction because dispersal from areas of similar native vegetation is more difficult over longer distances and populations are unlikely to be 'rescued' by immigration before they reach precariously small sizes (Brown & Kodric-Brown 1997). The nature or permeability of the surrounding matrix can significantly influence the 'effective isolation' of habitat patches, rendering them more or less isolated than simple distance models indicate (Cane 2001; Ricketts 2001). Matrix permeability is related to vertical vegetation structure, light environment and temperature (Ricketts 2001). In agricultural systems the inter-fragment area is cultivated with productive crop species, often as a monoculture. A matrix planted to smaller crops may be highly permeable to pollinators (Berge *et al.* 1998). In more obstructive forms of agriculture however, the intervening matrix may represent a physical barrier to most species, except to the strongest flyers such as birds, hawkmoths and possibly butterflies (Westerbergh & Saura 1994; Brown & Hutchings 1997). For example, in the Scandinavian mountains the presence of a dense spruce forest serves as a barrier to the movement of the muscid and syrphid fly pollinators of the herb, *Silene dioica* (Westerbergh & Saura 1994). Pollinator resource availability in the matrix may also influence the degree to which pollinators are attracted to flowering plants in habitat fragments. Becker *et al.* (1991) found no consistent relationship between forest patch size and euglossine abundance in the same Amazonian forest fragments where, five years earlier, Powell and Powell (1987) had found that male euglossine bee numbers at chemical baits declined with the size of a forest patch. This change was

attributed to the growth of secondary vegetation in the matrix (previously bare clearing), providing the euglossine bees with an environment which contains diverse nest sites and rich resin sources (Becker *et al.* 1991).

Habitat fragmentation may also counteract natural landscape-scale processes, potentially leading to changes in ecosystem structure or succession and a loss of species (Hansson & Angelstam 1991; Leach & Givnish 1996; Morgan 1999b). Fire for example can be important in preventing competitive exclusion between perennial plant species and maintaining a diverse flora as a whole, and the absence of regular fires has been implicated in the loss of plant biodiversity from isolated patches of fynbos (Bond *et al.* 1988), renosterveld (Kemper *et al.* 1999) and prairie vegetation (Leach & Givnish 1996).

EVIDENCE OF DISRUPTION OF PLANT-POLLINATOR MUTUALISMS AS A RESULT OF HABITAT FRAGMENTATION

A number of investigations have shown that habitat fragmentation can indeed disrupt plant-pollinator interactions, threatening the local persistence of the constituent species. In Sweden for example, visits by butterflies and flies to the self-compatible herb, *Dianthus deltoides* (Caryophyllaceae), declined by more than 50% in meadow fragments (isolated by 200 m of agricultural fields) compared to continuous habitat, directly resulting in much lower seed set, mostly the result of self-pollination (Jennersten 1988). Lindberg and Olesen (2001) found that individuals of *Passiflora mixta* (Passifloraceae, pollinated only by the sword-billed hummingbird *Ensifera ensifera*) inside a protected reserve had significantly higher fruit set than plants growing at a farmland site 40 km away. Trees of *Spondias mombin* (Anacardiaceae) found in small patches suffered significant reduction in fruit production and seed germination relative to large fragments or continuous forest (Nason & Hamrick 1977). Populations of the epiphytic orchid *Catasetum viridiflavum* on 10 islands created by construction of Panama canal were found to produce significantly less fruit per flower than populations at five sites in nearby large tracts of mainland forest (Murren 2002). Trees of *Enterolobium cyclocarpum* (Guanacaste) in continuous forest in Costa Rica are more likely to have pollen deposited on the stigma and six times more

likely to produce fruit and seeds with higher fitness levels (Rocha & Aguilar 2001). Significantly less pollen was received and fruit set was lower in highly fragmented linear vegetation remnants than in nearby reserves for two relatively common Australian shrub species, the insect-pollinated *Acacia brachybotrya* (Mimosaceae) and the bird-pollinated *Eremophila glabra* (Myoporaceae) (Cunningham 2000). Plants of a number of rare plant species in small and degraded fragments of pine rockland habitat in southern Florida experienced significantly lower pollinator visitation rates than those in intact sites (Koptur, unpublished data in Neal 1998). Reduced abundance and unpredictability of island pollinators resulted in reduced pollen dispersal, and occasionally in reduced fruit or seed set, in island populations of *Centrosema virginianum* (Fabaceae) and *Opuntia stricta* (Cactaceae) separated by less than 10 km of water from larger island or mainland populations on the western coast of Florida (Spears 1987).

Many of the above studies are based on investigations of one or two species and are thus limited in their interpretation; conclusions cannot be made regarding the generality of the findings or the contrasting responses of different plant and pollinator species to habitat fragmentation. Only a few multi-species studies have been conducted. Aizen and Feinsinger (1994) investigated the effects of habitat fragmentation on pollination and reproductive success of 16 plant species representing a range of pollination systems, breeding systems and growth forms in Argentinian Chaco dry forest. Number of pollen tubes per style, fruit set and seed production were found to decline with increasing fragmentation in 81, 73 and 79% of the species respectively. In three species the number of pollen tubes produced per pollen grain on the stigma declined with fragmentation. Fragment size was found to play a role; average percentage decline in pollination levels from continuous forest to small fragments (< 1 ha) was significantly greater than that from continuous forest to large fragments (> 2 ha) (Aizen & Feinsinger 1994). Median decreases in pollination levels and seed output from forest to fragments, isolated by only few tens or hundreds of metres, approached 20 percent (Aizen & Feinsinger 1994). An investigation of four plant species in the mallee woodlands of central New South Wales, Australia, revealed a significant decrease in reproductive output with fragmentation in two of the species, while no consistent effect was evident for a third species and another exhibited a significant increase in whole-plant seed production in the habitat

fragments (Cunningham 2000b). Fragmentation effects on pollination were apparently absent for 14 cloud forest plant species in the Colombian southwestern Andes (Murcia 1996).

DIFFERENTIAL VULNERABILITY OF PLANT SPECIES TO HABITAT FRAGMENTATION

Theory and empirical evidence quoted throughout this review suggest that plants and pollinators have attributes that lead to differential responses to the effects of fragmentation. Factors potentially contributing to the differential vulnerabilities of plant and pollinator species are summarized below.

Pollinator species

Pollinators differ in their sensitivity to fragmentation and to changes in resource availability depending on their physiological requirements, flight capacity and social and ecological constraints (Murcia 1996; Cane 2001). Highly vulnerable species are those which are dependent on specific resources (nectar, pollen, larval host plants, nesting or oviposition sites, mutualisms with other species), especially when these resources are unpredictable in time and space (Golden & Crist 1999; Kremen & Ricketts 2000). Such pollinators may have little flexibility to cope with a changing environment. The observed decline in fragments of butterflies and moths for example may be attributed partly to the highly specific habitat requirements of both adults and immatures (Baz & Garcia-Boyer 1995). Naturally rare endemic species may be vulnerable to elimination during the initial clearing of habitat during the fragmentation process (Noss & Csuti 1997). Summerville and Crist (2001) found that rare species of Lepidoptera were disproportionately affected by fragmentation in Ohio, USA. Wide-ranging species are vulnerable in that there is insufficient area in fragmented habitats to support them, while non-vagile species are susceptible to extinction because dispersal between fragments is severely limited and genetic diversity of small populations cannot be bolstered by the rescue effect (Noss & Csuti 1997; Schultz & Crone 2001). Pollinators capable of flying greater distances (usually dependent on body size) may be able to reduce the risk arising from dependence on particular resources by accumulating them from a number of fragments (Rathcke & Jules

1993). Long-lived pollinator species may be particularly vulnerable because their foraging seasons typically outlast the blooming period of any one host and these species thus require a diversity of floral species that bloom at different times of the year (Cane 2001).

Plant species

Plant species may be differentially susceptible to fragmentation depending on the characteristics of their pollination and breeding systems and their reliance on sexual reproduction for population growth and persistence, as outlined below.

Pollination system

A strong determinant of risk of mutualism collapse appears to be degree of specificity in the mutualism (Bond 1994, 1995; Johnson & Steiner 2000). There has been recent scepticism about the specialised nature of pollination systems (Ollerton 1996; Waser *et al.* 1996). Johnson and Steiner (2000) argue however that the increasing evidence of widespread generalisation in pollination systems comes mostly from the floras of Europe and the eastern and northern parts of North America where pollination is largely dominated by opportunistic social bees. Little is known about the majority of plants in the species-rich developing countries of the world and recent evidence from South Africa indicates the presence of numerous highly specialised pollination systems often involving just one pollinator (Johnson & Steiner 2000). It thus appears that plants can vary widely in their degree of interchangeability of effective pollinators and can be pollinated by anything from literally hundreds of pollinator species to just a single pollinator species (Johnson & Steiner 2000). Plants may also be specialised for pollination by a single functional type of pollinator, often involving closely related species, as opposed to a single species (Johnson & Steiner 2000).

Specificity in pollination systems is usually achieved through combinations of advertising by specific scents and colours, floral morphology that restricts access to nectar (complex shapes, large size, long corolla tubes and floral orientation), and in some cases, unusual rewards, e.g. oils, fragrances and resins) (Bond 1995; Johnson & Steiner 2000). A high degree of specialisation may increase extinction risk, since there are no effective alternative partners available should the pollinator or type of pollinator on which the plant specialises disappear

(Renner 1999; Johnson & Steiner 2000). The severely reduced reproductive success of *Ixianthes retzioides* (Scrophulariaceae) in areas where its specialist pollinator, the oil-collecting bee *Rediviva gigas* (Melittidae), has been lost through localised extinction, is an example of the risks associated with overspecialisation (Steiner 1993). Similarly, the India-rubber tree *Ficus elastica* (Moraceae) has been described as ecologically extinct in the wild due to the extinction of its highly specific wasp pollinator (Mawdsley *et al.* 1998). Plant species may be particularly vulnerable to mutualism collapse if they are specialised for pollination by a specific pollinator or pollinator type which is itself sensitive to the effects of fragmentation (Rathcke & Jules 1993). In the 1994 fragmentation study of Aizen and Feinsinger, the most severely affected plant species were those associated with relatively scarce pollinators.

Generalised pollination systems imply resilience to the effects of habitat fragmentation (Waser *et al.* 1996). They are less likely to be affected by loss of pollinator species, although there may be a reduction in pollination success if replacement pollinators are not as efficient (Rathcke & Jules 1993; Bronstein 1995; Didham *et al.* 1996). Pollination generalists that have become locally rare may also suffer from increased transfer of the wrong species pollen and other forms of competition for services of shared pollinators (Waser *et al.* 1996; Mahy *et al.* 1998). The prediction that a generalised plant would be less affected by a reduction in pollinator diversity is supported by the results of a study by Linhart and Feinsinger (1980) of two hummingbird-pollinated plant species on the differently sized islands of Trinidad and Tobago, where flower visitation frequencies, pollen dispersal and fruit set of the generalist *Justicia secunda* (Acanthaceae) were less affected by a reduction in pollinator diversity than that of the specialist *Mandevilla hirsuta* (Apocynaceae). The generalised pollination system of *Primula mistassinica* (Primulaceae) was also found to have mitigated the effects of island-induced reduction in pollinator diversity on the shorelines of Lake Huron, Canada (Larson & Barrett 1998).

Breeding system

One of the attributes leading to differential fragmentation effects is degree of reproductive dependence on the pollination interaction. Should the pollinators of a given plant species become scarce as a result of fragmentation of habitat, the

degree of reproductive dependence on the mutualism will affect the probability of successful reproduction and thus long term persistence of that plant species (Bond 1994).

The breeding system of a plant determines its dependence on pollinators (Dafni 1992; Bond 1995). Plant species capable of autonomous selfing (prior, competing or delayed) bypass the need for pollen vectors and sexual reproductive success is guaranteed against the uncertainties of pollinator availability, although the average genetic quality of the resulting offspring may be compromised (Goldingay & Whelan 1990; Schoen & Lloyd 1992; Karoly 1992; Bond 1994, 1995; Larson & Barrett 2000). Allogamous species on the other hand are entirely dependent on vector-mediated pollination for successful fertilization (Schoen & Lloyd 1992; Bond 1994, 1995). Allogamous species include obligately outbreeding self-incompatible species as well as self-compatible species in which autonomous self-pollination is prevented through separation of the anthers and stigma in space or time (herkogamy and dichogamy, respectively) (Richards 1986).

The compatibility status of a plant species may affect the probability of successful fertilization in the face of pollinator scarcity by affecting the pool of potential mates available. Self-incompatibility is a genetic mechanism that prevents fertilization from self or related pollen (Richards 1986). Any incompatibility system thus reduces the pool of potential pollen donors, increasing the probability of inadequate compatible pollen reception (Burd 1994; Ågren 1996). Pollinator fidelity and distance travelled between flowers are important to self-incompatible species (Arroyo 1976). The transfer of pollen of the same genetic identity or mating type onto the stigma of a self-incompatible species not only fails to fertilize the ovules of that flower, but may also clog the stigma or trigger stigmatic reactions that impair later fertilization by compatible pollen (Waser 1978). Self-incompatible species are thus predicted to be particularly vulnerable to the effects of habitat fragmentation (Renner 1999; Kéry *et al.* 2000). Two plant species of the Juan Fernández Islands for example are considered to be at severe risk of extinction in the near future because of their self-incompatible breeding systems, coupled with rarity due to human-induced disturbance (Anderson *et al.* 2001). Even more susceptible may be species like *Primula*

sieboldii (Primulaceae) with highly sophisticated breeding systems, such as heterostyly or dioecy, that further reduce the number of potential mating partners (Husband & Schemske 1996; Washitani 1996; Nishihira & Washitani 1998).

In small isolated fragment populations genetic drift may reduce the diversity of self-incompatibility alleles and thereby the frequency of cross-compatible mating combinations, thus limiting seed production for a large fraction of the plants in the population (Byers 1995; Ågren 1996). Smaller populations of the rare herb *Eupatorium resinosum* (Asteraceae) for example were found to be more cross-incompatible, with a higher variance in number of compatible matings, than a larger population (Byers 1995). In severe cases, the number of self-incompatibility alleles may fall below the number required for the breeding system to function. The last Illinois remnant population of the rare and self-incompatible endemic *Hymenoxys acaulis* var. *glabra* (Asteraceae) was found to be effectively extinct, despite the presence of pollinators, because the remaining plants belonged to the same mating type (Demauro 1993).

The capacity for self-fertilization decreases reliance on cross-pollination by pollinators and appears to buffer the effects of pollen limitation in times of pollinator scarcity (Bawa 1974; Karoly 1992; Ågren 1996; Zink & Wheelwright 1997; Larson and Barrett 2000). The lack of fruit and seed set differences between large and small populations of the prairie plant *Allium stellatum* Ker. (Liliaceae) for example, partly resulted from the ability of the plant to set seed from geitonogamous pollen (Molano-Flores *et al.* 1999). Within- or between-flower (geitonogamous) pollination also does not necessarily require pollinators which travel long distances between separate compatible plants and can be effected by a wider range of floral visitors, further increasing the probability of successful reproduction (Motten 1986). In several species however, self-pollination has been found to result in a lower seed production and a higher rate of abortion than pollination with outcross pollen, either as a result of partial self-incompatibility or inbreeding depression (Spears 1987; Lee 1988; Ågren 1996; Waser 1993; Van Treuren *et al.* 1994; Bosch & Waser 1999). As a result, changes in pollinator visitation may also affect self-compatible species, influencing the quality as well as the quantity of seeds produced, depending on

how the changes in pollinator visitation affect the deposition of outcross and self pollen (Jennersten 1988; Karoly 1992; Ågren 1996; Groom 1998; Oostermeijer *et al.* 1998).

Life history features

Recruitment in a plant species may be limited by the availability of seeds and of microsites (small-scale sites suitable for germination and survival of seedlings), or a combination of the two (Eriksson & Ehrlén 1992; Turnbull *et al.* 2000). A decrease in reproductive output as a result of pollinator scarcity or loss is likely to affect the extinction risk of a plant species when populations are seed-limited, but not if recruitment is microsite limited or if substantial self-thinning takes place (Bond 1995; Turnbull *et al.* 2000). Some plant species are also able to escape dependence on seeds through clonal or vegetative reproduction, as in the case of the shrub *Ixianthes* (Steiner & Whitehead 1996); long lifespans, as in *Aster furatus* (Asteraceae) (Les *et al.* 1991), and the capacity to resprout vegetatively (Bond 1995). The often moderate declines in seed production as a result of habitat fragmentation may thus have relatively little effect on plant population growth rates because the resources not expended on fruits and seeds may be reallocated to vegetative persistence (Thomson 2001).

TOWARDS ASSESSING VULNERABILITY AND EXTINCTION RISK

In order to fully understand the effects of habitat fragmentation and to adequately conserve threatened species there is a strong need for methods that can determine which species are more sensitive than others (Dettki 1998). Bond (1994) combined three measures (risk of pollinator or disperser failure, reproductive dependence on the mutualism and demographic dependence on seed) in a quantitative subjective index in an attempt to rapidly assess the extinction risk of plant species threatened by a decrease in pollinator or seed disperser abundance and diversity. Here we will be concerned only with the factors relating to the pollination interaction.

Bond's (1994) vulnerability index (VI) is:

$$VI = [BS \times PS] \times [SD]$$

where BS is breeding system (0 in asexual species to 1 in self-incompatible dioecious species), PS is pollinator specificity (0 in wind pollinated species to 1 in species dependent on a single species) and SD is seed dependence (0 for short-lived species producing few seeds and killed by disturbance to 1 for long-lived species capable of vegetative reproduction or resprouting) (Bond 1994, 1995). An analysis of case studies by Bond (1994) suggests that plants often compensate for high risk in one of the above three categories by low risk in another, but that compensation is not universal.

THE PRESENT INVESTIGATION

Habitat fragmentation and the grassland biome of South Africa

The Grassland Biome of South Africa is found chiefly on the high central plateau of the country and the inland areas of KwaZulu-Natal and the Eastern Cape (Low and Rebelo, 1996). Altitude varies from near sea level to 2850 m above sea level (Low and Rebelo, 1996). This biome is considered to have an extremely high biodiversity, second only to the Fynbos Biome, and is home to many rare plants, often endemic and threatened (Low & Rebelo 1996; Scott-Shaw 1999).

Worldwide however, grassland ecosystems have been ranked as one of the biomes likely to experience the greatest proportional reduction in biodiversity in the future (Sala *et al.* 2000). South Africa is no exception and the Grassland Biome is the biome most changed by human activity in the country (Macdonald 1989). The midland and upland regions of KwaZulu-Natal are prime agricultural and forestry areas (Armstrong *et al.* 1998). As a result large tracts of grassland have already been afforested with exotic trees and further afforestation activity is planned for the future (Meter *et al.* 1994). This large scale farming of trees has resulted in widespread reduction and fragmentation of grassland habitat, leaving only fragments of various sizes in many areas (Lombard 1995; Armstrong *et al.* 1998; Bredenkamp *et al.* 1999). In KwaZulu-Natal the removal of grassland

vegetation through afforestation has resulted in the extinction in the wild of three endemic plant species and the classification of another seven species as critically endangered or endangered (Hilton-Taylor 1996; Scott-Shaw 1999).

There has been little investigation into the general ecology and dynamics of grasslands in South Africa in general, let alone into the consequences of habitat fragmentation through commercial afforestation for the remnant grassland biota (Armstrong & van Hensbergen 1999; Johnson *et al.* 2002). Samways and Moore (1991) found that exotic tree plantations have a marked effect on grasshopper assemblages for many metres into surrounding grassland. In the grasslands of the Drakensberg in KwaZulu-Natal, Kamffer and Ferguson (1998) found that faunal diversity was strongly affected by fragmentation, true grassland butterflies being absent in habitat patches smaller than one hectare. Pryke and Samways (2002) compared adult butterfly species richness and abundance in interconnected remnant grassland linkages retained between commercial forest patches in the KwaZulu-Natal Midlands with that of a near-natural grassland outside the estate. Butterfly abundance and diversity of the lesser disturbed grassland remnants were found to be similar to that of sites in the natural grasslands, although species composition was sometimes altered (Pryke & Samways 2002). Nectar plants were the most significant variable explaining butterfly distribution (Pryke & Samways 2002). Level of disturbance was found to have a major effect on butterfly species richness and behaviour, with highly disturbed sites dominated by just a few generalist and vagile species, mostly in flight (attributed to low availability of floral nectar resources) (Pryke & Samways 2002). Beyond this, species characteristics responsible for variation in response to fragmentation effects were not explored. Winter and Morris (2001) investigated Natal Mistbelt Grassland fragmentation in the Umvoti conservancy, but the study was limited to a description of the plant species composition (excluding most non-grass species) and grass sward structure of the remnant grassland patches.

From the literature cited above there is limited evidence that habitat fragmentation has a detrimental effect on grassland plant and animal species. Nothing is said of the effects of fragmentation on the functioning of vital ecological interactions such as plant-pollinator mutualisms.

Site details

This study took place at Gilboa Estate (MONDI Forests Ltd) (29° 19'S, 30° 17'E), in the Karkloof mountain range of the KwaZulu-Natal Midlands of South Africa. Large areas of the natural moist upland grassland vegetation of the estate were planted with exotic pines (*Pinus patula* and *P. gregii*) and eucalypts (*Eucalyptus nitens* and *E. macarphurii*) at least 30 years ago (G. Hudson, P. Croft 2002, pers. comm.). This afforestation resulted in the formation of numerous fragments of native grassland habitat surrounded by an alien matrix of exotic tree plantation and a stark habitat mosaic radically different to the relatively low levels of habitat heterogeneity previously experienced by the grassland biota. Research was carried out in ten of these habitat fragments as well as at five sites located in the adjacent continuous and extensive grassland (>2090 ha) referred to here as the 'mainland' grassland. Study sites ranged from 1500 to 1760 m above sea level. Figure 1.1 is a map of MONDI Gilboa Estate showing the distribution of the mainland and fragment sites. Examples of study sites are shown in Fig 1.2. The area and isolation (measured as distance to the mainland grassland) of the grassland habitat fragments used in the study are listed in Table 1.1.

Table 1.1 Area and distance to the mainland of grassland habitat fragments used in this study.

Fragment site	Area (ha)	Distance to nearest M (km)
F1	12.21	3.38
F2	10.97	1.35
F3	9.42	1.46
F4	39.02	3.87
F5	6.68	3.55
F6	3.23	5.34
F7	1.95	0.14
F8	0.66	3.82
FW9	7.14	3.67
FW10	6.19	0.31

F = grassland fragment, M = mainland grassland, W = wetland.

Study species

Twenty four wildflower species were chosen for this study, encompassing six monocot and five dicot families and a diverse array of floral morphological characteristics (for species characteristics see Table 1.2 and Figures 1.3 to 1.9).

AIMS AND RATIONALE OF THE STUDY

Landscapes in South Africa and the world are being transformed yearly and ecosystems are being subjected to changes on an unprecedented scale (Armstrong *et al.* 1998; Novacek & Cleland 2001). It is inevitable that one day much of our natural heritage will exist as habitat remnants. As evident from the above review of the literature, the division of continuous habitats into disjunct fragments has the potential to disrupt vital ecological interactions such as pollination systems. The prevalence of these detrimental effects however cannot yet be determined. To determine this, and in order to fully grasp the effects of habitat fragmentation on plant-pollinator interactions and the nature and causes of the differential responses of the constituent plant and pollinator species, there is an urgent need for further investigation involving whole suites of species from a variety of ecosystems. Increased understanding may result in the development of predictive tools to determine which organisms are particularly susceptible and in the formulation of management strategies which will better ensure the long-term conservation of the plants and animals confined to existence within habitat fragments.

The aims of this study are thus:

- (1) to explore the range of pollination and breeding systems that characterise the wildflowers of KwaZulu-Natal Moist Upland Grasslands
- (2) to ascertain whether reproductive output of grassland wildflowers is limited by pollen availability and whether levels of pollen limitation are greater in habitat fragments than in mainland grasslands

(3) to determine whether habitat fragmentation resulting from large scale tree farming has a significant detrimental effect on the reproductive output of moist upland grassland wildflowers

(4) to test whether the magnitude of fragmentation effect for a given species can be predicted by breeding and pollination system characteristics.

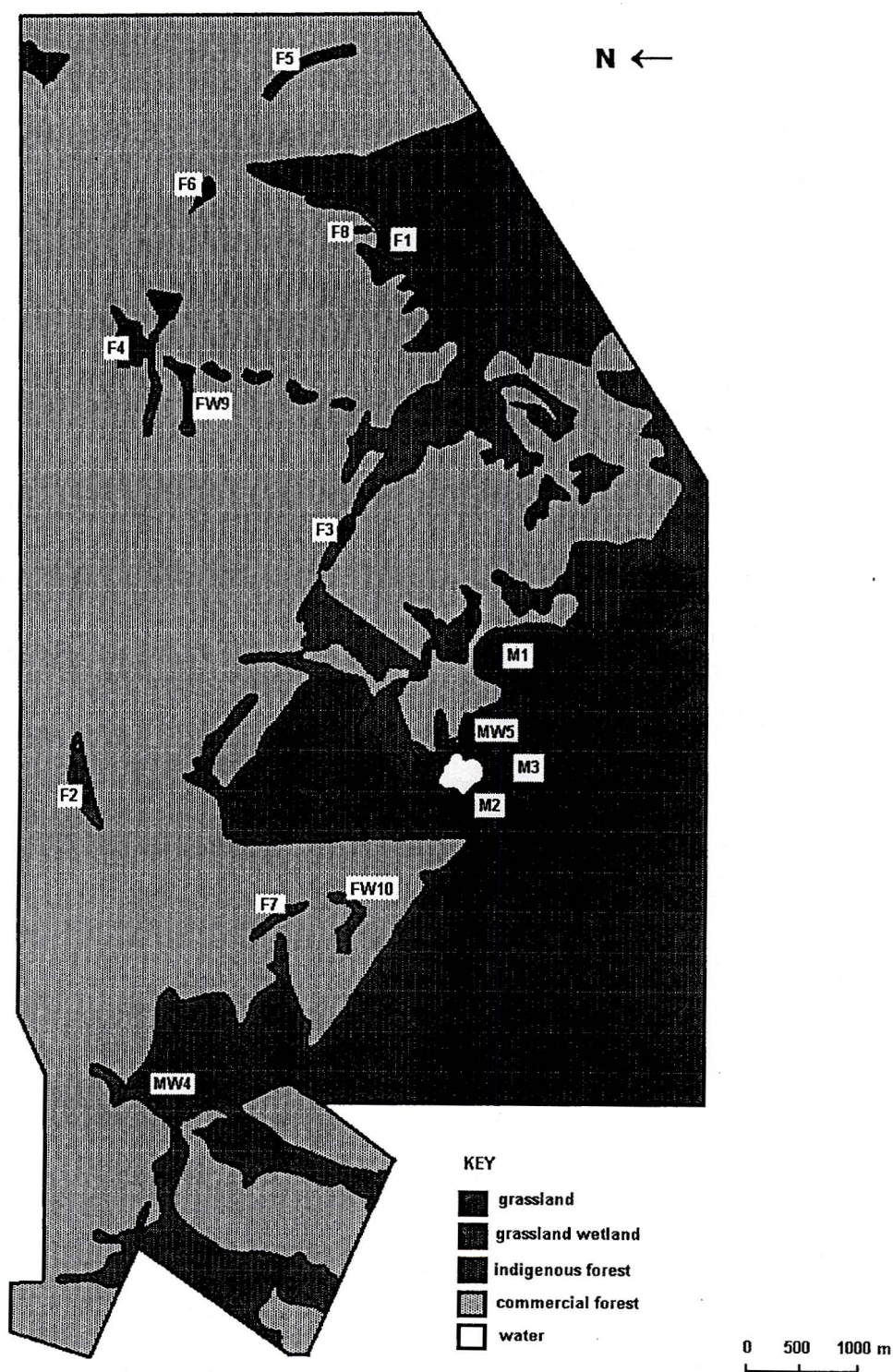


Figure 1.1 Layout of mainland (M) and habitat fragment (F) sites at MONDI Gilboa Estate. Mainland grassland continues beyond map boundaries. W=wetland habitat.

Figure 1.2 Study sites at Gilboa Estate. **A**, Mainland grassland (site M1) with view of afforestation and fragmented grasslands in the distance. **B**, Habitat fragment F1 surrounded on three sides by commercial afforestation and on one side by indigenous Afromontane Mistbelt Forest. **C**, Habitat fragment F2 after plantations were felled in 2001. **D**, Habitat fragment FW9, a wetland surrounded by pine plantations.

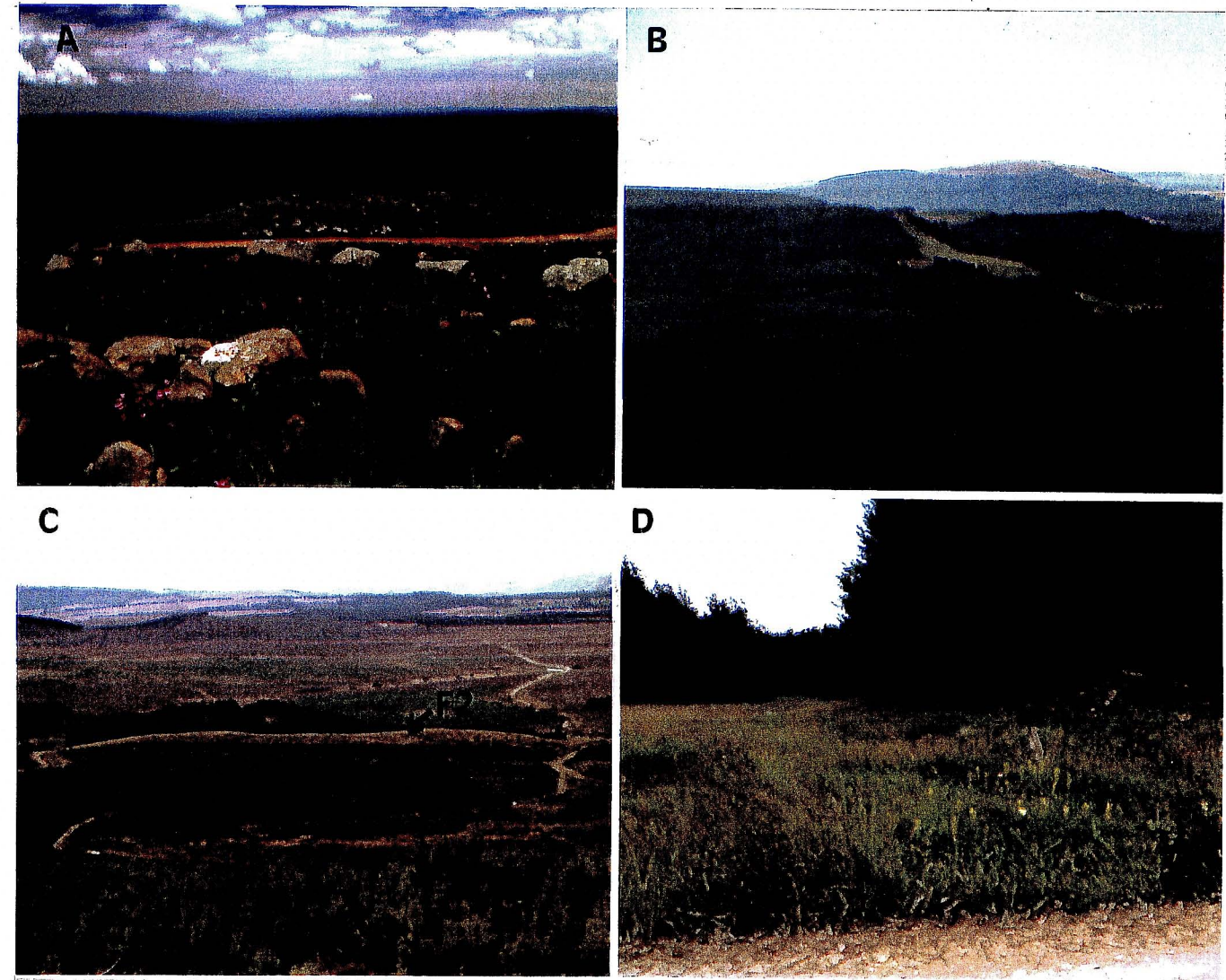


Table 1.2 Basic descriptions of the 24 grassland wildflower study species.

Plant family	Plant species	Spp abbr.	Basic floral characteristics	Mean tube length (mm)*	Nectar	Scent	Flowering time	Fig. no.
Iridaceae	<i>Gladiolus longicollis</i> Bak.	GL	Spike 1- to 3- flowered; flowers white to pale yellow; trumpet-shaped; very long perianth tube; open evenings	93.0	yes	carnation cloves	Oct - Feb	1.3A
Iridaceae	<i>Watsonia lepida</i> N.E. Br.	WL	Crowded inflorescence; flowers pink; funnel-shaped; long curved perianth tube	21.9	yes	no	Nov - Jan	1.3B
Iridaceae	<i>Dierama luteoalbidum</i> Verdoorn	DL	Drooping inflorescence, 1- to 3-branched; flowers white to pale creamy yellow; bell-shaped; short perianth tube; threatened Midlands endemic	7.4	yes	no	Oct - Dec	1.3C
Iridaceae	<i>Dierama dracomontanum</i> Hilliard	DD	Drooping inflorescence, 2- to 4-branched; flowers rose pink; bell-shaped; short perianth tube	4.4	yes	no	Nov - Feb	1.3D
Iridaceae	<i>Tritonia disticha</i> (Klatt) Bak.	TD	Inflorescence 4- to 12-flowered; flowers orange-red, yellow blotch on lower 3 lobes; funnel-shaped; short perianth tube	7.8	yes	no	Nov - Mar	1.4A
Iridaceae	<i>Moraea inclinata</i> Goldbl.	MI	Inflorescence 2- to 4-branched, leaning; flowers blue violet with yellow nectar guides; iris-like with flattened petaloid style branches, three separate entry points; short perianth "tube"	7.9	yes	no	Nov - Mar	1.4C
Iridaceae	<i>Aristea woodii</i> N.E. Br.	AW	Inflorescence well branched; flowers blue to mauve; open, cup-shaped; short perianth tube; open early, close midday	3.9	no	no	Sep - Mar	1.4B
Amaryllidaceae	<i>Brunsvigia undulata</i> Leighton	BU	Large, round inflorescence; flowers deep red; funnel-shaped; intermediate perianth tube	18.0	yes	no	Jan - Feb	1.4D
Alliaceae	<i>Agapanthus campanulatus</i> Leighton	AC	Round inflorescence; flowers light to dark blue, darker stripe along middle of each lobe; funnel-shaped; short perianth tube	8.6	yes	no	Dec - Mar	1.5C

Plant family	Plant species	Spp abbr.	Basic floral characteristics	Mean tube length (mm)*	Nectar	Scent	Flowering time	Fig. no.
Hyacinthaceae	<i>Eucomis autumnalis</i> (Mill.) Chitt.	EA	Raceme c 500 mm, 10-45 terminal bracts, pineapple-like; flowers light to darker green; open, cup-shaped; no perianth tube	0.0	yes	pineapple	Dec - Apr	1.5A
Aloaceae	<i>Aloe boylei</i> Bak.	AB	Inflorescence dense, flattish; flowers salmon pink; tubular (long perianth tube)	51.0	yes	no	Nov - Feb	1.5B
Asphodelaceae	<i>Kniphofia laxiflora</i> Kunth	KL	Inflorescence loosely arranged; flowers orange; tubular (long perianth tube)	38.3	yes	no	Feb - Mar	1.5D
Asphodelaceae	<i>Kniphofia ichopensis</i> Bak. ex Schinz	KI	Inflorescence lax; flowers bud yellow, flower yellow-green; tubular (long perianth tube)	41.7	yes	no	Dec - Mar	1.6B
Asphodelaceae	<i>Kniphofia fluviatilis</i> Codd	KF	Inflorescence dense; bud orange-red, hanging, flower yellow; tubular (long perianth tube)	40.2	yes	no	Nov - Dec	1.6A
Orchidaceae	<i>Satyrium longicauda</i> Lindl.	large morph SLL	Inflorescence relatively dense, tall; flowers white tinged with pink; hooded; long paired spurs	39.7	yes	sweet, evenings	Dec - Feb	1.6C
Orchidaceae		small morph SLS	Inflorescence relatively dense, short; flowers white; hooded; long paired spurs	-	yes	sweet, evenings	Dec - Feb	
Orchidaceae	<i>Disa versicolor</i> Reichenb. f.	DV	Inflorescence dense; flowers mottled pink turning brown; hooded; short spur	6.0	yes	indistinct	Dec - Feb	1.6D
Orchidaceae	<i>Disa stachyoides</i> Reichenb. f.	DS	Inflorescence dense; flowers purple, hooded; short spur	6.6	no	no	Dec - Feb	1.7A
Orchidaceae	<i>Eulophia zeyheriana</i> Sond.	EZ	Inflorescence; flowers pale blue-purple; hooded; short spur	2.3	no	no	Nov - Feb	1.7B
Fabaceae	<i>Eriosema distinctum</i> N.E. Br.	ED	Inflorescence short; flowers bright red with yellow markings; papilionoid; short perianth 'tube'	3.0	yes	no	Aug Mar	1.7C, D

Plant family	Plant species	Spp abbr.	Basic floral characteristics	Mean tube length (mm)*	Nectar	Scent	Flowering time	Fig. no.
Rubiaceae	<i>Pentania prunelloides</i> (Klotzsch ex Eckl. & Zeyh.)	PP	Inflorescence dense, round; flowers pale to deep purplish blue; trumpet-shaped; medium perianth tube; heterostylous (pin & thrum morphs)	14.4	yes	weak	Oct - Mär	1.8A, B
Scrophulariaceae	<i>Zaluzianskya natalensis</i> (Bernh. ex) Krauss	ZN	Inflorescence short, dense; flowers white inside, red beneath; trumpet-shaped; very long perianth tube; open at dusk	45.9	yes	cloves	Oct - Apr	1.8C
Gentianaceae	<i>Chironia krebisii</i> Griseb.	CK	Narrow terminal clusters; flowers deep pink; open, cup-shaped; short perianth tube; anthers porose	7.2	no	no	Oct - Jan	1.8D
Asclepiadaceae	<i>Xysmalobium parviflorum</i> Harv. ex Scott Elliot	XP	Inflorescence dense, round; flowers white to yellow; small, fleshy, goblet-shaped; no perianth 'tube'	2.7	yes	sickly sweet	Oct - Apr	1.9A, B
Asclepiadaceae	<i>Pachycarpus grandifloris</i> (L.f.) E. Mey.	PG	Inflorescence 2- to 6-flowered; flowers hanging, greenish yellow, dotted with purple; inflated and round; no tube; corona lobes spread to edge, incurve over	0.0	yes	decaying matter	Nov - Apr	1.9C, D

* tube length data from measurements in Chapter 2, *Spp abbr.* = species abbreviations.

Figure 1.3 Wildflower study species and their pollinators. **A**, Flowers of *Gladiolus longicollis* showing the elongated perianth tube. **B**, The long-tongued fly *Philoliche aethiopica* (Tabanidae) approaching a *Watsonia lepida* flower (photo: Steve Johnson). **C**, The drooping inflorescences of *Dierama luteoalbidum*. **D**, Halictid bees resting in a *Dierama dracomontanum* flower. Scale bars: A & B = 20 mm, C = 50 mm, D = 5 mm.

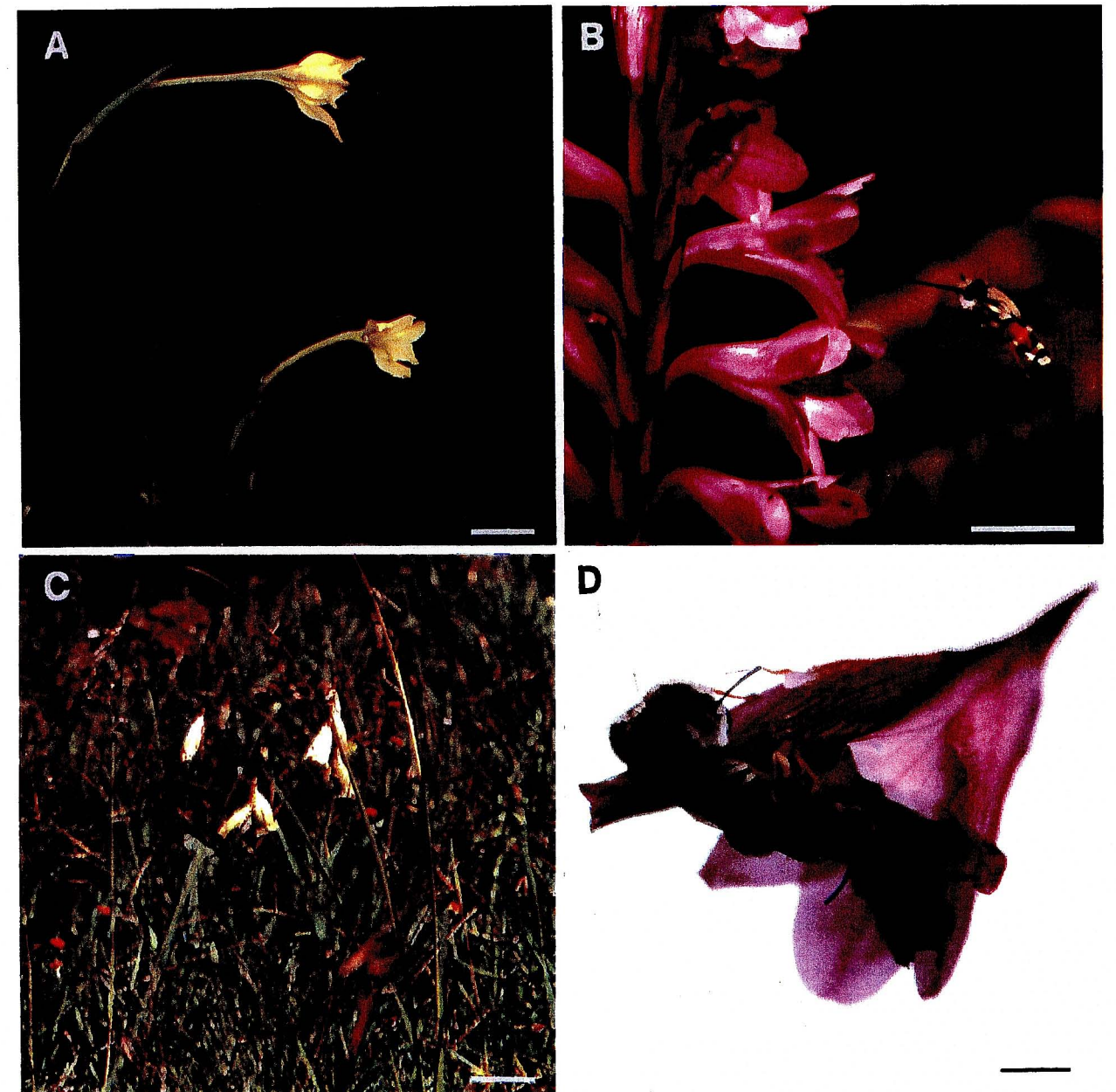


Figure 1.4 Wildflower study species and their pollinators. **A**, Halictid bees resting in a *Tritonia disticha* flower. **B**, Flowers of *Aristea woodii*. **C**, A bee foraging for nectar and/or pollen on *Moraea inclinata*. **D**, *Amegilla* sp (Anthophoridae) feeding on nectar from the mouth of the perianth tube of a *Brunsvigia undulata* flower. Scale bars: A, B & D = 10 mm, C = 2 mm.



Figure 1.5 Wildflower study species and their pollinators. **A**, The flowers of *Eucomis autumnalis* with a tabanid fly feeding on nectar and/or pollen. **B**, *Aloe boylei*. **C**, The long-tongued nemestrinid fly *Prosoeca* sp approaching a flower of *Agapanthus campanulatus*. **D**, The butterfly *Aeropetes tulbaghia* feeding on nectar from a *Kniphofia laxiflora* flower (note the pollen deposited on the proboscis). Scale bar: A, C & D = 10 mm, B = 50 mm.

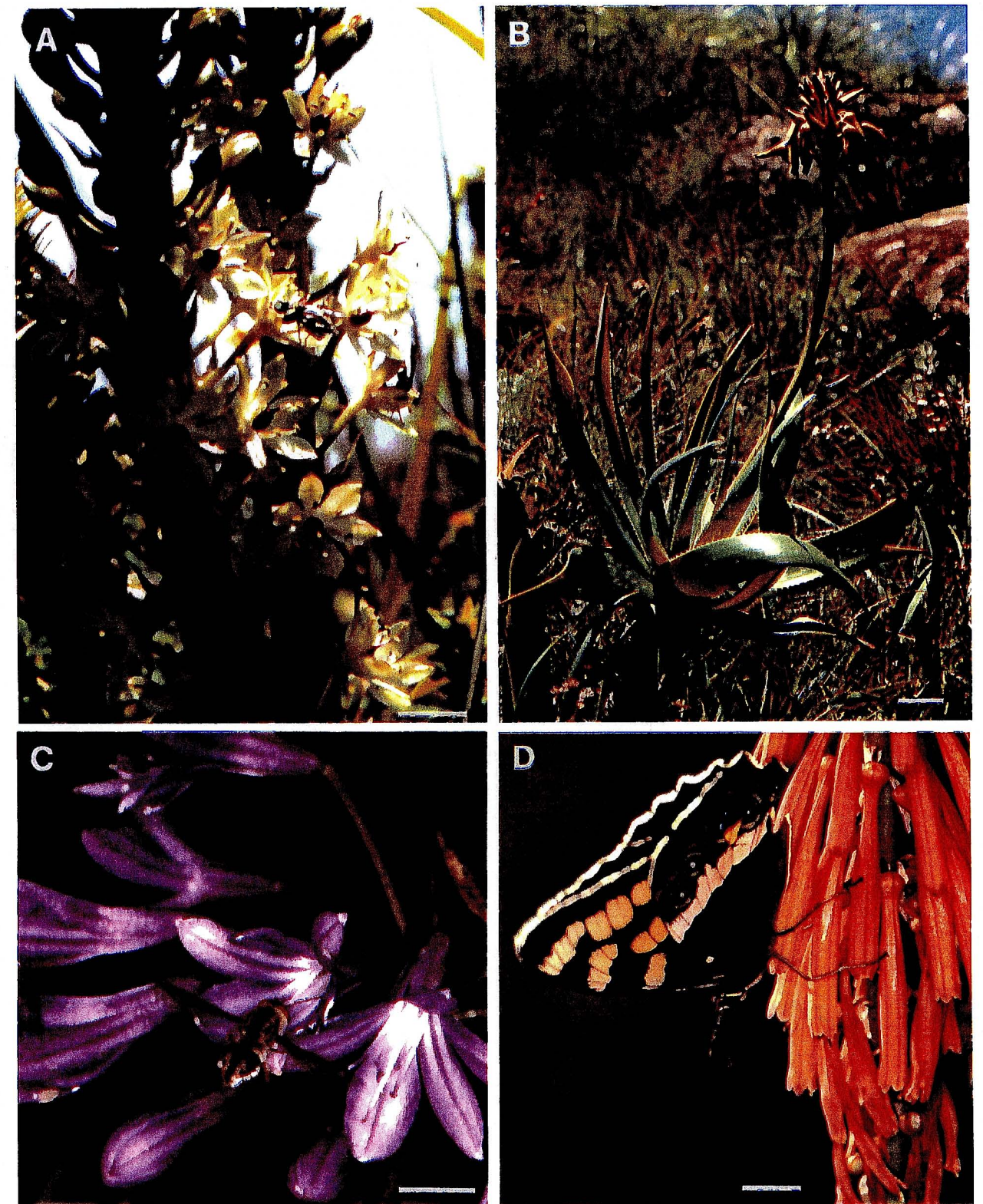


Figure 1.6 Wildflower study species and their pollinators. **A**, *Kniphofia fluviatilis*. **B**, *Kniphofia ichopensis*. **C**, A hawkmoth feeding on nectar from the spur of a *Satyrium longicauda* flower (note the pollinia attached to the proboscis) (photo: Steve Johnson). **D**, *Amegilla natalensis* (Anthophoridae) feeding on nectar from the short spur of a *Disa versicolor* flower (photo: Steve Johnson). Scale bars: A & B 100 mm, C & D = 10 mm.



Figure 1.7 Wildflower study species and their pollinators. **A**, *Disa stachyoides* (photo: Dave Thompson). **B**, *Eulophia zeyheriana*. **C**, *Chalicodoma* sp (Megachilidae) feeding on a flower of *Eriosema distinctum*. **D**, *Megachile* sp (Megachilidae) feeding on a flower of *E. distinctum*. Scale bars: A, C & D = 10 mm, B = 5 mm.

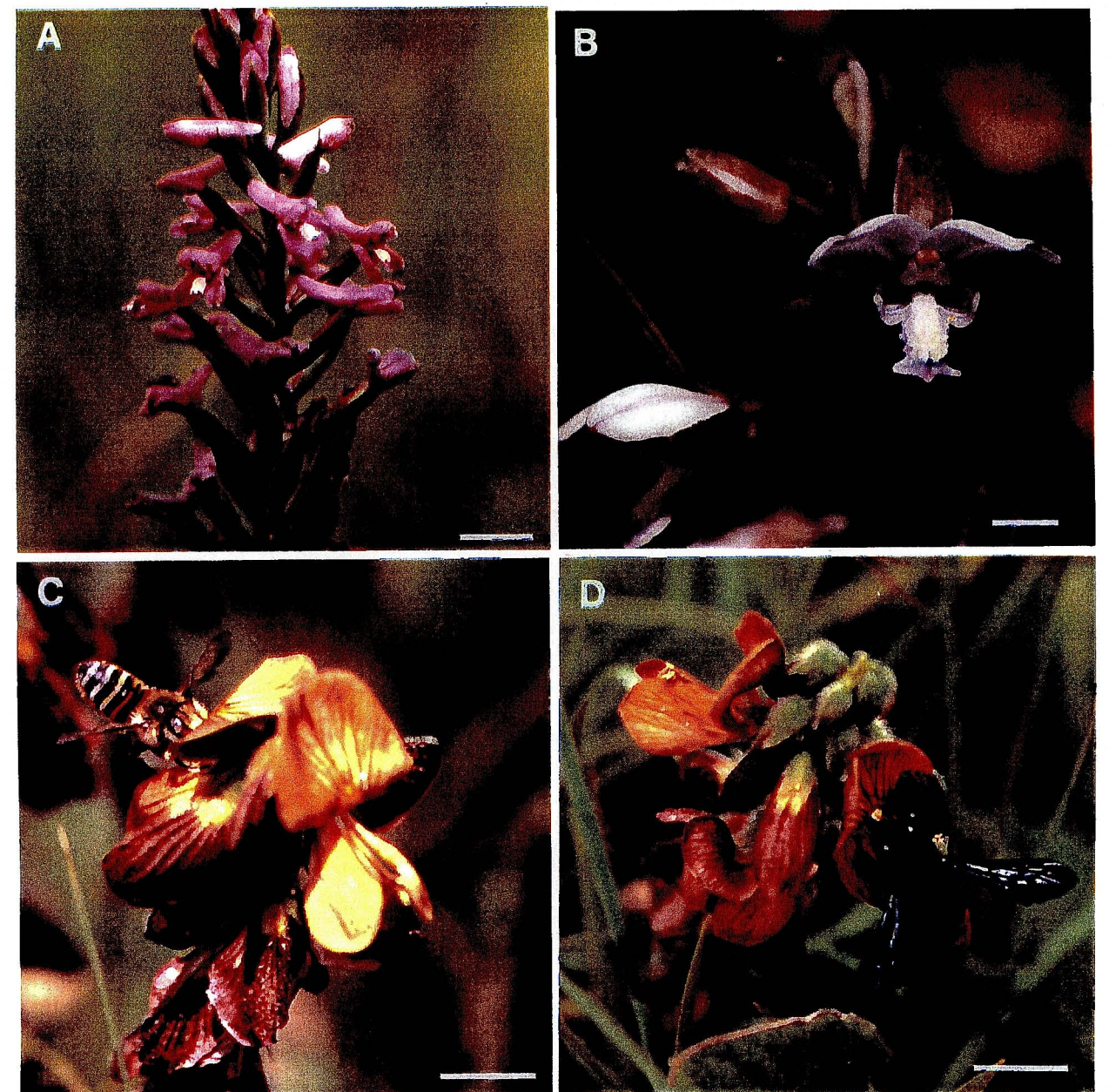
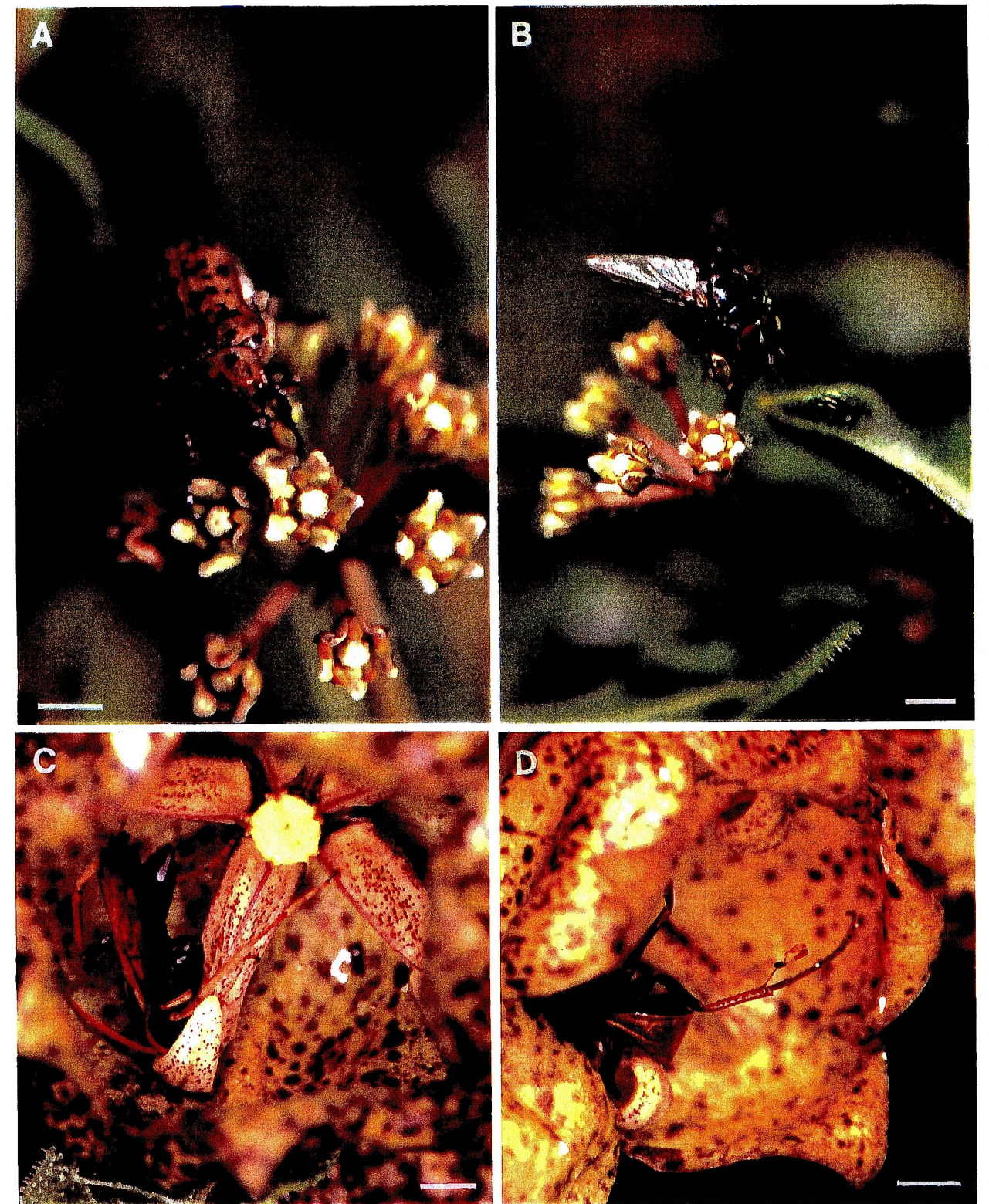


Figure 1.8 Wildflower study species and their pollinators. **A**, The butterfly *Belenois creona severina* probing a flower of *Pentanisia prunelloides* for nectar. **B**, *Systoechus* sp (Bombyliidae) feeding on nectar from a *Pentanisia prunelloides* flower. **C**, The hawkmoth *Basiothia schenki* probing *Zaluzianskya natalensis* flowers for nectar. **D**, *Chironia krebsii*. Scale bars: A & C = 10 mm, B = 5 mm, D = 20 mm.



Figure 1.9 Wildflower study species and their pollinators. **A**, The beetle *Atrichelephinis tigrina* feeding on the nectar of a *Xysmalobium parviflorum* flower. **B**, A short-tongued fly feeding on the nectar of *X. parviflorum* flowers. **C**, *Hemipepsis hilaris* (Pompilidae) feeding on nectar from the corona lobes of a *Pachycarpus grandifloris* flower. **D**, A dead wasp with a *P. grandifloris* pollinarium attached to its leg. Scale bars: A & B = 2 mm, C & D = 5 mm.



CHAPTER 2

PLANT-POLLINATOR MUTUALISMS

ABSTRACT

Plant-pollinator mutualisms of KwaZulu-Natal Moist Upland Grasslands have been largely unstudied. In order to explore the range of pollination systems characteristic of these grasslands and the vulnerability of these interactions to environmental perturbation, the degree of specificity in the pollination systems of 21 wildflower species was investigated. These wildflower species were found to support a rich and diverse pollinator community, involving long-tongued flies, hawkmoths and sunbirds and dominated by long-tongued solitary bees. Two thirds of the wildflower species appear relatively specialised in terms of pollination, although reserve pollinators may lend a degree of resilience to just under half of these. Six species are highly specialised, entirely dependent on a single species or functional pollinator type for pollination. The high degree of specificity in the pollination systems studied here suggests that the grassland community may be vulnerable to a decrease in the abundance and diversity of pollinators. The discovery of pollination specialists in grassland wildflowers adds to the numerous highly specialised pollination systems already discovered in South Africa.

INTRODUCTION

One of the key measures of risk of disruption of a given plant-pollinator mutualism may be the degree of specialisation or generalisation involved (Rathcke & Jules 1993; Waser *et al.* 1996; Johnson & Steiner 2000). Plant species vary from being pollinated by one or a few ecologically similar species to many species, possibly of diverse taxonomic origin (Johnson & Steiner 2000). As evident in Chapter 1, theory and empirical evidence suggest that pollination specialists are at greater risk of reproductive collapse and possible local extinction as a result of loss of pollinator species than are pollination generalists.

Knowledge of the differing efficacy of flower visitors as pollinators is important in determining the degree to which a given plant species is specialised for pollination and hence the vulnerability of that pollination system to environmental

perturbation. Assumptions about the generalisation of pollination systems have often been based on lists of floral visitors, rather than of effective pollinators (e.g. Waser *et al.* 1996). Although a plant species may be visited by a diverse array of species, often only a small fraction of these are effective pollinators and the species may be less resilient to disruption than previously thought (Lindsey 1984; Fishbein & Venable 1996; Kevan 1999). On the other hand, flowers apparently conforming to a particular syndrome can receive visits from different unexpected, opportunistic insects which may be able to effect successful fertilization and prevent reproductive collapse in the absence of the more 'classical' pollinator (Fishbein & Venable 1996; Olsen 1997; Mayfield *et al.* 2001). Pollen collecting bees for example appear to be responsible for the small amount of seed produced by *Ixianthes retzioides* (Scrophulariaceae) in areas where it has lost its highly specialised oil-collecting bee pollinator (Steiner & Whitehead 1996). Knowledge of the presence of such 'reserve' pollinators is essential if one is to assess the vulnerability of the pollination system at hand to disruption.

In order to effect fertilization, a pollinator must visit the flower in such a way that viable pollen is transferred from anther to stigma (Kevan 1999). The anatomical and behavioural fit of the visitor and the flower are important determinants of the efficiency of a given pollinator, affecting the amount of pollen deposited and removed per floral visit (Lindsey 1984; Kevan 1999; Herrera 2000). Also important are relative abundance on the host, degree of fidelity, flight distances between consecutive flower visits and the relative importance of within- versus between-plant interfloral flights (Lindsey 1984; Herrera 2000). It is thus essential to objectively discern between floral visitors and effective pollinators (Lindsey 1984; Johnson & Steiner 2000). Estimates of pollinator importance are ideally obtained from direct comparative studies of the efficiency of different pollinator species in terms of pollen removal, pollen deposition on the stigma and seed production (from single visits to virgin flower) (Lindsey 1984; Johnson & Steiner 2000). Indices (incorporating measures of relative abundance, pollen load size, host plant fidelity and morphological fit) are employed as estimates of pollinator efficacy where large numbers of visitor species are involved and direct comparisons are prohibitive.

In an attempt to learn more about the range of pollination systems characteristic of KwaZulu-Natal Moist Upland Grasslands and the vulnerability of these mutualisms to environmental perturbation, the pollinators of 21 wildflower species were determined based on:

- (1) relative abundance on the floral host
- (2) pollen load analysis (size and purity)
- (3) morphological fit between flower and visitor

MATERIALS AND METHODS

Plant measurements

Measurements were taken of the depth of the perianth tube or spur, the constricted portion of the flower that accommodates the tongue of the pollinator. Voucher specimens of wildflower species investigated are deposited in the Natal University Herbarium (NU). Basic characteristics of the wildflower species are summarized in Table 1.1.

Pollinator observations and collection

Field work took place over two flowering seasons (December 1998 through February 1999 and October 1999 through March 2000). Pollinator observations were carried out either at large flowering patches or while walking through most of the population at a given site. On some occasions multiple study species were observed simultaneously. Study sites and total observation time for each species are listed in Table 2.1. Records were made of each visit observed in which the stigmas and anthers appeared to be contacted. Where possible, a number of individuals of each visitor species were netted either while foraging on the flowers or once they had left. In addition to pollinator observations, a light trap equipped with a 250 W mercury-vapour lamp was used in the evenings to capture hawkmoths which are potential pollinators for *Gladiolus longicollis*, *Satyrium longicauda* and *Zaluzianskya natalensis*.

All captured insects were killed in individual killing jars or vials containing ethyl acetate. These specimens were then pinned and dried and identified to species where possible. Hymenoptera were identified by Dr F.W. Gess (Albany Museum,

Grahamstown), Prof V.B. Whitehead (S.A. Museum, Cape Town) and Prof D. Brothers (School of Botany and Zoology, University of Natal, Pietermaritzburg) and Diptera by Professors D.A. Barraclough and B.R. Stuckenberg of the Natal Museum, Pietermaritzburg. Classification of bees followed that of Michener (2000). At least one individual of each species is housed with the taxonomist responsible for identification. The rest of the collection is housed at the University of Natal, Pietermaritzburg.

Proboscis lengths (from the face to the tip of the extended tongue) of pollinators were measured where possible. To examine the size and purity of visitor pollen loads, pollen was removed from the body surface of 364 insects with cubes of fuschin glycerine jelly (Beattie 1971). This pollen was then compared to pollen samples in a reference collection from Gilboa Estate, using a compound light microscope. Scopal loads were removed using a dissecting needle. Pollen loads collected from more than one location on the body of a given insect were examined individually. The total number of conspecific pollen grains carried on the body of a given floral visitor was assigned to the following pollen load size classes: 0, 1-10, 10-50, 50-100, >100 grains. Because Orchidaceae and Asclepiadaceae package their pollen in large groups within pollinia and because pollinators of these species tend to carry far fewer pollinia than pollinators of other plant families carry pollen grains, the pollen load size classes were different for species from these families: 0, 1-3, 4-6, 7-9, ≥ 10 and 0, 1, 2, 3, ≥ 4 respectively. Pollen load purity was determined as the proportion of conspecific pollen grains that constitute the total pollen load. Pollen from areas that were unlikely to make contact with the stigma of the flower during foraging (such as that from scopal loads) was excluded from the above analyses.

Pollinator importance

In an attempt to determine the relative pollination importance of each visitor species to a given plant species, a pollinator importance index based on those of Lindsey (1984) and Hansen and Molau (1994) was calculated where possible.

Four basic components were measured:

1. Relative pollinator abundance (A) (Lindsey 1984): the proportion of a given floral visitor to all visitors observed or collected for a particular plant species, values from 0 to 1 (Lindsey 1984).

2. Relative pollen load index (PLI) (Hansen & Molau 1994): the median pollen load index of a given plant species' pollen, values from 0 to 1, constructed as follows: 0 = 0 pollen grains; 0.25 = 1-10 pollen grains; 0.50 = 10-50 pollen grains; 0.75 = 50-100 pollen grains; 1.00 = >100 pollen grains. The PLI is scored differently for orchid and asclepiad species, since a single pollinium is generally enough to fertilize all the ovules of a given flower and because of the low number of pollinia carried by asclepiad floral visitors. Values assigned are 0 (pollinia absent) and 1 (at least one pollinium or massulae).

3. Relative host plant fidelity (F) (Lindsey 1984): the median proportion of a species' body surface pollen load which contained host pollen, values from 0 to 1.

4. Pollination efficiency (E): a qualitative index, representing the probability that foraging activity of a given visitor results in pollination (Lindsey 1984). Since detailed measurements used by Lindsey (1984) of specific foraging behaviour were unavailable, pollination efficiency was based instead on a simple analysis of the morphological fit between flower (length of constricted portion of the perianth tube) and visitor (length of proboscis), and the likelihood that foraging behaviour resulted in contact with the anthers and stigmas. Values assigned were 0 (no contact), 0.5 (50% probability of contact) and 1 (consistent ensured contact).

As in Lindsey (1984) all four variables were multiplied to give a pollination importance value (PIV) for each floral visitor to a given plant species:

$PIV = A \times PLI \times F \times E$. Each floral visitor was then given a pollination importance index (PII) score, which was calculated as the percentage of total pollination importance values of all visitor species collected: $PII = PIV / \sum PIV$ (Lindsey 1984).

Pollination systems

Principal pollinators of the 21 wildflower species were determined on the basis of the above analyses. The main functional pollinator groups used here are short-tongued bee, long-tongued bee, wasp, short-tongued fly, long-tongued fly, butterfly, hawkmoth, beetle and bird. Pollination systems were considered to be 'highly specialised' when plant species are pollinated by a single species, 'specialised' when pollinated by a single functional type of pollinator, 'relatively specialised' when pollinated by few species limited to one or two orders and 'generalised' when pollinated by numerous species from many orders.

CASE HISTORIES

The grassland wildflowers studied here were found to support a relatively rich and diverse pollinator fauna and pollination systems varied greatly in terms of pollinator types and degree of specificity. Observations carried out on 21 species recorded a total of 1050 floral visitors from 116 species, 35 families and five orders (Table 2.1). The principal functional pollinator types involved in the pollination of each wildflower species and the number of pollinator taxa involved (a measure of specificity) are summarised in Table 2.1. Additional information was obtained from more recent studies carried out at mainland site M1 (Alexandersson & Johnson 2002; Johnson 2000; Johnson *et al.* 2002; Anna Hargreaves, unpublished data; Elisa Raulings & Steven Johnson 2001, pers. comm.; Steven Johnson & Paul Neal 2002, pers. comm.).

Monocotyledons

***Gladiolus longicollis* Bak. (Iridaceae)**

Observations

Observations at dusk carried out together with R. Alexandersson and S.D. Johnson at mainland site M1 revealed that the convolvulus hawkmoth, *Agrius convolvuli* (L.), was a consistent and frequent visitor to *G. longicollis* (usually around 7:45 pm). On a number of occasions, an ultraviolet light trap was set up in the evenings and captured hawkmoths were analysed for pollen. Twelve out of 15 captured *A. convolvuli* individuals as well as other captured hawkmoth species were found to carry *G. longicollis* pollen on and around the proboscis and frontally on the head (Table 2.2). Fertilization is effected through passive

removal and deposition of pollen as the hawkmoth thrusts its head into the mouth of the perianth tube in order to reach the nectar, thus contacting the floral sexual parts.

Discussion

The discovery that the pale whitish long-tubed flowers of *G. longicollis* (Fig. 1.3A.) are pollinated by night-flying hawkmoths is in keeping with the prediction of Goldblatt and Manning (1998) and with observations recorded in Goldblatt *et al.* (2001). *Agrius convolvuli*, with a mean proboscis length of 102.9 mm (SD = 16.2, n = 15), is best adapted to feed on the nectar located in the 93 mm long perianth tube (SD = 9, n = 289) and is the most consistent and efficient pollinator of *G. longicollis* (Alexandersson & Johnson 2002). The pollination system of *G. longicollis* is thus highly specialised, the species almost entirely dependent on a single species of hawkmoth for effective pollination (Table 2.1).

***Watsonia lepida* N.E. Br. (Iridaceae)**

Observations

By far the most consistent and frequently observed visitor to *W. lepida* flowers over both field seasons was the long-tongued tabanid fly, *Philoliche aethiopica* (Thunberg) (Fig. 1.3B.). Pollen is passively deposited onto and removed from the body of the insect as it forages. Captured flies were found to carry large amounts of relatively pure *W. lepida* pollen dorsally on the thorax and abdomen (Table 2.2). *Philoliche aethiopica* thus has a high pollinator importance index (PII) value of 98.59. Another fairly frequent flower visitor (although considerably more rare) is the anthophorid bee, *Amegilla natalensis* (Fries). Captured individuals were also found to carry *W. lepida* pollen dorsally, although in smaller, less pure amounts (Table 2.2), reflecting the more generalist foraging behaviour of the species. One *Amegilla aspergina* (Cockerell) individual was captured while visiting a *W. lepida* flower (Table 2.2).

Discussion

These findings are supported by Johnson (2000) who saw many hundreds of visits by *Philoliche aethiopica* to *W. lepida* at site M1 during 60 hours of observation in his study of Batesian mimicry in the orchid *Disa pulchra*. *Philoliche aethiopica*, with a mean proboscis length of 14.7 mm (SD = 0.8, n =

22), is the only horsefly species in the region that has a proboscis long enough to reach the nectar in the 21.9 mm long constricted portion of the *W. lepida* perianth tube (SD = 1.9, n = 14) (Johnson 2000). *Philoliche aethiopica* can thus be regarded as the most important pollinator of *W. lepida* in Gilboa Estate. *Watsonia lepida* forms part of a guild of flowers, including *Disa pulchra* (Orchidaceae) and *Cycnium racemosum* (Scrophulariaceae), that depend almost entirely on this fly for pollination (Johnson 2000). Although less abundant and possibly less efficient, *A. natalensis* is likely to effect a degree of fertilisation while foraging for pollen. Morphologically and phylogenetically similar to this species, *A. aspergina* may play a similar role in the pollination of *W. lepida*. Smaller bees, such as *A. mellifera* and various halictid bee species (Table 2.2) are unlikely to contact the stigma and contribute much to the effective fertilization of the species. Although apparently specialised for pollination by a single species, *W. lepida* appears to have reserve pollinators belonging to a different order (Table 2.1).

***Dierama luteoalbidum* Verdoorn (Iridaceae)**

Observations

The most consistent pollinator of *D. luteoalbidum* is the anthophorid bee, *Amegilla natalensis* (Friese), which is able to feed easily on the nectar housed in the 7.4 mm (SD = 0.7; n = 4) long constricted portion of the perianth (Table 2.2, Fig. 1.3C.). Pollen is passively deposited onto and removed from the insect body during the foraging process. Large amounts of *D. luteoalbidum* pollen were found to constitute about half the mixed pollen loads from both the dorsal and ventral surfaces of these bees (Table 2.2). The PII value of this species is calculated at 83.87. *Amegilla aspergina* (Cockerell) was also seen to visit *D. luteoalbidum* and captured individuals were found to carry small amounts of conspecific pollen, in less pure loads (Table 2.2). One visit by the long-tongued tabanid fly, *Philoliche aethiopica* (Thunberg), was observed and the captured individual was found to carry between 50 and 100 grains of *D. luteoalbidum* pollen exclusively (Table 2.2). Halictid bees and monkey beetles were also seen to visit the species (Table 2.2).

Discussion

The smaller bees and the monkey beetles (Table 2.2) are unlikely to be efficient pollinators of the species (visits are rare and these insects are unlikely to make consistent contact with floral sexual parts). Although *A. natalensis* is the principal pollinator of *D. luteoalbidum*, *A. aspergina* and *P. aethiopica* are able to effect a degree of fertilisation and can be considered as reserve pollinator species (Table 2.1).

***Dierama dracomontanum* Hilliard (Iridaceae)**

Observations

Time spent observing *D. dracomontanum* flowers was limited (Table 2.1). Visits by three halictid bee species as well as *Apis mellifera* were recorded (Table 2.2). The constricted portion of the perianth was measured at 4.38 mm (SD = 0.3, n = 3) and these bees are thus likely to be able to feed on the nectar contained there, passively picking up or depositing pollen as they forage. *Lasioglossum* species and *Halictus* sp. B13 (often found resting in the flowers, Fig. 1.3D.) were found to carry large amounts of *D. dracomontanum* pollen on the body as well as on the legs (pollen is also actively collected into scopae to provision offspring) (Table 2.2). *Dierama dracomontanum* pollen was also found in the mixed pollen loads analysed from the bodies of *Amegilla natalensis* individuals captured while visiting *D. luteoalbidum* flowers.

Discussion

Dierama dracomontanum appears to be pollinated by both large-bodied, long-tongued, nectar-foraging bees and small-bodied bees which actively collect pollen. *Apis mellifera* can be excluded as a potential pollinating species, since pollen was only found packed onto the legs where it is no longer accessible to the stigma (see also Westerkamp 1991). The single fly, Dipteran sp. BU, although carrying small amounts of *D. dracomontanum* pollen, is a rare visitor and is unlikely to contact the sexual parts of the flower. Although pollinated by species belonging to two families, the pollination system of *D. dracomontanum* is still relatively specialised, depending only on species of the order Hymenoptera (Table 2.1).

***Tritonia disticha* (Klatt) Bak. (Iridaceae)**

Observations

Halictid bees were the most frequently observed visitors to *T. disticha* flowers (Table 2.2). With their relatively short proboscides, these halictid bees may be restricted to pollen collection only. Five species were identified, the most common being *Halictus* sp. B13 (usually found resting in the flowers, Fig. 1.4A., Table 2.2). The single captured individual proved to be carrying between 10 and 50 grains of *T. disticha* pollen, almost exclusively (Table 2.2). Large amounts of pure *T. disticha* pollen were also found on the captured *Halictus* sp. AA individual. Smaller, less pure amounts of *T. disticha* pollen were found on the head and thorax of the *Lasioglossum* sp. individuals examined. Very little *T. disticha* pollen was found on *Halictus* sp. B10 and none was found on *Thrinchostoma* sp. (Table 2.2). *Amegilla natalensis* (Friese) and *Amegilla aspergina* (Cockerell) were also observed to visit *T. disticha* flowers. These species with their long proboscides (mean lengths of 9.4 mm and 7.8 mm, respectively) are easily able to access the nectar in the constricted perianth tube (mean length 7.75 mm, SD = 1.4, n = 3) and pollen is most likely passively deposited onto and removed from the insect body during feeding (large amounts of *T. disticha* pollen was found carried dorsally on the abdomen) (Table 2.2). The captured *A. natalensis* individual carried *T. disticha* pollen exclusively, while the median purity of the two *A. aspergina* individuals was only 0.35 (Table 2.2).

Discussion

Both the nectar- and pollen-foraging bees are likely to effect fertilization of *T. disticha*. The pollination system of *T. disticha* is relatively specialised, being limited to species of the order Hymenoptera (Table 2.1).

***Moraea inclinata* Goldbl. (Iridaceae)**

Observations

Pollinator observations carried out during this study revealed *M. inclinata* to be visited exclusively by Hymenoptera of the families Apidae, Megachilidae and Halictidae (Table 2.2). These bees are oriented by the bright floral nectar guides and push apart the tepal and style branch to climb towards the base where the nectar is located (Fig. 1.4C.). During this process pollen is passively removed from and deposited onto the insect body as the bee brushes first past the stigma

lobe and then the anther. Smaller bees may also actively collect pollen from the anthers, transferring it to the scopae while contacting receptive stigmas. Captured individuals of *Immanthidium immaculatum*, the most persistent visitor observed, were found to carry *M. inclinata* pollen exclusively on the body (Table 2.2). Also seen to visit the species were *Amegilla natalensis* (Friese), *Amegilla aspergina* (Cockerell) and individuals of the halictid species, *Halictus* sp. B15, *Halictus* sp. B10 and *Lasioglossum* sp, which all carried largely pure loads of *M. inclinata* pollen on the body as well as packed onto the legs (Table 2.2).

Discussion

The pollination system of *M. inclinata* is specialised with respect to order (Table 2.1). No single flower visitor species has a PII value that greatly exceeds that of any other however and it seems that the seven species (from three families) are equally able to effect successful fertilization of the species (although effectiveness may also depend on the behaviour of insects between flowers).

***Aristea woodii* N.E. Br. (Iridaceae)**

Observations

Time spent observing *A. woodii* populations was extremely limited and floral visitors were rare (Table 2.1). Visits made by the short-tongued *Lasioglossum* species (Halictidae) and by the Dipteran species BY and BZ were observed (Table 2.2). All except Dipteran species BY were found to carry relatively small, highly impure amounts of *A. woodii* pollen on the body and on the legs (Table 2.2).

Discussion

These limited observations support the prediction made by Bernhardt and Goldblatt (2000) that the pollination of the genus *Aristea* is dominated by species foraging for pollen. *A. woodii* is most likely relatively generalist in terms of pollination, the open shallow cup shape of the flower not restricting access to the pollen resource (Fig. 1.4B.). Breeding system experiments indicate that the species is capable of a high degree of autonomous self-pollination (see Chapter 3), explaining relatively high levels of fruit set despite the apparent rarity of floral visitors.

***Brunsvigia undulata* Leighton (Amaryllidaceae)**

Observations

The most common floral visitors recorded on *B. undulata* were the anthophorid bee species *Amegilla capensis* (Friese) and *Amegilla natalensis* (Friese) (Table 2.2). These bees were observed to contact the anthers and stigmas on their way to the base of the flower where they feed on floral nectar from the mouth of the c 18 mm long perianth tube (Fig. 1.4D.). All but one individual were found to carry *B. undulata* pollen, mostly dorsally for *A. capensis* and ventrally for *A. natalensis* (Table 2.2). The single captured *Amegilla aspergina* (Cockerell) individual was not found to carry *B. undulata* pollen (Table 2.2). Pollen was actively collected by *Allodapini* sp. (Apidae) and the captured individual was found to carry 50-100 pollen grains dorsally on the abdomen (Table 2.2). Butterflies were seen to visit *B. undulata* flowers but these visits were relatively infrequent and were restricted to three of the six sites in which observations were carried out. Relatively small amounts of *B. undulata* pollen were found in mixed pollen loads on the thorax and on and around the proboscides of two of the four butterflies examined (Table 2.2). On one occasion the Scarlet chested Sunbird, *Nectarinia senegalensis*, was observed feeding on nectar from *B. undulata* flowers, moving consistently from one inflorescence to another (Table 2.2).

Discussion

Although not all butterflies were found to carry pollen, they have been included as potential pollinating species since they are functionally and morphologically the same type of visitor. Likewise, *Amegilla aspergina* probably plays a similar role in the pollination of *B. undulata* as the other *Amegilla* species. Successful fertilization of *B. undulata* flowers may be effected by a wide range of at least 11 floral visitor species, including five families from the orders Hymenoptera, Lepidoptera and Passeriformes (Phylum Aves) (Table 2.1). The pollination system is thus a considerably generalist one.

***Agapanthus campanulatus* Leighton (Alliaceae)**

Observations

At all sites where observations were carried out, the most consistent and abundant visitors to the flowers of *A. campanulatus* are undoubtedly the

nemestrinid flies *Prosoeca* sp. 1 and *Prosoeca* sp. 2 (Fig. 1.5C., Table 2.2). The two species are very much alike and cannot be distinguished from one another unless captured. With average proboscis lengths of 12.4 mm (SD = 0.5 mm, n = 11) and 12.8 mm (SD = 0.5 mm, n = 4) respectively, these flies are easily able to feed on *A. campanulatus* nectar in the 8.64 mm long perianth tube (SD = 0.76, n = 9). All individuals examined carried large numbers of *A. campanulatus* pollen grains, mostly exclusively, from passive contact with the floral sexual parts during feeding (Table 2.2). Pollen was found mostly on and around the proboscis and the ventral surface of the body, but was also carried dorsally (Table 2.2). Butterflies also frequently visit *A. campanulatus* and 22 visits from eight species were recorded. Four of the butterfly species examined were found to carry *A. campanulatus* pollen on and around the proboscis (Table 2.2). *Agapanthus campanulatus* flowers were also visited by the anthophorid bee species *Amegilla natalensis* (Friese) (large amounts of pure pollen found on the ventral body surface and packed onto the legs), *Chalicodoma bombiformis* Gerstaecker, *Apis mellifera* (Linnaeus) and other smaller pollen-collecting halictid species (Table 2.2). On the 23 January 2000, a Scarlet chested Sunbird, *Nectarinia senegalensis*, was observed feeding in a clump of *A. campanulatus* flowers at site F5.

Discussion

With their elongated proboscides and large body size, *A. natalensis* and *C. bombiformis* are probably able to reach the nectar reward and effect passive fertilization with much the same efficiency as the nemestrinid flies, although visits are less frequent. The smaller halictids with their considerably shorter mouth parts are less likely to contact the stigma (probably restricted to pollen collection from the anthers) and are excluded as pollinating species. Although the two *Prosoeca* species tend to dominate the pollinator fauna of *A. campanulatus*, both in terms of efficiency and abundance, there is a wide variety of other floral visitors that are also able to effect fertilization (Table 2.1). The pollination system of *A. campanulatus* is thus a generalist one.

***Eucomis autumnalis* (Mill.) Chitt. (Hyacinthaceae)**

Observations

Observations at *E. autumnalis* populations revealed that the species is visited by a great diversity of insect species, including wasps, flies, bees, butterflies and beetles, which feed on the readily accessible and exposed nectar and/or pollen (Fig. 1.5A.). The most persistent visitor was the pompiliid wasp, *Hemipepsis hilaris* (Smith), followed by the tabanid fly, *Tabanocella denticornis* (Wiedemann), both of which feed on *E. autumnalis* nectar. Pollen is easily picked up from the anthers in the shallow open flowers and all captured individuals were found to carry *E. autumnalis* pollen, usually in large amounts (Table 2.2).

Discussion

The pollination system of *E. autumnalis* is a highly generalist one involving a great diversity of pollinator species from four different orders, all of which are able to effect fertilisation to some degree (Table 2.1).

***Aloe boylei* Bak. (Aloaceae)**

Observations

Although time spent observing flowering populations of *A. boylei* was extremely limited, frequent sightings were made of visits to the inflorescences by the Malachite Sunbird, *Nectarinia famosa*.

Discussion

These findings are supported by observations later carried out in 2002 by A. Hargreaves at site M1 in her study of the pollination systems of *Protea* spp (unpublished data). Numerous visits by the sunbirds to *A. boylei* were observed and all captured birds had extremely large *A. boylei* pollen loads (up to 3000 pollen grains) below the beak. *Aloe boylei* appears to be highly specialised, entirely dependent on a single species for pollination.

***Kniphofia laxiflora* Kunth. (Asphodelaceae)**

Observations

The flowers of *K. laxiflora* appear to be almost exclusively pollinated by the Mountain Pride butterfly, *Aeroptes tulbaghia* (Linnaeus), and hundreds of visits

by the species were observed. Twelve captured individuals proved to be carrying large (> 100 grains) pure loads of *K. laxiflora* pollen on the proboscis and sometimes also frontally on the head (Table 2.2). The mean proboscis length of *Aeropetes tulbaghia* is 32.3 mm (SD = 1.2 mm, $n = 8$) and the butterfly is thus well equipped to feed on the nectar located in the 38.3 ± 0.5 mm long perianth tube ($n = 10$). The butterfly feeds from a perched, upside down position, partially inserting its head into the mouth of the flower in order to reach the nectar with its proboscis, thus passively contacting the receptive stigma and dehiscent anthers (Fig. 1.5D.). *Aeropetes tulbaghia* is highly selective in its foraging and appears to be almost entirely dependent on *K. laxiflora* for nectar in this area (it was only seen to visit one other *Kniphofia* species not investigated in this study). *Catopsilia florella* (Fabricius) was occasionally also seen to visit the species and the two individuals examined carried small amounts of *K. laxiflora* pollen on and around the proboscis (Table 2.2). Five individuals of the bee species, *Hylaeus heraldicus* (Smith) (Colletidae), were seen collecting pollen from the anthers of *K. laxiflora* flowers and the single captured individual was found to be carrying small amounts of conspecific pollen (Table 2.2). On two occasions, a Malachite Sunbird, *Nectarinia famosa*, was seen to feed on *K. laxiflora* inflorescences.

Discussion

In terms of efficiency and abundance, *Aeropetes tulbaghia* is undoubtedly the most important pollinator of *K. laxiflora* and the reproductive success of the species would be considerably reduced in its absence. *Aeropetes tulbaghia* is known to be the near exclusive pollinator of about twenty South African plant species which have large red flowers (Johnson & Bond 1994). *Hylaeus heraldicus* however is also likely to effect a degree of pollination through passive contact with the exerted stigma and anthers, although the contribution of these bees to the pollination of the species is considerably less than that of *A. tulbaghia*. *Kniphofia laxiflora* is thus intermediately specialised in terms of pollination, with a number of reserve species from four different families available to effect a degree of fertilization (Table 2.1).

***Satyrium longicauda* Lindl. (Orchidaceae)**

Observations

Observations carried out at dusk during the 1999/2000 flowering season on a population of the taller *S. longicauda* morph at site M3 revealed the hawkmoth, *Basiothia schenki* (Möschler), to be the most frequent visitor (Table 2.1).

Pollinaria are passively removed and become attached to the moth's proboscis (42.0 mm, SD = 1.7, n = 3) as it passes over the column and into one of the floral spurs (mean length for Gilboa Estate populations 39.7 mm, SD = 2.0, n = 7) in which the nectar is concealed (Fig. 1.6C.). *B. schenki* was seen to move consistently between inflorescences, visiting a number of flowers on each.

Diurnal visits by butterflies were also witnessed, although these were rare and are unlikely to contribute much to the pollination of the species (Table 2.2). On one occasion the long-tongued fly Nemestrinid sp. 13 was seen to visit *S.*

longicauda inflorescences and capture of the individual revealed a *S. longicauda* pollinium attached to the elongated proboscis.

Discussion

Further observations carried out by S.D. Johnson and E. Raulings during the 2001 field season revealed numerous visits by *B. schenki* to *S. longicauda* inflorescences and support the observations of this study. Several individuals which had legitimate pollinaria attached to their proboscides were captured (Table 2.2). The pollination system of *S. longicauda* can be classified as highly specialised, the species being entirely dependent on *B. schenki* for pollination and fruit set at Gilboa Estate (Table 2.1).

The visit made by the long tongued nemestrinid fly is similar in nature to the observation made by Johnson (1997) in the grasslands of Verloren Valei Nature Reserve (Mpumalanga Province, South Africa) that the usually hawkmoth-pollinated, long-spurred form of *Satyrium hallackii* is also effectively pollinated by the long-tongued fly *Prosoeca ganglbauri* (Nemestiniidae). It may be interesting to investigate in the future whether there is a divergence in pollinator species between the different morphs of the *S. longicauda* complex.

***Disa versicolor* Reichenb. f. (Orchidaceae)**

Observations

Despite many hours of observation, only one visit made by a bee belonging to the genus *Amegilla* (Anthophoridae) was observed. On a separate occasion, an individual of the species *Amegilla natalensis* (Fries), captured while visiting *Tritonia disticha*, was found to have a *D. versicolor* pollinarium attached to its mouthparts (Table 2.2).

Discussion

The above observations are in keeping with those carried out in Dullstroom and in the Drakensberg Mountains of Lesotho and KwaZulu-Natal where *A. natalensis* was found to be a frequent visitor to *D. versicolor*, often accumulating large loads of pollinaria on the mouthparts (Johnson 1995). Investigations revealed the elongated proboscis of the bee (10.0 mm, $n = 14$) to be well suited to feeding on the nectar contained in the *D. versicolor* spur (9.8 mm, $s = 0.8$, $n = 11$) (Johnson 1995). The bees were observed to hang down from the dorsal sepal by their front legs while feeding (Fig. 1.6D., Johnson 1995). The galea, a rigid non-retractable portion of the mouthparts, is inserted into the dorsal sepal chamber, where pollinaria become attached to the ventral surface and then the true tongue (glossa) is extended into the downwardly curved spur (Johnson 1995). Later general pollinator observations carried out by S.D. Johnson and P. Neal at site M1 revealed numerous visits by *A. natalensis* to *D. versicolor*, four of the nine captured individuals carrying pollinia (S.D. Johnson 2002, pers. comm.; Table 2.2). *Disa versicolor* is highly specialised in terms of pollination, entirely dependent on *A. natalensis* for fruit set (Table 2.1).

***Eulophia zeyheriana* Sond. (Orchidaceae)**

Observations

No floral visits were witnessed during the 1999/2000 flowering season despite many hours of observation carried out at a number of *E. zeyheriana* populations at the different study sites.

Discussion

Although no floral visitors were observed in this study, later observations by G. Anderson, C. Peter and S.D. Johnson in the 2002 flowering season at site M1

revealed the species to be pollinated by a single species of the halictid bee genus *Nomia*. Nine individuals carrying *E. zeyheriana* pollinaria were captured while sheltering in the flowers of a species of *Wahlenbergia* (Craig Peter, unpublished data). *Eulophia zeyheriana* is non-rewarding and probably mimics flowers of *Wahlenbergia* sp. (flowers of both species have similar reflectance patterns) (C. Peter, unpublished data). The presence of *Wahlenbergia* sp. plants in the vicinity of *E. zeyheriana* appears to facilitate the pollination of *E. zeyheriana*, with individuals growing in the absence of *Wahlenbergia* sp. experiencing depressed levels of fruit set (C. Peter, unpublished data).

Eulophia zeyheriana is highly specialised for pollination by a single species, the halictid bee *Nomia* sp. (Table 2.1). The mutualism between *E. zeyheriana* and *Nomia* sp. may be particularly fragile because of its partial dependence on the presence of a second plant species.

DICOTYLEDONS

***Eriosema distinctum* N.E. Br. (Fabaceae)**

Observations

Eriosema distinctum flowers were visited by a great diversity of Hymenoptera (18 species from four different families: Megachilidae, Apidae, Halictidae, Vespidae) (Table 2.2). Legitimate access to the nectar located in the perianth tube is achieved through tripping the wing petals with the feet of the insect, which pushes the anthers and style onto the ventral surface of the body, thus passively depositing and picking up pollen. The most common, legitimate pollinators (all found to carry *E. distinctum* pollen ventrally) were *Megachile* sp. 1 (Fig. 1.7D.), *Megachile gratiosa* Gerstaecker, *Chalicodoma bombiformis* Gerstaecker (Fig. 1.7C.), and *Immanthidium immaculatum* (Smith) (Table 2.2). *Apis mellifera*, one of the most frequent flower visitors, was only seen illegitimately robbing nectar by biting through the corolla at the base of the flower, thus contributing nothing to pollination of the species. *E. distinctum* flowers were also visited by smaller butterfly species and bombyliid flies (Table 2.2).

Discussion

Although the butterfly and bombyliid species are likely to be less effective pollinators than the bees because they do not trip the flowers, a small degree of fertilization may still be effected as their proboscides move over the sexual structures to reach the nectar. *Eriosema distinctum* has a relatively generalist pollination system involving at least 18 insect species from three orders (Table 2).

***Pentanisia prunelloides* (Klotzsch ex Eckl. & Zeyh.) (Rubiaceae)**

Observations

Many butterflies were observed to visit and consistently move between *P. prunelloides* inflorescences during the 1999 flowering season (43 butterfly visits by 15 species were recorded at the various study sites). The most common butterfly species were *Junonia octavia sesamus natalensis* (Trimen), *Vanessa cardui* (Linnaeus) and *Belenois creona severina* (Stoll) (Fig. 1.8A.), all examined individuals of which were found to carry relatively large amounts of *P. prunelloides* pollen mostly exclusively on and around the proboscis (Table 2.2). *Pentanisia prunelloides* pollen was also found on other species of Lepidoptera examined (Table 2.2). Another common and persistent visitor to *P. prunelloides* inflorescences was the bombyliid fly, *Systoechus* sp. (Table 1, Fig. 1.8B.). These flies spent large amounts of time foraging in *P. prunelloides* patches and were found to carry mostly pure loads of *P. prunelloides* pollen on and around the proboscis. A much less frequent visitor to the species is the long-tongued tabanid fly, *Philoliche aethiopica* (Thunberg). The long proboscis of this species allows easy access to the nectar contained in the 14.44 mm long perianth tube (SD = 0.9, n = 9) and the fly is most likely an efficient pollinator, despite its rarity (Table 2.2). Other Diptera and Hymenoptera were seen to visit *P. prunelloides* flowers (Table 1). Larger bee species such as *Amegilla natalensis* (Friese) and *Amegilla aspergina* (Cockerell) are probably able to effect pollination of the species whilst foraging for nectar with their long proboscides. The smaller bees and flies with their shorter mouthparts are probably restricted to foraging for pollen and are unlikely to make effective contact with thrum morph stigmas (located within the perianth tube).

Discussion

The bombyliid fly, *Systoechus* sp., is equipped with a long, sucking proboscis which invaginates into the head capsule. Grimaldi (1988) calculated the proportion of the total proboscis length that retracted into the head for both *Bombylius pygmaeus* and *B. major* at 28%. It is also mentioned that the proboscis of *B. lancifer* can be extended nearly twice its resting length (Grimaldi 1988). Using this information to correct for the invaginated portion of the proboscis of pinned *Systoechus* sp. specimens, the maximum functional proboscis length of the species is estimated at between 8.14 and 11.8 mm. A proboscis of this length would just enable access to the nectar contained within the *P. prunelloides* perianth tube. Although *Systoechus* sp. may contribute a fair amount to the successful fertilization of the species, it is probably reasonable to assume that butterflies are more important pollinators here, as they tend to travel greater distances between visits, which may be important for the transfer of compatible pollen in heterostylous species. The pollination system of *P. prunelloides* is a considerably generalist one, with a greatly diverse pollinator fauna available to effect successful fertilisation of the species (Table 2.1).

***Zaluzianskya natalensis* (Bernh. ex) Krauss (Scrophulariaceae)**

Observations

Observations carried out at *Z. natalensis* populations at dusk at sites M1 and M3 during the 1999/2000 flowering season revealed the hawkmoth, *Basiothia schenki* (Möschler), to be the most consistent and frequent visitor to the flowers (Fig. 1.8C.).

Discussion

Further observations carried out by Johnson *et al.* (2002) revealed frequent visits by hawkmoths, mostly by the species *Hippotion celerio* (L.). Trapping for hawkmoths at night yielded a number of hawkmoth species carrying large amounts (100-3000 grains) of *Z. natalensis* pollen frontally on the head and on the proboscis (Johnson *et al.* 2002; Table 2.2). The moths *Basiothia schenki* and *Hippotion celerio* (L.), with their long proboscides (40.0 ± 2.8 mm, $n = 2$ and 39.3 ± 1.4 , $n = 23$ mm respectively), are well suited to feeding on the nectar concealed in the 45.9 mm long (SD = 2.6, $n = 22$) perianth tube (Johnson *et al.* 2002). Pollen is passively deposited onto and picked up from the proboscis and

head of the moth as it feeds. With proboscis lengths more than double that of the perianth tube, *Agrius convolvuli* (L.) and *Coelonia mauritii* (Butl.) may be less efficient pollinators of the species (pollen will be deposited on the proboscis only and not on the face) (Table 2.2). The pollination system of *Z. natalensis* is specialised, with the species entirely dependent on a single specific functional type of pollinator for successful reproduction (Table 2.1).

***Chironia krebsii* Griseb. (Gentianaceae)**

Observations

Observations for *C. krebsii* pollinators were limited and visits to the flowers were infrequent. Two halictid bee species, *Halictus* sp. B10 and *Lasioglossum* sp, and three fly species, Eristalinae species 1, 3 and 4 were captured (Table 2.2).

Discussion

The porose nature of the anthers suggests the possibility of buzz pollination by carpenter bees, as in *Chironia jasminoides* (Johnson 1997). None were seen to visit *C. krebsii* however and the exposed anthers seem to dehisce automatically, indicating, together with the open cup-shape of the perianth (Fig. 1.8D.), that the species may be a generalist with respect to pollination (few morphological mechanisms to exclude any low-efficiency pollen foragers) or that the species is autogamous (supported by the high capacity for autonomous self-pollination evident in the breeding system experiments of Chapter 3).

***Xysmalobium parviflorum* Harv. ex Scott Elliot (Asclepiadaceae)**

Observations

Xysmalobium parviflorum flowers were visited by large numbers of flies of the families Muscidae, Tachinidae and Sarcophagidae (Fig. 1.9B.); the most common being *Orthellia* sp. (Muscidae) (Table 2.2). These flies come in large numbers to feed on *X. parviflorum* nectar, attracted by the sickly sweet scent of the flowers. Pollinaria are occasionally picked up by the proboscides of these flies as they lick or suck the nectar from the flowers. Of the 15 captured *Orthellia* sp. individuals examined, five had at least one *X. parviflorum* pollinarium attached to their mouthparts (Table 2.2). Four pollinaria were found attached to the mouthparts of the single captured individual of *Sarcophaga* sp. BG. Large numbers of the generalist cetoniid beetle, *Atrichelephinis tigrina*, also visited the flowers of *X.*

parviflorum (Fig. 1.9A.) and four individuals were found to carry pollinaria on their legs (Table 2.2). Other visitors observed were butterflies and halictid bees. None of these were found to carry pollinaria (Table 2.2).

Discussion

Although some of the fly species were not found to carry pollinaria, they are functionally and morphologically the same flower visitor type foraging for the same resource and here they have all been included as potential pollinator species. The butterflies and bees however have been excluded due to their rarity and lack of pollinaria. The pollination system of *X. parviflorum* is intermediately specialised with the species dependent on a number of pollinating species from the orders Diptera and Coleoptera (Table 2.1).

***Pachycarpus grandifloris* (L.f.) E. Meyer (Asclepiadaceae)**

Observations

Pachycarpus grandifloris flowers were consistently visited throughout the flowering season by the pompiliid wasp *Hemipepsis hilaris* Smith (both males and females) (Table 2.2). These large, strong-flying wasps have short mouthparts and come in great numbers to feed on the small amounts of nectar which collect at the ends of the corona lobes, under the sharply incurved apex (Fig. 1.9C.). During the course of feeding or moving between lobes within the inflated, round corolla, the back legs of the wasp generally come into contact with the staminal column and may occasionally pick up or deposit a pollinarium. On two occasions, a wasp was found dead in a flower with its leg trapped by the corpusculum (Fig. 1.9D.). Of the 30 captured wasps however, only two individuals were found to carry a *P. grandifloris* pollinarium attached to the back leg (Table 2.2). A single *P. grandifloris* pollinarium was found attached to the middle leg of a captured beetle, *Lycus* sp. 2 (Lycidae), a few of which were seen to visit the flowers. The beetle, *Atrichelephinis tigrina*, was also present in large numbers in *P. grandifloris* flowers at site F4 but individuals were not found to carry pollinaria.

Discussion

Because only two individuals were found to carry pollinaria, one may question whether *H. hilaris* is the principal pollinator of *P. grandifloris*. The low number of pollinaria carried is however in keeping with the low pollination success and

natural fruit set of the species (see Chapter 5) and of the plant family in general (Wyatt & Broyles 1994). A slow turnover of pollinaria may be important in terms of long distance pollen dispersal, in the deposition of compatible pollen and in maintaining genetic diversity (Wyatt & Broyles 1994). Alternatively, the lack of pollinaria may be the result of rapid turnover, suggesting that *H. hilaris* is in fact a highly effective pollinator of the species.

Although the beetle *Lycus* sp. 2 may well effect fertilization, it is reasonable to assume that this species is a less efficient pollinator than *H. hilaris*, being less abundant, less selective in its foraging and weaker in flight ability. The pollination system of *P. grandifloris* can thus probably be considered relatively specialised, with the species almost entirely dependent on the wasp for successful pollination but with the added flexibility of a reserve pollinator in *Lycus* sp. 2 (Table 2.2).

GENERAL DISCUSSION

The degree of specialisation for pollination was found to vary widely among the 21 grassland wildflower species investigated here with the number of pollinator species per plant ranging from one to as many as 20 from ten different families (Table 2.1).

Of the 21 plant species for which potential pollinators were observed, six were found to be highly specialised in terms of pollination with reproductive success entirely dependent either on a single species (*D. versicolor*, *E. zeyheriana*, *S. longicauda*, *G. longicollis*, *A. boylei*) or on a specific functional type of pollinator from a single family (*Z. natalensis*). One would expect these species to be particularly susceptible to reproductive collapse should the pollinator fauna of Gilboa Estate be disrupted. Five species are apparently highly specialised for pollination by long-tongued flies (*W. lepida*), long-tongued bees (*D. luteoalbidum*), wasps (*P. grandifloris*), short-tongued flies (*X. parviflorum*) and butterflies (*K. laxiflora*), but also have 'reserve' pollinators belonging to different orders and sometimes even different phyla. These wildflower species may be slightly more resilient to disruption, although the degree to which these

considerably less abundant pollinator species may compensate for the loss of the more 'classical' pollinator has yet to be determined. The irid species *D. dracomontanum*, *T. disticha* and *M. inclinata* have relatively specialised, two-tiered pollination systems involving both pollen- and nectar-foraging bees.

The remaining species have pollination systems more generalist in nature. The pollination system of *E. distinctum*, although still dominated by bees, involves considerably more species and families and also butterflies and flies. *Chironia krebsii* and *A. woodii* seem to be generalist species pollinated by polylectic short-tongued bees and flies and have a high capacity for autonomous self-pollination. *Brunsvigia undulata*, *A. campanulatus*, *E. autumnalis* and *P. prunelloides* are highly generalist and are pollinated by numerous taxa. These seven species are probably particularly well buffered against reproductive collapse should habitat fragmentation cause changes in the abundance, diversity and species composition of pollinator fauna.

Two thirds of the wildflower species studied are thus specialised to some degree (although reserve pollinators may lend a degree of resilience to just under half of these). Degree of specialisation in plant species appears to be higher here than in the wildflower communities of forests of North Carolina, Illinois and New Brunswick studied by Motten (1986), Schemske *et al.* (1978) and Barrett and Helenurm (1987) respectively. Similar investigations of wildflower pollination systems carried out by Motten (1986) revealed only three of the 12 species investigated to be relatively specialised, according to the criteria used in this study (p 43). Schemske *et al.* (1978) found four of seven wildflower species to be pollination specialists (three species visited by a single pollinator species). Five of the 12 species investigated by Barrett and Helenurm (1987) were found to be relatively specialised for pollination.

The large proportion of plants with apparently specialised pollination systems in this study suggests that the plant-pollinator community of KwaZulu-Natal Moist Upland Grasslands may be vulnerable to disruption. The discovery of specialised pollination systems involving hawkmoths, solitary anthophorid bees and long-tongued flies adds to the numerous highly specialised pollination systems already discovered in South Africa and supports the claim that pollination in the largely

unstudied developing world may be more specialised than in parts of Europe and North America (see Johnson & Steiner 2000).

The repercussions resulting from the loss of a given pollinator species will vary according to the importance of that pollinator species to the various plant species, i.e. the number of plant species dependent on that pollinator for successful fertilization and reproductive output, and the degree to which those plant species are specialised. Observations carried out here reveal numerous diverse pollinator species with high indices of pollinator importance (PII) whose loss may consequently result in the reproductive collapse of one or a few wildflower species (Table 2.2). Important pollinator taxa include long-tongued anthophorid bees, short-tongued bees, wasps, hawkmoths, butterflies, long-tongued-flies, short-tongued flies and sunbirds.

Hymenoptera are the most important floral visitors in the pollination systems studied here both in terms of abundance (34% of the total number of diurnal floral visitors observed) and the number of wildflower species dependent on them for pollination. Long-tongued solitary bees are involved in the pollination of 59% of the plant species studied and comprise more than half of the diurnal floral visitors observed. One of the more important pollinators of the wildflowers investigated is the anthophorid bee *Amegilla natalensis*, the sole known pollinator of *D. versicolor* (PII = 100) and the primary pollinator of *D. luteoalbidum* (PII = 83.9). The bee is also important in the pollination of *D. dracomontanum* and *B. undulata* (*A. natalensis* and *A. capensis* have a combined PII of 92.2 for this species). Another important bee is the short-tongued halictid *Nomia* sp, the sole known pollinator of *E. zeyheriana*. Although wasps are not involved in the pollination of many species, *P. grandifloris* is almost entirely dependent on *Hemipepsis hilaris* for pollination (PII = 90.9).

Lepidoptera are also important in the grassland pollination systems studied here. Two hawkmoth species, *Agrius convolvuli* and *Basiothia schenki*, have pollinator importance indices of 100 and are the sole pollinators of *G. longicollis* and *S. longicauda* respectively. Hawkmoths are the only known pollinators of *Z. natalensis*. *Kniphofia laxiflora* is almost entirely dependent on the butterfly *Aeropetes tulbaghia* for pollination (PII = 99.8). Long-tongued flies constitute 18% of the total number of diurnal floral visitors observed and the tabanid

Philoliche aethiopica is almost entirely responsible for the pollination of *W. lepida* (PII = 98.5). The short-tongued *Orthelia* sp. (Muscidae) is important in the pollination of *X. parviflorum* (PII = 66.5). *Aloe boylei* is entirely dependent on the Malachite Sunbird *Nectarina famosa* for pollination.

The wildflower communities of temperate deciduous forests studied by Motten (1986), Schemske *et al.* (1978) and Barrett and Helenurm (1987) appear to support a substantially less diverse pollinator fauna than that of the moist upland grasslands studied here. As in this study the most important pollinators in the forests of piedmont North Carolina and Illinois are Hymenoptera, making up 57 and 75% of the total number of floral visitors observed (Schemske *et al.* 1978; Motten 1986). Other important pollinators in these systems are Diptera (Bombyliidae and Syrphidae). These systems also appear to have fewer pollinator species that are entirely responsible for the pollination of one or a few plant species. No pollinator species was found to be solely responsible for the pollination of a plant species in Motten's (1986) study. The highest relative abundance values were 0.79 for *Andrena nigrihirta* visiting *Uvularia sessilifolia* (Liliaceae) and 0.63 for *Bombylius major* visiting *Viola papilionaceae* (Violaceae). In the study of Schemske *et al.* (1978) two pollinator species (the syrphid *Metasyrphus americanus* and the bumblebee *Bombus griseocollis*) are solely responsible for the pollination of one and two plant species respectively. Species of *Bombus* are the most important pollinators of the wildflower community in New Brunswick and are involved in the pollination of 67% of the plant species investigated (Barrett & Helenurm 1987). Also common in their study were syrphids, bombyliids, and various solitary bees. The mayfly *Ephemerella* sp. was the only species observed visiting *Medeola virginianum* (Liliaceae) (Barrett & Helenurm 1987).

Fidelity was found to vary among the pollinators captured in this study (Table 2.2). Median purity of the pollen loads of all *Amegilla natalensis* individuals examined was found to be 0.9. This suggests that, although *A. natalensis* is involved in the pollination of nearly half the plant species studied here, it tends to be fairly constant in its foraging, collecting pollen from only one or a few species at least within the peak flowering periods of those species. Plant species specialised for pollination by *A. natalensis* should thus suffer little from

deposition of foreign pollen. The lower median pollen load purity of the smaller, short-tongued halictid bees examined (0.6) reflects the more generalist foraging behaviour of these species. Long-tongued flies of all species appear to be highly selective foragers, with the median pollen load purity of examined individuals calculated at 1.0. The butterfly species studied here also appear to be fairly constant in their foraging, at least temporally, with a grouped median pollen load purity of 1.0. In contrast, hawkmoths were observed to visit a variety of wildflower species in addition to the specialised species studied here (also evident in pollen loads, R. Alexandersson 2002, pers. comm.). Malachite Sunbirds also regularly and consistently visited species of *Protea* in the area as well as the specialist *A. boylei* (A. Hargreaves, unpublished data).

Fidelity in the grassland pollinators encountered in this study thus seems to be variable, although the majority of important pollinator species tend to show temporal specialisation at least. This contrasts with the results of similar pollinator observations and pollen load analyses carried out by Motten (1986) in forests of piedmont North Carolina where most individual visitors to wildflowers were found to forage on more than one host. *Bombylius major* was found to be the least discriminating host, contrasting with the high degree of fidelity of the bombyliid species studied here. The somewhat greater host fidelity of bees was apparently forced by the abundance of one flowering species and a paucity of other simultaneously blooming nectar sources at the time (Motten 1986). This is an unlikely explanation for the temporal specialisation of pollinators in this study given the large number of species found blooming concurrently at any one time during the study season. The study of Schemske *et al.* (1978) in the woodlands of Illinois found that most floral visitors were constant foragers, visiting only one species of wildflower during a foraging trip.

CONCLUSION

Due to the limited scope of the study (dealing with 24 plant species over two field seasons), it is likely to be only an approximation of the full range of interactions in the community (Bronstein 1995; Corbet 1997). Nevertheless, this is the first attempt at an exploration of plant-pollinator mutualisms in moist upland

grasslands of KwaZulu-Natal, a region threatened by commercial afforestation. The discovery of a rich and diverse plant-pollinator community, involving unusual and previously undescribed species (*Prosoeca* sp. 1, *Prosoeca* sp. 2, Nemestrinid sp. 13; D.A. Barraclough 2001, pers. comm.) warrants further investigation and greater publicity for this biome. The relatively high levels of specialisation suggest that the wildflower species of moist upland grasslands may be vulnerable to pollinator disruption resulting from habitat fragmentation. The diversity of pollinator types may however lend a degree of resilience to the system as a whole.

Table 2.1 Summaries of the pollination systems of the 24 wildflower study species with details of study sites used and time spent observing the species.

Plant species	Principal pollinators	No. of pollinator taxa			Study sites	Obs. time
		S	F	O		
<i>Gladiolus longicollis</i>	Hawkmoth	1	1	1	M1	3 + 4 ¹ ev.
<i>Watsonia lepidia</i>	LT fly, LT bee	3	2	2	M1, M2, F1	5 + 60 ² h
<i>Dierama luteoalbidum</i>	LT bee, LT fly	3	2	2	M1, M3	6 h
<i>Dierama dracomontanum</i>	LT bee, ST bee	4	2	1	M1	4 h
<i>Tritonia disticha</i>	LT bee, ST bee	6	2	1	M1, M2, F2, F6	20 h
<i>Moraea inclinata</i>	LT bee, ST bee	7	3	1	M1, M2, F1	3 h
<i>Aristea woodii</i>	ST fly, ST bee	≥3	≥3	≥2	M1, F6	2 h
<i>Brunsvigia undulata</i>	butterfly, LT bee, sunbird	11	5	3	M1, M3, MW5, F1, F4	20 h
<i>Agapanthus campanulatus</i>	LT fly, butterfly, LT bee, sunbird	14	9	4	M1, F1, F5	20 h
<i>Eucomis autumnalis</i>	ST fly, wasp, ST bee, butterfly, beetle	11	9	4	MW5, FW9, F4	8 h
<i>Aloe boylei</i>	sunbird	1	1	1	M1	2 h
<i>Kniphofia laxiflora</i>	butterfly, LT bee, sunbird	5	5	3	M3, F7	10 h
<i>Satyrium longicauda</i> (lrg morph)	Hawkmoth	1	1	1	M3	3 + 3 ³ ev.
<i>Disa versicolor</i>	LT bee	1	1	1	M1	4 + 1 ⁴ h
<i>Eulophia zeyheriana</i>	ST bee	1	1	1	M1, F1	5 h + ⁵
<i>Eriosema distinctum</i>	LT bee, ST bee, LT fly, butterfly, wasp	18	6	3	M1, M2, F2, F4	20 h
<i>Pentanisia prunelloides</i>	butterfly, LT fly, LT bee	20	10	3	M2, F1, F2	20 h
<i>Zaluzianskya natalensis</i>	Hawkmoth	8	1	1	M1, M3	4 + 6 ⁶ ev.
<i>Chironia krebsii</i>	ST fly, ST bee	≥5	≥2	≥2	MW4, FW9	3 h
<i>Xysmalobium parviflorum</i>	ST fly, beetle	8	4	2	M2, F4	5 h
<i>Pachycarpus grandifloris</i>	pompilid wasp, beetle	2	2	2	M2, F1, F4	8 h

S = Species, F = families, O = orders, obs. time = observation time, h = hours, ev. = evenings

1 (Alexandersson & Johnson 2002)

2 (Johnson 2000)

3 (E. Raulings & S.D. Johnson 2001, pers. comm.)

4 (S.D. Johnson & P. Neal 2002, pers. comm.)

5 (C. Peter, unpublished data)

6 (Johnson *et al.* 2002)

Table 2.2 The identity and relative importance of floral visitor species to the 21 wildflower species as determined from abundance, median pollen load size and purity and morphological fit.

Family	Species	N			no. of individuals in pollen load classes					Pollinator Importance						location of pollen	mean length mouthparts x ± SD (n)
		obs.	capt.	ex.	0	1-10	10-50	50-100	> 100	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
GLADIOLUS LONGICOLLIS 1# (tube length 93.0 ± 9.0 mm, n = 289)																	
Lepidoptera																	
Sphingidae	Theretra cajus (Cramer)	-	3	3	2	1	0	0	0	0.00	-	-	1.00	0.00	0.00	pb +	27.0±2.7 (3)
Sphingidae	Theretra capensis (L.)	-	1	1	1	0	0	0	0	0.00	-	-	1.00	0.00	0.00	pb +	35.0
Sphingidae	Agrius convolvuli (L.)	-	15	15	3	10	2	0	0	0.25	-	-	1.00	-	100.00	pb +	102.9±16.7 (15)
Sphingidae	Basiothla schenki (Möschler)	-	6	6	4	2	0	0	0	0.00	-	-	1.00	0.00	0.00	pb +	39.5±2.9 (6)
Sphingidae	Hippotion celerio (L.)	-	8	8	4	3	1	0	0	0.00	-	-	1.00	0.00	0.00	pb +	36.8±3.8 (8)
Sphingidae	Nephele sp	-	3	3	3	0	0	0	0	0.00	-	-	1.00	0.00	0.00	pb +	37.0±3.0 (3)
WATSONIA LEPIDA (tube length 21.9 ± 1.9 mm, n = 14)																	
Diptera																	
Tabanidae	Philoliche aethiopica (Thunberg)	c 80	9	3	0	1	0	2	0	0.75	0.80	0.72	1.00	0.43	98.46	th; ab (d)	14.7±0.8 (22)
Hymenoptera - Apoidea																	
Apidae - Apinae	Amegilla aspergina (Cockerell)	1	1	0	0	0	0	0	0	-	-	0.01	1.00	-	-		9.1 ±0.6 (8)
Apidae - Apinae	Amegilla natalensis (Friese)	15	7	4	1	2	1	0	0	0.25	0.20	0.14	1.00	0.01	1.54	bd	9.4±0.5 (10)
Apidae - Apinae	Apis mellifera (Linnaeus)	1	1	1	1	0	0	0	0	0.00	0.00	0.01	ND	-	-	lg	3.4±0.2 (11)
Apidae - Xylocopinae	Allodapini sp	1	1	1	0	1	0	0	0	0.25	0.01	0.01	ND	-	-	lg	3.8±0.3 (4)
Halictidae	Lasiglossum sp (subgenus Ctenonomia)	3	3	3	0	2	0	0	0	0.25	0.20	0.03	ND	-	-	bd (d); lg	2.6±0.5 (7)
Halictidae	Hallctus sp AH	9	1	1	0	0	1	0	0	0.50	0.01	0.08	ND	-	-	lg	-
Megachilidae - Megachilinae	Megachile konowiana Friese ♂	1	1	1	1	0	0	0	0	0.00	0.00	0.01	ND	-	-		>5.0 (1)

Family	Species	N			no. of individuals in pollen load classes					Pollinator Importance						location of pollen	mean length mouthparts x ± SD (n)
		obs.	capt.	ex.	0	1-10	10-50	50-100	> 100	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
DIERAMA LUTEOALBIDUM (tube length 7.4 ± 0.7 mm, n = 4)																	
Hymenoptera - Apoidea																	
Apidae - Apinae	<i>Amegilla natalensis</i> (Friese)	11	3	3	0	0	0	1	2	1.00	0.45	0.58	1.00	0.26	83.87	bd; lg	9.4±0.5 (10)
Apidae - Apinae	<i>Amegilla aspergina</i> (Cockerell)	3	3	3	0	2	0	0	1	0.25	0.30	0.16	1.00	0.01	0.03	bd; lg	9.1 ±0.6 (8)
Halictidae	<i>Halictus</i> sp AG	1	1	1	0	0	0	0	1	1.00	1.00	0.05	ND	-	-	bd (v)	-
Halictidae	<i>Halictus</i> sp B19	1	1	0	0	0	0	0	0	-	-	0.05	ND	-	-		-
Megachilidae - Anthidiini	<i>Immanthidium immaculatum</i> (Smith)	2	1	1	1	0	0	0	0	0.00	0.00	0.11	ND	0.00	0.00	bd	3.0±0 (10)
Diptera																	
Tabanidae	<i>Philoliche aethiopica</i> (Thunberg)	1	1	1	0	0	0	1	0	0.75	1.00	0.05	1.00	0.04	0.13	th (d); bd (v)	14.7±0.8 (22)
Coleoptera																	
Scarabaeidae - Rutellinae	Hopliini sp AU	8	8	0	0	0	0	0	0	-	-	0.42	-	-	-		-
DIERAMA DRACOMONTANUM (tube length 4.4 ± 0.3 mm, n = 3)																	
Hymenoptera - Apoidea																	
Halictidae	<i>Halictus</i> sp AE	2	2	0	0	0	0	0	0	-	-	0.11	1.00	-	-		-
Halictidae	<i>Halictus</i> sp B13 (sleeping)	3	1	1	0	0	0	0	1	1.00	1.00	0.17	1.00	0.17	40.48	bd (v)	3.0 (1)
Halictidae	<i>Lasiglossum</i> sp (subgenus Ctenonomia)	10	5	3	0	0	1	1	1	0.75	0.60	0.56	1.00	0.25	59.52	Bd; lg	2.6±0.5 (7)
Apidae - Apinae	<i>Apis mellifera</i> (Linnaeus)	2	2	1	0	0	1	0	0	0.50	0.80	0.11	1.00	0.00	0.00	lg	3.4±0.2 (11)
Diptera																	
Unidentified dipteran family	Dipteran sp BU	1	1	1	0	1	0	0	0	0.25	0.20	0.06	ND	-	-	bd (v)	-

Family	Species	N			no. of individuals in pollen load classes					Pollinator Importance						location of pollen	mean length mouthparts x ± SD (n)
		obs.	capt.	ex.	0	1-10	10-50	50-100	> 100	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
TRITONIA DISTICHA (tube length 7.8 ± 14 mm , n = 3)																	
Hymenoptera - Apoidea																	
Apidae - Apinae	<i>Amegilla aspergina</i> (Cockerell)	2	2	2	0	0	1	0	1	0.75	0.35	0.09	1.00	0.02	6.25	ab (d)	7.8±0.4 (2)
Apidae - Apinae	<i>Amegilla natalensis</i> (Friese)	1	1	1	0	0	0	1	0	0.75	1.00	0.05	1.00	0.04	12.50	ab (d)	9.4±0.5 (10)
Halictidae	<i>Halictus</i> sp B13	9	1	1	0	0	1	0	0	0.50	0.99	0.41	1.00	0.20	62.50	bd	3.0
Halictidae	<i>Halictus</i> sp B10	2	2	2	1	0	0	1	0	0.38	0.05	0.09	1.00	0.00	0.06	lg	2.3±0.4 (2)
Halictidae	<i>Thrinchostoma</i> sp	1	1	1	1	0	0	0	0	0.00	0.00	0.05	1.00	0.00	0.00		-
Halictidae	<i>Halictus</i> sp AA	1	1	1	0	0	0	0	1	1.00	1.00	0.05	1.00	0.05	15.63	bd	-
Halictidae	<i>Lasioglossum</i> sp (subgenus Ctenonomia)	3	3	3	1	1	0	1	0	0.25	0.40	0.14	1.00	0.01	3.13	hd; th	2.6±0.5 (7)
Hymenoptera - Vespoidae																	
Sphecidae - Philanthinae	<i>Philanthus histrio</i> Fabricius ♀	2	2	1	0	0	1	0	0	0.50	0.10	0.09	ND	-	-	hd; th	-
Diptera																	
Syrphidae	<i>Asarkina</i> sp 2	1	1	0	0	0	0	0	0			0.05	ND	-	-		
MORAEA INCLINATA (tube length 7.9 ± 0.3 mm, n = 3)																	
Hymenoptera - Apoidea																	
Apidae - Apinae	<i>Amegilla natalensis</i> (Friese)	1	1	1	0	0	1	0	0	0.50	0.95	0.04	1.00	0.02	5.44	bd; lg	9.4±0.5 (10)
Apidae - Apinae	<i>Amegilla aspergina</i> (Cockerell)	3	2	2	1	0	0	1	0	0.38	0.90	0.12	1.00	0.04	11.59	bd; lg	7.8±0.4 (2)
Apidae - Apinae	<i>Apis mellifera</i> (Linnaeus)	5	5	3	0	0	2	1	0	0.50	1.00	0.20	1.00	0.10	28.61		3.4±0.2 (11)
Apidae - Xylocopinae	<i>Xylocopa flavorufa</i> (De Geer) ♀	1	1	1	1	0	0	0	0	0.00	0.00	0.04	1.00	0.00	0.00		-
Halictidae	<i>Halictus</i> sp B10	1	1	1	0	0	0	0	1	1.00	0.95	0.04	1.00	0.04	10.87	ab (v); lg	2.3±0.4 (2)
Halictidae	<i>Halictus</i> sp B15	5	5	3	2	1	0	0	0	0.00	0.90	0.20	1.00	0.00	0.00	bd	1.5 (1)

Family	Species	N			no. of individuals in pollen load classes					Pollinator Importance						location of pollen	mean length mouthparts $\bar{x} \pm SD$ (n)
		obs.	capt.	ex.	0	1-10	10-50	50-100	> 100	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
Halictidae	<i>Lasiglossum</i> sp(subgenus Ctenonomia)	7	7	4	0	1	1	0	2	0.75	0.85	0.16	1.00	0.10	29.18	ab (v); lg	2.6±0.5 (7)
Megachilidae - Anthidiini	<i>Immanthidium immaculatum</i> (Smith)	5	3	2	1	0	1	0	0	0.25	1.00	0.20	1.00	0.05	14.31	bd	3.0±0 (10)
ARISTEA WOODII (tube length 3.9 ± 0.2 mm, n = 5)																	
Diptera																	
Calliphoridae	BY	1	1	1	1	0	0	0	0	0.00	0.00	0.33	1.00	0.00	0.00		-
Unidentified dipteran family	BZ	1	1	1	0	1	0	0	0	0.25	0.10	0.33	1.00	0.01	2.20	bd (v)	-
Hymenoptera - Apoidea																	
Halictidae	<i>Lasiglossum</i> sp(subgenus Ctenonomia)	1	1	1	0	1	0	0	0	0.25	0.01	0.33	1.00	0.00	0.22	bd (v); lg	2.6±0.5 (7)
BRUNSVIGIA UNDULATA (tube length 18 mm)																	
Hymenoptera - Apoidea																	
Apidae - Apinae	<i>Amegilla natalensis</i> (Friese)	c35	10	9	1	1	0	3	4	0.75	0.99	0.38	1.00	0.28	45.53	bd (v); lg	9.4±0.5 (10)
Apidae - Apinae	<i>Amegilla capensis</i> (Friese)	c35	5	5	0	0	1	2	2	0.75	0.99	0.38	1.00	0.33	53.66	ab (d); lg	9.1±0.6 (8)
Apidae - Apinae	<i>Amegilla aspergina</i> (Cockerell)	1	1	1	1	0	0	0	0	0.00	0.00	0.01	ND	0.00	0.00		7.8±0.4 (2)
Apidae - Xylocopinae	<i>Allodapini</i> sp	1	1	1	0	0	0	1	0	0.75	0.60	0.01	ND	-	-	ab (d)	3.8±0.3 (4)
Hymenoptera - Vespoidae																	
Sphecidae - Philanthinae	<i>Cerceris latifrons</i> Bingham ♂	1	1	1	1	0	0	0	0	0.00	0.00	0.01	ND	0.00	0.00		-
Lepidoptera																	
Hesperiidae	Hesperiid sp (unidentified)	1	0	0	0	0	0	0	0	-	-	0.01	ND	-	-		-

Family	Species	N			no. of individuals in pollen load classes					Pollinator importance						location of pollen	mean length mouthparts x ± SD (n)
		obs.	capt.	ex.	0	1-10	10-50	50-100	> 100	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
Papilionidae	<i>Princeps ophidicephalus</i> (Oberthür)	1	0	0	0	0	0	0	0	-	-	0.01	1.00	-	-		24.5 (1)
Papilionidae	<i>Princeps nireus lyaeus</i> (Doubleday)	5	0	0	0	0	0	0	0	-	-	0.05	1.00	-	-		22.8±1.8 (2)
Papilionidae	<i>Princeps euphranor</i> (Trimen)	2	1	1	1	0	0	0	0	0.00	0.00	0.02	1.00	0.00	0.00		21.5 (1)
Pieridae	<i>Belenois gidica</i> (Godart) ♀	1	1	1	0	1	0	0	0	0.25	1.00	0.01	1.00	0.00	0.49	pb +	9.9±0.5 (4)
Pieridae	<i>Catopsilla florella</i> (Fabricius)	3	1	1	0	1	0	0	0	0.25	0.25	0.03	1.00	0.00	0.33	th (v)	16.5 (1)
Pieridae	<i>Appias epaphia contracta</i> (Butler)	1	1	1	1	0	0	0	0	0.00	0.00	0.01	1.00	0.00	0.00		22.5 (1)
Coleoptera																	
Scarabaeidae - Cetoniinae	Cetoniinae sp AW	3	3	0	0	0	0	0	0	-	-	0.03	ND	-	-		-
Meloidae	<i>Mylabris</i> sp AX	1	1	0	0	0	0	0	0	-	-	0.01	ND	-	-		-
Passeriformes (Phylum Aves)																	
Nectariniidae	<i>Nectarinia senegalensis</i>	1	1	0	0	0	0	0	0	-	-	0.01	ND	-	-		-
AGAPANTHUS CAMPANULATUS (tube length 8.6 ± 0.8 mm, n = 9)																	
Lepidoptera																	
Acraeinae	<i>Acraea</i> sp 1	4	2	2	1	0	0	0	1	0.50	1.00	0.03	1.00	0.02	3.51	pb +	11.0 (1)
Acraeinae	<i>Acraea horta</i> (Linnaeus) ♂	1	1	1	1	0	0	0	0	0.00	0.00	0.01	1.00	0.00	0.00		10.0 (1)
Nymphalinae	<i>Junonia octavia sesamus natalensis</i> (Trimen) ♂	2	1	1	0	0	0	1	0	0.25	0.80	0.02	1.00	0.00	0.70	pb +	14.0 (1)
Papilionidae	<i>Princeps nireus lyaeus</i> (Doubleday) ♀	8	3	3	0	2	1	0	0	0.25	1.00	0.07	1.00	0.02	3.51	pb +	22.8±1.8 (2)
Papilionidae	<i>Princeps euphranor</i> (Trimen) ♂	1	1	1	1	0	0	0	0	0.00	0.00	0.01	1.00	0.00	0.00		21.5 (1)
Papilionidae	<i>Princeps ophidicephalus</i> (Oberthür)	4	1	1	0	1	0	0	0	0.25	0.80	0.03	1.00	0.01	1.05	pb +	24.5 (1)

Family	Species	N			no. of individuals in pollen load classes					Pollinator Importance						location of pollen	mean length mouthparts x ± SD (n)
		obs.	capt.	ex.	0	1-10	10-50	50-100	> 100	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
Pieridae	<i>Colias electo electo</i> (Linnaeus) ♂	1	1	1	1	0	0	0	0	0.00	0.00	0.01	1.00	0.00	0.00		10.0 (1)
Pieridae	<i>Belenois creona severina</i> (Stoll) ♀	1	1	0	0	0	0	0	0			0.01	1.00	-	-		9.9±0.5 (4)
Diptera																	
Nemestrinidae	<i>Prosoeca</i> sp 1	c 40*	16	6	0	0	0	4	2	0.75	1.00	0.34	1.00	0.26	45.70	pb +; bd (v)	12.4±0.5 (11)
Nemestrinidae	<i>Prosoeca</i> sp 2	c 40*	6	3	0	1	0	1	1	0.75	1.00	0.34	1.00	0.25	43.94	pb +; bd (v)	12.8±0.5 (4)
Tabanidae	<i>Philoliche aethiopica</i> (Thunberg)	4	3	0	0	0	0	0	0	-	-	0.03	1.00	-	-		14.7±0.8 (22)
Hymenoptera - Apoidea																	
Apidae - Apinae	<i>Apis mellifera</i> (Linnaeus)	1	1	1	0	0	0	0	1	1.00	1.00	0.01	ND	-	-	lg	3.4±0.2 (11)
Apidae - Apinae	<i>Amegilla natalensis</i> (Friese)	3	3	2	0	0	0	0	2	1.00	1.00	0.01	1.00	0.01	1.58		9.4±0.5 (10)
Apidae - Xylocopinae	<i>Allodapini</i> sp	2	2	1	0	0	1	0	0	0.50	0.40	0.02	ND	-	-	hd; bd (v)	3.8±0.3 (4)
Halictidae	<i>Lasioglossum</i> sp(subgenus Ctenonomia)	1	1	1	0	0	0	0	1	1.00	0.99	0.01	ND	-	-	bd	2.6±0.5 (7)
Halictidae	<i>Halictus</i> sp AJ	1	1	1	0	0	0	0	1	1.00	1.00	0.01	ND	-	-	bd (v); lg	-
Megachilidae - Megachilinae	<i>Chalicodoma bombiformis</i> Gerstaecker	1	0	0	0	0	0	0	0	-	-	0.01	1.00	-	-		-
Passeriformes (Phylum Aves)																	
Nectariniidae	<i>Nectarinia senegalensis</i>	1	1	0	0	0	0	0	0	-	-	0.01	ND	-	-		-
EUCOMIS AUTUMNALIS (tube length 0.0 ± 0.0 mm)																	
Diptera - Tabanidae																	
Sarcophagidae	<i>Sarcophaga</i> sp BI	1	1	0	0	0	0	0	0	-	-	0.03	1.00	-	-		-
Tabanidae	<i>Tabanocella denticornis</i> (Wiedemann)	7	7	4	0	1	1	0	2	0.75	1.00	0.20	1.00	0.15	21.47	bd	-

Family	Species	N			no. of individuals in pollen load classes					Pollinator Importance						location of pollen	mean length mouthparts $\bar{x} \pm SD$ (n)
		obs.	capt.	ex.	0	1-10	10-50	50-100	> 100	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
Tabanidae	<i>Tabanus taeniatatus</i> (Macquart)	2	2	1	0	0	0	1	0	0.75	1.00	0.06	1.00	0.04	6.16	th (d/v)	
Hymenoptera - Vespoidea																	
Pompilidae - Pepsinae	<i>Hemipepsis hilaris</i> Smith	10	10	5	0	0	0	0	5	1.00	1.00	0.29	1.00	0.29	41.10	bd; lg	
Hymenoptera - Apoidea																	
Apidae - Apinae	<i>Apis mellifera</i> (Linnaeus)	1	1	1	0	0	0	0	1	1.00	0.99	0.03	1.00	0.03	4.07	bd	3.4±0.2 (11)
Halictidae	<i>Halictus</i> sp AH	1	1	0	0	0	0	0	0	-	-	0.03	1.00	-	-		-
Lepidoptera																	
Nymphalidae	<i>Junonia octavia sesamus natalensis</i> (Trimen)	1	1	1	0	1	0	0	0	0.25	0.50	0.03	1.00	0.00	0.51	pb +	11.8±1.2 (4)
Satyrinae	<i>Stygionympha vigilans</i> (Trimen)	1	1	1	0	1	0	0	0	0.25	0.50	0.03	1.00	0.00	0.51	pb +	8.3±1.8 (2)
Coleoptera																	
Lycidae	<i>Lycus</i> sp 1	5	5	2	0	0	0	1	1	0.88	1.00	0.14	1.00	0.10	14.40	bd	-
Scarabaeidae - Cetoniinae	<i>Atrichelephinis tigrina</i>	5	5	2	0	0	0	1	1	0.88	0.65	0.14	1.00	0.08	11.69	bd	-
Scarabaeidae - Cetoniinae	Cetoniinae sp AT	1	1	1	0	1	0	0	0	0.25	0.10	0.03	1.00	0.00	0.10	hd (d)	-
ALOE BOYLEI (tube length 51.0 ± 1.7 mm, n = 3)																	
Passeriformes (Phylum Aves)																	
Nectariniidae	<i>Nectarinia famosa</i>	4	0	0	-	-	-	-	-	-	-	-	-	-	-		
KNIPHOPHIA LAXIFLORA (tube length 38.3 ± 0.5 mm, n = 10)																	
Lepidoptera																	
Satyrinae	<i>Aeropetes tulbaghia</i> (Linnaeus)	>100	13	12	1	0	1	1	9	1.00	1.00	0.94	1.00	0.94	99.83	pb +	32.3±1.2 (8)
Pteridae	<i>Catopsilia florella</i> (Fabricius)	2	2	2	0	2	0	0	0	0.25	0.50	0.01	1.00	0.00	0.17	pb +	16.5 (1)

Family	Species	N			no. of individuals in pollen load classes					Pollinator Importance					location of pollen	mean length mouthparts x ± SD (n)	
		obs.	capt.	ex.	0	1-10	10-50	50-100	> 100	PLI	pollen purity	relative abundance	pollinator efficiency	PIV			PII
Hymenoptera - Apoidea																	
Colletidae - Hylaeinae	<i>Hylaeus heraldicus</i> (Smith)	5	1	1	0	1	0	0	0	0.25	-	0.03	ND	-	-	hd; ab (v)	-
Apidae - Apinae	<i>Apis mellifera</i> (Linnaeus)	1	1	1	1	0	0	0	0	0.00	0.00	0.01	ND	0.00	0.00		3.4±0.2 (11)
Passeriformes (Phylum Aves)																	
Nectariniidae	<i>Nectarinia famosa</i>	2	0	0	0	0	0	0	0	-	-	0.01	ND	-	-		-

Family	Species	N			no. of individuals in pollinia classes					Pollinator Importance						location of pollen	mean length mouthparts x ± SD (n)
		obs.	capt.	ex.	0	1-3	4-6	7-9	≥10	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
SATYRIUM LONGICAUDA 2# (tube length 39.7 ± 2.0 mm, n = 7)																	
Lepidoptera																	
Nymphalinae	small yellow butterfly	1	0	0	0	0	0	0	0	-	-	0.04	1.00	-	-		
	<i>Junonia octavia sesamus natalensis</i> (Trimen)	3	0	0	0	0	0	0	0	-	-	0.11	1.00	-	-		11.8±1.2 (4)
Nymphalinae	<i>Vanessa cardui</i> (Linnaeus)	1	0	0	0	0	0	0	0	-	-	0.04	1.00	-	-		10.3±0.4 (2)
Sphingidae	<i>Basiothia schenki</i> (Möschler)	c 20	3	3	0	1	0	1	1	1.00	1.00	0.77	1.00	0.77	100.00	pb+	42.0±1.7 (3)
Diptera																	
Nemestrinidae	Species 13	1	0	0	0	0	0	0	0	-	-	0.04	1.00	-	-		-
DISA VERSICOLOR 3 (tube length 5.98 ± 0.8 mm, n = 3)																	
Hymenoptera - Apoidea																	
Apidae - Apinae	<i>Amegilla natalensis</i> (Friese)	12	11	10	5	5	0	0	0	0.25	1.00	1.00	1.00	0.25	100.00	mp	9.4±0.5 (10)

Family	Species	N			no. of individuals in pollen load classes					Pollinator Importance						location of pollen	mean length mouthparts x ± SD (n)
		obs.	capt.	ex.	0	1-10	10-50	50-100	> 100	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
ERIOSEMA DISTINCTUM (tube length 3.0 ± 0.4 mm, n = 4)																	
Hymenoptera - Apoidea																	
Apidae - Apinae	<i>Amegilla natalensis</i> (Friese)	1	1	1	0	0	0	0	1	1.00	0.90	0.01	1.00	0.01	1.84	ab (v)	9.4±0.5 (10)
Apidae - Apinae	<i>Amegilla punctifrons</i> (Walker) ♂	1	1	1	1	0	0	0	0	0.00	0.00	0.01	1.00	0.00	0.00		
Apidae - Apinae	<i>Amegilla capensis</i> (Friese)	1	1	1	1	0	0	0	0	0.00	0.00	0.01	1.00	0.00	0.00		9.1±0.6 (8)
Apidae - Apinae	<i>Apis mellifera</i> (Linnaeus)	47	11	2	1	1	0	0	0	0.13	0.00	0.36	0.00	0.00	0.00	lg	3.4±0.2 (11)
Apidae - Xylocopinae	<i>Pithitis</i> sp	2	2	1	0	0	0	0	1	1.00	1.00	0.02	1.00	0.02	4.08	bd (v)	-
Apidae - Xylocopinae	<i>Allodapini</i> sp	1	1	1	1	0	0	0	0	0.00	0.00	0.01	1.00	0.00	0.00		3.8±0.3 (4)
Halictidae	<i>Nomia</i> sp	3	3	1	0	0	1	0	0	0.50	0.95	0.02	1.00	0.01	2.91	bd	-
Megachilidae - Megachilinae	<i>Megachile planatipes</i> Cockerell	4	4	3	0	0	2	0	1	0.50	0.99	0.03	1.00	0.02	4.04	bd	5.5±0 (3)
Megachilidae - Megachilinae	<i>Chalicodoma bombiformis</i> Gerstaecker	10	2	2	0	1	0	0	1	0.50	1.00	0.08	1.00	0.04	10.20	bd	-
Megachilidae - Megachilinae	<i>Megachile lydenbergiana</i>	1	1	0	0	0	0	0	0	-	-	0.01	1.00	-	-		-
Megachilidae - Megachilinae	<i>Megachile</i> sp 1	21	11	7	1	1	1	0	4	1.00	1.00	0.16	1.00	0.16	42.84	hd/ ab (v); lg	3.6±0.9 (7)
Megachilidae - Megachilinae	<i>Megachile gratiosa</i> Gerstaecker	13	9	3	0	1	0	0	2	1.00	0.90	0.08	1.00	0.09	23.50	ab (v); bd (d)	3.8±0.3 (3)
Megachilidae - Megachilinae	<i>Creightonella ianthoptera</i> Smith	3	2	2	0	1	1	0	0	0.38	0.50	0.02	1.00	0.00	1.15	hd; th (d)	4.5 (1)
Megachilidae - Megachilinae	<i>Creightonella dorsata</i> Smith	1	1	1	0	0	0	0	1	1.00	0.99	0.01	1.00	0.01	2.02	bd (v); lg	-
Megachilidae - Megachilinae	<i>Megachile konowiana</i> Friese	2	2	1	0	0	0	0	1	1.00	0.95	0.02	1.00	5.11	4.96	bd (d)	>5 (1)
Megachilidae - Megachilinae	<i>Creightonella rufa</i> (Friese)	3	1	1	0	0	1	0	0	0.50	0.80	0.02	1.00	2.52	2.44	bd (v)	-

Family	Species	N			no. of individuals in pollen load classes					Pollinator importance						location of pollen	mean length mouthparts x ± SD (n)
		obs.	capt.	ex.	0	1-10	10-50	50-100	> 100	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
Megachilidae - Anthidiini	<i>Immanthidium immaculatum</i> (Smith)	9	5	4	2	2	0	0	0	0.13	0.01	0.07	1.00	0.00	0.01	bd (d)	3.0±0 (10)
Hymenoptera - Vespoidea																	
Vespidae - Eumeninae	<i>Stellepiona stellenboschensis</i> (Cameron)	3	1	0	0	0	0	0	0	-	-	0.02	ND	-	-	-	-
Diptera																	
Unidentified dipteran family	CA	1	1	0	0	0	0	0	0	-	-	0.01	ND	-	-	-	-
Asilidae	Asilidae BW	1	1	0	0	0	0	0	0	-	-	0.01	ND	-	-	-	-
Bombyliidae	<i>Systoechus</i> sp	3	1	0	0	0	0	0	0	-	-	0.02	ND	-	-	-	8.1±0.5 (6)
Lepidoptera																	
Lycaenidae	<i>Cupidopsis cissus</i> (Godart) ♀	1	1	0	0	0	0	0	0	0.00	0.00	0.01	ND	0.00	0.00	-	-
PENTANISIA PRUNELLOIDES (tube length 14.4 ± 0.9 mm, n = 9)																	
Lepidoptera																	
	Butterfly species (unidentified)	5	0	0	0	0	0	0	0	-	-	0.06	1.00	-	-	-	-
Danainae	<i>Danaus chrysippus aegyptius</i> (Schreber)	1	1	1	0	0	1	0	0	0.50	1.00	0.01	1.00	0.01	2.35	pb +	10.50 (1)
Hesperiidae	Hesperiid sp 4	3	1	1	1	0	0	0	0	0.00	0.00	0.03	1.00	0.00	0.00	pb +	9.00 (1)
Hesperiidae	Hesperiid sp 2	5	3	1	0	1	0	0	0	0.25	1.00	0.06	1.00	0.01	3.92	-	14.00 (1)
Hesperiidae	Hesperiid sp 3	1	1	0	0	0	0	0	0	-	-	0.01	1.00	-	-	-	-
Hesperiidae	Hesperiid sp 1	2	2	0	0	0	0	0	0	-	-	0.02	1.00	-	-	-	12.50 (1)
Hesperiidae	Hesperiid sp 5	1	1	0	0	0	0	0	0	-	-	0.01	1.00	-	-	-	19.00 (1)
Lycaenidae	<i>Lepidochrysops ignota</i> (Trimen)	1	1	0	0	0	0	0	0	-	-	0.01	1.00	-	-	-	-
Nymphalinae	<i>Catacroptera cloanthe cloanthe</i> (Stoll)	1	1	1	0	0	0	1	0	0.75	1.00	0.01	1.00	0.01	3.14	pb +	-

Family	Species	N			no. of individuals in pollen load classes					Pollinator importance						location of pollen	mean length mouthparts x ± SD (n)
		obs.	capt.	ex.	0	1-10	10-50	50-100	> 100	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
Nymphalidae	<i>Junonia octavia sesamus natalensis</i> (Trimen)	9	6	5	0	1	2	0	2	0.50	1.00	0.10	1.00	0.05	19.61	pb +	11.8±1.2 (4)
Nymphalidae	<i>Vanessa cardui</i> (Linnaeus)	5	2	2	0	0	1	1	0	0.63	1.00	0.06	1.00	0.04	15.69	pb +	10.3±0.4 (2)
Papilionidae	<i>Priniceps euphranor</i> (Trimen)	1	0	0	0	0	0	0	0	-	-	0.01	1.00	-	-		21.5 (1)
Papilionidae	<i>Priniceps nireus lyaeus</i> (Doubleday)	2	2	2	0	1	1	0	0	0.38	0.85	0.02	1.00	0.01	2.75	pb +	22.8±1.8 (2)
Pieridae	<i>Belenois creona severina</i> (Stoll) ♀	4	4	3	0	0	0	3	0	0.75	1.00	0.04	1.00	0.03	11.76	pb +	9.9±0.5 (4)
Satyrinae	<i>Stygionympha vigilans</i> (Trimen)	2	2	2	2	0	0	0	0	0.00	0.00	0.02	1.00	0.00	0.00		8.3±1.8 (2)
Diptera																	
Syrphidae	<i>Asarkina</i> sp 1	3	3	2	0	0	0	0	2	1.00	0.99	0.03	ND	-	-	hd; mp	5.5 (1)
Syrphidae	<i>Eristalis</i> sp 1	1	1	1	0	0	0	0	1	1.00	0.95	0.01	ND	-	-		-
Syrphidae	<i>Eristalis</i> sp 2	1	1	0	0	0	0	0	0	-	-	0.01	ND	-	-		-
Asilidae	Asilidae BT	1	1	1	1	0	0	0	0	0.00	-	0.01	0.50	-	-		-
Bombyliidae	<i>Systoechus</i> sp	21	12	9	0	5	3	0	1	0.25	1.00	0.23	1.00	0.06	23.53	pb +; hd	8.1±0.5 (6)
Tabanidae	<i>Philolche aethiopica</i> (Thunberg)	4	4	3	1	0	0	0	2	1.00	1.00	0.04	1.00	0.04	15.69	pb +; hd	14.7±0.8 (22)
Tabanidae	big black horsefly	1	0	0	0	0	0	0	0	-	-	0.01	1.00	-	-		
Hymenoptera - Apoidae																	
Andrenidae - AndreninaeND	<i>Andrena</i> sp AI	1	1	0	0	0	0	0	0	-	-	0.01	ND	-	-		-
Apidae - Apinae	<i>Thyreus oxaspi</i> (Cockerell)	1	1	1	0	1	0	0	0	0.25	0.30	0.01	ND	-	-		-
Apidae - Apinae	<i>Amegilla natalensis</i> (Friese)	2	1	1	0	0	0	1	0	0.75	0.50	0.02	0.50	0.00	1.57	lg	9.4±0.5 (10)
Apidae - Apinae	<i>Amegilla aspergina</i> (Cockerell)	1	1	1	0	1	0	0	0	0.25	0.05	0.01	0.50	0.00	0.02	mp +	7.8±0.4 (2)

Family	Species	N			no. of individuals in pollen load classes					Pollinator importance						location of pollen	mean length mouthparts $\bar{x} \pm SD$ (n)
		obs.	capt.	ex.	0	1-10	10-50	50-100	> 100	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
Apidae - Apinae	<i>Apis mellifera</i> (Linnaeus)	4	4	1	0	1	0	0	0	0.25	1.00	0.04	0.00	0.00	0.00	bd (d)	3.4±0.2 (11)
Apidae - Xylocopinae	<i>Allodapini</i> sp	1	1	1	0	1	0	0	0	0.25	0.01	0.01	0.00	0.00	0.00	lg	3.8±0.3 (4)
Halictidae	<i>Halictus</i> sp AJ	4	2	2	0	1	1	0	0	0.38	0.15	0.04	0.00	0.00	0.00	bd (v)	3.0 (1)
Halictidae	<i>Halictus</i> sp B13	1	1	1	1	0	0	0	0	0.00	0.90	0.01	0.00	0.00	0.00		3.0 (1)
ZALUZIANSKYA NATALENSIS 4# (tube length 45.9 ± 2.6 mm, n = 22)																	
Lepidoptera																	
Sphingidae	<i>Hippotion celerio</i> (L.)	-	23	23	3	4	4	1	11	0.75	1.00	-	1.00	0.75	18.75	pb +	39.3±1.4 (23)
Sphingidae	<i>Agrius convolvuli</i> (L.)	-	2	2	0	1	0	0	1	0.63	1.00	-	ND	-	-	pb +	110.0±19.7 (2)
Sphingidae	<i>Basiothia schenki</i> (Möschler)	-	2	2	0	0	1	0	1	0.75	1.00	-	1.00	0.75	18.75	pb +	40.0±2.8 (2)
Sphingidae	<i>Hippotion eson</i> (Cramer)	-	1	1	0	0	0	0	1	1.00	1.00	-	1.00	1.00	25.00	pb +	47.0 (1)
Sphingidae	<i>Theretra cajú</i> (Cramer)	-	1	1	0	0	1	0	0	0.50	1.00	-	1.00	0.50	12.50	pb +	30.0 (1)
Sphingidae	<i>Hyles lineata llvornica</i> (Esper)	-	1	1	0	0	1	0	0	0.50	1.00	-	1.00	0.50	12.50	pb +	30.0 (1)
Sphingidae	<i>Dellephila nervii</i> (L.)	-	1	1	0	0	1	0	0	0.50	1.00	-	1.00	0.50	12.50	pb +	42.0 (1)
Sphingidae	<i>Coelonia mauritii</i> (Butl.)	-	1	1	0	1	0	0	0	0.25	1.00	-	ND	-	-	pb +	86.0 (1)
CHIRONIA KREBSII (tube length 7.2 ± 1.3 mm, n = 6)																	
Hymenoptera - Apidae																	
Halictidae	<i>Halictus</i> sp B10	1	1	1	0	0	0	0	1	1.00	0.70	0.17	1.00	0.12	31.82	bd	2.3±0.4 (2)
Halictidae	<i>Lasioglossum</i> sp (subgenus Ctenonomia)	1	1	1	0	0	0	0	1	1.00	0.80	0.17	1.00	0.13	36.36	bd	2.6±0.5 (7)
Diptera																	
Syrphidae	Eristalinae sp 1	1	1	1	0	0	0	0	1	1.00	0.70	0.17	1.00	0.12	31.82	bd	-
Syrphidae	Eristalinae sp 3	1	1	1	1	0	0	0	0	0.00	0.00	0.17	1.00	0.00	0.00		-
Syrphidae	Eristalinae sp 4	2	2	2	2	0	0	0	0	0.00	0.00	0.33	1.00	0.00	0.00		-

Family	Species	N			no. of individuals in pollinaria classes					Pollinator importance						location of pollen	mean length mouthparts x ± SD (n)
		obs.	capt.	ex.	0	1	2	3	4	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
XYSMALOBIMUM PARVIFLORUM (tube length 2.7 ± 0.3 mm, n = 5)																	
Diptera																	
Muscidae	Orthellia sp	20	15	15	10	4	1	0	0	1.00	1.00	0.51	1.00	0.51	66.54	mp	-
Muscidae	Helina sp BF	1	1	1	1	0	0	0	0	0.00	0.00	0.03	1.00	0.00	0.00		-
Tachinidae	Cuphocera sp BE	1	1	1	1	0	0	0	0	0.00	0.00	0.03	1.00	0.00	0.00		-
Sarcophagidae	Sarcophaga sp BG	3	1	1	0	0	0	0	1	1.00	1.00	0.08	1.00	0.08	10.04	mp	-
Sarcophagidae	Sarcophaga sp BH	1	1	1	1	0	0	0	0	0.00	0.00	0.03	1.00	0.00	0.00		-
Sarcophagidae	Sarcophaga sp BJ	1	1	1	1	0	0	0	0	0.00	0.00	0.03	1.00	0.00	0.00		-
Sarcophagidae	Sarcophaga sp BK	1	1	1	1	0	0	0	0	0.00	0.00	0.03	1.00	0.00	0.00		-
Coleoptera																	
Scarabaeidae - Cetoniinae	Atrichelephinis tigrina	7	7	7	3	3	1	0	0	1.00	1.00	0.18	1.00	0.18	23.42	lg; ab	-
Lepidoptera																	
Nymphalinae	Vanessa cardui (Linnaeus)	1	0	0	0	0	0	0	0	-	-	0.03	ND	-	-		10.3±0.4 (2)
Hesperiidae	Hesperid sp 6	1	1	1	1	0	0	0	0	0.00	0.00	0.03	-	0.00	0.00		-
Hymenoptera - Apoidea																	
Halictidae	Halictus sp AG	2	2	2	2	0	0	0	0	0.00	0.00	0.05	1.00	0.00	0.00		-
PACHYCARPUS GRANDIFLORIS (tube length 0.0 ± 0.0 mm)																	
Hymenoptera - Vespoidea																	
Pompilidae - Pepsinae	Hemipepsis hilaris Smith	55	30	30	28	2	0	0	0	1.00	1.00	0.50	1.00	0.50	90.90	lg	-
Tiphiidae	Tiphiidae sp AO	15	3	3	3	0	0	0	0	0.00	0.00	0.14	ND	0.00	0.00		-
Diptera																	
Sarcophagidae	Sarcophaga sp BI	1	1	1	1	0	0	0	0	0.00	0.00	0.01	ND	0.00	0.00		-
Coleoptera																	

Family	Species	<i>N</i>			no. of individuals in pollinaria classes					Pollinator importance						location of pollen	mean length mouthparts x ± SD (n)
		obs.	capt.	ex.	0	1	2	3	4	PLI	pollen purity	relative abundance	pollinator efficiency	PIV	PII		
Scarabaeidae - Cetoniinae	<i>Atrichelephinis tigrina</i>	35	0	0	0	0	0	0	0	-	-	0.32	ND	-	-		-
Lycidae	<i>Lycus</i> sp 2	5	1	1	0	1	0	0	0	1.00	1.00	0.05	1.00	0.05	9.10	lg	-

obs. = number of individuals captured and/or observed visiting flowers; *capt.* = number of individuals captured ; *exm.* = number of individuals examined for pollen; *PLI* = Pollen load index; pollen purity = median purity of pollen loads examined; *pb* = proboscis; *mp* = mouth parts; *hd* = head; *th* = thorax; *ab* = abdomen; *lg* = legs; *bd* = body; + = and around; *ND* = no data; tube length x ± SD mm, # = species for which floral visitors were captured using light traps, where number of individuals observed represents the number of individuals found to carry conspecific pollen

1 from Alexandersson and Johnson (2002)

2 from S. Johnson and E. Raulings (2001, pers. comm.)

3 from S. Johnson and P. Neal (2002, pers. comm)

4 from Johnson *et al.* (2002)

CHAPTER 3

BREEDING SYSTEMS

ABSTRACT

The degree to which a plant species is dependent on pollen vectors in order to reproduce sexually may be important in determining the vulnerability of that species to environmental perturbations. In order to determine the extent to which reproductive output of grassland wildflower species is dependent on animal-mediated pollen transfer, the capacity for autonomous selfing and the self-compatibility status of 14 species were determined with a range of controlled hand-pollination and bagging experiments. The capacity for autonomous selfing was inferred for an additional six species. The majority of species (90%) were found to be dependent on pollinators for fruit and seed set. Self-incompatibility is operative in at least six species. The apparent rarity of autonomous selfing in the grassland wildflower community could potentially indicate that natural pollination levels in this community are, and have been in the past, consistently high. The high degree of dependence on pollinators for reproductive output potentially renders the wildflower community of KwaZulu-Natal Moist Upland Grasslands vulnerable to a decrease in the abundance and diversity of pollinators.

INTRODUCTION

Despite the fact that higher plants are essentially stationary and mostly rely on independent vectors for the transfer of pollen between their sexual organs, pollen is not dispersed onto stigmas of the same or different flowers in an uncontrolled manner (Richards 1986). In hermaphrodite plant species pollen may be transferred from anthers to stigmas within the same flower (autogamy) or between different flowers (alogamy), either of the same plant (geitonogamy) or of different plants (xenogamy or outcrossing) (Richards 1986). Within-flower pollen transfer may be mediated by pollinators or may take place in the absence of flower visitors. In some flowers self pollen is deposited onto the stigma as the corolla wilts and drops off, ensuring seed production by selfing in the absence of cross-pollination (Kearns & Inouye 1993). The degree of separation that occurs between the anthers and the stigmas in time (dichogamy) and space (herkogamy) partly determines the amount of autogamy that takes place. The

number of pollen transfers that result in geitonogamous as opposed to xenogamous crosses depends on patterns of pollinator movement between flowers, on the size of the plant and on the number of flowers open together on the plant (Richards 1986). Successful pollination does not automatically translate into successful fertilization and in many species related pollen is rejected through a mechanism known as self-incompatibility (Richards 1986). Through these mechanisms plant species are able to manipulate the amount of selfing and crossing taking place and ultimately the relative genetic contribution to the next generation of individuals within a species (Wyatt 1983).

In self-compatible species however changes in the abundance or species composition of the pollinator fauna may affect the proportion of outcross and self pollen deposited, potentially resulting in reduced quantity and quality of offspring produced through the harmful effects of inbreeding depression (Chapter 1). Features which minimise levels of inbreeding and promote allogamy, such as self-incompatibility and herkogamy or dichogamy, may simultaneously substantially increase the risk of reproductive failure should the pollinators on which that plant species depends become locally extinct. Breeding systems thus also govern the degree of reproductive dependence on the pollination mutualism and may in turn affect the probability of successful reproduction and long term persistence of a plant species in the face of decreased pollinator abundance and diversity (Chapter 1). Dependence varies from none in asexual or autonomously selfing species to obligate in self-incompatible species (Bond 1994, 1995). Degree of self-compatibility will also affect the probability of successful fertilization by determining the pool of potential mates available (Chapter 1).

In order to determine the degree to which quantitative reproductive output is dependent on animal-mediated pollen transfer for a suite of grassland wildflower species, the capacity for autonomous selfing and the self-compatibility status of 14 species were determined with a range of controlled hand-pollination and bagging experiments. The importance of these attributes in assessing the risk of reproductive failure and extinction of these species due to fragmentation-induced pollinator disruption will be investigated in Chapter 5.

MATERIALS AND METHODS

To determine the self-compatibility status and reproductive dependence on vector-mediated pollination flowers of each species were bagged with fine mesh nylon bags during the bud stage and later randomly assigned to one of the following treatments: (1) unmanipulated, to test for autonomous self-pollination, (2) self-pollinated and (3) cross-pollinated. Pollen for self-pollination was taken from the same flower or another flower in the same plant and for cross-pollination from individuals occurring at least 10 m away from the pollen recipient (sometimes a mix of two or three other plants). Pollen was applied to the stigma using forceps and the anther itself. Flowers were marked by means of coloured wool and immediately rebagged after controlled pollinations to prevent pollen contamination and herbivory. Experiments were performed on several individuals of each species and different treatments were done on flowers within the same inflorescence. Due to time constraints, full breeding experiments could not be carried out for all of the study species and for some the capacity for autonomous selfing was determined by comparing reproductive output of bagged flowers from the unmanipulated treatment with that of flowers which were naturally pollinated in the field.

Fruit set (percentage flowers that set fruit), seed set per fruit and seed set per flower were later determined for each treatment. Where breeding system tests were carried out over two field seasons, treatment results were pooled for analysis. Percentage seed viability was determined for *Satyrium longicauda*: sub-samples of pooled seed from each treatment were examined under a dissecting microscope for the presence of embryos (capsules from each treatment accidentally dehisced into a common vial). Fruit set data from breeding system tests were analysed using contingency tables and the Chi-square statistic (Statgraphics Plus Version 7 1993). When significant differences were found, non-parametric Tukey-type multiple comparisons for proportions were employed (Zar 1996, p 560) in order to determine differences among treatments. Non-proportional data (seed set per fruit and seed set per flower) were analysed using Student's *t*-test or One Way Analysis of Variance (ANOVA) (Statgraphics Plus Version 7 1993). Where significant differences occurred between the three treatments, the Tukey Highly Significant Difference multiple

range test was carried out to determine where these differences occurred (Statgraphics Plus Version 7 1993). Non-normally distributed data were subjected to the Mann-Whitney *U*-test or the Kruskal-Wallis *H*-test for nonparametric data (Statgraphics Plus Version 7 1993) and the nonparametric equivalent to the Tukey HSD test (Zar 1996, p226).

Plant species producing significantly fewer fruits and seeds from self-pollination compared to cross-pollination were classified as self-incompatible and obligately xenogamous (following Jaimes & Ramirez 1999). When the results were not statistically different the species was considered self-compatible. Self-compatible species were then categorised into those capable of a degree of autonomous selfing (no significant differences in fruit and seed set between the unmanipulated and hand self-pollinated treatments) and those dependent on pollen vectors for fruit set (significantly fewer fruits and seeds produced from the unmanipulated treatment). Such analyses could not be carried out on the three species for which reproductive output was compared between the bagged unmanipulated treatment and flowers which were naturally pollinated in the field. A significant difference between the two treatments reveals only that reproductive output for these species would be significantly reduced in the absence of pollen vectors.

Two ratios were calculated for each species in order to obtain alternative measures of the degree of self-incompatibility and the capacity for autonomous selfing. The index of self-incompatibility (ISI) was calculated as fruit set from self-pollinated flowers divided by fruit set from cross-pollinated flowers following Zapata and Arroyo (1978), Dafni (1992) and Jaimes and Ramirez (1999). ISI values range from 0 (fully self-incompatible) to 1 (fully self-compatible). An index of autonomous self-pollination (IAS) was obtained by comparing fruit set resulting from no manipulation and that resulting from hand cross-pollination. IAS values range from 0 (completely dependent on pollen vectors for fruit set) to 1 (capable of 100% fruit set through autonomous selfing).

It should be noted that a high fruit set resulting from the unmanipulated treatment may also be the result of agamospermy although this condition is relatively unusual (Richards 1996). In addition, a significant reduction in

reproductive output in the self- compared to the cross-pollinated treatment can arise either from genetic self-incompatibility or inbreeding depression (Charlesworth 1985; Charlesworth & Charlesworth 1987; S.C.H. Barrett 2000, pers. comm.). In the absence of evidence of seed abortion suggesting inbreeding depression, a significantly lower reproductive output from the selfing compared with the outcrossing treatment was taken here as evidence of self-incompatibility.

RESULTS

Breeding system information was obtained for 14 species (eleven full breeding experiments, three bagging experiments only) and is presented in Tables 3.1 and 3.2. The capacity for autonomous self-pollination was inferred where possible for the eight species for which results were inconclusive or not available.

Three species (*Gladiolus longicollis*, *Zaluzianskya natalensis* and *Kniphofia laxiflora*) were found to be completely self-incompatible and obligately xenogamous (fruit set from both the unmanipulated and self-pollination treatments is negligible and the indices of self-incompatibility and autonomous self-pollination are 0). These species are thus obligately dependent on pollen vectors for sexual reproductive output.

Dierama luteoalbidum, *Tritonia disticha* and *Watsonia lepida* were also found to be partially self-incompatible, with fruit set resulting from self-pollinations significantly lower than that produced from hand cross-pollination. The indices of self-incompatibility are correspondingly low (0.14, 0.32 and 0.33 respectively). Although number of seed per fruit was not found to be significantly different between hand cross- and self-pollination in *D. luteoalbidum* and *T. disticha*, significant differences were evident in seed set per flower and these species appear to be largely self-incompatible. Despite the small amount of fruit resulting from the unmanipulated and hand self-pollination treatments of these species (this may represent partial self-compatibility or a breakdown or leak in the self-incompatibility system), they are still largely dependent on pollinators for

Table 3.1 Results of full breeding system experiments where bagged flowers were either left unmanipulated (control), self-pollinated (self) or cross-pollinated (cross). A statistically significant difference ($P < 0.05$, denoted by 'a' and 'b') between self and cross treatments was considered evidence of self-incompatibility. For self-compatible species no significant differences between self and control treatments indicates the capacity for autonomous self-pollination.

Species	Indices		Percentage fruit set \bar{x} (n)			Seed set per fruit $\bar{x} \pm SD$ (n)			Seed set per flower $\bar{x} \pm SD$ (n)		
	ISI	IA	cross	self	control	cross	self	control	cross	self	control
Self-incompatible, xenogamous											
<i>Z. natalensis</i> #	0	0	100.0 (9) a	0.0 (8) b	0.0 (37) b	36.1 \pm 23.7 (9)	0.0 \pm 0	0.0 \pm 0	36.1 \pm 23.7 (9)	0.0 \pm 0 (8)	0.0 \pm 0 (37)
<i>G. longicollis</i>	0	0	86.7 (15) a	0.0 (15) b	0.0 (20) b	112.1 \pm 42.8 (15)	0.0 \pm 0	0.0 \pm 0	93.4 \pm 58.3 (15)	0.0 \pm 0 (15)	0.0 \pm 0 (20)
<i>K. laxiflora</i>	0	0	70.0 (10) a	0.0 (10) b	0.4 (242) b	10.6 \pm 4.8 (10)	0.0 \pm 0	0.0 \pm 0	6.6 \pm 6.6 (10)	0.0 \pm 0 (10)	0.0 \pm 0 (242)
<i>D. luteoalbidum</i>	0.14	0.10	80.0 (10) a	11.1 (9) b	7.8 (64) b	14.3 \pm 7.7 (8) a	9.0 \pm 7.1 (2) ab	5.0 \pm 4.8 (7) b	11.4 \pm 9.1 (10) a	1.8 \pm 4.5 (9) b	0.5 \pm 2.1 (64) b
<i>T. disticha</i>	0.32	0.07	94.1 (17) a	30.0 (10) b	6.5 (31) b	11.2 \pm 4.5 (13) a	5.7 \pm 3.2 (3) ab	2.0 \pm 1.4 (2) b	10.4 \pm 5.2 (17) a	1.7 \pm 3.1 (10) b	0.1 \pm 0.6 (31) b
<i>W. lepidia</i>	0.33	0.24	66.7 (21) a	22.2 (27) b	16.1 (39) b	15.0 \pm 4.7 (12) a	9.3 \pm 2.0 (6) b	4.2 \pm 3.2 (13) c	9.0 \pm 8.4 (21) a	2.0 \pm 4.0 (27) b	0.6 \pm 1.9 (39) b
Self-compatible, incapable of autonomous self-pollination											
<i>A. campanulatus</i>	0.77	0.14	90.0 (10) a	70.0 (10) a	12.8 (141) b	19.6 \pm 2.4 (9) a	18.0 \pm 2.5 (7) a	12.3 \pm 5.0 (16) b	17.6 \pm 2.4 (10) a	12.6 \pm 8.9 (10) a	1.4 \pm 4.3 (141) b
<i>S. longicauda</i>	1.00	0.06	100.0 (11) a	100.0 (12) a	5.6 (108) b	70.5 \pm 10.7 a *	18.3 \pm 5.1 b *	14.5 \pm 10.5 b *			
Self-compatible, capable of autonomous self-pollination											
<i>D. dracomontanum</i>	0.23	0.02	38.9 (18) a	9.1 (22) ab	0.7 (136) b						
<i>E. zeyheriana</i>	0.45	0.05	83.3 (6) a	37.5 (8) ab	4.0 (25) b						
<i>C. krebsii</i>	-	0.96	100.0 (12) a	-	95.5 (22) a						

ISI = index of self-incompatibility, IAS = index of autonomous self-pollination.

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* percentage filled ovules

pollen transfer in order to effect fertilization and have low indices of autonomous self-pollination (0.10, 0.07 and 0.24 respectively).

Satyrium longicauda and *Agapanthus campanulatus* are classified as self-compatible with high ISI values (1.00 and 0.77 respectively) and no significant differences were evident between reproductive output from self- and cross-pollination treatments. Examination of percentage seed viability (number of seeds with embryos) resulting from pollination treatments on *S. longicauda* however revealed that selfed fruits had a significantly lower percentage of viable seeds than those arising from outcrossed flowers (Table 3.1). Both *S. longicauda* and *A. campanulatus* have low indices of autonomous self-pollination (IAS) (0.06 and 0.14 respectively) and reproductive output is significantly lower in the unmanipulated treatments than in hand self-pollination treatments. Thus, despite the strong self-compatibility of *S. longicauda* and *A. campanulatus*, these species are still dependent on pollinators for successful fertilization.

Dierama dracomontanum and *Eulophia zeyheriana* were found to be self-compatible and capable of some autonomous selfing with no significant differences in fruit set occurring between the self- and cross-pollination treatments and the self- and unmanipulated treatments. The ISI of *D. dracomontanum* is unexpectedly low at 0.23 as opposed to 0.45 for *E. zeyheriana*. Fruit set in open-pollinated *D. dracomontanum* plants is much higher at 54% (Chapter 4) than the 38.9% observed from the hand cross-pollination, suggesting error in the treatment (possibly from inviable pollen or missed stigma receptivity). The low IAS values of *D. dracomontanum* (0.18) and *E. zeyheriana* (0.05) indicate that these species are only capable of a small amount of autonomous self-pollination and that pollinators still play a major role in the transfer of pollen.

Chironia krebsii is fully self-compatible with an IAS value very close to 1 at 0.96. It is the only species that did not produce significantly more seeds in the cross-pollinated treatment than in the unmanipulated treatment (Table 3.1) and *C. krebsii* is thus capable of nearly 100% fruit set in the absence of pollen vectors.

Eucomis autumnalis, *Kniphofia ichopensis* and *Aristea woodii* produced significantly less fruit and seed in the absence of pollen vectors than in their presence (Table 3.2). The low levels of fruit set in the bagged unmanipulated treatment and consequent low IAS values in *E. autumnalis* (0.08) and *K. ichopensis* (0.25) suggest that these species are not capable of autonomous selfing to any great degree. The much higher IAS value for *A. woodii* (0.58) implies a high capacity for autonomous selfing and a low dependence on pollen vectors.

Table 3.2 Results of breeding system experiments in which reproductive output was compared between bagged, unmanipulated (control) and naturally pollinated (open) flowers. Significant differences between treatments ($P < 0.05$) are denoted by 'a' and 'b'.

Species	IAS	Fruit set $\bar{x} \pm SD$ (n)		Seed set per fruit $\bar{x} \pm SD$ (n)	
		open	control	open	control
<i>E. autumnalis</i>	0.08	87.9 \pm 7.3 (15) a	6.9 \pm 5.4 (3) b	7.4 \pm 3.9 (15) a	1.5 \pm 0.7 (3) b
<i>K. ichopensis</i>	0.25	29.0 \pm 16.7 (29) a	7.3 \pm 9.0 (11) b	10.4 \pm 2.9 (26) a	8.3 \pm 1.3 (4) a
<i>A. woodii</i>	0.58	80.7 \pm 11.6 (21) a	47.1 \pm 28.8 (9) b		

IAS = index of autonomous self-pollination.

Aloe boylei and *Moraea inclinata* are considered incapable of autonomous selfing on the basis of evidence of self-incompatibility in the literature (Riley & Majumdar 1979; Goldblatt 1986) and in the lack of fruit set in bagged inflorescences. Autonomous self-pollination is prohibited in *Xysmalobium parviflorum* and *Pachycarpus grandifloris* by the structure of the asclepiad gynostegium (herkogamy) (Wyatt & Broyles 1994). Similarly, autonomous selfing is mechanically prohibited by floral structure in *Disa versicolor* (Johnson 1995). The heterostylous flowers of *Pentanisia prunelloides* are considered incapable of autonomous selfing because fertilization generally cannot occur within the same floral morph (Kearns & Inouye 1993) and no fruits were set in the few inflorescences bagged. Flowers of *Eriosema distinctum* were so heavily infested with caterpillars that no conclusions as to breeding system could be drawn. The species has been described as having a clonal nature and extremely poor seed set by Stirton (1975), suggesting that *E. distinctum* may be self-incompatible. The results for breeding system tests of *Brunsvigia undulata* were inconclusive with high levels of seed abortion and parthenocarpy in both seasons.

DISCUSSION

The 14 species tested here exhibited a range of dependency on external pollen vectors from those which are obligately outbreeding to those capable of a high degree of autonomous selfing (Tables 3.1, 3.2). Only *C. krebsii* (IAS 0.96) and *A. woodii* (IAS 0.58) were found to be clearly capable of autonomous self-pollination. The remaining 12 species were found to be largely dependent on pollen vectors for fruit set (at least six of these were also self-incompatible and obligately outbreeding). Of the additional eight species for which data were unavailable, complete dependence on pollen vectors could be inferred for six. Ninety percent of the species investigated here are thus dependent on pollinators for the production of fruit and seed.

The predominance of wildflower species dependent on pollinators for sexual reproduction in moist upland grassland is similar to that found in the study of Jaimes and Ramirez (1999) of the breeding systems of trees, shrubs and herbs in secondary deciduous forest in Venezuela (73.5% of the 49 species are dependent on vector-mediated pollination). Capacity for autonomous self-pollination was also found to be relatively rare in the breeding systems of the Cape flora, although this is mainly due to the prevalence of monoecy and dioecy in the flora (Steiner 1987). In the current study around half of the wildflower species dependent on pollinators were found to be self-incompatible, similar again to the results of Jaimes and Ramirez (1999) and of Barrett and Helenurm in their (1987) study of 12 boreal forest herbs.

Unreliable pollinator service can be a potent selective force and plants may respond by evolving the capacity for autonomous self-pollination (Kevan 1972; Wyatt 1983). The apparent rarity of this capacity in the grassland wildflower community studied here could thus potentially indicate that natural pollination levels in this community are consistently high and that plant species are seldom limited by pollen availability (supported by the results of Chapter 4). High levels of autogamy and self-compatibility in the breeding systems of the Juan Fernández Islands flora (Anderson *et al.* 2001) and of the spring wildflower community of a temperate deciduous forest (Motten 1986) may be the evolutionary result of high risk of pollen limitation due to rarity of floral visitors

on the Juan Fernández Islands (Anderson *et al.* 2001) and high levels of pollinator competition in the spring wildflower community (Motten 1986).

As previously mentioned, reduced fruit and seed set following self-pollination may in fact be the result of strong inbreeding depression (Charlesworth 1985; Charlesworth & Charlesworth 1987). It is suspected to be the reason for the reduced seed viability of selfed fruit in *Satyrium longicauda* (Table 3.1), since self-incompatibility is not common among orchids (Dressler 1981). *Satyrium longicauda* may be susceptible to inbreeding depression because it most probably naturally experiences a high degree of outcrossing as a result of the long distance foraging ability of its hawkmoth pollinator (Chapter 2) (Janzen 1983; Ellstrand & Elam 1993; Van Treuren *et al.* 1994).

It is interesting to compare the two methods used here to estimate the compatibility status and capacity for autonomous self-pollination of the grassland wildflower species. The statistical comparison of reproductive output of the different treatments provides a clear-cut and objective method of distinguishing between species which are capable of self-pollination or self-fertilization and those which are not, without having to use arbitrarily chosen ratios as did Bawa (1974) and Zapata and Arroyo (1978). In Bawa (1974), for example, those species in which cross-pollinations yielded five times more fruits than self-pollinations were considered to be self-incompatible. The indices of self-incompatibility and autonomous self-pollination are probably better measures of the natural situation however, since many species lie between the extremes of obligately autogamous and obligately outbreeding (Bawa 1974; Waser 1993). Caution must be exercised in the interpretation of these indices because of the potential compounded error associated with deriving a ratio by combining two estimates.

In addition, experimental conditions may have resulted in higher levels of autonomous self-pollination, and thus higher IAS values, in bagged flowers than is true for the natural situation. Bagging may increase the amount of self-pollination taking place by altering the natural position of the anthers and stigma in the flower and bringing the stigma into contact with self pollen (directly from the anthers or from where it has settled on the petals or the bag itself) as a

result of windy conditions or constriction (Dafni & Dukas 1986; Schoen & Lloyd 1992; Steiner 1993; Zink and Wheelwright 1997). This effect may be made worse by the large amounts of self pollen present in bagged flowers since it cannot be removed by insects from the dehiscent anthers (Zink & Wheelwright 1997).

An alternative method would have been to classify the wildflower species into mating system categories according to the pollen-ovule ratios of the species following Cruden's classical (1977) study. The counting of pollen-ovule ratios may have been prohibitive for a large range of species however. In addition, we are concerned here primarily with the potential of a given species to set seed in the absence of pollen vectors and not with estimating the actual levels of selfing and outcrossing taking place in a natural population. Cruden's work has also been criticised for suggesting that pollen production is only to ensure ovule fertilization (Charnov 1979). Finally, plant breeding systems have been incorrectly classified based on pollen-ovule ratios, as evident in the two facultatively selfing species of the Juan Fernández Islands flora that were wrongly classified as obligate outbreeders (Anderson *et al.* 2001).

CONCLUSION

If the 20 species investigated here are representative of the grassland wildflower community as a whole, then the apparent high reproductive dependence on pollinators suggests that the community may potentially be highly susceptible to habitat fragmentation should pollinator abundance and diversity be disrupted. The risk of local extinction of these species may in turn depend on the ability of the species' pollinators to persist in a fragmented landscape, the degree of specificity of the plant-pollinator mutualism (investigated in Chapter 2) and the degree of demographic dependence on seeds (Bond 1995; Turnbull *et al.* 2000). An understanding of breeding systems and pollination of threatened plant species is thus crucial for the development of effective conservation or restoration programmes. Many initiatives may fail if the mutualistic relationships of the plant species with their pollinators are not simultaneously preserved (Kearns & Inouye 1998; Weller 1994).

CHAPTER 4

POLLEN LIMITATION

ABSTRACT

Female reproductive success in plants may be limited either by the availability of pollen or physical resources such as water and nutrients. In order to determine whether reproductive output in grassland wildflower species is pollen-limited in undisturbed habitats, supplemental hand-pollination experiments were performed on nine species. Degree of pollen limitation was inferred for an additional five species by comparing reproductive output from natural pollination with that from hand cross-pollination of bagged flowers. The relative rarity of pollen limitation in these wildflower species (evident in only four species) suggests that moist upland grasslands are characterised by high levels of pollinator activity. In order to determine whether pollen limitation is more severe in habitat fragments, supplemental hand pollination experiments were carried out simultaneously in mainland and fragmented sites for five species. Greater pollen limitation of reproductive output in fragments was evident in two species, suggesting that decreased reproductive output in habitat fragments is the result of a change in the quantity and/or quality of pollinator services for these species.

INTRODUCTION

Female reproductive success in plants may be limited either by the quantity and quality of pollen received, or by the availability of physical resources, such as water and nutrients (Burd 1994; Wilson *et al.* 1994). Most recent reviews point to pollen availability as the more prevalent factor limiting reproductive success (Burd 1994; Larson & Barrett 2000; Bierzychudek 1981; Gill 1989; Wilson *et al.* 1994). Burd's (1994) review of literature published on hand-pollination experiments found pollen limitation to be a widespread phenomenon in undisturbed natural plant populations, with 62% of the 258 species studied exhibiting significant pollen limitation effects of some sort. Burd (1994) suggests that pollen limitation of female reproductive success is common in a stochastically fluctuating pollination environment as the outcome of an adaptive strategy that allows individuals to adjust reproductive output in response to unpredictable variations in pollinator abundance.

Inadequate pollination can reduce plant reproductive success at different levels, either by decreasing the number of flowers that set fruit or by decreasing seed-set per fruit (both in turn affecting seed set per flower) (Motten 1986). The first effect is a result of flowers remaining unpollinated or receiving illegitimate pollen, while the second is a result of transfer of insufficient quantities of pollen grains, a decrease in the quality of pollen genotypes provided or some combination of the two (Motten 1986; Karoly 1992).

Most of the investigations into the occurrence of pollen limitation in natural plant populations are based on one or a few plant species. In order to understand the ecological correlates of pollen limitation (which taxa or habitats are particularly susceptible to pollen limitation) more community-wide investigations involving whole suites of plant species are required (Johnson & Bond 1997). In one such study, Motten (1986) found that pollinator service is not a major source of reduced fecundity in the wildflower community of mesic deciduous forests in piedmont North Carolina. In a study of conservation target plant species in the Western United States, V.J. Tepedino *et al.* are also finding pollen limitation to be uncommon, occurring in only 10-15% of the more than 30 species investigated thus far (unpublished data in Thomson 2001). In contrast, pollen-limitation of fecundity was found to be relatively common in populations of temperate montane wildflower species studied by Campbell (1987) and in spring woodland herb species in woodlands of Illinois studied by Schemske *et al.* (1978). Johnson and Bond (1997) also found that pollen-limitation of fruit set occurs widely in a whole suite of wildflower species in the Cape, South Africa.

Little is known of the general ecology and dynamics of the plant-pollinator community of KwaZulu-Natal Moist Upland Grasslands, let alone the role of pollen availability in determining the quantity of plant reproductive output. Because of the relatively high degree of specialisation found in the pollination systems of these wildflowers (Chapter 2) and a predominance of species dependent on vector-mediated pollination for seed set (Chapter 3), one may expect the incidence of pollen limitation of reproductive output to be relatively high in these moist upland grasslands. The weather conditions of Gilboa Estate during the flowering season are seldom adverse however and pollinator activity levels may be sufficiently high to avoid pollen deficits in grassland wildflowers.

The fragmentation of habitats can cause changes in the abundance, diversity and species composition of the pollinator fauna as a result of changes in resource availability, plant population attributes, abiotic conditions and habitat continuity (Chapter 1). Decreases in the abundance and diversity of pollinators may exacerbate pollen limitation, with a decrease in either the quantity or quality of pollen deposited (Goldingay & Whelan 1990; Burd 1994).

Species that are limited by pollen availability in the undisturbed systems may experience particularly severe pollen limitation in fragmented habitats. On the other hand, such species may have evolved compensatory traits in response to a prior history of low pollination; many pollen-limited species are also capable of extensive asexual reproduction (Pleasants 1980; Barrett 1982; Motten 1986; Bond 1994). This may mean that species not already experiencing pollination deficits in undisturbed habitats are more vulnerable to a decrease in pollinator services, since they do not possess buffering mechanisms with which to decrease the likelihood of local extinction.

Investigations of pollen limitation in fragmented habitats are relatively few. Jennersten (1988) found that the decrease in natural seed set of *Dianthus deltoides* (Caryophyllaceae) in the fragmented area compared to the mainland site in Sweden could be explained by a decrease in pollinator service in the habitat fragment (where hand pollination increased seed set up to 4.1 times). Evidence of pollen limitation in *Acacia brachybotrya* (Mimosaceae) and *Eremophila glabra* (Myoporaceae) in highly fragmented habitats in Australia provided a causal link between declining levels of pollination and previously documented depressed fruit production (Cunningham 2000). Spears (1987) found reproductive output of *Centrosema virginianum* (Fabaceae) to be pollen-limited in populations of the small distant island separated by less than 10 kilometres of water from larger island or mainland populations on the western coast of Florida.

In order to determine the extent to which fruit and seed set is a function of pollen receipt in the wildflower community of undisturbed moist upland grasslands, hand pollen supplementation experiments were carried out here on a number of plant species in the undisturbed grassland mainland. In order to

determine whether pollen limitation is exacerbated in fragmented habitats as a result of changes in the pollinator fauna, supplemental hand pollinations were also carried out simultaneously in mainland and fragmented sites for selected species.

MATERIALS AND METHODS

Pollen limitation in the mainland

In order to determine whether fruit and seed production in grassland wildflower populations is limited by pollen availability, supplemental hand-pollination experiments were performed on nine species in the grassland mainland. Plants were randomly assigned to the hand-supplementation and control treatments. The pollen supplementation treatment involved the addition of pollen to inflorescences from other individuals at least 10 m away to achieve maximum pollination. As many flowers as possible on a given plant were hand-pollinated and inflorescences were revisited in order to treat newly receptive flowers. Reproductive success of these plants (measured as fruit set, seed set per fruit and seed set per flower) was compared with that of control plants in which natural pollination was allowed to occur.

The method of adding pollen to only part of an inflorescence in the supplementation treatment has been criticised since the plant may reallocate its resources to the flowers with greater pollen, resulting in higher seed set for those flowers at the expense of unmanipulated flowers (Burd 1994; Byers 1995; Ågren 1996). However these effects are cancelled out when female reproductive success is measured at the whole plant level. The use of mean fruit and seed set values for whole plants is also important to prevent pseudo-replication, since the flowers on a given plant may share the same resource pool (Johnson & Bond 1997).

In five species for which controlled supplemental hand pollinations were not performed, the degree of pollen limitation was inferred by comparing fruit and seed set resulting from naturally pollinated flowers with that resulting from

flowers that were hand-pollinated with cross pollen in the breeding system experiments described in Chapter 3.

Treatments were compared using the Student's *t*-test or Mann-Whitney *U*-test (non-normally distributed data) (Statgraphics version 7.0). A significant increase in female reproductive success associated with pollen supplementation is considered evidence of pollen limitation.

Where a species was found to be pollen-limited over two seasons, an index of pollen limitation (*L*) was calculated according to Larson and Barrett (2000): $L = 1 - (P_o/P_s)$ where P_o is the reproductive output of open or naturally pollinated controls and P_s is the reproductive output of plants that received supplemental cross pollen. $L = 0$ indicates no pollen limitation in the population under study (Larson & Barrett 2000).

Pollen limitation in habitat fragments

In order to determine whether pollen limitation is more severe in habitat fragments, supplemental hand pollinations were also carried out simultaneously in mainland and fragmented sites for *Tritonia disticha*, *Gladiolus longicollis*, *Chironia krebsii*, *Eriosema distinctum* and *Brunsvigia undulata*. Data were analysed as above.

RESULTS

Pollen limitation in the mainland

Few of the species investigated showed evidence for pollen limitation of fruit and seed output. Hand pollen supplementation significantly increased reproductive output of *Tritonia disticha* at the whole plant level in both seasons, indicating pollen limitation of fecundity in the mainland grassland for this species (Fig. 4.1). In the 1998/1999 season all three measures of reproductive output were found to be pollen-limited whereas in the 1999/2000 season pollen supplementation did not significantly affect seed set, suggesting that the flowers that were visited received sufficient amounts of pollen for maximum fertilization. The index of pollen limitation for fruit set was 0.2 for both seasons.

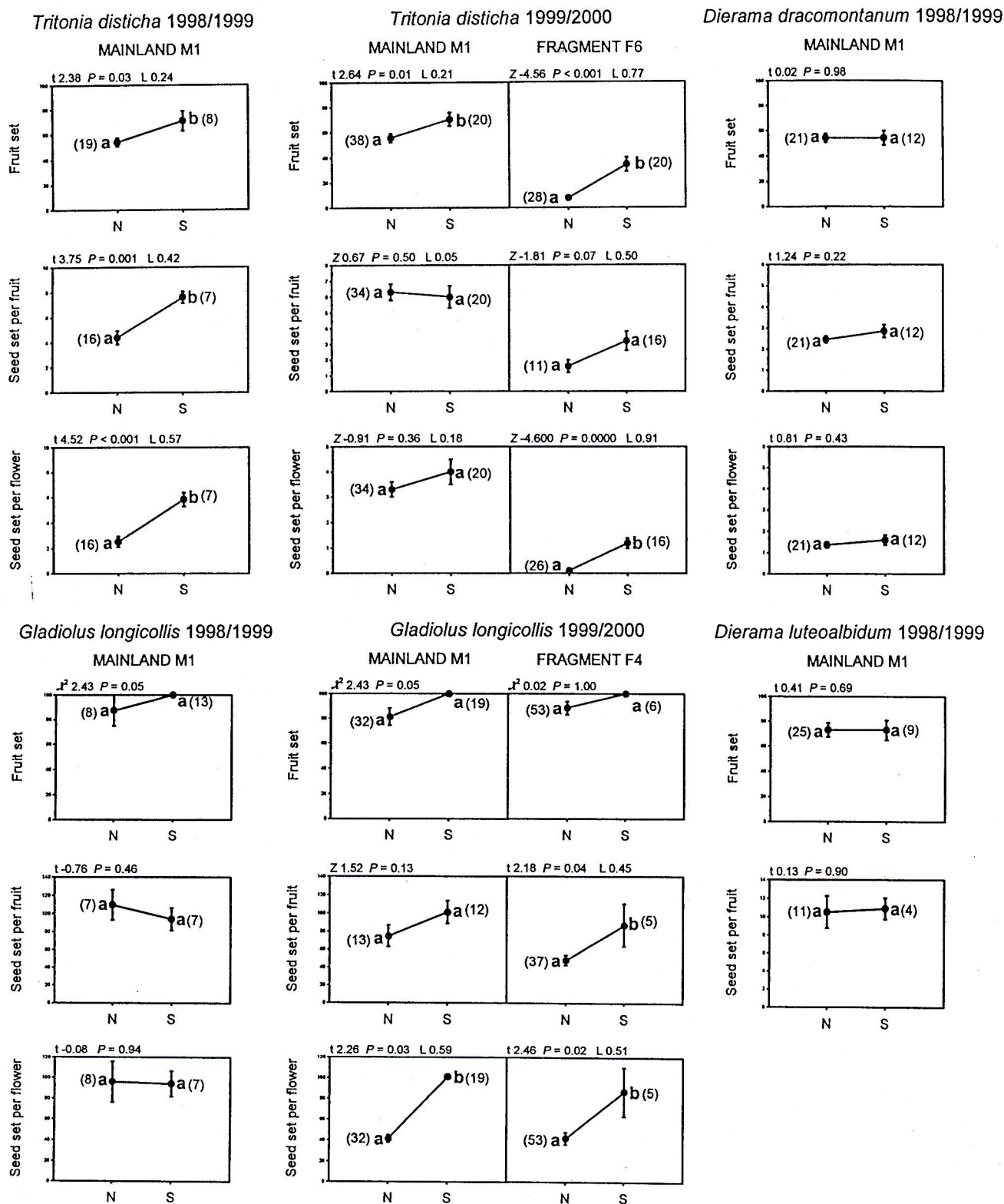


Figure 4.1 Effect of pollen supplementation on mean plant reproductive output in mainland (M) and fragment (F) habitats. A significantly greater whole plant reproductive output ($P < 0.05$, denoted by 'a' and 'b') resulting from pollen supplementation (S) compared to naturally pollinated plants (N) is considered evidence for pollen limitation in the species. Error bars denote standard error of the mean, bracketed numbers indicate sample size, t = Student's t -test statistic, Z = normal approximation to the Mann-Whitney U-test statistic.

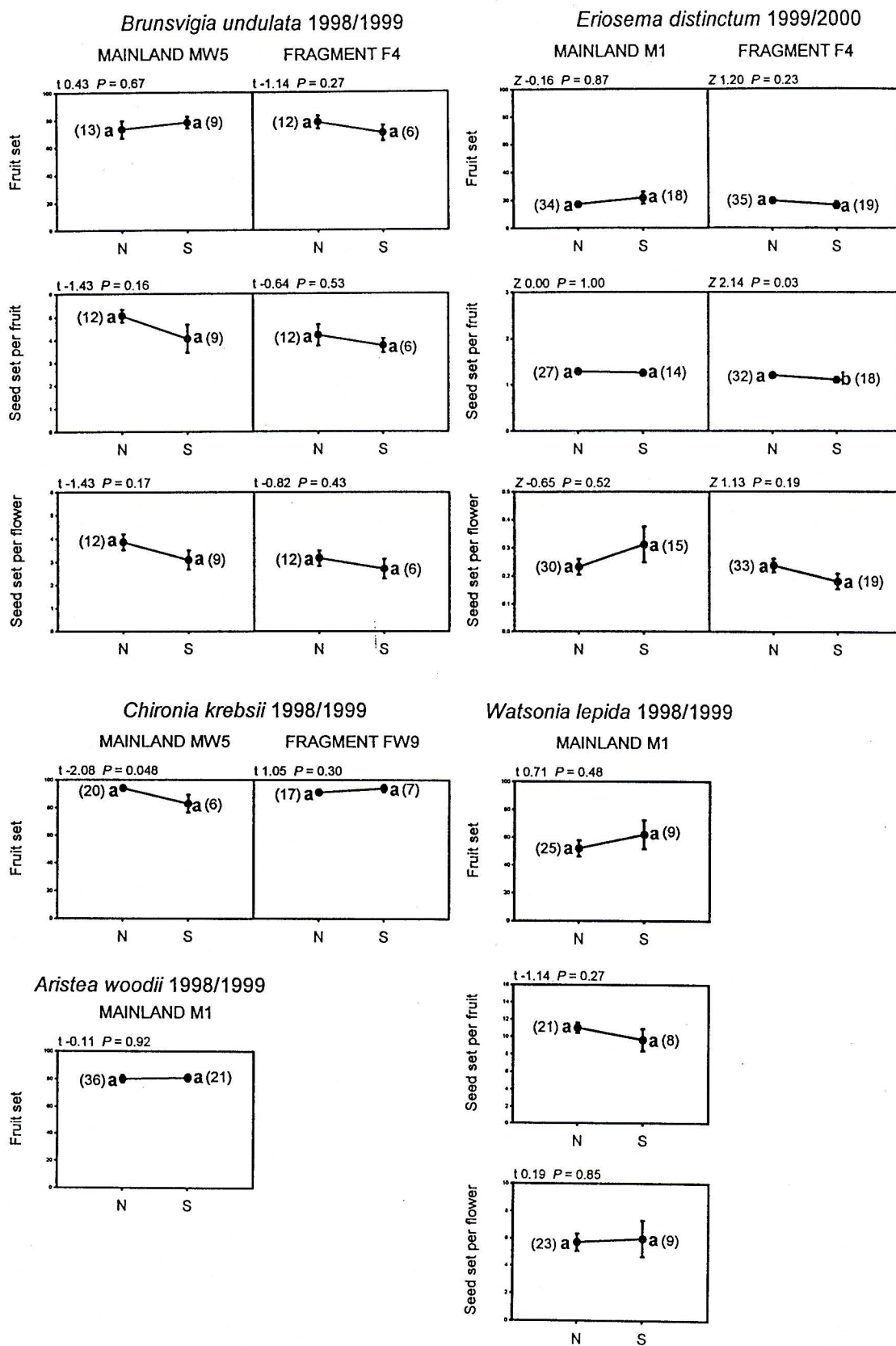


Figure 4.1 continued.

No significant increases in fruit set and seed set per fruit with hand pollen supplementation were evident in *Gladiolus longicollis* for both seasons (Fig. 4.1). Seed set per flower was however found to be significantly greater in the pollen supplementation treatment in the 1999/2000 season (101.1 versus 41.6; $t = 2.26$, d.f. = 49, $P = 0.03$, Fig. 4.1), suggesting that less pollen was deposited per flower in naturally pollinated flowers (less visits per flower or smaller pollen loads) or that the pollen transferred was incompatible.

No increases in reproductive output at the whole plant level with hand pollen supplementation were found in *Watsonia lepida*, *Dierama luteoalbidum*, *Dierama dracomontanum*, *Aristea woodii*, *Eriosema distinctum*, *Brunsvigia undulata* and *Chironia krebsii* (Fig. 4.1).

Statistical comparison of fruit set from naturally pollinated flowers and from flowers that were hand cross-pollinated in the breeding system experiments of Chapter 3 (9 vs 83%) indicates that *Eulophia zeyheriana* is severely limited by pollen availability at this site (Table 4.1). Statistical comparison of fruit set from naturally pollinated flowers and bagged unmanipulated flowers from the breeding system experiments reveals no significant difference ($\chi^2 = 0.21$, d.f. = 1, $P = 0.64$). This suggests that natural levels of fruit set of *E. zeyheriana* for the 1999/2000 flowering season are largely the result of autogamy and that these flowers were seldom visited by pollinators, if at all.

Statistical comparison of reproductive output from natural and hand cross pollination of *Agapanthus campanulatus* flowers revealed no significant differences for fruit set but indicated that mean seed set per fruit is significantly lower in naturally pollinated flowers (Table 4.1).

Comparisons of reproductive output from natural and hand cross pollinations revealed no significant differences in fruit and seed set for *Zaluzianskya natalensis*, *Satyrium longicauda* and *Kniphofia laxiflora* (Fig. 4.1), suggesting that reproductive output in these species is not limited by the availability of pollen.

Table 4.1 Plant species for which reproductive output was compared between flowers that were naturally pollinated (natural) and flowers that received supplemental pollination as part of a breeding system experiment (outcrossed). A significantly greater reproductive output in the outcrossed treatment ($P < 0.05$, denoted by 'a' and 'b') is perceived as indirect evidence of pollen limitation.

Species	Fruit set \bar{x} (n)		Seed set per fruit $\bar{x} \pm SD$ (n)	
	natural	outcrossed	natural	outcrossed
<i>S. longicauda</i>	82 (618) a	100 (11) a	-	-
<i>Z. natalensis</i>	88 (467) a	100 (9) a	77.3 \pm 32.8 (173) a	36.1 \pm 23.7 (17) a
<i>K. laxiflora</i>	44 (5962) a	70 (10) a	10.9 \pm 6.3 (429) a	10.6 \pm 4.8 (5) a
<i>A. campanulatus</i>	60 (957) a	90 (10) a	7.4 \pm 3.8 (200) a	19.6 \pm 2.4 (9) b
<i>E. zeyheriana</i>	9 (495) a	83 (6) b	-	-

Pollen limitation in habitat fragments

Greater pollen limitation of plant reproductive output in fragmented habitats was evident in two of the five species investigated. Increased fruit set with pollen supplementation was evident in both mainland and fragment populations of *Tritonia disticha* (Fig. 4.1). Significant increases in seed set per fruit and seed set per flower were also evident in the habitat fragment (Fig. 4.1). The indices of pollen limitation of fruit set clearly reveal the more severe levels of pollen limitation in the habitat fragment ($L = 0.8$ vs 0.2 in the mainland).

While only seed set per flower exhibited a significant increase with hand pollen supplementation in the mainland for *Gladiolus longicollis*, both seed set per fruit and seed set per flower were found to be limited by pollen availability in the habitat fragment (Fig. 4.1). Degree of pollen limitation measured as seed set per flower was similar for both sites ($L = 0.6$ and 0.5 for the mainland and fragmented sites respectively).

Significantly greater reproductive output with pollen supplementation was not found for fragment populations of *Chironia krebsii*, *Brunsvigia undulata* and *Eriosema distinctum* (Fig. 4.1).

DISCUSSION

Pollen limitation in the mainland

Less than a third (29%) of the species investigated exhibited pollen-limited reproductive output in the mainland grassland of Gilboa Estate (Fig. 4.1, Table 4.1). Later studies carried out at the same site (M1) have revealed further evidence for pollen limitation in *Gladiolus longicollis* (both fruit and seed set) (Alexandersson & Johnson 2002) and in *Watsonia lepida* (Paul Neal, unpublished data), as well as in the non-rewarding orchid *Disa pulchra* (Johnson 2000) and a species of *Protea* (A. Hargreaves, unpublished data). Species that were found to experience pollen-limited reproductive output do not appear to have any common ecological features that distinguish them as a group from the rest of the species examined.

The lack of pollen limitation of reproductive output in *A. woodii* and *C. krebsii* (Fig. 4.1) may be the result of these species' high capacity for autonomous self-pollination. The rest of the species for which breeding system data is available are incapable of autonomous selfing to any great degree and lack of pollen-limited fruit and seed set in these species suggests high levels of pollinator activity in the mainland grassland or the existence of some other more important factor which limits female plant reproductive success, such as water or nutrients. The incidence of pollen-limited reproductive output discovered here is comparable with that found in the wildflower community of a temperate North American forest studied by Motten (1986) in which only three of the 12 species examined exhibited pollen limitation. The apparent rarity of pollen-limited fecundity in the North American forest seems to be a result of the prevalence of floral biology traits like autogamy and self-compatibility, extended receptivity and pollination by a variety of visitor types (Motten 1986). From the previous two chapters however, it is evident that capacity for autonomous self-pollination and generalised pollination systems are not common in KwaZulu-Natal Moist Upland Grasslands. From Larson and Barrett's (2000) observation that pollen limitation seems to be less intense in self-compatible and autogamous species one may expect pollen limitation to be more prevalent in grasslands than was discovered here. Johnson and Bond (1997) suggest that the frequent occurrence of pollen limitation in Cape wildflower species may be attributed to the scarcity of

pollinators in the region. Following on from this hypothesis, the rarity of pollen limitation in KwaZulu-Natal Moist Upland Grasslands may suggest high pollinator abundance and activity in the community. Johnson and Bond's (1997) claim that pollen limitation of fruit set occurs widely in Cape wildflower species was however based on data from only two plant families, Amaryllidaceae and Orchidaceae, the latter family possibly being prone to pollen limitation (see Nilsson 1992).

Reproductive output is commonly pollen-limited in orchids with their specialised floral morphology and characteristically low pollinator visitation rates (Nilsson 1992; Calvo 1993; Johnson & Bond 1997; Larson & Barrett 2000). In keeping with this trend, fruit set was found to be severely pollen-limited in the non-rewarding species *Eulophia zeyheriana* (Table 4.1) and *Disa pulchra* (Johnson 2000). In strong contrast, fruit set in the nectar-producing orchid *Satyrium longicauda* was not significantly affected by pollen supplementation (Table 4.1). The differential response between these species supports the hypothesis that non-rewarding orchid species are particularly susceptible to pollen limitation (Johnson & Bond 1997; Neland & Wilcock 1998).

The technique of hand pollination may produce artifacts and introduce error through mechanisms such as mechanical damage to stigmas, pollination with non-viable or local incompatible pollen grains from a single donor, pollen tube crowding, pollen removal or stigma damage by pollen thieves (high pollen loads on stigmas may attract pollen thieves) or pollinators and missed stigma receptivity (Young & Young 1992). Such error may negate the potential advantage of pollen addition in supplementation experiments (Thomson 2001). Low sample sizes for the supplementation treatments may also result in a Type II error and the masking of a significant pollen limitation effect (Young & Young 1992). It is possible that the lack of evidence for pollen limitation in *E. distinctum* and *B. undulata* is a result of poor pollination technique. The period of receptivity of legume stigmas is often brief and can easily be missed. It was also extremely difficult to access the stigma without depositing self pollen or damaging the flower (the significantly lower seed set per fruit in the pollen supplementation treatment of *E. distinctum* in the habitat fragment (Fig. 4.1) suggests that the hand supplementation treatment may have had a detrimental effect on this species). Hand pollination of *B. undulata* flowers may have

involved similar error, judging from the inconclusive breeding system results (Chapter 3). The conclusion that pollen limitation is relatively rare in grassland wildflowers should thus be viewed with caution.

Pollen limitation in habitat fragments

Greater pollen limitation of reproductive output in the habitat fragments was evident in *Tritonia disticha* and *Gladiolus longicollis*, two of the five species investigated in both mainland and fragment sites (Fig. 4.1). Both of these species were also found to experience strong effects of fragmentation on reproductive output (Chapter 5, Fig. 5.6). Results suggest that reduced reproductive output of these species in the habitat fragments is a result of a change in the quantity and/or quality of pollinator services there.

Due to a shortage of pollen in the habitat fragment (high levels of floral and pollen predation), pollen for the supplementation treatment of *T. disticha* had to be brought in from the mainland site, more than 5.3 km away. The considerably lower reproductive output of *T. disticha* individuals in the hand supplemented treatment of the fragment compared to the mainland (Fig. 4.1) may thus be the result of increased predation at the site and possibly also a degree of outbreeding depression (Waser 1993; Waser & Price 1991). The disparity between sites for the same treatment may also partly be the result of greater resource limitation in the habitat fragment.

The absence of pollen limitation in both mainland and fragmented sites for the remaining three species investigated (Fig. 4.1) correlates with the relatively weak effects of habitat fragmentation on plant reproductive output experienced by these species (Chapter 5, Fig. 5.6). For *Chironia krebsii* this may be a result of a high capacity for autonomous self-pollination. *Eriosema distinctum* and *Brunsvigia undulata* are pollination generalists (Chapter 2) and the lack of pollen limitation may be the result of the large number of pollinator species potentially able to effect fertilization in these species (increasing the probability that at least one suitable pollinator species survives in a given habitat fragment).

It is difficult to speculate on the frequency of pollen-limited reproductive output in grassland habitat fragments from limited observations. Pollen limitation in

fragments does however appear to correlate with strong habitat fragmentation effects on reproductive output and with the occurrence of pollen limitation in mainland populations. This does not preclude the possibility that species that are not pollen-limited in the mainland will be pollen-limited in fragments.

CONCLUSION

The need to understand more thoroughly the degree to which particular plant species or communities are limited by pollen availability is becoming increasingly urgent with escalating claims of widespread declines in pollinator populations around the world (Nabhan 1996; Allen-Wardell *et al.* 1998; Kearns *et al.* 1998). Numerous investigations involving whole suites of plant species in a range of different ecosystems are required. Little is known about the ecological correlates of pollen limitation (Johnson & Bond 1997) and none of the plant traits investigated by Larson and Barrett (2000) in their review of 224 flowering plant species were singularly unambiguous predictors of pollen limitation. A greater understanding in this regard is important for the grassland wildflower species studied here, since there do not appear to be any distinctive ecological characteristics shared by the species found to experience pollen limitation.

CHAPTER 5

CONSEQUENCES OF HABITAT FRAGMENTATION FOR PLANT REPRODUCTIVE OUTPUT

ABSTRACT

The fragmentation of habitat can disrupt plant-pollinator interactions, resulting in lower and inferior pollinator visitation and thus depressed reproductive output for plants in remnant habitat fragments. In an attempt to determine whether habitat fragmentation has a detrimental effect on plant-pollinator interactions and hence quantitative reproductive output of wildflowers of KwaZulu-Natal Moist Upland Grasslands, the fruit and seed set of 24 plant species was compared between mainland and fragment sites. Significant detrimental effects on reproductive output were evident to some degree in two thirds of the wildflower species, with 94% of the species exhibiting overall declines in seed set per fruit from the average mainland site to the average habitat fragment. Median decline in seed set per fruit was 33.0%. Significantly more species experienced overall declines in reproductive output than would have been expected by chance alone. In an attempt to determine whether magnitude of decline in reproductive output can be predicted from breeding and pollination system characteristics, univariate and multiple regression analysis was used to examine how magnitude of fragmentation effect is related to capacity for autonomous self-pollination, degree of self-compatibility and pollination specificity. Only specificity of the pollination interaction in terms of number of pollinator taxa involved was found to be significantly related to percentage change in seed set from mainland to fragment habitats. This effect was diminished when other factors were included in a multiple regression. Results support Bond's (1994) hypothesis that degree of specificity in pollination systems is important in determining extinction risk of a given plant species.

INTRODUCTION

The fragmentation of natural habitat is often considered to be one of the greatest threats to terrestrial biodiversity worldwide and has the potential to severely disrupt plant-pollinator interactions and threaten the local persistence of the constituent species (Jennersten 1988; Rathcke & Jules 1993; Kearns & Inouye 1997; Kearns *et al.* 1998). Habitat fragmentation can cause changes in the abundance, diversity, species composition and behaviour of pollinator fauna in

the remnant habitat fragments as a result of changes in resource availability, plant population attributes, abiotic conditions and habitat continuity (Chapter 1). A change in the pollinator fauna may result in lower and inferior pollinator visitation and depressed reproductive output for plants in remnant habitat fragments compared to mainland populations, potentially resulting in a reduced plant population size and increased risk of extinction (Chapter 1). Empirical evidence quoted in Chapter 1 reveals this to be true for a number of species in different systems around the world.

Theory and increasing empirical evidence reveal however that not all plant species decline towards extinction as a result of fragmentation of their habitat and suggest that both plants and pollinators have attributes that lead to differential effects of fragmentation (Aizen & Feinsinger 1994; Bond 1994; Murcia 1996; Berge *et al.* 1998; Bosch & Waser 1999; Golden & Crist 1999; Cunningham 2000b; Davies *et al.* 2000). Bond (1994, 1995) combines three factors in a subjective index for the rapid assessment of extinction risk of a plant species threatened by a decrease in pollinator abundance and diversity: (1) the probability of a mutualism failing due to demise of mutualist partners (depending on the probability of a particular mutualist being lost and the specificity of the pollination interaction), (2) the degree of reproductive dependence on the mutualism (depending on the capacity for autonomous self-pollination and the degree of self-compatibility) and (3) the importance of seeds in the demography of the plant (Bond 1994, 1995).

Previously, testing of Bond's hypothesis was precluded in the majority of fragmentation studies because only one or a few species were included (Spears 1987; Jennersten 1988; Gigord *et al.* 1999; Cunningham 2000; Costin *et al.* 2001; Lindberg & Olesen 2001). In one of the few multi-species investigations Aizen and Feinsinger (1994) tested whether breeding system and pollination guild type are important in determining sensitivity to fragmentation in Argentinian Chaco dry forest. While they found some evidence that plants belonging to different pollination guilds might differ in their sensitivity to fragmentation, breeding system was not found to significantly affect the severity of fragmentation effect (Aizen & Feinsinger 1994).

The reproductive biology of the 24 grassland wildflower species studied here has been described in earlier chapters. The species were found to support a relatively rich and diverse pollinator fauna, including as yet undescribed species. Two thirds of the pollination systems investigated are specialised to some degree, with six species entirely dependent on a single species or specific type of pollinator from a single family for seed set. Only 10% of the species tested are capable of autonomous self-pollination to any substantial degree, with the majority dependent on pollen vectors for reproductive output. According to Bond's (1994) hypothesis, these results suggest that a large proportion of grassland wildflower species may be highly susceptible to the effects of environmental perturbation.

A significant decrease in reproductive output of wildflower species in the habitat fragments compared to the mainland sites would indicate that habitat fragmentation has a detrimental effect on grassland wildflower species, most probably through changes in the pollinator community, and that these species may decline in habitat fragments over time. A relationship between the severity of fragmentation effect, or magnitude of decline in reproductive output, and the pollination and breeding system of the wildflower species would support Bond's (1994) hypothesis that these attributes are important in determining the differential responses of species to the same environmental perturbation. Because size and degree of isolation of a given habitat fragment have been found to be important in determining the severity of fragmentation effect (Chapter 1) and because the 24 wildflower species are unevenly distributed across sites, the effects of these attributes on magnitude of fragmentation effect also need to be explored (e.g. a species may appear particularly sensitive simply because its reproductive output was measured in smaller, more isolated fragments).

The objectives of this chapter are:

(1) to determine whether habitat fragmentation as a result of commercial afforestation has a significant detrimental effect on the reproductive output of wildflower species of KwaZulu-Natal Moist Upland Grasslands

(2) to test the hypothesis that specificity of the pollination interaction and degree of reproductive dependence on the mutualism are important in determining the magnitude of fragmentation effect on plant reproductive output.

MATERIALS AND METHODS

Consequences of habitat fragmentation for plant reproductive output

A number of individuals (c 20) of each of the 24 wildflower species were marked in as many mainland and fragment sites as possible. Plants were later collected and reproductive output analysed in terms of fruit set and, where possible, seed set per fruit and seed set per flower. Pollination success was also quantified more directly in some of the orchid and asclepiad species as these have pollen packed into large and easily visible units, allowing the percentage of open flowers on a given individual that had at least one pollinarium removed or deposited to be determined.

Reproductive output data for each species at the respective sites were subjected to the Kolmogorov-Smirnov one sample test for goodness-of-fit to determine whether the data set was normally distributed. Normally distributed data were then compared between sites using the Student's *t*-test or One Way Analysis of Variance (ANOVA) (Statgraphics Plus Version 7 1993). Percentage data were square root arcsine transformed prior to analysis. In instances where significant differences occurred between more than two sites, the Tukey Highly Significant Difference (HSD) multiple range test was carried out to determine where these differences occurred (Statgraphics Plus Version 7 1993). Where the data set was not found to be normally distributed (mostly because of a large number of zero values) it was subjected to the Mann-Whitney *U*-test or the Kruskal-Wallis *H*-test for non-parametric data (Statgraphics Plus Version 7 1993) and a non-parametric equivalent to the Tukey HSD test (for data with tied ranks and different sample sizes) (Zar 1996, p 226).

Magnitude of fragmentation effect

In order to assess the overall effect of fragmentation on these 24 grassland wildflower species, percentage change from the continuous mainland grassland to grassland fragments was determined for each species in each reproductive

variable following Aizen and Feinsinger (1994). Percentage change was calculated as the difference between the mean fragment reproductive output and the mean mainland reproductive output, divided by the latter term and expressed as a percentage. For species that were studied over two seasons, mean percentage change was taken from those calculated for each season using only the sites common to both years. A negative percentage change represents a decrease in reproductive output from the average mainland site to the average fragment. To determine whether the percentage change value for a given species represents a significant overall fragmentation effect, grouped data from the mainland sites and the habitat fragments were compared for each species, using the Student's *t*-test or the Mann-Whitney *U*-test (non-normally distributed data) (Statgraphics Plus Version 7 1993). Following Aizen and Feinsinger (1994) percentage change in reproductive output was compared between self-compatible and self-incompatible species using the Mann-Whitney *U*-test (Statgraphics Plus Version 7 1993). In the absence of an overall effect of fragmentation, equal numbers of species should show negative and positive changes in reproductive output, and the median percentage change value of the set of study species should be statistically indistinguishable from 0. Percentage change data were subjected to the Wilcoxon signed-rank test for each of the reproductive variables (Statgraphics Plus Version 7 1993).

Factors important in determining magnitude of fragmentation effect

Univariate regression was used to determine the relationship between mean fruit set for all study species at a fragment and the size and degree of isolation (distance to the mainland) of that fragment. Mean fruit set was square root arcsine transformed prior to analysis. The uneven distribution of plant species across the habitat fragments could however mean that the particular mix of species at a given site may affect the relationship between fragment size and isolation and mean fruit set. Because of this a different approach was also used: the median size and degree of isolation of the fragments, in which the reproductive success of a given species was recorded, was used as an independent variable in the regressions described below.

Univariate and multiple regressions were used to determine how magnitude of fragmentation effect (percentage change in reproductive output) of a given plant

species is related to pollination specificity, capacity for autonomous self-pollination, degree of self-compatibility and the physical attributes of the habitat fragments in which that species was investigated. Independent variables used were number of taxa involved in the pollination interaction (species, families, orders) and floral tube length (both measures of the pollination specificity), the index of autonomous self-pollination (IAS), the index of self-incompatibility (ISI) and the median area and median distance to the mainland of the fragmented sites in which the wildflower species was studied. Pearson's product-moment test was used to test for correlation between the variables. Univariate regression was also used to determine whether floral tube length is related to specificity of the pollination interaction measured in terms of number of pollinator taxa involved in the interaction. In order to determine how the response of wildflower species to habitat fragmentation in KwaZulu-Natal Moist Upland Grasslands compare to other systems in the world, similar regressions were used on data from a study carried out in Argentinian Chaco dry forest by Aizen and Feinsinger (1994). All proportions were arcsine square-root transformed prior to analysis.

RESULTS

Consequences of habitat fragmentation for plant reproductive output

When reproductive outputs for all species in all sites and years were examined together (Fig. 5.1-4.) significant detrimental fragmentation effects are evident to some degree in two thirds of the plant species studied. For the majority of the species studied here a significantly lower seed set per flower in a fragmented site was the result of a significant decrease in both fruit set and seed set per fruit. The most severely affected species were *Tritonia disticha*, *Zaluzianskya natalensis*, *Kniphofia laxiflora*, *Dierama dracomontanum* and *Aloe boylei*, where all populations in habitat fragments exhibited consistently and significantly lower reproductive success with respect to all mainland populations (Fig. 5.1-4.). *Tritonia disticha* appears to be the worst affected with both fruit and seed set negligible in the fragments for both seasons. Relatively strong fragmentation effects were evident in *Kniphofia fluviatilis*, *Pachycarpus grandifloris*, *Aristea woodii*, *Kniphofia ichopensis* and *Disa versicolor* (1999/2000 season) where reproductive success in one or more fragment was significantly lower than that of the mainland site (Fig. 5.1-4.). Intermediate fragmentation effects, where the

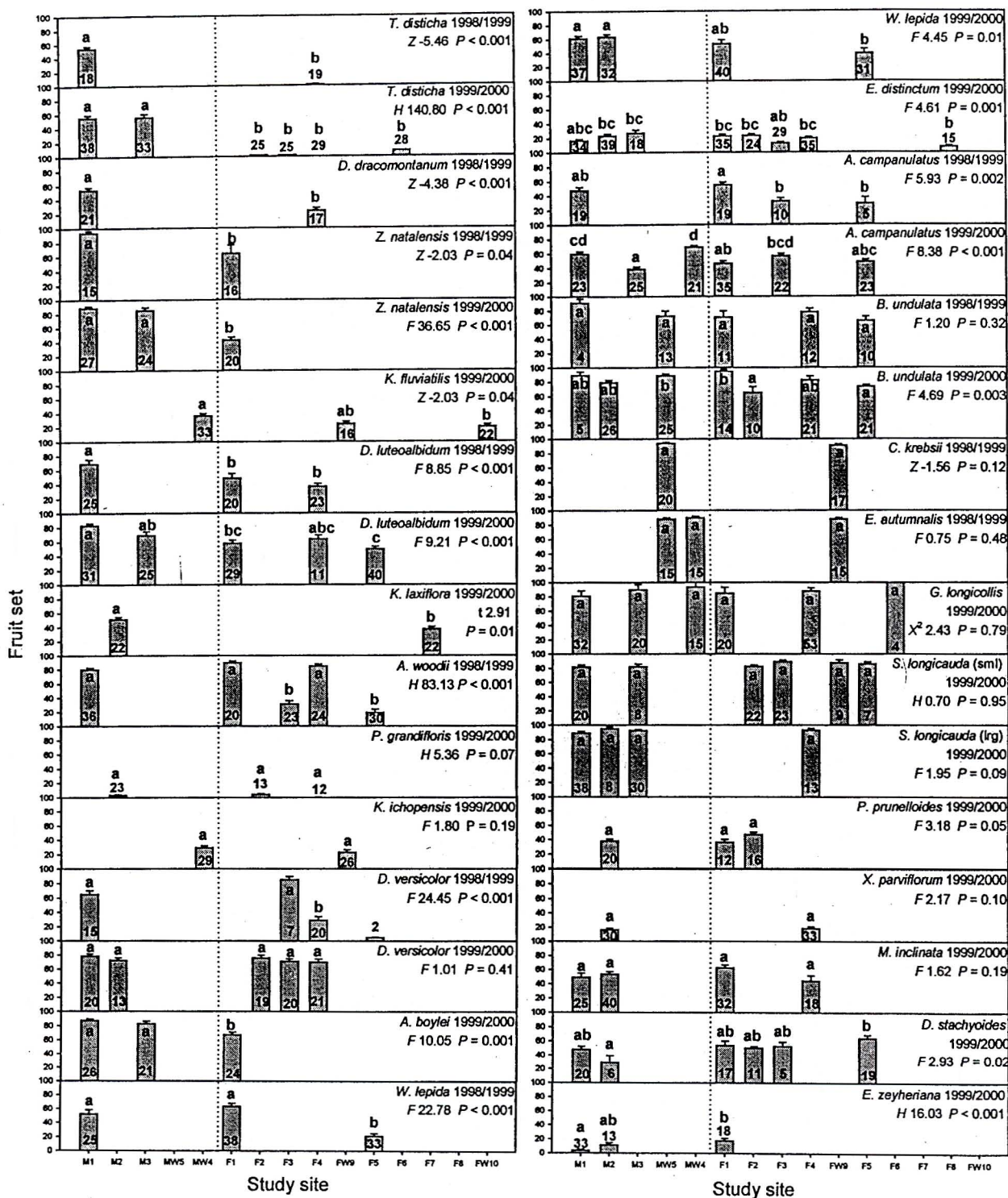


Figure 5.1 Mean fruit set for each study site in which the 24 wildflower species were investigated. Significant differences between sites are denoted by letters in bold type. Error bars denote standard error of the mean, numbers at the base of the bars indicate sample size, t = Student's t -test statistic, Z = normal approximation to the Mann-Whitney U -test statistic, F = ANOVA test statistic, H = Kruskal-Wallis H -test statistic, χ^2 = Chi-square test statistic.

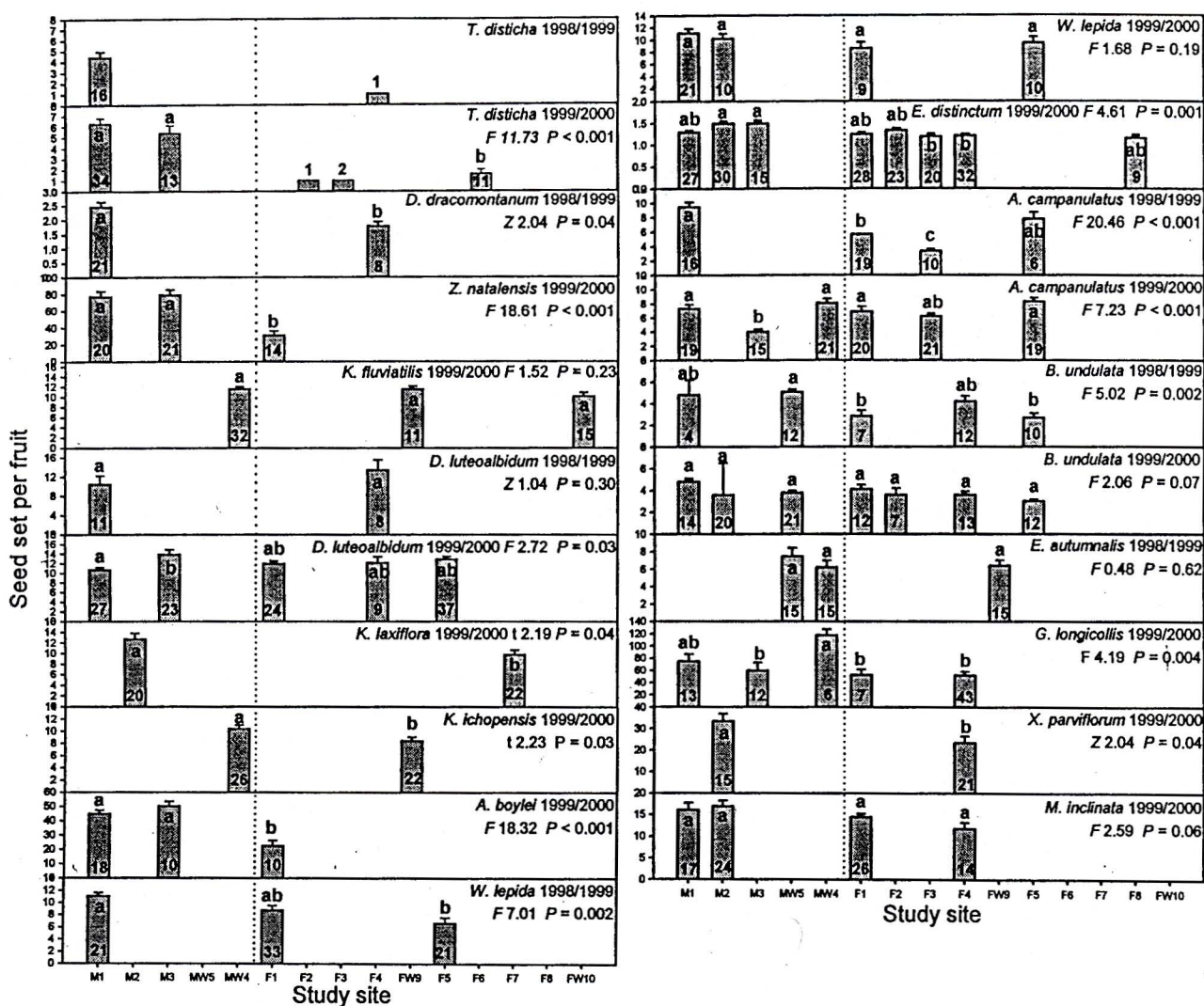


Figure 5.2 Mean seed set per fruit for each study site in which the 24 wildflower species were investigated. Symbols and abbreviations as for Fig. 5.1.

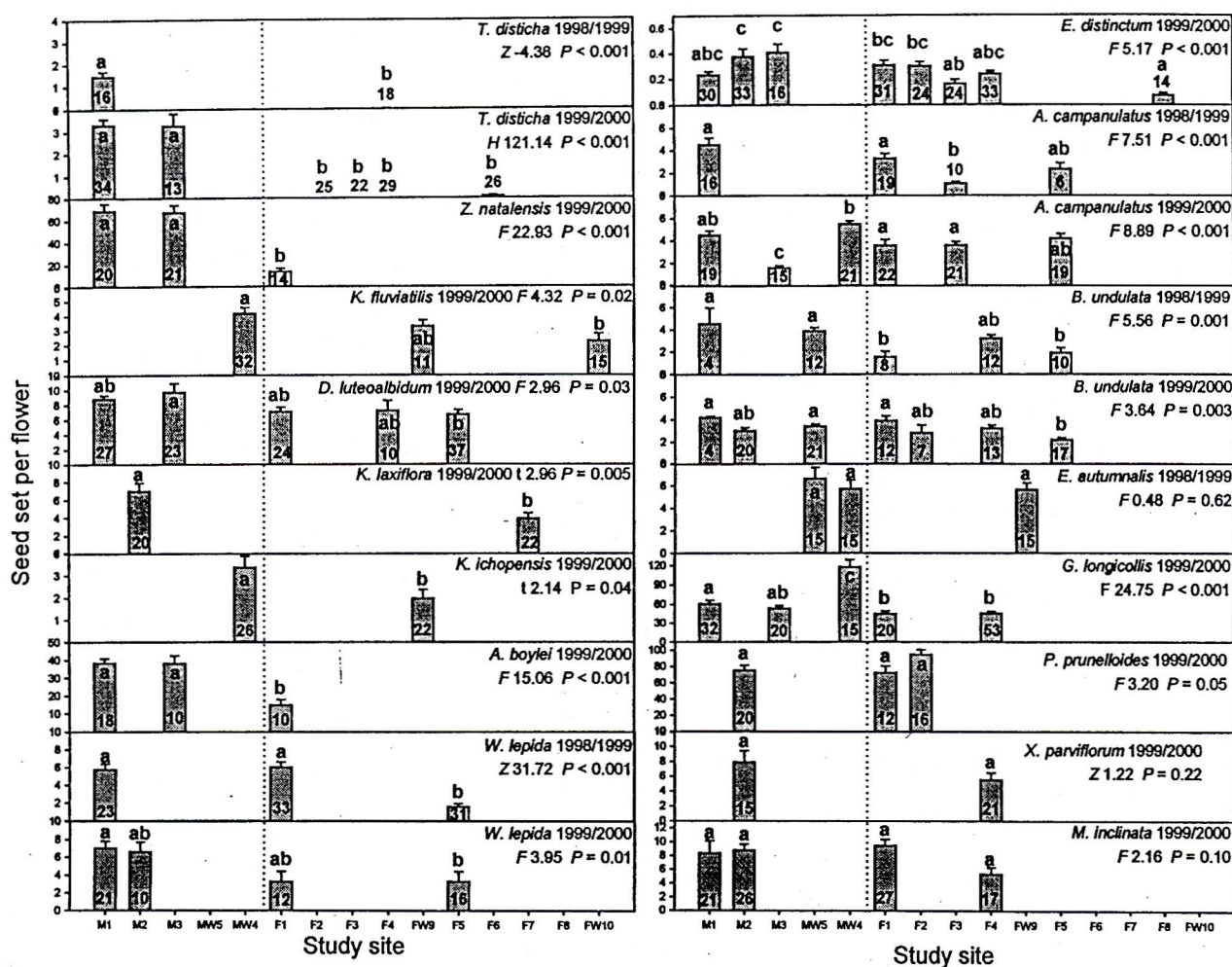


Figure 5.3 Mean seed set per flower for each study site in which the 24 wildflower species were investigated. Symbols and abbreviations as for Fig. 5.1.

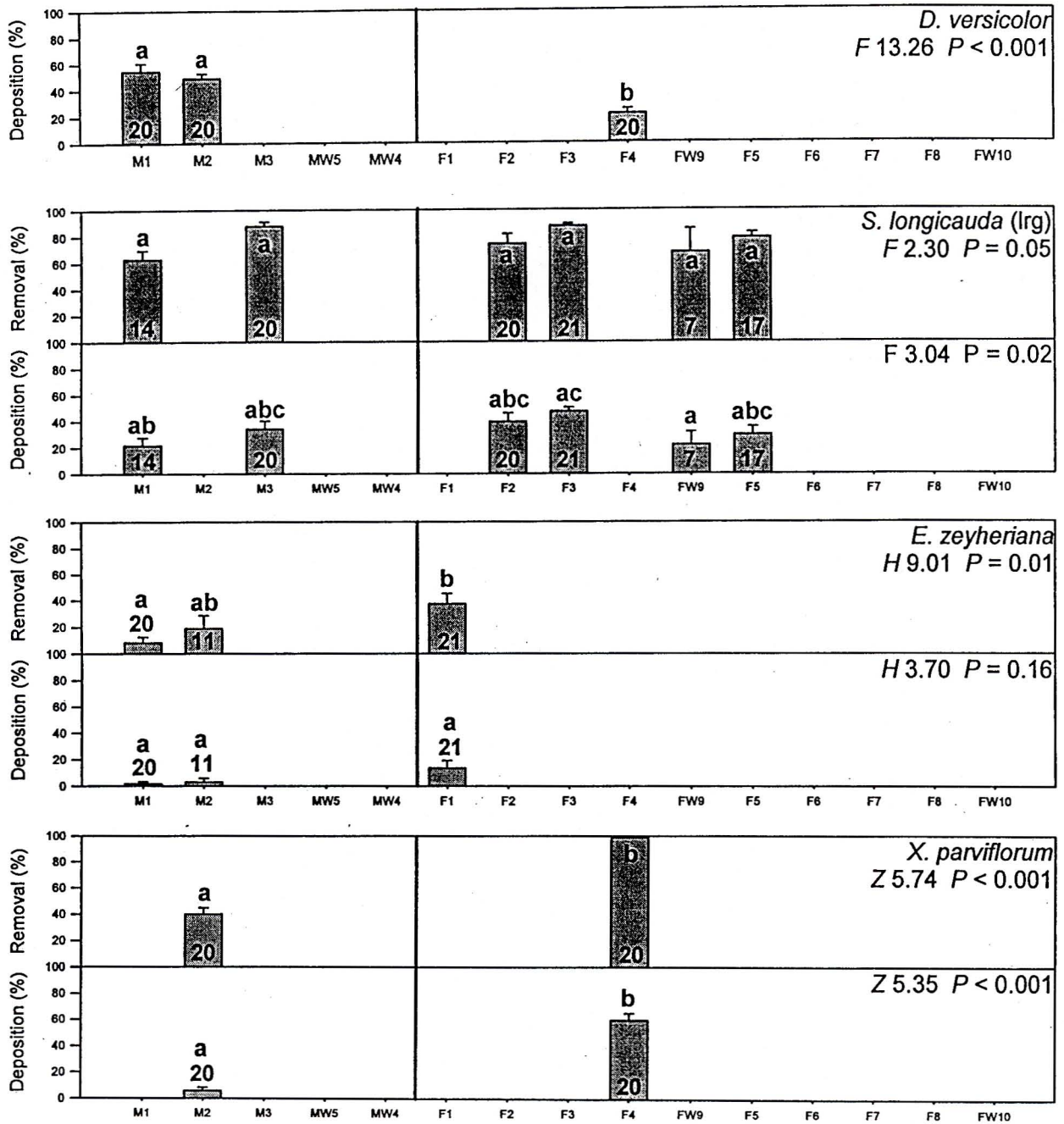


Figure 5.4 Mean percentage open flowers with at least one pollinarium removed or deposited for each study site in which the four orchid and asclepiad species were investigated. Symbols and abbreviations as for Fig. 5.1.

population in one or more fragmented sites is significantly lower than that of at least one mainland site, were evident in *Dierama luteoalbidum*, *Watsonia lepida*, *Eriosema distinctum*, *Agapanthus campanulatus*, *Gladiolus longicollis* and *Brunsvigia undulata* (Fig. 5.1-4). Twenty nine percent of the species studied did not experience any significant fragmentation effects and exhibited variable or no significant differences across the sites (Fig. 5.1-4.). In *Eulophia zeyheriana*, the reproductive output of the fragment population was found to be unexpectedly and significantly greater than that of one of the two mainland sites (Fig. 5.1).

For *S. longicauda* and *E. zeyheriana*, trends in pollinarium removal and deposition across the sites mirrored those of reproductive output (Fig. 5.4). For *D. versicolor* and *X. parviflorum* however significantly different pollination success recorded in the habitat fragment compared to mainland sites did not translate into significantly different levels of fruit set at that site (Fig. 5.1-4.). Percentage flowers with at least one pollinarium removed was significantly lower in the habitat fragment than in the mainland sites for *D. versicolor* (Fig. 5.4). In *X. parviflorum* both the percentage flowers with pollinaria removed and with pollinaria deposited were significantly greater in the habitat fragment (Fig. 5.4). When reproductive output was analysed for the species, only seed set per fruit was significantly different in that habitat fragment (in the opposite direction to that expected from pollination success) (Fig. 5.1-3.).

Because comparison of reproductive success across sites was carried out over two seasons for seven of the study species, reproductive output and degree of fragmentation effect can be compared between years. Only *D. versicolor* experienced substantial differences in fragmentation effect between seasons, with one of the fragment populations in the 1998/1999 season exhibiting significantly lower fruit set compared to the mainland population, while the fragment populations appeared to be unaffected in the 1999/2000 season. Three other species (*A. campanulatus*, *B. undulata* and *D. luteoalbidum*) also exhibited slightly more severe fragmentation effects in the 1998/1999 flowering season.

Magnitude of fragmentation effect

Figure 5.5 shows the decline in reproductive output from the average mainland site to the average fragment site for most of the wildflower species studied. An

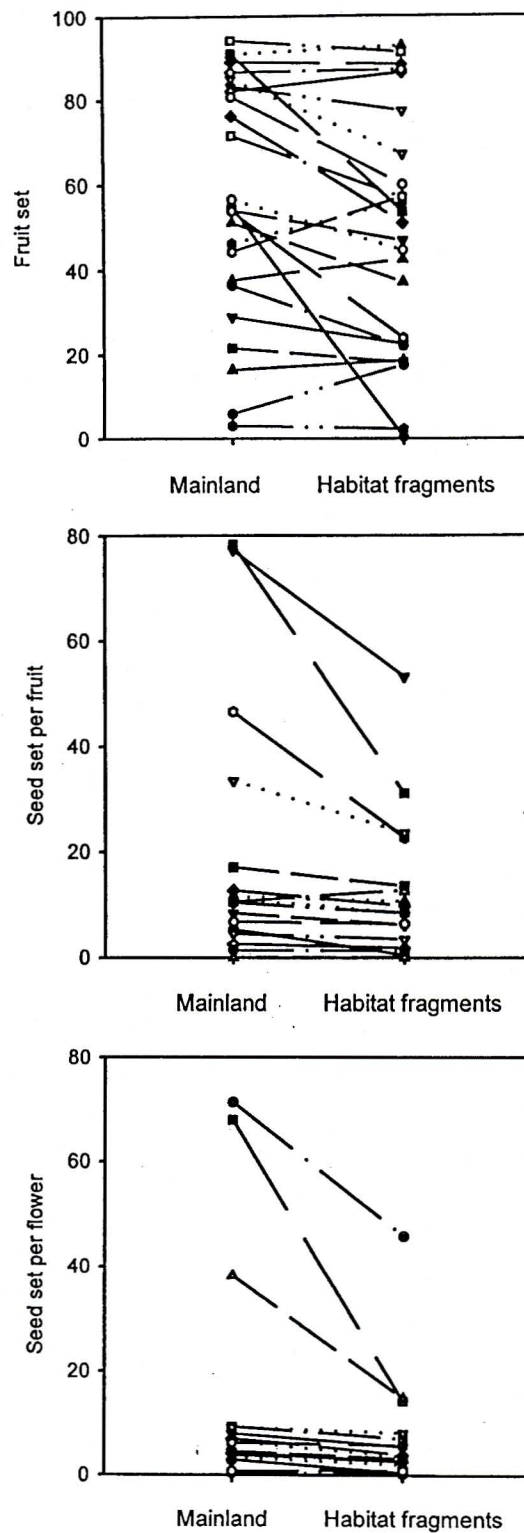


Figure 5.5 Effect of habitat fragmentation on reproductive output of 24 wildflower species measured as fruit set, seed set per fruit and seed set per flower. Each line represents a different wildflower species.

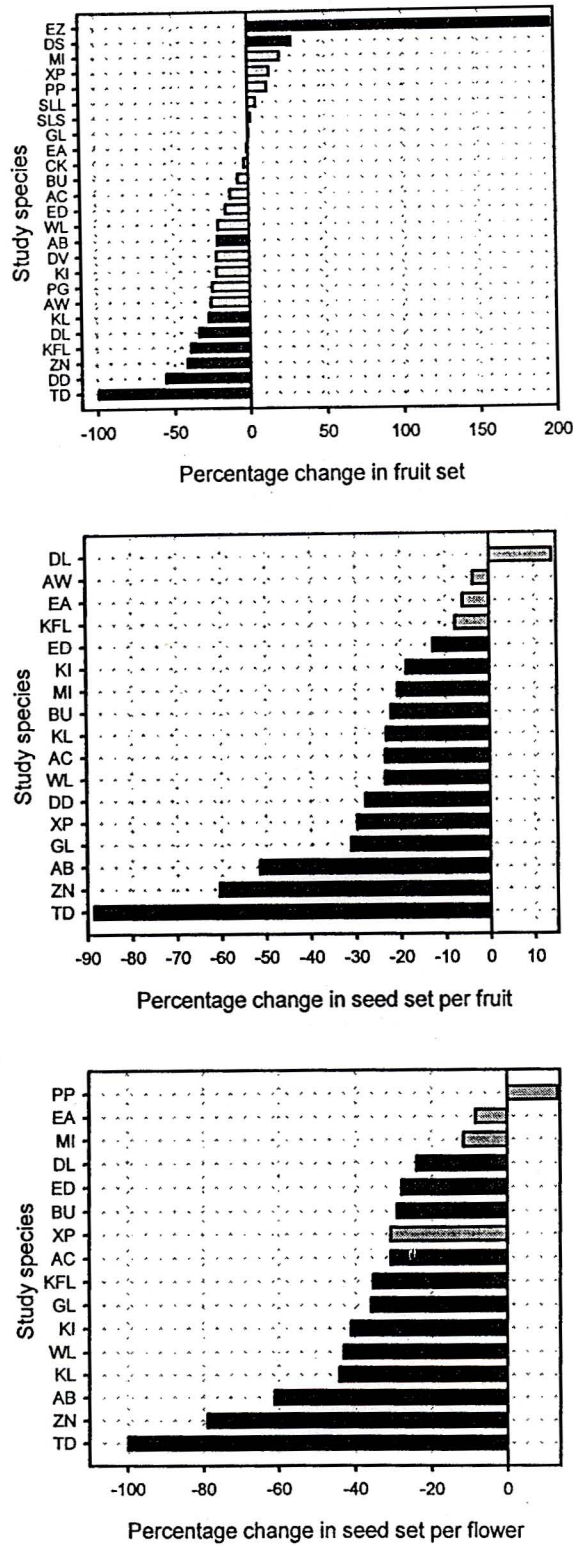


Figure 5.6 Percentage change in grouped reproductive output from mainland to fragment sites for each plant species, ranked in descending order, measured as fruit set, seed set per fruit and seed set per flower. Dark grey bars indicate significant differences ($P < 0.05$) in grouped mean reproductive output between mainland and fragment sites.

overall decline in fruit set from average mainland site to average habitat fragment occurred in 17 wildflower species (significantly in 7) and an increase occurred in seven (significantly in 2) (Fig. 5.6). Percentage change in fruit set varied from -99.3% in *Tritonia disticha* to +197.6% in *Eulophia zeyheriana*, with a median percentage change of -18.2 (Fig. 5.6). Percentage change in seed set per fruit showed an overall decline between mainland and fragmented sites in 16 out of 17 species (significantly in 13) and varied from -88.6% in *T. disticha* to +14.0% in *Dierama luteoalbidum*, with a median percentage change of -23.1 (Fig. 5.6). Percentage change in seed set per flower varied from -99.8% in *T. disticha* to +13.3% in *Pentanisia prunelloides*, with a median percentage change of -33.0 (Fig. 5.6). Of the 16 species, only *P. prunelloides* exhibited an increase (not significant) in seed set per flower with fragmentation. Significant overall declines in seed set per flower occurred in 12 species. Percentage change did not differ significantly between self-compatible and self-incompatible species for all three reproductive variables (Table 5.1).

Table 5.1 Statistical comparison of percentage change in reproductive output between self-compatible (SC) and self-incompatible (SI) species using the Mann-Whitney *U*-test.

Reproductive variable	sample median (n)		statistics	
	SC	SI	Z	P
Fruit set	-7.7 (8)	-30.3 (6)	1.61	0.11
Seed set per fruit	-13.2 (5)	-27.3 (6)	1.81	0.07
Seed set per flower	-30.7 (3)	-43.8 (6)	1.42	0.15

Z = Normal approximation to the Mann-Whitney *U*-test.

When all species were taken into account, the median percentage change in reproductive output for each variable was found to be significantly different from 0 using the Wilcoxon signed-rank test (Table 5.2). When only those species that exhibited significant changes between mainland and fragmented sites were used, median percentage change in reproductive output was still significantly different from 0 for seed set per fruit and seed set per flower data.

Table 5.2 Results of Wilcoxon signed-rank test carried out on percentage change in reproductive output of the 24 wildflower species (Null hypothesis median = 0). A statistically significant difference (indicated by bold type) between median reproductive output and the H_0 median suggests that more species experienced detrimental effects of habitat fragmentation than would be expected from chance alone.

Reproductive variable	All species		Only species showing significant overall increase or decline			
	median (n)		statistics		median (n)	
		Z	P		Z	P
Fruit set	-18.2 (25)	2.18	0.03	-33.4 (9)	1.30	0.19
Seed set per fruit	-23.1 (17)	3.41	<0.001	-23.4 (13)	3.21	0.001
Seed set per flower	-33.0 (16)	3.25	0.001	-38.4 (12)	3.10	0.002

Z = Wilcoxon signed-rank test statistic.

Because five of the nine (56%) species which were not found to exhibit fragmentation effects happened to have minute seeds, these species are not included in the percentage change in seed set per fruit and seed set per flower data sets, thus biasing them somewhat in the direction of overall detrimental effect. This effect is mitigated to some degree however by the fact that 25% of the species exhibiting significant fragmentation effects were also excluded.

Factors important in determining magnitude of fragmentation effect

Mean plant reproductive output is not related to fragment size or degree of isolation (linear regression, $R^2 = 9.60$, $P = 0.38$, $N = 10$ and $R^2 = 14.24$, $P = 0.28$, $N = 10$ respectively). The three measures of percentage change in reproductive output from the average mainland site to the average fragment are not related to capacity for autonomous self-pollination, degree of self-compatibility, floral tube length, fragment size or degree of fragment isolation (Table 5.3, Fig. 5.7). Overall percentage change in seed set per flower is significantly and positively related to pollination specificity in terms of number of pollinator families and orders involved (Table 5.3, Fig. 5.7). Marginally significant positive relationships are also evident between percentage change in seed set

Table 5.3 Results of univariate regressions carried out to determine the relationship between percentage change in reproductive output (fruit set, seed set per fruit and seed set per flower) and various breeding system, pollination system and fragment attributes. Significant relationships ($P < 0.05$) are denoted by bold type.

Dependent variable: fruit set				
Independent variable	R^2	F	P	N
IA	0.07	0.01	0.92	19
ISI	4.38	0.37	0.56	10
Tube length	1.95	0.44	0.51	24
No species	0.71	0.12	0.73	19
No families	0.01	0.00	0.97	19
No orders	0.26	0.04	0.84	19
Area	2.46	0.56	0.46	24
Distance to M	1.14	0.25	0.62	24

Dependent variable: seed set per fruit				
Independent variable	R^2	F	P	N
IA	17.95	2.41	0.15	13
ISI	0.18	0.01	0.92	8
Tube length	5.58	0.89	0.36	17
No species	3.64	0.45	0.51	16
No families	17.31	2.51	0.14	16
No orders	27.67	4.59	0.05	16
Area	4.10	0.64	0.44	17
Distance to M	0.21	0.03	0.86	17

Dependent variable: seed set per flower				
Independent variable	R^2	F	P	N
IA	0.00	0.00	0.98	12
ISI	1.16	0.06	0.82	7
Tube length	8.18	1.25	0.28	16
No species	23.99	3.79	0.08	15
No families	42.58	8.90	0.01	15
No orders	30.34	5.23	0.04	15
Area	0.54	0.08	0.79	16
Distance to M	0.00	0.00	0.98	16

Table 5.4 Results of univariate regressions carried out to determine the relationship between pollination system specificity (measured as number of pollinator species, families and orders involved) and floral tube length.

Dependent variable	<i>R</i> ²	<i>F</i>	<i>P</i>	<i>N</i>
No species	9.29	1.74	0.20	19
No families	11.1	2.12	0.16	19
No orders	9.87	1.86	0.19	19

Table 5.5 Multiple regression models for factors influencing percentage change in reproductive output between mainland and fragment sites for the 24 wildflower species studied at Gilboa.

Dependent variable	Partial regression coefficients				Model			
	IAS	No Families	Area	Distance	<i>F</i>	<i>r</i> ²	<i>P</i>	<i>N</i>
Fruit set	-0.18	0.01	-0.23	0.28	0.17	0.06	0.95	16
seed set per flower	-0.05	0.75*	-0.07	0.19	1.79	0.54	0.25	11

* *P* = 0.04

Table 5.6 Results of univariate regressions carried out on the data of Aizen and Feinsinger (1994) to determine the relationship between percentage change in reproductive output (seed output per plant and number of pollen tubes per style) and breeding and pollination system.

Dependent variable: Seed output				
Independent variable	<i>R</i> ²	<i>F</i>	<i>P</i>	<i>N</i>
no orders	3.89	0.45	0.52	13
IAS	1.31	0.15	0.71	13

Dependent variable: Number of pollen tubes				
Independent variable	<i>R</i> ²	<i>F</i>	<i>P</i>	<i>N</i>
no orders	6.78	0.80	0.39	13
IAS	14.54	1.87	0.20	13

Table 5.7 Multiple regression models for factors influencing percentage change in reproductive output between mainland and fragment sites for the 16 plant species studied in Argentinian Chaco dry forest by Aizen and Feinsinger (1994).

Dependent variable	Partial regression coefficients		Model			
	IAS	No Orders	<i>F</i>	<i>r</i> ²	<i>P</i>	<i>N</i>
Seed output	-0.07	-0.18	0.22	0.04	0.80	13
No. pollen tubes	0.48	-0.39	2.01	0.29	0.19	13

per flower and number of pollinator species, and percentage change in seed set per fruit and number of pollinator orders and families (Table 5.3). In this case the positive correlation between magnitude of fragmentation effect and pollination specificity means that an increase in number of taxa involved leads to a decrease in severity of fragmentation effect. Overall percentage change in fruit set was not related to pollination specificity however (Table 5.3). Specificity of the pollination interaction was not found to be related to floral tube length (Table 5.4).

Multiple regression showed that variation in overall percentage change in fruit set and seed set per flower between mainland and fragment sites cannot be explained by a model including capacity for autogamy, number of pollinator families, fragment size and fragment isolation, although change in seed set per flower is still significantly related to number of pollinator families ($P = 0.04$) (Table 5.5).

Analysis of the data of Aizen and Feinsinger (1994) revealed that overall percentage change in reproductive output (seed output per plant or number of pollen tubes per stigma) is not related to degree of self-compatibility or number of pollinator orders involved in the pollination interaction (Tables 5.6 and 5.7, Fig. 5.8).

DISCUSSION

Consequences of habitat fragmentation for plant reproductive output

Results show that fragmentation of grassland habitat through commercial afforestation has a significant detrimental effect on the reproductive success and potentially the long-term persistence of the wildflower species investigated (Fig. 5.5 and 5.6). Significant detrimental effects are evident in two thirds of the 24 plant species studied (Fig. 5.1-4.), suggesting that a large fraction of the grassland wildflower community might be susceptible to the effects of habitat fragmentation. Significantly more species experienced overall declines in reproductive output than would have been expected by chance alone. Evidence of more severe pollen-limitation of reproductive output in habitat fragments

compared to mainland sites (Chapter 4) and the fact that not all species followed the same trends in reproductive output across the study sites, suggest that these

decreases in fruit and seed set are largely the result of changes in the pollinator service in the fragments.

The percentage of species exhibiting overall significant or non-significant declines in fruit set from the average mainland site to the average habitat fragment is similar to that found in a study carried out by Aizen and Feinsinger (1994) on a set of 16 species in a Chaco dry forest in northwestern Argentina (74 vs 73%), but is much greater here for seed set (94 vs 79%). Whereas overall decline in pollination levels and seed output from continuous forest to the most fragmented patches was found to approach 20% for a Chaco dry forest (Aizen & Feinsinger 1994), the median decline in seed set per flower for the range of grassland wildflowers studied here was 33%.

There are no data available to indicate whether or not recruitment in grassland wildflower species is sensitive to declines in seed production of the magnitude discovered here. The importance of seeds in the demography of the plant, the third component in Bond's (1994) vulnerability index, is also unknown for these plant species. As evident in Chapter 1, a decrease in reproductive output as a result of pollinator scarcity or loss is likely to affect the extinction risk of a plant species when populations are seed-limited, but not if recruitment is microsite limited (Bond 1995; Turnbull *et al.* 2000). Eriksson and Ehrlén (1992) suggest that the importance of seed limitation in plant populations has been underestimated. From a sowing experiment with plant species native to grasslands in south-eastern Sweden, Jakobsson and Eriksson (2000) found 45 out of 50 species to be seed-limited, suggesting that seed limitation may be important in grassland plants and that the species studied here may be vulnerable to local extinction in fragmented landscapes. The ability to escape dependence on seeds through vegetative persistence or a long-lived seed bank also needs to be investigated. The high proportions of bulbous plant species in grasslands suggest that these species may be able to persist vegetatively for some time.

Factors important in determining magnitude of fragmentation effect

From statistical comparisons of percentage change in reproductive output of species with different breeding system attributes (Table 5.1), and from simple and multiple regressions carried out with these variables (Tables 5.3, 5.5, Fig. 5.7), it is evident that only degree of specificity of the pollination system (number of pollinator taxa involved) is important in determining the severity of fragmentation effect for a given plant species.

This discovery supports previous evidence for the vulnerability of pollination specialists to the detrimental effects of environmental perturbations (Linhart & Feinsinger 1980; Steiner 1993; Weller 1994; Ladley & Kelly 1995; Mawdsley *et al.* 1998; Robertson *et al.* 1999; Lindberg & Olesen 2001) and Bond's (1994) prediction that a high degree of specialization will increase the risk of mutualism collapse. The fact that the relationship does not hold true when percentage change in fruit set is used as a measure of fragmentation effect (Table 5.3) indicates that the relationship is not particularly strong however. The effect of pollination specificity on magnitude of fragmentation effect (fruit set, seed set per flower) was also diminished when other factors were included in a multiple regression (Table 5.5), although this may be the result of a lack of data on all of these factors for some of the species and thus reduced power of the analysis. Pollination specificity was also not a significant determinant of fragmentation effect (percentage change in seed output or in number of pollen tubes per stigma) when using data from Aizen and Feinsinger's (1994) study of 16 plant species from Argentinian Chaco dry forest (Tables 5.6, 5.7, Fig. 5.8).

Number of families or orders of pollinators involved are likely to be more meaningful measures of the degree of specificity of the pollination system than is number of species; different pollinator species from the same family may well react to or be affected by environmental perturbations in the same way, so that a pollination system with many pollinator species from a single family may be just as vulnerable as those specialised for pollination by a single species (Johnson & Steiner 2000). Pollinators from different families or orders have different behaviours and environmental requirements (nesting, larval and food resources) and may well be very differently affected by the same environmental disruption. An increase in the number of families or orders involved in the pollination of a

given plant species may thus increase the probability that at least one pollinator species able to effect fertilization survives environmental perturbation, decreasing the likelihood of reproductive collapse in its host plant species.

Aizen and Feinsinger (1994) found some evidence in their study that plants belonging to different pollination guilds might differ in their sensitivity to fragmentation. It would be interesting to determine whether grassland wildflower species specialised for pollination by particular types of pollinators are more or less vulnerable to the effects of habitat fragmentation than others. The wide variety of pollination systems documented here however meant that we were unable to determine whether visitor category, e.g. moths or birds, has an effect on magnitude of fragmentation effect (few plant species belong to each category resulting in sample sizes too small for statistical comparison).

Specificity of the pollination interaction was not found to be related to floral tube length (Table 5.4), suggesting that floral morphological characteristics cannot be used as a substitute for field studies of specificity in pollination systems (see also Waser *et al.* 1996; Larson & Barrett 2000). This probably explains the lack of relationship between floral tube length and magnitude of fragmentation effect (Table 5.3).

The apparent lack of relationship between breeding system and differential decline in plant reproductive output between mainland and fragmented sites, even when controlling for degree of specificity of the interaction and fragment size and isolation in multiple regression (Table 5.3, 5.5, Fig. 5.7), contradicts Bond's (1994) hypothesis that obligately outbreeding species should be more vulnerable to the effects of fragmentation than those which are able to set seed in the absence of pollinator visitation. Multiple regressions were constrained here however by the lack of breeding system data for some species and it is possible that an effect may have been detected had the sample sizes been somewhat greater and the range of species been more balanced between those capable of autonomous self-pollination or self-fertilization and those not. Degree of self-compatibility was also not found to be significantly related to magnitude of fragmentation effect when regressions were carried out using data from Aizen and Feinsinger's (1994) study (Tables 5.6, 5.7, Fig. 5.8). Further similar studies

are required in order to determine the generality of this lack of relationship between breeding system and magnitude of fragmentation effect.

As mentioned above, the lack of relationship between the size and degree of isolation of a habitat fragment on mean percent fruit set for all species in that fragment may be the result of the uneven distribution of species across the fragment sites; a fragment containing species which typically produce few fruit may have a considerably lower mean fruit set than one containing a higher proportion of species which typically set many fruit. However, when multiple regressions were carried out using median size and isolation of the habitat fragments in which the reproductive output of a species was recorded, magnitude of fragmentation effect was still not related to these attributes (Table 5.5). The power of the regressions may however have been reduced by the limited range of fragment sizes and degrees of isolation, and by the lack of adequate site replication (fragment attributes could not easily be controlled for when selecting sites).

Results from other investigations reveal that changes may occur in the pollinator community in response to changes in habitat quality, disturbance frequency and other physical features at different sites, apparently independently of the degree of fragmentation (Moody-Weis & Heywood 2001; Pryke & Samways 2002). Disturbance frequency can also affect plant recruitment (see Jakobsson and Eriksson 2000). Because of this, and because of the wide spatial and temporal variation naturally inherent in plant-pollinator systems (Campbell 1987; Horvitz & Schemske 1988; Johnson & Bond 1992; Aizen & Feinsinger 1994; Roubik 2001), adequate replication of 'treatments' is crucial in order to ascribe changes in plant reproductive output to a single factor such as habitat fragmentation. Further investigations, including additional mainland and fragment sites from other moist upland grassland areas, are required to increase our understanding of the effects of habitat fragmentation on the plant-pollinator mutualisms occurring there.

CONCLUSION

This is one of the few studies to show an overall detrimental effect of habitat fragmentation on the reproductive output of a whole suite of species in a single vegetation type. It is also the first fragmentation study to test the hypothesis of Bond (1994) regarding factors important in assessing extinction risk of a given plant species due to the disruption of pollinator fauna. Findings tentatively support Bond's (1994) prediction that pollination specificity increases risk of extinction, but contradict well established theory regarding the importance of breeding system in determining magnitude of extinction risk.

CONCLUSIONS

FINDINGS OF THE STUDY

The moist upland grassland wildflowers studied here were found to support a rich and diverse pollinator community, involving long-tongued flies, hawkmoths and sunbirds and dominated by long-tongued solitary bees (Chapter 2). Two thirds of the wildflower species appear relatively specialised in terms of pollination, although reserve pollinators may lend a degree of resilience to just under half of these. Six species are highly specialized, entirely dependent on a single species or functional pollinator type for pollination (Chapter 2). The majority of species (90%) were found to be incapable of autonomous self-pollination and thus dependent on pollinators for fruit and seed set (Chapter 3). Self-incompatibility is operative in at least six species. Pollen limitation of plant reproductive output was found to be relatively rare in the wildflower species studied, suggesting that KwaZulu-Natal Moist Upland Grasslands are characterised by high levels of pollinator activity (Chapter 4).

Habitat fragmentation as a result of large scale tree farming was found to have an adverse effect on the reproductive output of a suite of 24 grassland wildflower species (Chapter 5). Significant detrimental effects on reproductive output were evident in two thirds of the wildflower species, with 94% of the species exhibiting overall declines in seed set per fruit from the average mainland site to the average habitat fragment. Median decline in seed set per fruit was 33.0%. Significantly more species experienced overall declines in reproductive output than would have been expected by chance alone. Evidence of more severe pollen limited fecundity in habitat fragments compared to mainland sites for some species (Chapter 4) suggests that these declines in reproductive output are a consequence of changes in the pollinator service in the habitat fragments.

There is evidence that the magnitude of fragmentation effect on reproductive output is, at least in part, related to the degree of specialisation of the pollination system, with overall percentage change in seed set significantly and positively related to number of pollinator taxa involved in the pollination interaction

(Chapter 5). No significant relationships were found between magnitude of fragmentation effect and capacity for autonomous self-pollination, degree of self-compatibility, floral tube length or fragment size and isolation. The effect of pollination specificity on magnitude of fragmentation effect was diminished when these other factors were included in a multiple regression. However lack of data on all of these factors for some of the species means that the power of the multiple regression was reduced.

Findings thus tentatively support Bond's (1994) hypothesis that pollination specificity increases risk of extinction but not that plants dependent on pollinators for fruit set will be more vulnerable to fragmentation effects. The generality of these results is difficult to determine. Analyses carried out on data from a similar study in Argentinian Chaco dry forest (Aizen & Feinsinger 1994) did not reveal significant relationships between magnitude of fragmentation effect and either pollination specificity or degree of self-compatibility.

IMPLICATIONS FOR THE FUTURE OF KWAZULU-NATAL MOIST UPLAND GRASSLANDS

Large areas of the species-rich moist upland grasslands of KwaZulu-Natal have been afforested with exotic trees, resulting in severe habitat fragmentation in many areas (Armstrong *et al.* 1998). Study results confirm that this habitat fragmentation has a definite adverse effect on the reproductive output of grassland wildflower species. This has implications for the continued existence of these species in an increasingly fragmented landscape, particularly if they are found to be seed-limited.

The relatively moderate fragmentation effects on plant reproductive output (median decline in seed set per flower of 33%) suggest however that the fragments already formed may still play an important role in the conservation of this endangered vegetation type. Plant species may be able to persist locally at these reduced levels of fecundity. The grassland fragments, in conjunction with the adjoining undisturbed grassland mainland, may prove to be valuable repositories of significant numbers of moist upland grassland flora and fauna and

their genetic diversity and sources for recolonization of nearby disturbed areas (Turner & Corlett 1996; Borgelia *et al.* 2001).

FUTURE RESEARCH

The results of this study strongly reveal the need for further investigation into the differential susceptibility of different species and communities to the effects of habitat fragmentation. Studies should involve whole suites of species in a range of different environments around the world. Sites need to be adequately replicated because of the wide spatial and temporal variation naturally inherent in plant-pollinator systems (Campbell 1987; Horvitz & Schemske 1988; Johnson & Bond 1992; Aizen & Feinsinger 1994; Roubik 2001). Knowledge of which factors are important in determining the risk of extinction of a given plant species may allow for the development of predictive tools for the rapid assessment of the vulnerability of species or communities threatened by habitat alteration.

With respect to moist upland grasslands of KwaZulu-Natal, further investigations including additional mainland and fragment sites from other moist upland grassland areas may increase our understanding of the effects of habitat fragmentation on the plant-pollinator mutualisms occurring there. The demographic dependence of grassland wildflower species on seeds needs to be investigated in order to determine whether the decreases in reproductive output in these species will lead to reduced population growth and threaten local persistence of the species. Pollination systems also need to be investigated further: the efficacies of the less 'classical' reserve pollinators of wildflower species need to be determined directly in order to assess whether these pollinators impart a degree of resilience and flexibility to the system. The abundance and diversity of pollinators across sites should be measured directly (through timed observations or use of traps) in order to confirm that declines in plant reproductive output in the habitat fragments are the result of changes in the pollinator fauna at these sites. Direct measures of pollinator abundance would also determine which pollinator types are more susceptible to the effects of habitat fragmentation.

Further research needs to be carried out on management strategies that can be used to mitigate the disruption of mutualisms and loss of species in already-fragmented grasslands. Corridors for example are popular tools to mitigate fragmentation and conserve biodiversity (Hess & Fischer 2001). Collinge (2000) found that corridors slightly decreased the rate of loss of some arthropod species in prairie fragments. Studies would have to be conducted to determine the types of corridors required. Pryke and Samway's (2001) study found that local and endemic grassland butterfly species would only make use of corridors linking grassland habitat in a commercial tree plantations if the width was greater than 250 m. Research is also required to determine the optimal management programmes for these habitat remnants, since habitat fragmentation tends to disrupt natural disturbance regimes such as fire which may be important in the maintenance of plant population dynamics. Some grassland wildflower species are dependent on regular fires for persistence and may eventually die if the grass canopy is not removed for extended periods of time (Tainton & Mentis 1984). Too frequent burning at inappropriate times of the year can also be detrimental (Armstrong & van Hensbergen 1996). Research into the effects of different fire regimes on pollinator communities is urgently required. Areas would need to be left unburnt at any given time so that fire sensitive species can survive in these refuges and later recolonise burnt areas (Tainton & Mentis 1984). Further research of this nature may one day provide guidelines for timber companies on how best to lay out new tree plantations so as to minimise the adverse effects of habitat fragmentation on ecological interactions and biological diversity.

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