



Biology and conservation of the threatened Karkloof blue butterfly

***Orachrysops ariadne* (Butler) (Lepidoptera: Lycaenidae)**

by

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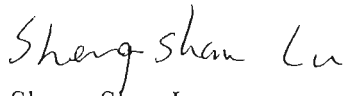
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I declare that this thesis is my own original work. Where use has been made of the work of others, it has been duly acknowledged in the text. I have not submitted this material, either whole or in part, for a degree at this or any other institution.


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ABSTRACT

The Karkloof blue butterfly, *Orachrysops ariadne* (Butler), is endemic to the Mistbelt grassland of KwaZulu-Natal province, South Africa, and is currently Red-listed as 'Vulnerable'. *O. ariadne* is univoltine and on the wing in March and April, when it utilizes eight species of nectar plants. This study confirmed that the larval hostplant is *Indigofera woodii* H. Bol. var. *laxa* H. Bol., an erect variety. It was also confirmed that this butterfly is ant-dependent, with the young larva being taken into the nest of *Camponotus natalensis* (F. Smith) where development continues, including pupation.

This study compares the ecological conditions at the four known locations so as to make informed decisions regarding its conservation. A large proportion of the grassland in KwaZulu-Natal has been aforested and cultivated, and at least 92% of the Mistbelt has been transformed, with only about 1% in good condition remaining. Predictions on the habitat and habitat requirements of this species are necessary for developing a conservation strategy and action plan. Here, we propose *O. ariadne* as an indicator species for Mistbelt grassland. Saving enough of the remaining Mistbelt grassland is crucial, not just for the survival of the Karkloof blue, *O. ariadne*, but also for the Mistbelt grassland community as a whole.

The population structure and movement of *Orachrysops ariadne* and *O. subravus* were studied by mark-release-recapture methods in 1999. There were 290 *O. ariadne* marked over 48 days between March and April, 124 (42.8%) were recaptured at least once. Of 631 *O. subravus* marked over two months between September and November, 311 (49.3%) were recaptured at least once. Both species exhibited protandry, male appearance about one to two weeks earlier. The sex ratio of *O. ariadne* is heavily male biased 5.6:1 (246 males and 44 females), and the sex ratio of *O. subravus* is 1.6:1 (387 males and 244 females). The Jolly-Seber model was used to

estimate daily population numbers (N_i), survival rates (ϕ_i), recruitment rates (B_i), proportion of marked animals in the total population (α_i), and the number of marked animals at risk (M_i). Average residence times of male adults were generally similar in both species in the range of 5.36-5.44 days, and were slightly longer for male than for female *O. subravus* (by 4.09 days). *O. ariadne* is a strong and rapid back and forth flier, covering mean recapture distances of 157 m, almost twice that of *O. subravus*, principally in search of scarce nectar sources. The extreme rarity of *O. ariadne* is not so much to do with behaviour, survivorship or longevity, but rather the butterfly is limited in distribution by suitable habitat for both larva and adult.

The aim of management is to optimize the habitat so that it best meets the present and future needs of the butterfly. The effects of the current fire regime on the butterfly, host plant and ant host were evaluated here. It is recommended that burning only take place after the larvae have hatched and gone underground with the ant host. Using GPS and GIS, core, quality habitat characteristics were defined. In cooperation with the landowner at one site, alien invasive plants are being removed to increase the area of quality habitat. Availability of host plants is limiting for success of the butterfly in the field. Guidelines are provided for propagation and introduction of the host plant, so as to provide the butterfly with more oviposition sites.

Chapter 1. General Introduction

1.1 Biodiversity conservation in South Africa

Worldwide, natural ecosystems have been diminishing at alarming rates. Never before have so many species gone extinct in such a short period on this human-dominated planet (Vitousek *et al.*, 1997). It is clear that human transformation of Earth is growing substantially, and the threats to biodiversity are accelerating from the rapid growth of human population. As a consequence, conservation biology has arisen as a response to the biodiversity crisis.

The Convention on Biological Diversity, at the Earth Summit in Rio de Janeiro in 1992, is a landmark treaty on the conservation of biodiversity. The main objectives are the conservation of biodiversity and the sustainable use of its components. It is the first time that biodiversity was comprehensively addressed, and became a common concern for humankind (Glowka *et al.*, 1994).

South Africa has rich and diverse fauna and flora (Myers, *et al.*, 2000). These extremely diverse plant communities have a high insect species richness, although not proportionately. The number of South African endemic insects is also very high (Samways, 1995). Among them are many rare, endemic butterflies which only occur at single localities (Henning and Henning, 1989).

South Africa ratified the Convention on Biological Diversity in 1995, which obligates the government to maintain the country's biodiversity. The Convention led the 'White Paper on the Conservation and Sustainable Use of South Africa's Biological Diversity' in 1997. Among other things, the White Paper mentions that:

A substantial proportion of natural habitat has been transformed –largely by agriculture, urban developments, afforestation, mining and dams. In addition to habitat loss and degradation, the overexploitation of certain species, the introduction of exotic species, and the toxification of the soil, water and atmosphere have had major effects on South Africa's terrestrial, freshwater and marine biodiversity. Already 3435 (15%) of South Africa's plant species, 102 (14%) of bird, 72 (24%) of reptile, 17 (18%) of amphibian, 90 (37%) of mammal, and 142 (22%) of butterfly species are listed as threatened in the South African Red Data Books, which indicate the conservation status of

threatened species and ecosystems... (cf. 'The Brenton Blue Saga', (Steenkamp and Stein, 1999)).

The Convention on Biological Diversity has a significant influence on the campaign for the threatened Brenton blue butterfly *Orachrysops niobe* (Trimen), and is a good case study for South African species conservation. The Brenton blue has received considerable attention as there is an imminent threat to its last known colony by housing development (Henning and Henning, 1995; Edge and Pringle, 1996; Williams, 1996). It was the first time that this law (Section 31A of the Environmental Conservation Act) was used to halt a development at Brenton-on-Sea, near Knysna. After a couple of years work, the campaign reached the national level, and finally led to the establishment of the Brenton blue butterfly reserve (Steenkamp and Stein, 1999).

In the real world of conservation biology as a 'crisis discipline' (Soulé, 1985), it is not possible to conserve biodiversity species by species. The time and resources for the conservation of biodiversity are limited, and unless we take fast and effective conservation action, much biodiversity will soon be lost. We could not expect all the threatened or rare species to receive such attention as *O. niobe*. To some extent, *O. ariadne* has also been considered. The first known colony at The Start, KwaZulu-Natal was registered as a Natural Heritage Site in 1996 by the forestry company South African Pulp and Paper Industries (SAPPI). However, until now, very little information has been available on the biology of the species.

1.2 From species to landscape levels

Biodiversity is the variety and variability of all living organisms, and recognizes a hierarchy from genetic, population-species, community-ecosystem to landscape levels (Noss, 1990). A comprehensive approach to biodiversity conservation must address these multiple levels of organization and at different spatial scales. The traditional species approach to maintaining biological diversity has generally been to proceed species by species and threat by threat. Single-species management is well established in Europe, North America, Australia, Japan and South Africa, where knowledge on habitat management and target species ecology are relatively advanced. But in tropical

areas, where the great majority of species occur, this approach is impractical and often inappropriate (New *et al.*, 1995).

The single-species approach can be of value in protecting small parcels of land, especially for certain localized insects which can occur within one hectare, such as the Brenton and Karkloof blues. The Endangered Species Act of U. S. and the Convention on International Trade in Endangered Species (CITES) both are based on the species-level for protection of biodiversity (Meffe and Carroll, 1994). Indeed, conservation of rare species is an important issue of the preservation of biodiversity in certain regions. In practice, most other insects are also preserved through conservation of rare species, as we can be considered as ‘flagship species’ or ‘umbrella species’. However, in the greater picture, a single-species approach can secure only a tiny fraction of overall biodiversity, and must be combined with protection of habitats and ecosystems (Meffe and Carroll, 1994).

The primary threat to species throughout the world is the alteration and destruction of habitats in which they live. Conservation of species can best be done through conservation of their habitats, especially in the case of insects (Collins and Thomas, 1991; Henning *et al.*, 1997). In practice, most species of insects are protected by preservation of their physical and biotic biotopes and ecosystems (Samways, 1994). Furthermore, the protection of ‘hot spots’ of biodiversity should be a high priority, to ensure that all areas and ecosystems rich in species diversity are adequately managed.

When species-oriented conservation programmes are undertaken while also valuing the habitat as a whole, then the conservation of many species can be achieved by focusing on the needs of the few (Pullin, 1997). A habitat conservation approach that includes species-interaction management are complementary efforts. For example, when the interaction between the large blue *Maculinea arion* Linnaeus and its host ant was restored and managed, other vulnerable and endangered species found in the same habitat also made a resurgence (New *et al.*, 1995)

Time is short for conserving as much as possible of the world’s biota. The most cost-effective approach to preserving as many insect species as possible is to conserve a variety of landscapes (Samways, 1994). The landscape is a sufficiently large scale and of a fairly well-definable structure and composition for it to be a good umbrella for preservation of smaller scale units and processes. Concern for ecosystems and landscapes is therefore of great importance for all its individuals, species and

interactions. This landscape approach also creates an awareness and an ethic that all landscapes are linked by biogeocycles (Samways, 1994).

Human modification of the landscape is growing at an increasing rate, and even with reserve protection, there is no guarantee for the survival of all species (Sutherland, 1995). This is emphasized by many of Britain's rare species of butterflies not being conserved effectively under the current site protection system (Warren, 1993). This is partly because habitat fragmentation has a profound influence on the probability of any one species' survival, with the loss and isolation of natural habitat through fragmentation being a major threat to biodiversity. For conservation of biodiversity in fragmented landscapes there should be emphasis on the management of habitat fragments and better integration of protected areas into the surrounding landscape (Warren, 1993).

1.3 Butterfly conservation and the importance of conserving lycaenid mutualisms

Despite insects representing the major component of terrestrial biodiversity, the idea of conserving insects is still strange for most people. In turn however, when there is mention of conservation of butterflies sympathy is often generated. Butterflies have received a great deal of attention, as being aesthetically and culturally important to conservation (New, 1997). The alert to butterfly loss is an initial concern for insect conservation (Samways, 1994), for example, loss of the large copper *Lycaena dispar* and large blue *Maculinea arino* in Britain, and the Xerces blue *Glaucopsyche xerces* in USA. This has led to more studies on butterfly conservation than on any other groups of insects, especially in Europe and North America.

There are two main issues when considering butterfly conservation. The first issue revolves around butterflies as the specific targets of conservation. Many butterflies around the world have declined, as well as many other terrestrial forms of wildlife, through four main factors: habitat destruction, pollution, alien species invasion and commercial exploitation (New, 1993). This has resulted in particular populations of rare species being threatened by some form of immediate or imminent habitat change, thus requiring urgent crisis management to conserve them (New, 1997). Examples from Europe, North America, and elsewhere exemplify increasing global interest in butterfly conservation (New *et al.*, 1995; New, 1997)

The second issue is the use of butterflies as indicator of habitat or community health. Butterflies are one group of insects for which relatively full data may be available, and meet the criteria useful for indicator groups: conspicuous, day-flying, taxonomically tractable with most species recognizable, widespread, relatively diverse, many with precise ecological requirements and many known to respond to particular changes in habitat parameters (New, 1997).

Probably the most widely-appreciated butterflies are the Papilionidae (e.g. swallowtails and birdwings). Although many other butterflies are much less well-known than the Papilionidae, substantial conservation efforts have been directed to the largest family of butterflies, the Lycaenidae (New, 1993). Lycaenidae exemplify a wide spectrum of concerns: populations are often extremely localized, with colonies often occupying a few hectares or less. Many are also associated with early successional stages of vegetation in grasslands or particular herb associations. Many also participate in subtle ecological association with ants or (more rarely) Homoptera (New, 1993).

The Lycaenidae is the largest and most diverse butterfly family in southern African (Henning, 1983). Over 860 species have been recorded in the subregion (Williams, 1996). The lycaenids comprise nearly half (47%) of all butterfly species in the region (Pringle *et al.*, 1994), yet they are also the poorest known group, especially the immature stages (Williams, 1996). This variety coupled with lack of knowledge makes them as a group difficult to conserve. This is emphasized as Lycaenidae comprise 75% of the threatened species in South Africa, with two extinct (Samways, 1993). This clearly illustrates that much more attention should be given to the conservation of the Lycaenidae.

Over 80% of South African lycaenids have an association with ants at some stage during their life history (Fiedler, 1991 *cf.* Heath and Classens, 2000). These myrmecophilous (ant-associated) lycaenids have received a great deal of attention. In fact, the butterflies most at risk in South Africa are these myrmecophilous lycaenids (Henning and Henning, 1989). Furthermore, these myrmecophilous species are often very localized and rare, and as they are often confined to a limited area, often smaller than a hectare in size, these species are particularly vulnerable to any disturbance of their preferred habitat.

Myrmecophilous Lycaenidae require both the presence of the host ant and host

plant, as well as optimal climatic conditions. In the case of the butterfly *M. arion* in Britain, the host ant is also sensitive, with only a slight change in microclimate making survival of both species precarious. Species loss may thus have a cascade effect, which inevitably affects the integrity of the whole ecosystem (Samways, 1998). The interdependence (exploitive or mutualistic) of rare species may mean that the removal of one rare species could result in the loss of several other, dependent species, such as specialist parasitoids (Hochberg *et al.*, 1998).

Henning *et al.* (1997) points out that ‘habitat conservation is the only solution’ in his review of butterfly conservation in South Africa. This is especially in the case in South Africa, because many butterflies are Red-listed (Henning and Henning, 1995). Proclamation of the ‘Ruimsig Entomological Reserve’ in 1985 in Gauteng which was the first reserve devoted to a threatened butterfly in the southern hemisphere, and the ‘Brenton Blue Butterfly Reserve’ in 1998 at the Brenton-on-Sea in the Western Cape, clearly illustrate the value of reserve acquisition for conserving particular lycaenid species.

1.4 Aims of the present study

Studies in many countries show the complexity of management needs for single species (New *et al.*, 1995). New (1997) illustrated the management scheme for practical conservation of a species. The key to protecting and managing a rare or endangered species is to understand its natural history or autecology (Primack, 1993). Primack (1993) also points out that habitat management is the key to success in the conservation of endangered butterflies. In other words, conservation of the Karkloof blue relies on knowledge of the species' life cycle, each developmental stage, behaviour, distribution, habitat requirements, population structure and so on. Unfortunately, we know almost nothing about the biology and ecology of this butterfly, despite the fact that it was first discovered over a century ago.

This study aims to elucidate the life history of the threatened Karkloof blue butterfly, its ecological and habitat requirements, and its population structure and movement parameters. The sibling species *O. subravus* (Grizzled blue) will also be considered, for comparison. Effects of the current fire regime on this butterfly are also given attention as currently the fire management practice is without any research foundation. Gathering information on these aspects means that we can then take more effective conservation action, and thus recommendations and guidelines for future conservation management are also proposed.

Chapter 2. Life history of the threatened Karkloof blue butterfly *Orachrysops ariadne*

2.1 Abstract

The behaviour and life history of the highly threatened Karkloof blue butterfly *Orachrysops ariadne* (Butler) (Lepidoptera: Lycaenidae) were studied between 1997 and 1999. The butterfly is now known from four sites in KwaZulu-Natal province, South Africa. *O. ariadne* is univoltine and on the wing in March and April, when it utilizes eight species of nectar plants. The oviposition and host plant is *Indigofera woodii* H. Bol. var. *laxa* H. Bol., an erect variety. This butterfly is ant-dependent, with the young larva being taken into the nest of *Camponotus natalensis* (F. Smith) (Hymenoptera: Formicidae) where development continues, including pupation. Indications are that the critical factor limiting population levels is oviposition/host plant availability.

2.2 Introduction

The Karkloof blue butterfly, *Orachrysops ariadne* (Butler, 1898) (Lepidoptera: Lycaenidae), is a highly localized endemic South African species, that is currently Red-listed as 'Vulnerable' (Henning and Henning, 1995). The butterfly was discovered in the Karkloof area, KwaZulu-Natal province, in 1897, and described by A. G. Butler in 1898. After that, it remained unrecorded until K. M. Pennington collected one male near the Karkloof Falls in March 1928. In March 1936, Pennington and R. C. Wood discovered a breeding colony in a small valley on the farm The Start, near Karkloof. Another colony was found near Michaelhouse, Balgowan in 1941, but this population was extirpated some time after 1945 (Henning and Henning, 1989). The Karkloof blue is an extremely rare species, with The Start colony being the only well-known location, and the focal population for this study. Except for a few specimens from Nkandla in northern KwaZulu-Natal, it is thought that this species only exists in the KwaZulu-Natal Midlands (Pringle *et al.*, 1994), where it appears always to have been rare (Swanepoel, 1953; Pringle *et al.* 1994).

Very little is known about the biology of this species. Although Henning and Henning (1994) described the adult morphology in detail, there are only a few brief descriptions of its habitat and behaviour, and these only from The Start (Swanepoel, 1953; Henning and Henning, 1989). Some preliminary observations of the Karkloof blue butterfly were conducted (Manning, 1982; Parry, 1983), but its life history has remained an enigma for more than a century.

All species in the genus *Orachrysops* Vari, 1986, are endemic to South Africa. Three species were transferred from *Lepidochrysops* (*niobe* (Trimen), *ariadne* (Butler) and *lacrimosa* (Bethune-Baker)) and seven new species and one new subspecies were described by Henning and Henning (1994). A further new species was described by Heath (1997). Currently, we have a very incomplete knowledge of the life history of *Orachrysops* (Henning and Henning, 1994; Heath, 1997; Heath and Claassens, 2000). Apart from the fact that they feed on the genus *Indigofera* (Fabaceae) during their early larval instars. It is assumed that older larvae are carried, or make their way, into an ant's nest, as in the case of the closely-related genus *Lepidochrysops* Hedicke (Henning and Henning, 1994). However, there is as yet no evidence to support this contention. The only species of *Orachrysops* for which the life history is known is *O. niobe* which has been bred in captivity (Edge and Pringle, 1996).

The larvae of *O. lacrimosa*, *O. niobe* and *O. brinkmani* have tubercles and dorsal nectar organ (DNO) from the second instar onwards (Clark and Dickson, 1971; Edge and Pringle, 1996; Heath, 1997), which is morphological evidence that they may all have a relationship with ants. However, again, no host ant has yet been found that is directly associated with these species. The young larva of *O. lacrimosa* and *O. mijburghi* feed on the leaves of the oviposition plant *Indigofera* sp., but die shortly after it confined to just this plant (Clark and Dickson, 1971; Williams, 1996). A few larvae of the Brenton blue (*O. niobe*) that were successfully reared to adults in the laboratory were phytophagous in all instars (Edge and Pringle, 1996).

The key to protecting and managing a rare or threatened species is to understand its life history (Primack, 1993). Detailed knowledge about the life history of a threatened species is an important requirement for effective conservation. For an endopterygote, this means understanding the requirements of both the immature stages and of the adult. The immature stages are important in butterfly conservation, because developmental polymorphism means, effectively, that the immature stages have

different habitat requirements and behaviours (Samways, 1994). Clearly, we need to know the life history of *O. ariadne*, especially the details of the behaviour of the immature stages.

The aim of this study was to elucidate the life history of the Karkloof blue butterfly *O. ariadne*, as the baseline for its effective conservation. For survival of this species, we need to know the requirements of each developmental stage. Specifically, the objectives of this study were to: (1) describe the nectar sources that are used and adult behaviour during the flight period; (2) investigate the morphology and development of the egg stage and the oviposition host plant; (3) investigate the larval and pupal stage, and to identify the possible host ant; (4) to use these findings to make conservation management suggestions for the species.

2.3 Study sites

Currently, there are four known localities for this butterfly (Fig. 1). Most of the studies conducted here were taken from the farms The Start and Wahroonga, because the other two colonies at the farm Stirling were only discovered during this study in 1999, and the Nkandla site was visited the same year.

The Start (29°24'S, 30°17'E, elevation \pm 1080m a.s.l.) is in the Karkloof area, 12 km north of Howick. The forestry company, South African Pulp and Paper Industry (SAPPI), is the custodian of this area, and in 1996 registered the site as a Natural Heritage Site (8.9 ha) specifically to secure the future of this butterfly. The butterfly inhabits only 1 ha of steep dense grassland on a south-facing hillside near a small patch of indigenous forest. On the steep, south-facing slope the tall-grass *Hyparrhenia* spp. grows, amongst which the host plant *Indigofera woodii* is found (Pringle *et al.*, 1994). The site lies within the Mistbelt area, where the rainfall varies from 700 mm to 1300 mm per annum, and the average temperature is 13°C in July and 21°C in January (Schulze, 1997). Pine plantations, maize fields and cattle-pastures surround the butterfly colony.

The site of the Wahroonga colony (29°36'S, 30°07'E) is a fine example of Mistbelt grassland, owing to its unique and rare floral diversity, and was registered as Natural Heritage Site (36 ha) in 1990. The butterfly inhabits a 10-ha area of rank grassland on the south- to southwest-facing slope, adjacent to a strip of Mistbelt forest.

The average rainfall is about 900 mm. The elevation ranges from 1320 m to 1440 m a.s.l.. The surrounding land consists of *Pinus* and *Eucalyptus* plantations with cattle-grazing.

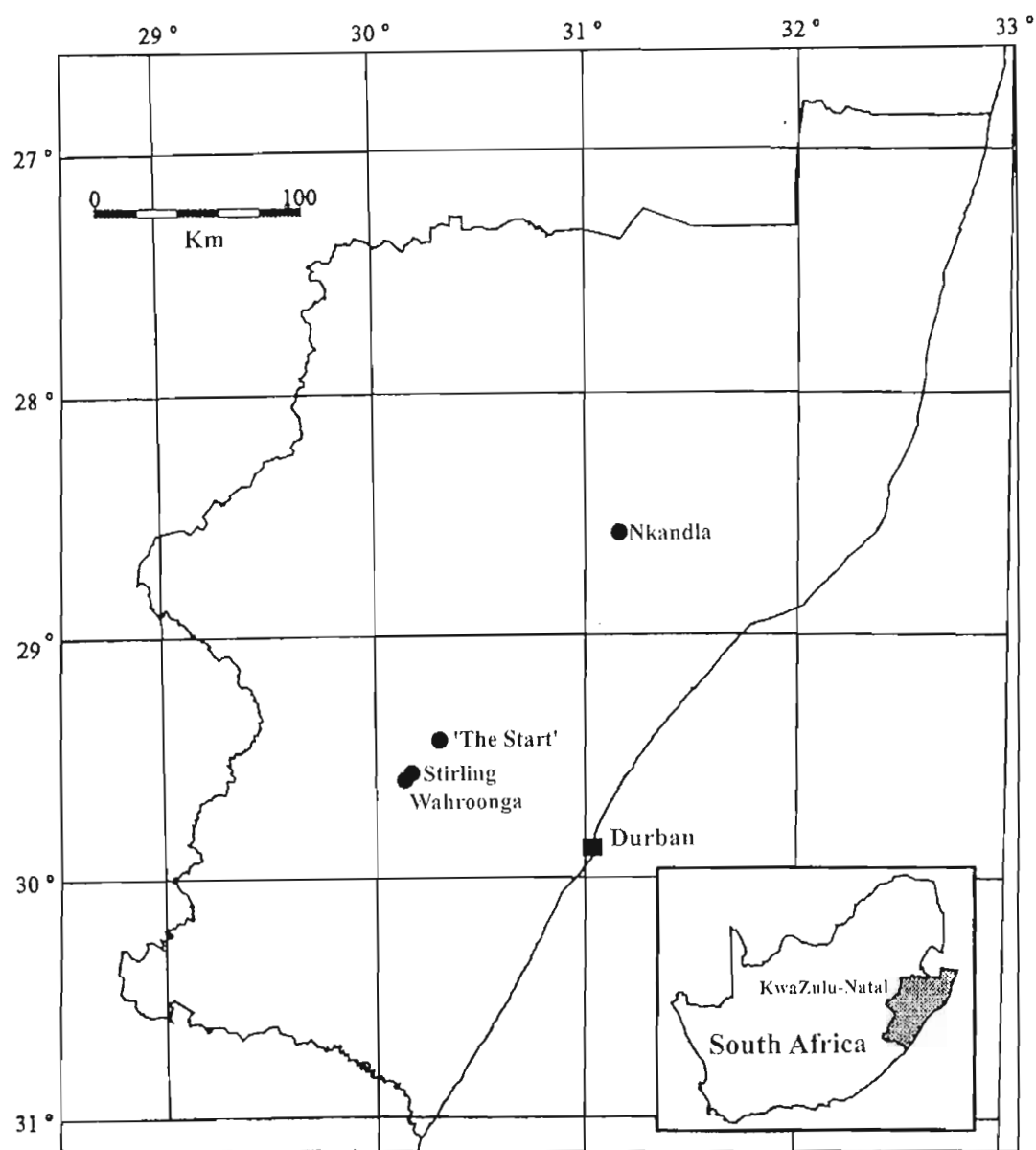


Figure 1. Current geographical distribution of the Karkloof blue butterfly *Orachrysops ariadne*.

The farm Stirling (29°35'S, 30°08'E), adjacent to Wahroonga, also lies within Mistbelt grassland, at an elevation of 1460 m a.s.l.. The butterfly was first recorded here in this study on 25 March 1999. At this site, the butterfly was flying on a south-facing, tall-grass slope, adjacent to a stand of wattle (*Acacia mearnsii* De Wild). The site is surrounded by cattle pastures.

There is an early record of a Karkloof blue colony at Nkandla (28°42'S, 31°08'E), near Nkandla Forest Reserve (Pringle *et al.*, 1994). The elevation ranges from 1100 m to 1200 m a.s.l.. This colony was rediscovered on 10 April 1999 with the help of Mr. Don Quinn. This area is currently under protection by KwaZulu-Natal Wildlife, along with a number of adjacent and nearby Forest Reserves, and regarded as a conservation unit comprising a 3792 ha. The butterfly was flying on a south- to southwest-facing slope, adjacent to gullies where the oviposition host plant was abundant.

2.4 Material and Methods

Adult stage

Owing to the topography and dense vegetation, the butterfly is difficult to follow and observe. Nevertheless, using paths and binoculars, data gathering was possible. The behaviour was observed and recorded throughout the adult flight period from March to April at The Start from 1997 to 1999, and at Wahroonga in 1998 and 1999. Additional observations were made at Stirling and at Nkandla in 1999. A detailed record was kept of the adult behaviour, especially that on the nectar plants and host plant. *Ad libitum* sampling was used for preliminary observation, while focal-sampling and behaviour-sampling were used to record particular individuals or behavioural events (Martin and Bateson, 1986). *Ad libitum* sampling means that the observer simply notes down whatever is visible at the time and seems relevant. Focal-sampling and behavioural-sampling means observing an individual for a specified amount of time and watching a particular type of behaviour (Martin and Bateson, 1986).

Egg stage

The adults were monitored, paying particular attention to egg laying. By observing oviposition, it was possible to recognize the egg of this species. The host plant was

surveyed for eggs from March to June at The Start, from 1997 to 1999, along a 400 m x 2m transect. In 1999, additional surveys were done at Wahroonga and at Nkandla. When eggs were found, the supporting twig was marked with a serial number using plastic electrical tape as a tag. The tag lasted several months, which was long enough to cover the whole season. This labelling avoided duplicative sampling, and made records on each egg possible. By random searching of the host plant, egg position on it, status (see description below), and number of eggs per stem (bud) and per host plant were recorded.

Egg counts were taken every two or three days at The Start and Wahroonga, weather permitting. The 200 eggs at Nkandla were observed only twice, in May and June owing to the distance of the colony, and only having been found in 1999. The status of the egg was identified at each visit, and each egg was assigned to one of three categories, i.e. hatched, damaged or completely disappeared. The eggs whose fate was not known were identified and classified as ‘Unknown’.

When the larva emerged, the chewed eggshell was then discarded. A round hole indicated that the larva had hatched successfully (Fig. 2). It was relatively easy to differentiate the eggshells that had been damaged by predators, or from other causes, from healthy ones using a 10X or 20X hand lens. The eggs with labels were checked until they could be classified as hatched, damaged or disappeared. A few of eggs were used to examine the ultra-structure using a scanning electron microscope (SEM).

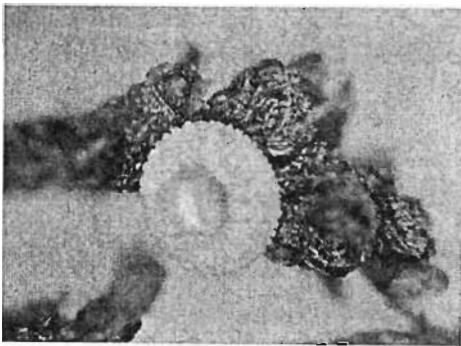


Figure 2. A round hole indicated that the larva had hatched successfully.

Larval stage

As this is an extremely rare and threatened species, a compromise had to be found to minimize collection of material from the field while ensuring that scientifically meaningful results were obtained. Thirty eggs were collected. When the larva hatched, it was placed in a small container (3 x 3 x 5 cm) and fresh new shoots were provided every one or two days. Some of larvae were also examined using a SEM. Detailed

surveys were conducted on host plants, searching for larvae after they had hatched. Ten larvae from the laboratory were also released into the field for further behavioural observation. Hatching larvae were also observed in the field when encountered. One older larva and two ants were collected from Wahroonga on 9 December 1999, and were inspected under the microscope to observe myrmecophilous behaviour. The base of the host plant was carefully dug out to search for the larvae and pupae.

2.5 Results

Adult stage

Flight behaviour

This butterfly is univoltine. The adult flight period begins in either March or April. When the adults flew a short distance, it was possible to follow them, unless they stopped to rest in the dense grass, where they were then lost from sight. They were only seen again when they resumed flight. The adults fly mainly for the purpose of searching for food (nectar sources) and to mate. In addition, an important factor for the female is searching for the host plant for oviposition. They either rest or bask between these activities.

Flower visitation (Fig. 3)

Eighty-five observations on flower-visiting were made at the four colonies. Eight different nectar sources were recorded (Table 1). They were *Hebenstretia dura* (n=36), *Tephrosia polystachya* (n=20), *Athrixia phyllicoides* (n=11), *Epilobium salignum* (n=9), *Vernonia neocorymbosa* (n=5), *Psoralea pinnata* (n=2), *Senecio*



Figure 3. Visiting flowers of *Tephrosia polystachya*

paludaffinis (n=1), *Cineraria deltoidea* (n=1). *H. dura* (42.4%, n=85) and *T. polystachya* (23.5%, n=85) are the two main nectar plants. *H. dura* is the most preferred nectar source and found at Wahroonga and Stirling in 1999, but not at The Start or Nkandla. *T. polystachya* and *A. phyllicoides* were the two most preferred nectar sources at The Start. *P. pinnata* was only recorded at Nkandla.

Table 1. The nectar plants utilized by *Orachrysops ariadne* at the four sites; n=number of flower visitations observed.

Nectar plants	The Start	Wahroonga	Stirling	Nkandla	n
<i>Hebenstretia dura</i> Choisy (Scrophulariaceae)	-	+	+	-	36
<i>Tephrosia polystachya</i> E. Mey (Fabaceae)	+	+	-	-	20
<i>Athrixia phylloides</i> D.C. (Asteraceae)	+	?	-	-	11
<i>Epilobium salignum</i> Hausskn (Onagraceae)	-	+	-	-	9
<i>Vernonia neocorymbosa</i> Hilliard (Asteraceae)	?	+	?	-	5
<i>Psoralea pinnata</i> L. FP. (Fabaceae)	-	-	-	+	2
<i>Senecio paludaffinis</i> Hilliard (Asteraceae)	-	+	-	-	1
<i>Cineraria deltoifea</i> Sond. (Asteraceae)	-	+	-	-	1

+: nectar plants utilized by the butterfly.
?: nectar plants presence but not seen to be utilized by the butterfly.
-: nectar plants absent from site.

Mating behaviour (Fig. 4)

Mating was observed on two occasions. The first was on 31 March 1998 at The Start. Beginning at 12h40, the two adults flew back and forth past the same spot, then made contact, and then fell into the dense grass several times. The mating pair then rested on the grass. They continued to copulate for 10 min, and then separated. They attempted to copulate again, but failed, then flew away.

The second observation was made while doing a mark-release-recapture study on 6 April 1999 at Wahroonga. A marked female was resting, after having been released from the captured point. A male was also released, after having also just been marked. They immediately began mating at 12h27. Copulation lasted for 20 min, then they separated. The abdomens of the pair could be seen to be constricting as if sperm was being transferred. In both cases the couple’s wings were brilliant, possibly due to the fact that the individuals had recently emerged.

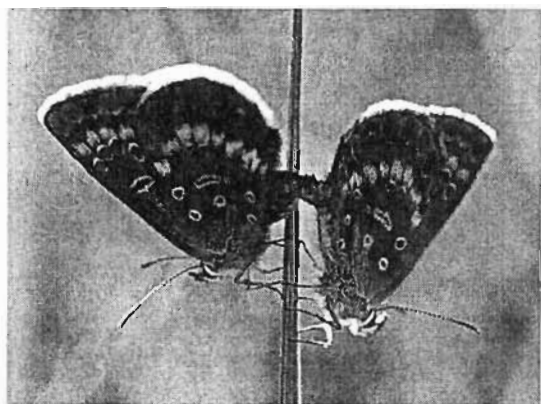


Figure 4. A mating pair of *Orachrysops ariadne*. Left side is female, and right is male.



Figure 5. A female laying eggs on the host plant.

Oviposition behaviour (Fig. 5)

Females searched for the host plant *I. woodii* var. *laxa* for oviposition. When the female landed on the host plant bud, she used antennal palpation and her front legs drummed as if confirming identification. She curved her abdomen and immediately deposited an egg. The periods between oviposition were usually accompanied by resting and/or visitation of a flower. Usually, a single egg or a pair of eggs were laid on each bud. One female was followed for 15 min. Fourteen eggs were laid in total (six pairs and two single eggs) on four individual host plants (3-5 m apart).

The host plant was confirmed by repeatedly noting the female's oviposition behaviour in the field. Formerly misidentified as *Indigofera astraglina* DC (Swanepoel, 1953; Pringle *et al.* 1994), the host plant was confirmed as *Indigofera woodii* Bolus (Manning, 1982). The herbarium specimens of *I. woodii* at the University of Natal are all prostrate, except for the one collected from The Start by Manning (1982), which was about 1 m in height. This erect, long-stemmed *I. woodii* H. Bol. var. *laxa* H. Bol. was confirmed here as the oviposition host plant.

Resting and basking behaviour (Fig. 6 & 7)

The adults rest primarily on *Miscanthus* sp. (Gramineae), which abounds in the habitat, or else on other flat-leaves near the host plant. While resting, the adult is very sensitive to the closeness of the observer. Also, when a flying insect, such as a skipper or a brown butterfly passed, the Karkloof blue sometimes pursued them. Vertical movement of the hindwings regularly occurred in the resting adults, as is common in many other members of the Lycaenidae.

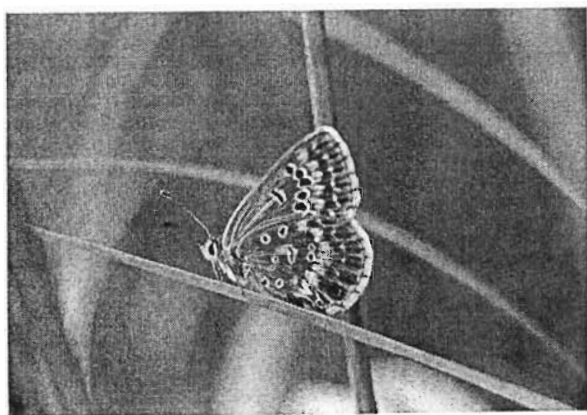


Figure 6. A resting adult butterfly.



Figure 7. A basking male butterfly.

This species flies only on warm and sunny days. During rainy or overcast days, individuals rest and are difficult to find. Cloudy days with occasional sunshine offer the greatest chance of observing basking behaviour. Observations here showed that the basking behaviour lasted for several hours, until the sky cleared, and then the butterflies flew off. At the beginning of basking (or when at rest), when it was cloudy, the wings of the butterfly were closed. The wings were slightly or half-opened when the sun appeared. The butterfly also adjusted its position and orientation according to the direction of the sun's ray to receive maximum sunlight. When it became cloudy again the wings were closed. They usually repeated this behaviour several times.

Another type of basking behaviour was also observed. The wings were closed but perpendicular to the direct sunlight, where the underside of one wing was orientated to receive maximum sunlight. After a few minutes, the adults moved around to expose the other underside of the other wing. This was quite different behaviour from when the closed wings were parallel to the sunlight, which appeared to be a response to avoid receiving too much radiant in hot conditions. Under cloudy conditions, the butterfly closed its wings, presumably to avoid losing body heat and to increase camouflage.

Predation by hangingflies (Mecoptera: Bittacidae)(Fig. 8)

On 20 April 1999, at Wahroonga, one individual Karkloof blue butterfly was preyed on by a hangingfly (*Bittacus kimminsi* Tjeter 1956) at 12h50. The butterfly flapped its wings but was unable to escape. The hangingfly attacked the abdomen, paralyzing the butterfly. It then proceeded to suck haemolymph from both the abdomen and head. This activity lasted until 13h24, when the butterfly was released (but by now dead and with a distinct depression in the abdomen). The butterfly and hangingfly were both on the nectar plant *H. dura*.



Figure 8. Predation by a hangingfly
Bittacus kimminsi.

Egg stage

Morphology

The egg is 0.7 mm in diameter and 0.35 mm high. The colour of the newly-laid egg is pale green for up to one week, becoming whiter before the larva hatches. Fig. 9 is an SEM micrograph of the egg. The egg of this species resembles that of other species in the genus *Orachrysops* (Clark and Dickson, 1971; Edge and Pringle, 1996). It is discoidal with very fine rib reticulation. The upper surface is slightly concave, the micropyle being sunken. The rib pattern on the upper surface consisted of five or six concentric rings of irregular compartments. The rib pattern around the sides is prominent (Fig. 10).

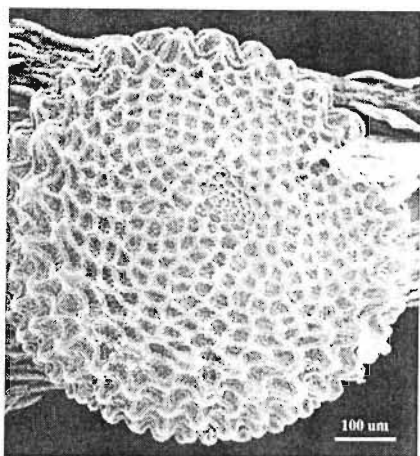


Figure 9. SEM view of an *Orachrysops ariadne* egg.

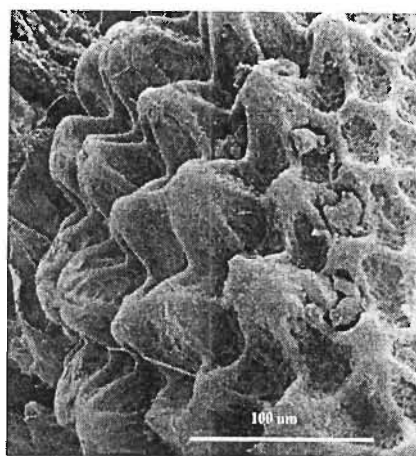


Figure 10. The prominent rib pattern around the egg.

Egg status

Annual egg counts at The Start are given in Table 2. In the first year (1997), some of the marked eggs were lost owing to the dense and rank grassland vegetation. A similar situation occurred at Wahrenonga in 1999. These eggs are classified as 'Unknown' in Table 2. Numbers of egg at The Start remained remarkably constant from year to year. The low figure in 1999 may have been a result of smothering of the host plant by other vegetation. The hatching rates were similar in different years, varying between 42.7% and 55.7% at The Start. Damage levels were also similar, both from year to year (Table 2) and from site to site (Table 3). About 20% of the eggs disappeared without trace at The Start (Table 2). The colony at Nkandla had a higher hatch rate and lower damage rate, compared with the other two colonies (Table 3). The first few days was the time when the newly-laid eggs were most vulnerable to

attacked from predators. No egg parasitoids were recorded.

Table 2. Status of eggs of *Orachrysops ariadne* (number and percentage) from March to May from 1997 to 1999 at The Start.

Year	Hatched	Damaged	Disappeared	Unknown	Total
1997	94(42.7)	53(24.1)	41(18.6)	32(14.6)	220(100)
1998	122(55.7)	49(22.4)	48(21.9)	--	219*(100)
1999	90(55.2)	44(27.0)	29(17.8)	--	163(100)

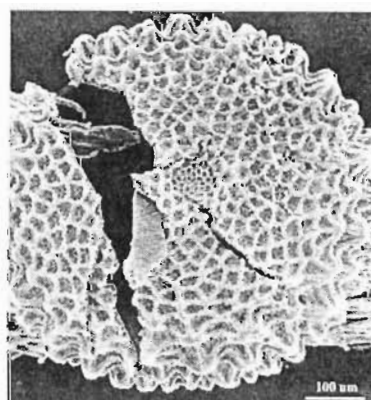
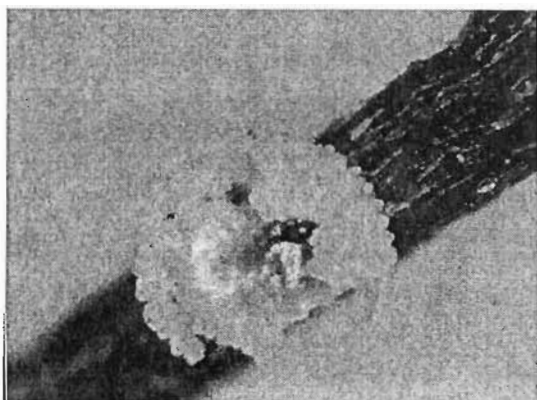
* 30 eggs were collected and taken into the laboratory early in the laying season, and are not included here.

Table 3. Status of eggs of *Orachrysops ariadne* (number and percentage) at three sites in 1999.

Site	Hatched	Damaged	Disappeared	Unknown	Total
The Start	90(55.2)	44(27.0)	29(17.8)	--	163(100)
Wahroonga	170(57.2)	61(20.5)	32(10.8)	34(11.5)	297(100)
Nkandla	137(68.5)	30(15.0)	13(6.5)	20(10)	200(100)

Types of damage

The hatching larva left a round exit hole with a clean edge, clearly different from a hole resulting from damage to the egg. There were various kinds of damage, the most common being a ragged opening with a variable amount of the shell remaining (Fig. 11), sometimes with only a fragment of the shell being left behind. The other common form of damage was an irregular, minute crevice on the surface (Fig. 12). Some of the eggs shrank or had a deep depression in the region of the micropyle. Some had a minute round hole on the side. Some eggs did not hatch and yet had no visible damage. These were possibly unfertilized eggs or had been damaged by other unknown factors. In the case of a few eggs, the larva did not complete the hatching process.



Figures 11 & 12. The two different types of damage.

Egg density

Egg density was measured as number of eggs per plant. The egg densities per host plant are given in Table 4. It was 7.3 ± 9.7 ($n=30$, range 1-38) in 1997, 4.4 ± 6.4 ($n=56$, range 1-35) in 1998 and 2.1 ± 1.7 ($n=78$, range 1-12) in 1999 at The Start (Table 4). Interestingly, the maximum number of eggs per plant in 1997 ($n=38$ eggs) and 1998 ($n=35$ eggs) was in the same individual host plant. But in 1999, the host plants withered, mainly due to overgrowth by other vegetation. As a result only four eggs were observed on that host plant that year. The dispersion of eggs was unequal with respect to host plants. On one experimental patch (2m x 4m) at The Start, five host plants received 81 eggs (32.53%, $n=249$) in 1998 (Fig. 13). In 1999, the egg density was 2.8 ± 3.1 ($n=108$, range 1-16) at Wahroonga, and 13.3 ± 19.1 ($n=15$, range 1-66) at Nkandla (Table 4).



Figure 13. Local distribution of eggs at The Start.

Table 4. Egg densities per host plant (n=number of plants sampled) and hatching period of *Orachrysops ariadne* at three sites.

	Egg density (mean \pm S.D.)	Egg hatching period
The Start (1997)	7.3 \pm 9.7 (n=30, range 1-38)	4 April-19 May
The Start (1998)	4.4 \pm 6.4 (n=56, range 1-35)	21 March-24 May
The Start (1999)	2.1 \pm 1.7 (n=79, range 1-12)	4 April-23 May
Wahroonga (1999)	2.8 \pm 3.1 (n=108, range 1-16)	14 April-28 May
Nkandla (1999)	13.3 \pm 19.1 (n=15, range 1-66)	--

Eggs were mostly laid singly (n=629 eggs) or in pairs (n=94 eggs) on the buds of the host plant, but 27 triplets or more were also occasionally found (Fig. 14). Most of the pairs or clusters of eggs had synchronized hatching time, but for some pairs, hatching time was different. There were also some eggs that were laid at different times, probably by different females, on the same host-plant bud.

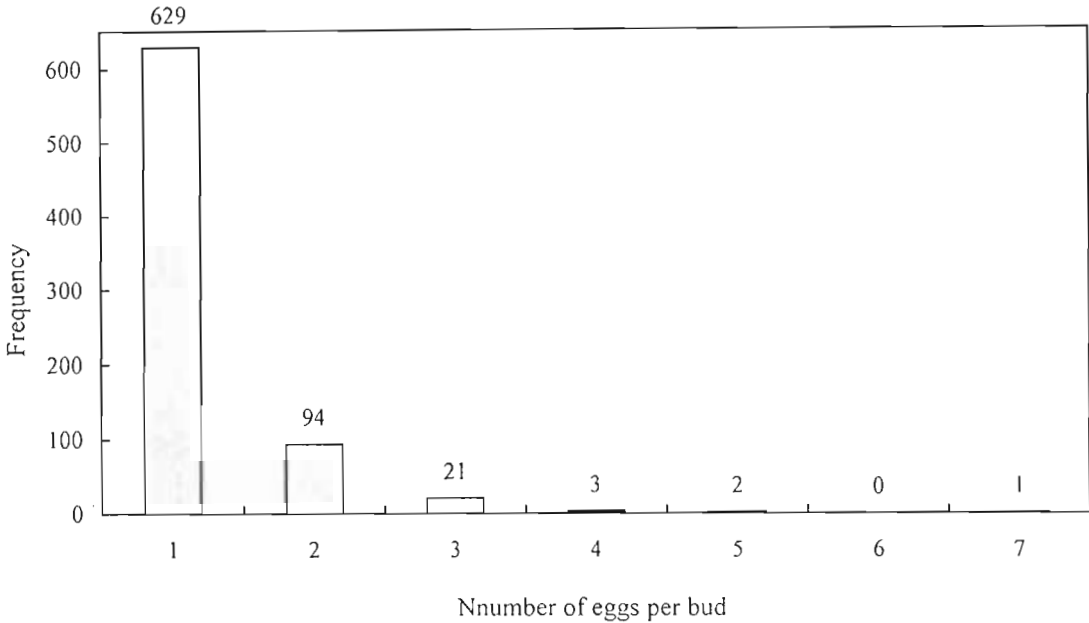


Figure 14. Frequency of number of eggs per bud.

Egg incubation period

The egg hatching period is given in Table 4. Field egg development time ranged from 18 (n=3) to 30 (n=1) days. Hatching time for field-collected, newly-laid or pale green (young) eggs when brought into the laboratory range from 8 to 27 days (Fig. 15). Eggs laid early in the season generally hatched early than those laid later in the season. Of 15 eggs laid between 21 and 24 March 1998, 12 hatched within 8-9 days, two in 13 days and one in 15 days. By contrast, of the 13 eggs laid between 1 April 1998 and 5 May 1998, only five hatched in <10 days, while eight hatched between 11 and 27 days, with the two last eggs taking 23 and 27 days to hatch.

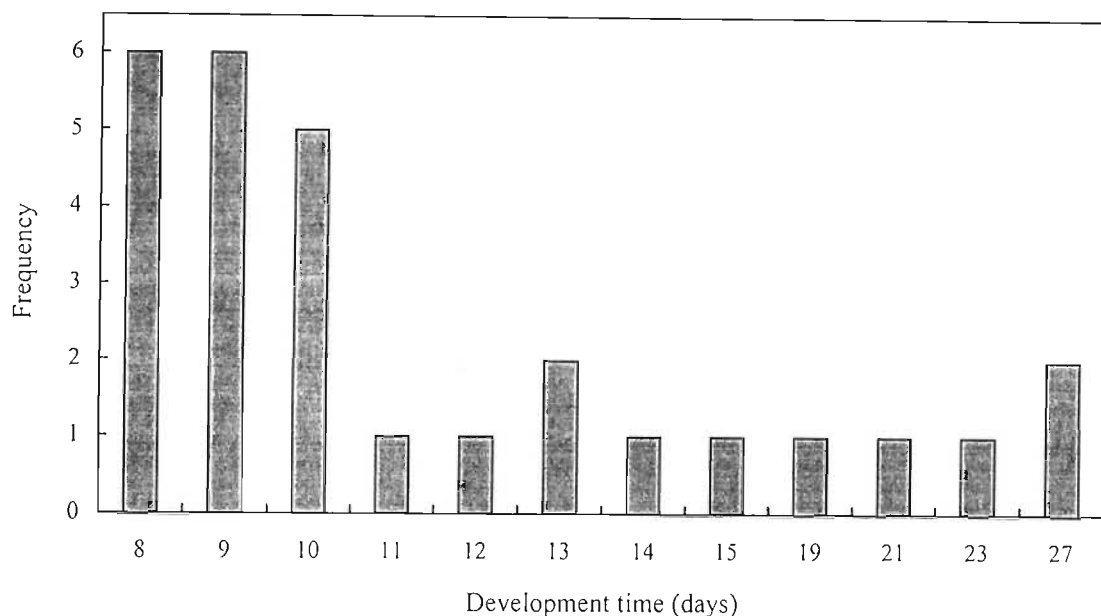


Figure 15. Incubation time of 28 newly-laid or young (pale green) eggs when maintained in the laboratory.

Larval stage

Morphology

The newly-hatched larva was about 1mm long, and was dark green, resembling the colour of the host plant. It has long setae (Fig. 16), which become shorter from the second instar onwards. There is no dorsal nectar organ (DNO) in the first-instar larva. From the second instar, the larva became yellow-green (Fig. 17). The morphology is similar to that of the larva of Brenton Blue *O. niobe* (Edge and Pringle, 1996). A DNO (Fig. 18) and a pair of tubercles are present from the second instar onwards. From this stage, the tubercles were sometimes seen to move in and out as occurs in *O. niobe* (Edge and Pringle, 1996). The DNO at times had a drop of liquid, with 1-2 drops produced in 10 min.

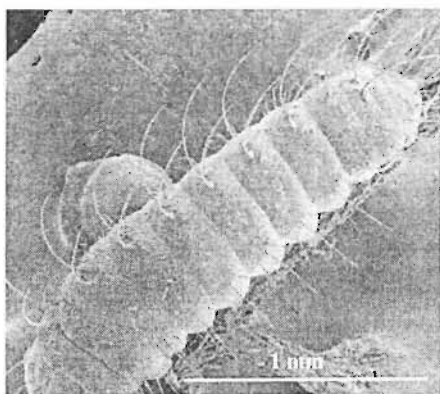


Figure 16. The newly-hatched larva has long setae.

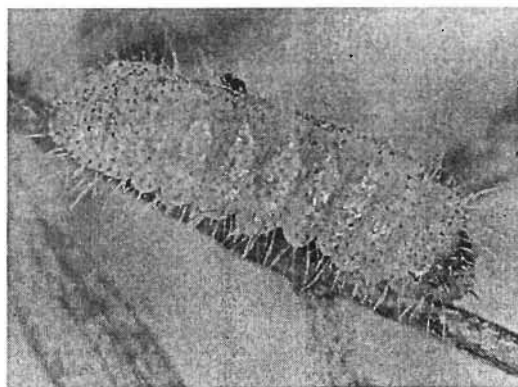


Figure 17. Third instar larva with yellowish green colour.

Behaviour

When larvae hatched in captivity, they fed on young shoots of the host plant. In the beginning, the newly hatched larva fed on the surface of the leaflet but made no hole. After one or two days, the larva fed deeper into the plant tissue and created holes in the leaflet. From the second instar, it also fed on the surface of the stem. The larva ceased feeding for one or two days before moulting.

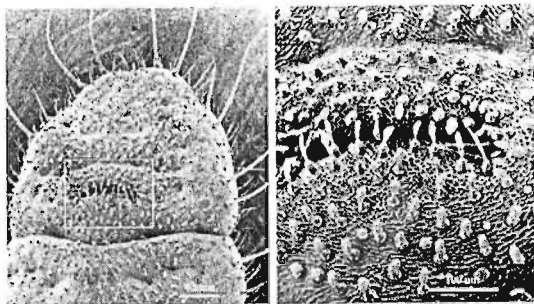


Figure 18. SEM views of the dorsal nectar organ (DNO).

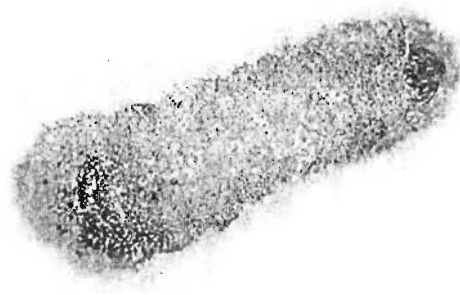


Figure 19. A fungal infection (dark patch) on a larva, especially around the DNO.

Larvae in captivity were able to survive for up to three months, feeding on the host plant shoots, but finally all died from infection with an unknown fungus (one possible fungus was identified of *Cladosporium cladosporioides* (Fresenius) de Vries). The area nearest the DNO was the area most obviously infected (Fig. 19). The infected larva was still able to grow and moult two or three times, and was 5-8 mm long before it died. In the field, most of the larvae disappeared from the host plant after they hatched. To confirm this, night work was also carried out, but no larva were found return to the host plant to feed at night.

Eighteen observations of the larvae emerging from the egg were made. The larvae slowly chewed a hole in a clockwise direction at the top of the egg. After making a small piece of opening in the egg, some of the larvae emerged immediately, but others took one or two hours to do so. The chewed eggshell was then discarded and the central piece with the micropyle was discarded when the larva emerged. The eggshell inside was shiny. A clean-edged, round hole indicated that the larva had hatched successfully. Occasionally, the hole was crescent-shaped where a central piece of the shell remained.

The larvae emerged mostly between 10h10 and 15h30 based on fifteen records from the field and one from captivity. Two captive larvae emerged between 07h00 and 08h00. On hatching in the field, the larvae crawled to a nearby leaflet mid-rib, resting

or basking for one or two hours, sometimes even longer. The larvae periodically fed on the surface of the leaflet and faeces could be seen at the rear end of the larvae. No larvae could be found on the leaflet in the field the next day.

Larval microhabitat

Larvae were extremely difficult to find in the field. In 1997, all efforts made to locate older larva failed. Some larvae from the laboratory were released onto the host plant for further observation. Being so tiny, the larvae were difficult to observe and they soon disappeared and could not be found again. They were followed for one to two hours but they were eventually lost from sight. They were last seen crawling down the stem towards the soil. In response to this behaviour, an attempt was made to dig out the larvae from around the root of the host plant. One species of ant, *Camponotus natalensis* (F. Smith) was often present among the roots of the host plant. One or two soldiers and several workers of this ant were usually in nests or as a group without brood beneath the host plant. It was very difficult to dig without damaging the host plant. Nevertheless, the bases of host plants with eggs of *O. ariadne* were very carefully dug, and the ant *C. natalensis* was usually found when disrupting the soil surface. Another ant, *C. irredux* Forel was also found active on the aerial parts of the host plant, where *C. natalensis* was not active. *C. irredux*, however, did not nest beneath the host plant.

On 8 June 1998 at The Start, one host plant (#1) with 24 eggs, 14 of which had successfully hatched, was dug up. One *C. natalensis* soldier and two workers were found. A larva of *O. ariadne* (about 7mm long) was also present in the chamber, about 10 cm below the ground. A *C. natalensis* worker picked up the larva, and transported it into a deeper chamber. Further digging revealed no ant brood and there were only three workers. Concern that damage might be done to the host plant and that the disturbed ants may also cause damage to the larva, led to only a few attempts being made to further dig up this host plant on following date. The hole was carefully covered by the excavated soil and leaf litters after each digging.

Host plant #1 was dug up again on 1 July 1998 and 6 October 1998, each time only two or three ants were found. On 7 November 1998, one larva (about 13mm long) was found. On digging up the nest, there were 12 ant individuals, including two soldiers. On 6 December 1998 and 18 January 1999 further digging at the base of the

same host plant revealed only about 10 ants, but no larvae. On 28 February 1999, one larva (about 14mm in length) was found under the soil (1 cm below the ground), resting on the surface of the root (Fig. 20). There were about five *C. natalensis* workers around this larva and one or two workers stroked it with their antennae. This was the last time that this larva was found, and no pupa was subsequently located.



Figure 20. Larva at the base of stem associated with host ant *Camponotus natalensis*.

Further searches in 1999 revealed five larvae at Wahroonga, as well as two at The Start, and one at Nkandla. All these larvae were found beneath the host plants, about 3-5 cm below the soil surface, and were all associated with the ant *C. natalensis*. The larvae were covered after the disturbance, but most of them could not be found again. The details are as follow.

On 8 December 1999, one larva (about 15mm long) and about ten ants were found at The Start. The larva was 5cm below the soil surface. Another larva (about 14mm long) was found at the same depth under another host plant. The posterior end of this second larva was buried by the soil that had been dug up, and the ants attempted to pull the larva out of the soil. Finally, the host ant removed the soil from the body of the larva, and then pulled the larva free. The following day, neither larvae could be found.

At Wahroonga, on 21 November 1999, one larva (about 9mm long) and three *C. natalensis* workers were found. On 5 December 1999, at the same site, one larva (about 15mm long) was attached in some unknown way, head-down, to the root of the

host plant, about 5cm below the surface. The host ants touched the larva with their antennae, and placed their mandibles against the body in the region of the DNO. On 9 December 1999, one larva (about 14mm long) was on the main root and was attached head-down to the root of the host plant. On 23 December 1999, two larvae (about 15mm long) were 3-5cm below the surface. One of them was in association with the same individual host plant on 21 November as mentioned above. At Nkandla, on 16 December 1999, one larva (about 15mm long) and two *C. natalensis* workers were found 5cm below the surface. In January 1999, two more, unsuccessful attempts were made to find the larva.

Regardless of the number of eggs found on individual host plants, only one larva was ever found on the root of the host plant. There was never any ant brood associated with the larva, although it was always attended by *C. natalensis* workers and sometimes soldiers.

Pupal stage

In March 1998 and 1999, just before the adult butterfly flight period, pupae were searched for on and under the host plants where the larvae had previously been found, as well as on nearby host plants. On 11 March 1999, two pupae were found in association with two different host plants, after a total of thirty host plants were thoroughly searched. One pupa was adjacent to the root of the host plant, about 2cm below the ground. No ants were associated with this pupa. This pupa colour was dark



Figure 21. The pupa of *Orachrysops ariadne*.

brown (Fig. 21) and it was 13mm in length. Another pupa was just beneath the base of stem. The pupa was creamy yellow and was 15mm in length. On being disturbed, the pupa was quickly picked up by the host ant *C. natalensis* and taken to a deeper chamber.

Myrmecophilous behaviour

On 27 April 1999 at The Start, a newly-hatched larva crawled down to the base of

one stem of the host plant. The host ant antennated the larva there. Due to the fact that the larva was so tiny and the ground cover so dense, it was not possible to observe further details of the interaction. The host ant was nesting beneath the plant.

One larva (about 14mm long) and two ants (one worker and one soldier) were collected from Wahroonga on 9 December 1999. Interactions were observed under a dissecting microscope, where the larval tubercles were seen to come out and be withdrawn very briefly. Both ants showed much interest in the larva, although sugar-water was also available. They crawled over the larva and antennated its surface, particularly the area nearest the DNO. There were drops secreted from the DNO and these were immediately imbibed by the ants (Fig. 22). After the honey from the larva was obtained, both ants underwent a process of self-grooming and regurgitation. Trophallaxis (liquid food exchange) was observed between the two ants. The ants seemed not to be satisfied and repeatedly solicited liquid food. The pair of tubercles was extruded and withdrew independently, and the ants either investigated or attacked the emerging tubercles. The larva grasped the surface of the stem and occasionally fed on the stem surface, but the larva demonstrated no interest in the foliage. The larva did not appear to receive any direct advantage from the ants during this observation.

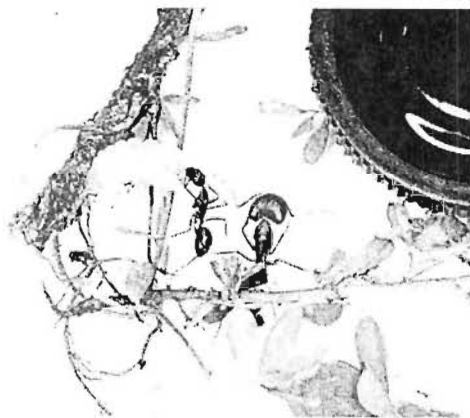


Figure 22. The larva and associated host ant, note the ant sucking the honeydew from the larva.

2.6 Discussion

Host plant and nectar plants

The distribution of both the host plant and the nectar plants may be the two severest limiting factors for the distribution of this butterfly (Murphy *et al.*, 1984; Schultz and Dlugosch, 1999), bearing in mind that the host ant is widely-distributed in South Africa (from western Cape to the eastern seaboard). The erect host plant *I. woodii* var. *laxa* is clearly different in form from the prostrate *I. woodii* var. *woodii*, and is characteristically up to 1 m in height. The variety *woodii* is typically only 20 cm in height. The variety *laxa* has a larger flower (4.0-4.5mm long) than the variety

woodii (3.0-3.5mm long) (Schrire, pers. comm). *O. ariadne* only oviposits on variety *laxa*.

Interestingly, the variety *woodii* is the host plant of the closely-related species *O. subravus* (G. A. Henning and S. F. Henning) (personal observation). *O. subravus* is a more abundant and widespread species, and its host plant, variety *woodii*, also has a wider distribution. Although the distribution of the host plant could potentially be used to find new colonies of *O. ariadne*, variety *laxa* appears to be very rare, and no further colonies of either this plant variety (except one more site, which had no *O. ariadne*) or *O. ariadne* were found (other than of the four sites here), despite intensive searches.

In the adult stage, nectar sources may play an important role in the survival and reproduction of this species. Eight species of nectar source were recorded for the four colonies. Each colony was associated with a different taxonomic range of nectar plants, with the butterflies opportunistically using whatever nectar plants were available (Table 1). Flight paths along ridge lines were characteristically near to, or in association with, nectar sources.

Egg stage

Clark and Dickson (1956, 1971) suggested using the egg and first-larval instar for the classification of South African Lycaenidae. Development of the SEM has greatly enhanced the effectiveness of this approach. Indeed, it has been shown here that the egg ribs are prominent and the pattern of this species distinct from that of *O. lacrimosa* and *O. niobe* (Clark and Dickson, 1971; Edge and Pringle, 1996) (Fig. 10).

Owing to different host plant densities and inaccessible topography, it was not possible to directly compare population levels at each of the four sites (in 1999). In the Palaearctic Region, annual egg-count monitoring has proved important for assessing population levels of *Maculinea rebeli* (Dolek *et al.*, 1998). In this study on *O. ariadne* it was possible to undertake comparative annual counts at one site, The Start. Eggshell remains were found to be particularly valuable for assessing egg status (Lu and Samways, 1999). Eggshell remains have some benefits for assessing the presence and population level of this species. This is a more practical alternative to assessing adults, which have a short flight period and rapid elusive flight behaviour over dense vegetation, making them difficult to record. Also, the eggs are good

surrogates and have great potential for plotting the local distribution of the breeding sites (Fig. 13), as well as for locating new colonies.

Different types of egg damage probably reflect different types of predators or parasitoids (Spoor and Nickles, 1994). None of these were found, which suggests that damage possible either took place rapidly and/or at night. The hatch rate and damage rate were similar, but Nkandla had a higher hatch rate and a lower damage rate (Table 3). The stable damage rates may play a role in population regulation. This includes the substantial figure of 20% of eggs that disappeared with no trace.

Fresh eggs were most frequently attacked by predators within the first few days, which suggests there may be strong selection to increase protection of these eggs. This may be one reason why they are strongly ribbed. The droplets of morning dew also present an obstacle for small predators, which would have to overcome the surface tension effects. Protecting the newly laid eggs would also increase larval survival. This has important implications for conservation and protection of the species. Egg collection should be done early on and the newly-hatched larvae released back to their colonies or perhaps, after due consideration, translocated to potential new sites.

Most of the eggs were positioned near the host plant buds or new shoots, with over 75% being placed on the underside of the leaf. Although insect eggs are sometimes laid on the underside to avoid direct exposure to sun or predators (Hinton, 1981), the suitable physical structure of the undersides of the host plant for egg attachment may also be important. The surface of the underside is more densely spined than the upper side, making attachment easier. Also, the underside of the leaflet may be easier to receive the egg as the female curves her abdomen.

Eggs were laid mainly singly or in pairs. The older host plants had more buds and were therefore capable of receiving more eggs. Overcrowding of the eggs on the host plant may have a detrimental effect on larval development and survival. For example, the larvae of orange-tip butterfly *Anthocharis cardamines* can be cannibalistic (Dempster, 1997) although this does not appear to be the case with *O. ariadne*.

Most of the pairs or clusters of eggs had synchronized hatching times, indicating that they were likely to have been laid at the same time by the same female. However, for some pairs of eggs hatching times were different, suggesting that there was repeated egg-laying. The Nkandla site had a high egg density, up to a mean of 13.3 per host plant. This was considerably higher than at the other sites (Table 4). Of

conservation concern is that there appeared to be insufficient suitable host plants at each site for the full egg-laying potential to be reached.

Eggs laid early in the oviposition season, when conditions were warmer, had a shorter development time. Extrapolating from time of the first-hatched egg, suggested that the first adult appeared earlier than the record suggested. Similarly, the last adult probably disappeared later than actually recorded. The important point is thus that all the eggs hatched before the end of May, which is before severe dews, frosts and prior to the winter fires. This had management implications for the timing of the fire regime (see details in Chap. 5).

Larval stage

Larvae mostly emerged between 10h10 and 15h30. In the field, eggs were frequently exposed to dew in the early morning and this dew sometimes persisted until noon in a wet, shady site. Dew frequently forms a surface tension trap that damages small insect (Samways, 1989). This is highly likely to be the case for newly-hatched *O. ariadne* larvae which are only 1.0 mm long. Indeed, the larvae did not appear when there was dew, and only emerged once it had evaporated.

The larva disappeared from view after hatching, and it was assumed that they were carried or made their way into an ant's nest, as in the closely related genus *Lepidochrysops* (Henning and Henning, 1994). There are a variety of reasons for the larva being in the ground with the host ant. Some of the benefits of the association include avoiding parasitoids and catastrophic events, and having a safe shelter as well as stable food resources (Claassens and Dickson, 1980; DeVries, 1991; Eastwood and Fraser, 1999; Fiedler, 1996; Fiedler *et al.*, 1992; Fiedler *et al.*, 1996; Pierce, 1985; Pierce and Mead, 1981). Results from this study indicate that most of the larvae hatched before the end of May, just before the first frost. In the study area, this period is followed by the fire season from May to November. This means that most of the larvae can escape damage from frost and fire when underground.

Ant nests had to be carefully excavated to avoid damaging host plants and larvae. Although fewer than a dozen larvae were found in association with ants in the field, it was confirmed that the host ant is *Camponotus natalensis*. The ant was associated with both the larvae and the pupae. The larvae were found beneath the host plant, a few centimeters below the surface. It is not known if the host ant carries the larvae up

to the surface on warm, sunny days as in the case *Lepidochrysops* species (Claassens, 1976) or if they surfaced by themselves.

After excavating an ant nest, the larvae usually disappeared although the host ant was still present. This made it difficult to continue monitoring larvae in the field. No ant brood was found together with any of the larvae. Some visible host ant mounds with abundant ant brood, were excavated, but no larva of *O. ariadne* were found. It is still not known what the food source is for the larva that reside underground with the host ant. Further work needs to be done on the host ant in the future to investigate their biology. This needs to be done very carefully indeed so as to avoid harming this threatened species.

A variety of lycaenid feeding habits are associated with ants. Some larvae are entirely herbivorous but need the presence of ants to complete their life cycle. Others are predaceous, feeding on honeydew-producing Homoptera or ants, or feeding only on secretions from ants or Homoptera, or from both (Henning, 1983). The *Orachrysops* species were originally thought to be phytophagous in some instars and predacious in others. *Lepidochrysops* spp., for example, feed on the flower-heads of the host plant in the first two instars. On entering the ant nest, the young larvae feed on the silken materials of cocoons, and the older larvae feed on ant larvae and pupae (Clark and Dickson, 1971; Claassens, 1972, 1976; Henning, 1983).

Work done on *O. ariadne* and *O. subravus* (personal observation) or finding of other researchers on *O. lacrimosa* (Clark and Dickson, 1971), *O. mijburghi* (Williams, 1996) and *O. brinkmani* (Heath, 1997) has shown that the young larva feeds on the leaves of the host plant *Indigofera* when in captivity. Interestingly, the larvae of *O. subravus* was found to become cannibalistic when more than two larvae were placed together in captivity (personal observation). Field observation on the same species showed that the first two larval instars feed on the host plant (personal observation). *O. ariadne* differs from *O. subravus* in that the first-instar larvae disappeared from foliage of the host plant after they had hatched in the field.

In captivity, the larvae of *O. ariadne* were able to feed on new shoots of the host plant for up to three months, but they finally died following a fungal infection. This situation is common and has been encountered by other researchers attempting to rear the larvae of *Orachrysops* species (Edge and Pringle, 1996; Williams, 1996) as other species also (Fiedler and Saam, 1995). Even in *O. niobe*, which has been successfully

reared in captivity, and is phytophagous in all instars, fungal infection was the main factor for high larval mortality in the laboratory (Edge and Pringle, 1996). Another consideration, is that the long (over 10 months) larval development stage of *O. ariadne* makes it more difficult to rear. The infection could be reduced using distilled water to wash the larva, as was done when breeding *Maculinea* species (Wardlaw *et al.*, 1998).

The larva of *O. ariadne* has tubercles and a DNO from the second instar onwards. This is morphological evidence of a relationship with ants. Indeed, observation here showed that *C. natalensis* imbibes honeydew from the larva. In captivity, the region of the DNO was the area that was most badly infected by fungus. This phenomenon might indicate that the host ant has the function of tending the larva and plays a cleaning role by removing the honeydew or fungal spores, which would otherwise be a site for of fungal spores germination (Samways, 1983). Such “hygienic effect” of was not always effective. Indeed, the presence of a DNO on the larval body offers an additional opportunity for pathogenic fungi to attack lycaenid caterpillars (Fiedler and Saam, 1995).

Reared adults of *O. niobe* showed dwarfism, with wingspans of 18-20 mm, compared with to the full size of 26-30 mm (Edge and Pringle, 1996). Such dwarfs of *O. niobe* were observed both in the field as well as in captivity. This can possibly be explained by the fact that the second brood of larva could not access the flowers of the host plant late in the season (Edge and Pringle, 1996). From the observations here on *O. ariadne*, there was no evidence that the larvae had particular floral requirements. In captivity, the larvae of *O. ariadne* and *O. subravus* (personal observation) only fed on the leaves and young shoots of the host plant. Indeed, *O. ariadne* oviposited on the leaves of the host plant, in contrast to *Lepidochrysops* spp. where oviposition occurs on the flowers. The flowers of the host plant do not appear to be an essential component of larval food for the *Orachrysops* species. Rather, the relationship with the host ant seems to plays a more important role in larval development.

With our limited knowledge of larval feeding in *Orachrysops* spp., we need perhaps to look to the much better-known *Maculinea* spp., to postulate the possible feeding habits. There are two known larval feeding strategies for *Maculinea* spp.. *Maculinea arion* is an obligate predator on an ant brood, whereas *M. rebeli* and *M. alcon* mimic ant larvae and feed directly from the worker ants (Thomas and Elmes, 1998, and references therein).

The *M. arion* final instar larvae gain more than 99% of their ultimate biomass in the host ant nest (*Myrmica sabuleti*) (Thomas and Wardlaw, 1992). They feed on the largest ant larvae available. When the *Myrmica sabuleti* nest adopted more than two larvae of *M. arion*, mortality of *M. arion* larva is high as a result of scramble competition and starvation (Thomas and Wardlaw, 1992). By contrast, *M. rebeli* and *M. alcon* exploit *Myrmica* in a more efficient way, through trophallaxis fed by nurse ants (Thomas and Elmes, 1998). These species increase their chances of survival when the host nests are overcrowded, because nurse ants support as many butterfly larvae as they can feed. Many (4.7-fold) more larvae of *M. rebeli* and *M. alcon* are supported per ant colony than of *M. arion* (Thomas and Elmes, 1998). The largest number of final-instar larvae in South African lycaenids ever recorded in one ant nest is seven, for *Lepidochrysops methymna* and *L. trimeni* (Claassens, 1976). In the case of *O. ariadne*, no matter how many eggs are laid on the host plant, only one larva or pupa was associated with the host ant's nest at the base of the plant.

Although this study confirms that the host ant for *O. ariadne* is *C. natalensis*, this is only the first step towards elucidating the complex relationship between the larva and the host ant. It is extremely difficult to find larvae in the field, making this a major task. The impact that the carrying capacity of the host ant nest has on larval survival is not known. Once we know the feeding habits of *O. ariadne*, and the capacity of the host ant nests to support *O. ariadne* larvae, it may be possible to increase larval survival rate by collecting the newly laid eggs, as mentioned previously.

Fiedler (1998) conducted comparative work, tracing the *Maculinea*-ant interaction. He mentioned an unexplained parallelism in polyommata genera from Africa. One is the 'free-living mutualistic' genus *Orachrysops*, in which all species feed on Fabaceae. The other is the 'parasitic' genus *Lepidochrysops*, in which all members feed on inflorescences, mostly of Lamiaceae, but also some species of Verbenaceae, Scrophulariaceae, and Geraniaceae, in their early larval instars. This suggests that we should compare these two genera in southern Africa. Indeed, understanding the evolutionary patterns and processes which underlie the transition from mutualism to parasitism would be of general biological interest (Fiedler, 1998). The extinction of every species is the deletion of another data point as the source of potential information (Clemmons and Buchholz, 1997), and conservation should thus be a priority for the threatened *O. ariadne*.

Chapter 3. Behavioural ecology of the Karkloof blue butterfly *Orachrysops ariadne* relevant to its conservation

3.1 Abstract

The Karkloof blue butterfly, *Orachrysops ariadne*, is endemic to the Mistbelt grassland of KwaZulu-Natal province, South Africa, and is currently Red-listed as 'Vulnerable'. This study compares the ecological conditions at the four known colonies to assist in making informed decisions regarding its conservation. All the four sites lie within the Mistbelt grassland on a south-facing slope. The host plant *I. woodii* H. Bol. var. *laxa* and host ant *C. natalensis* were present at all four sites. This study has elucidated aspects of the ant's behavioural ecology that have a bearing on the conservation of the butterfly. A large proportion of the grassland in KwaZulu-Natal has been aforested and cultivated, and at least 92% of the Mistbelt has been transformed, with only about 1% in good condition remaining. Predictions on the habitat requirements of this species are necessary for developing a conservation strategy and action plan. Here, we also propose *O. ariadne* as an indicator species for quality Mistbelt grassland. Saving enough of the remaining Mistbelt grassland is crucial, not just for the survival of *O. ariadne*, but also for the Mistbelt grassland community as a whole.

3.2 Introduction

The Karkloof blue butterfly, *Orachrysops ariadne* (Butler) is a threatened and rare species. It is endemic to KwaZulu-Natal province, South Africa, and is currently Red-listed as 'Vulnerable' (Henning and Henning, 1995). *Orachrysops ariadne* has always been rare (Swanepoel, 1953; Pringle *et al.* 1994) and is known from only four sites, despite intensive searches over the past 100 years. It is not clear what the specific habitat requirements are that may make this butterfly so rare. The present contribution aims to compare these four sites in an attempt to elucidate aspects of the behaviour and ecology of the species that may help in making informed decisions regarding its conservation.

This study follows an earlier one that described the butterfly's lifecycle (Chap. 2). The aim here is to examine its oviposition behaviour, availability of nectar resources, and the foraging behaviour of the host ant. In the previous study (Chap. 2), it was clear that oviposition sites were a limiting factor. But for meaningful conservation planning, it is important to ask whether there are other limitations faced by the butterfly that increase risks to its long-term survival.

3.3 Study sites

The current distribution of *O. ariadne* is within the Moist Midlands Mistbelt in KwaZulu-Natal (Camp, 1999). There are four known sites: The Start (29°24'S, 30°17'E, elevation \pm 1080m), Wahroonga (29°36'S, 30°07'E, elevation 1320-1440m), Stirling (29°35'S, 30°08'E, elevation \pm 1460m) and Nkandla (28° 42'S, 31°08'E, elevation 1100-1200m). The colony at Nkandla is in Ngongoni Veld (Acocks, 1988), and is 100 km from the other three sites, which are closely clustered.

The Mistbelt area has a temperate climate, receiving most rain in summer. This has made it suitable for large-scale afforestation (Armstrong *et al.*, 1998) and intensive farming (Acocks, 1988). Mists are common and provide additional moisture, and thunderstorms are frequent in summer and autumn. Climatic hazards include occasional, short-duration droughts, hail, frosts and excessive cloudiness (Camp, 1999). Hot, north-westerly winds, followed by sudden low temperature or cold fronts, make for unpredictable weather conditions, especially in spring and early summer (Camp, 1999).

Average rainfall (over 27 years) and average temperature (over 24 years) at Evendon Estate, 8 km from the best-known *O. ariadne* site, The Start, is illustrated in Fig. 23. Average rainfall (over 19 years) and average temperature (over 13 years, only minimum temperature records) at Wahroonga are given in Fig. 24. The third site Stirling, adjoins the Wahroonga site. The average rainfall varies from 700 to 1300 mm per annum, and average temperature is 13°C in July and 21°C in January. The first frosts usually come in late May.

Soil characteristics necessary for the butterfly, host plant, or ant community are not known. However, the soil's clay percentage ranged from 37 to 64%, with a pH between 3.83 and 4.82 at the four known sites. The soils differed markedly in the acid saturation, with the three southern sites having low, and Nkandla high, acid saturation. There were

differences in soil acid saturation between the south-facing slope (with a butterfly colony) and the north-facing slope (without a colony) at The Start. The south-facing slope, without the host plant, had a higher acid saturation than The Start site (Table 5).

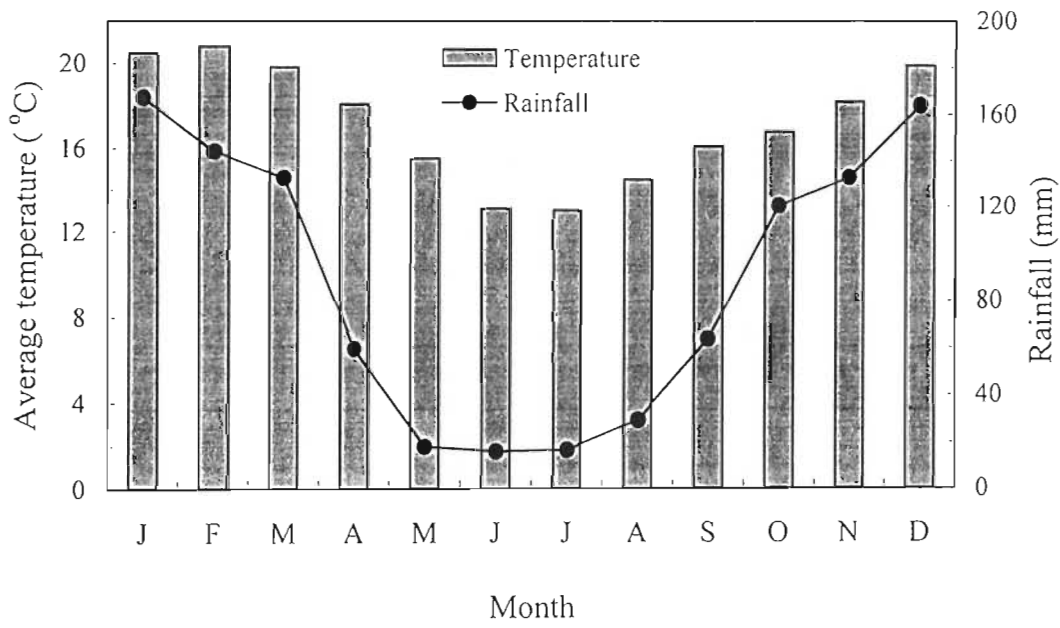


Figure 23. Average rainfall (over 27 years) and temperature (over 24 years) from Evendon Estate, eight km from The Start.

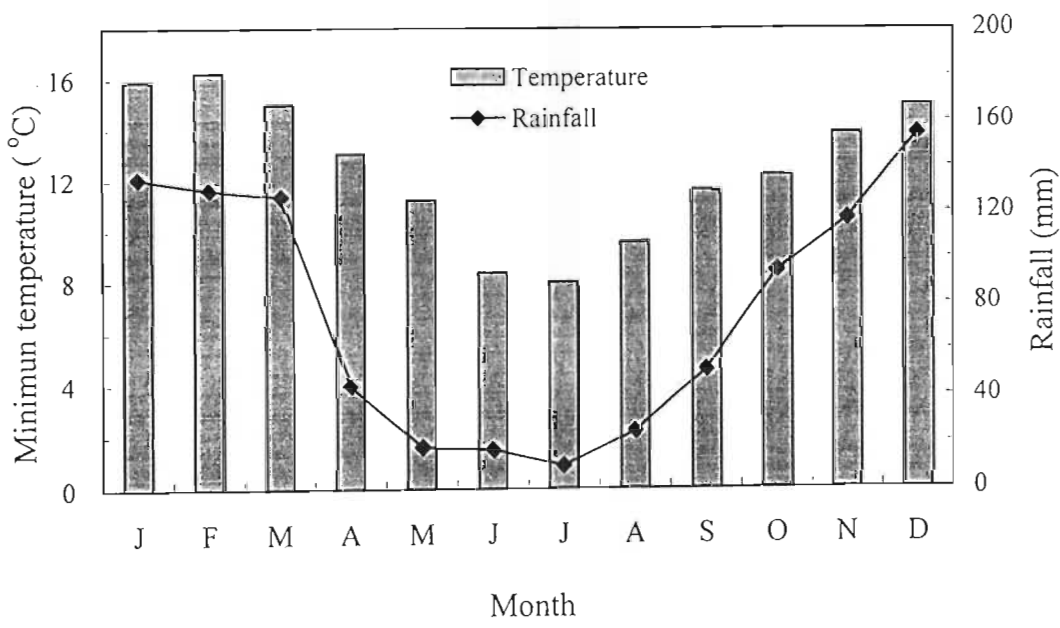


Figure 24. Average rainfall (over 19 years) and average temperature (over 13 years, only minimum temperature records) at Wahroonga.

Table 5. Soil acid saturation and pH at the four colonies of *Orachrysops ariadne*.

Sample sites (n=3)	Acid saturation (%)		pH
	Mean± 1 S.D.	Range	Mean± 1 S.D.
The Start, south-facing with host plant	9.31±2.88	7.17-12.59	4.28±0.04
Wahroonga, south-facing with host plant	0.97±0.5	0.48-1.48	4.62±0.08
Stirling, south-facing with host plant	0.24±0.06	0.18-0.29	4.73±0.09
Nkandla, south-facing with host plant	29.36±10.67	18.03-39.21	4.03±0.21
The Start, north-facing without host plant	25.93±18.24	10.56-46.09	4.17±0.15
The Start, south-facing without host plant	29.76±18.65	8.54-43.55	4.25±0.09
Wahroonga, without host plant	3.82±2.36	1.71-6.37	4.57±0.06

3.4 Materials and methods

Habitat comparisons

The four known sites, The Start, Wahroonga, Stirling and Nkandla, were compared in terms of aspect, elevation, annual rainfall and soil properties. The presence of the host plant *I. woodii* H. Bol. var. *laxa* (Fabaceae), host ant *Camponotus natalensis* (F. Smith) and nectar plants were also recorded. The phenology of the host plant was observed at The Start from 1997 to 1999 and at Wahroonga in 1999. The host plant variety *laxa* is upright, whereas variety *I. woodii* H. Bol. var. *woodii* is prostrate.

The position of eggs on the host plant were measured from March to May 1998-1999 at The Start. At Wahroonga and Nkandla, egg measurements were only made in 1999. The position of each egg was marked using plastic electrical tape, with a serial number, wrapped around the twig. This avoided duplicate sampling and enabled the taking of individual records. Two distances for each egg position were measured: one from the ground to the egg, and the other from the tip of each supporting bud to the egg. T-tests were used to compare the data in 1998 and 1999 at The Start, while ANOVA was used to compare the three study sites in 1999.

Flower visitations by adult were observed throughout the flight season between March and April at The Start, from 1997 to 1999, and at Wahroonga in 1998 and 1999. Additional observations were made at Stirling and at Nkandla in 1999 only, as the colonies were only discovered that year. Species of nectar plants and the frequency of butterfly utilization of each nectar plants were recorded.

Biological observations of the host ant

Nesting and foraging behaviour of the host ant *C. natalensis* was recorded at 20 exposed ant mounds after the prescribed fire at The Start in 1998. Seasonal activity of the host ant was assessed using pitfall traps in April, May, July, October 1998 and January 1999 at The Start. Glass test tubes (25 x 150 mm) containing a mixture of 70% ethyl alcohol and glycerol (3:1) to a depth of *ca.* 20 mm served as individual pitfall traps (Majer, 1978). Four test tubes in a 1m x 1m configuration were defined as one sampling unit. A total of 12 of these sampling units was placed along a line transect, each separated by 20-40 m intervals adjacent to the host plants, for five days.

Additional observations were made using sugar-water baits as an attractant (Fig. 25), and without harming the ants at weekly intervals, i.e. on 1, 8, 15, 22 and 29 May 1999. Four sugar-bait samples, each representing a sampling unit, were placed near the host ant nest entrance. Each sampling unit was represented by three shallow containers (two 3.5 cm and one 9.0 cm in diameter) spaced by 50 cm apart. Each sampling unit was observed for 15 min each hour at 17h00, 18h00 and 19h00 and the maximum number of ants recorded. The containers were refilled with sugar-water after each observation period. The number of ants attracted by all four sampling units were pooled.



Figure 25. Sugar-water baits being used to attract the host ant.

Daily ant activity was recorded from 15h00 overnight to 09h00 on 1-2 May using sugar-water baits. The baits were set up one hour prior to observation. There were four sampling units, each represented by three shallow containers as described above. Each sampling unit was observed for 15 min, each hour, from 15h00 to 09h00 and the maximum number of ants recorded. The containers were refilled with sugar-water after each observation period. The mean of four sampling units was used for analysis.

3.5 Results

Habitat comparison

The vegetation at The Start consists of dense grassland on a south-facing hillside near a small patch of indigenous forest (Swanepoel, 1953). The other three sites are also south-facing slopes, although slightly from south- to southwest-facing at the two sites Wahroonga and Nkandla. At three of the sites dense, long grass and forbs, 1-2 m high, dominate, while the Nkandla site has shorter vegetation only 50 to 80 cm high. The host plant *I. woodii* H. Bol. var. *laxa* and host ant *C. natalensis* were present at all four sites.

Host plant

The host plant *I. woodii* H. Bol. var. *laxa* (Fig. 26) is an upright variety, that grows to 1 m tall, unlike the prostrate variety *I. woodii* H. Bol. var. *woodii* (Fig. 27) which is normally only 20 cm high (see detail in Chap. 2). The variety *laxa* inhabits rank



Figure 26. The host plant *I. woodii* H. Bol. var. *laxa*.

The prostrate variety *woodii* occurs throughout KwaZulu-Natal, although it is concentrated in the southern part of the province in the Midlands (Manning, 1982). Despite intensive searches for the variety *laxa*, it was found only at the extant butterfly colonies. The low apparency of the variety *woodii* was emphasized by the herbarium

grassland on south-facing to southwest-facing slopes, often adjacent to natural forest. The prostrate variety *woodii* inhabits rocky grassland with exposed boulders. The prostrate *woodii* was present at The Start and Wahroonga, but did not overlap spatially with variety *laxa*.



Figure 27. The prostrate variety *I. woodii* H. Bol. var. *woodii*, which is not utilized by *Orachrysops ariadne*.

collection at the University of Natal and KwaZulu-Natal Wildlife, which lacked the variety *laxa*, with the exception of one of specimen collected by J. Manning at The Start in 1982 (voucher specimen in the herbarium of the University of Natal).

The host plant *I. woodii* var. *laxa* is an indigenous, perennial legume. The phenology of the host plant and the butterfly are given in Table 6. The host plant may flush all year, although it partially wilts during the dry months of June and July. During anthropogenic burning of grassland between July and October (see details in Chap. 5), above-ground foliage and stems of the host plant die. The new stem flushes from the old host plant rootstock and grows vigorously after the burn, especially with the spring rains. Flowering is from December to April, but the peak is from January to March. Seed pod production occurs from March to May, followed by dehiscence during May and June. Seedlings appear from September to December, depending on the occurrence of fire (see details in Chap. 5).

Table 6. Annual life cycle of the Karkloof blue butterfly *Orachrysops ariadne* and the host plant *Indigofera woodii* var. *laxa*.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Host plant
Butterfly													
	+++++	+++++	+++++	+++++	+++++	++		+++++	+++++	+++++	+++++	+++++	Flushing
Adults			••••	••••									
	+++++	+++++	+++++	+++							+++	+++++	Flowering
Eggs			•••	•••••	••								
				+++++	+++++	++							Seed production
Larvae	•••••	•••••	••	•••••	•••••	•••••	•••••	•••••	•••••	•••••	•••••	•••••	
					+++++	+++++	++						Dehiscence
Pupae		•	•••••	•									
	+++++								+++	+++++	+++++	+++++	Seedling appearance

Positioning of the eggs

Most of the eggs are positioned on the host plants near the buds or new shoots (Table 7). At The Start, the height above ground of egg was significantly ($t=4.77$, $p<0.0001$) greater in 1998 than in 1999 (Table 7). In 1999, the height of eggs above ground was significant ($t=2.64$, $p<0.001$) lower at Wahroonga than at The Start and significant ($t=16.77$, $p<0.001$) lower still at Nkandla. The heights of eggs from the ground in different years, and different sites, were significantly correlated with the heights of host plant buds ($r=0.988$, $p<0.001$).

Table 7. Height of *Orachrysops ariadne* eggs from the ground, and distance of the egg from the tip of the host plant at three sites (two-year data at The Start).

Sites	Height (cm)	Distance to tip (cm)
The Start(1998)	84.5±17.7 (n=249, range 40-147)	1.1±0.8 (n=249, range 0.1-7)
The Start(1999)	76.8±17.5 (n=159, range 44-125)	0.8±0.6 (n=159, range 0.2-5)
Wahroonga(1999)	73.1±10.7 (n=291, range 44-105)	1.0±1.0 (n=297, range 0.1-7)
Nkandla (1999)	49.1±12.1 (n=200, range 24-78)	1.4±1.9 (n=200, range 0.2-13)

Nectar plants

Eight different nectar sources were recorded at the four sites (see details in Chap. 2). In 1998, of the thirty observations made on nectar plant visitation at The Start and Wahroonga, butterflies utilized *T. polystachya* (n=20) twice as often as *A. phyllicoides* (n=10). *T. polystachya* was visited between 16 and 28 March at The Start (n=10), and from 3 to 29 March at Wahroonga (n=10). Few *T. polystachya* flowers remained at the end of March, resulting in the butterfly switching to *A. phyllicoides* from 13 to 22 April at The Start (n=10), when *A. phyllicoides* began to flower. In 1999, at Wahroonga and Stirling, by contrast, the main nectar plant was *H. dura*.

Biology of the host ant

The host ant *C. natalensis* occurred at the base of the host plant, although entrance holes were not generally visible. When the soil surface was scarified, often there were ant chambers just beneath the host plant. This chamber contained no ant larvae or pupae. From October to January, excavated soil particles were present, forming loose mounds of soil pellets, which indicated the entrance of a nest (Fig. 28). Ant trails were also present between the ant chambers beneath the host plants, other nearby plants and other subterranean nests during this time. Sometimes a round entrance hole, about 10 mm in diameter, was present.

Another sign indicating the presence of the host ant were its mounds. Although there were co-occurring mounds of other ant species, the mounds of the host ant could be determined by the particular soil texture. The mounds were mostly adjacent to boulders or intermingled with grasses and forbs, usually covered by long grass that made them difficult to detect. When fire removed the covering vegetation, the mounds were exposed (Fig. 29), showed that some reached a height of 50cm. Sometimes, nests of the host ant were under stones.



Figure 29. The above-ground mounds of host ant *Camponotus natalensis*.



Figure 28. Excavated soil indicates the presence of the host ant *Camponotus natalensis*.

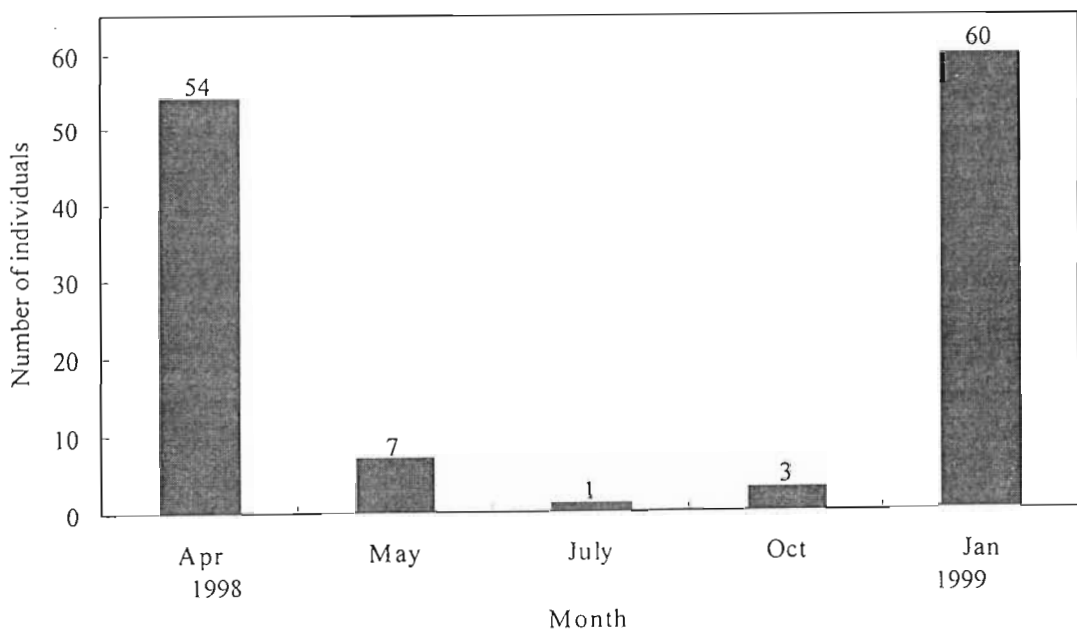


Figure 30. Abundance of the host ant *Camponotus natalensis* sampled using pitfall trapping between April 1998 and January 1999 at The Start.

Seasonal and daily activity

By the end of May, most of the butterfly larvae had hatched, and ant abundance had also dropped (Fig. 30). This was verified by results from the sugar-water baits (Fig. 31). The important point is that the timing of the butterfly larvae hatch at the time when the host ant is most active.

Observations from nest excavation indicated that the host ant *C. natalensis* is very active at night, removing soil from evening until dawn. The ants' daily activity, as record using the sugar-water baits, showed that they were attracted to the baits after 15h00, when recruitment of more foraging individuals took place. However, the maximum number of ants were attracted to the bait at night between 21h00 to 03h00, after which time the number decreased (Fig. 32).

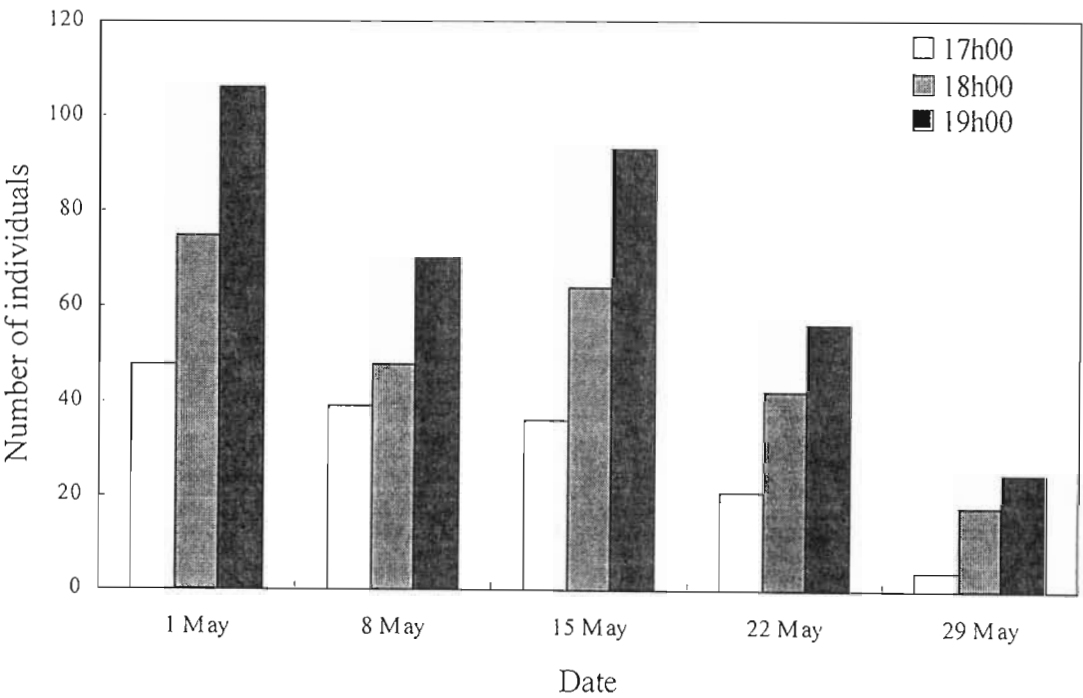


Figure 31. Abundance (mean±1SE) of the host ant *Camponotus natalensis* attracted by sugar-water baits in May 1999 at The Start.

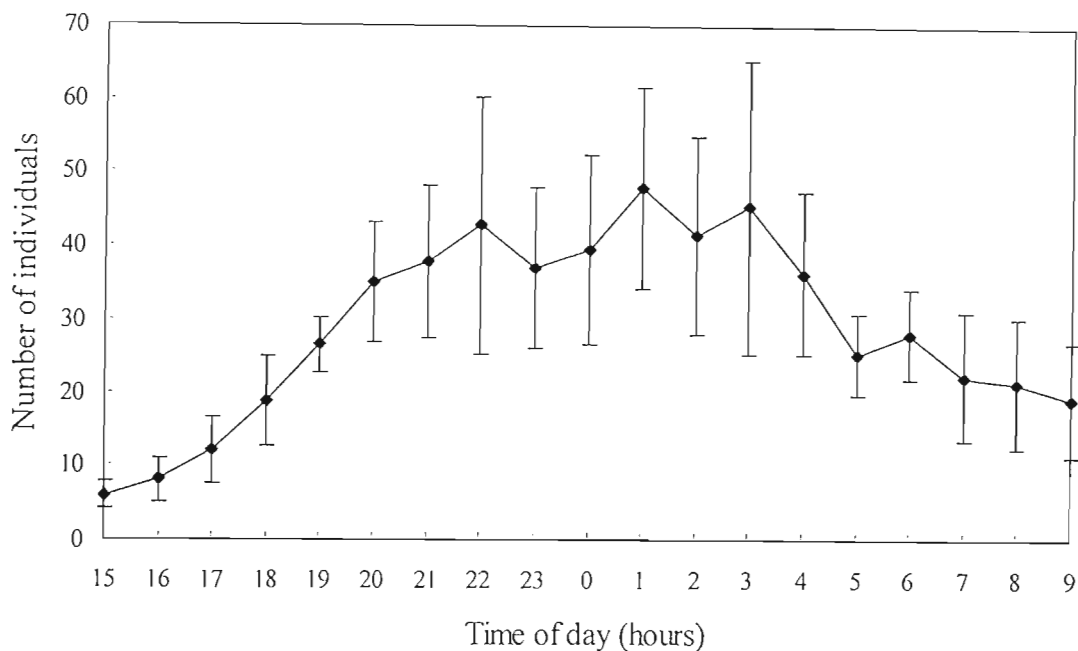


Figure 32. Daily activity (mean \pm 1SE) of the host ant *Camponotus natalensis* on 1 and 2 May 1999 at The Start.

Foraging behaviour

Little is known of the feeding habits of the host ant, although clearly the ant is attracted to sucrose. The host ant usually forages on the ground, rarely doing so during the day. Foraging individuals were mostly encountered on cloudy rather than sunny days. Nevertheless, few prey items were seen to be taken. On one occasion, on 9 December 1998 at The Start, an amphipod, *Talitriator africana* (Bate) individual was taken. Further observations on 14 January 1999 showed the ant to be feeding directly on the host plant by sucking liquid from a damaged stem. On 6 May 1999, host ants used antennae to stroke an *O. ariadne* larva near the base of the host plant. At this same location the ants were also sucking the base of the stem that had been physically damaged.

Of particular note is that the host ant regularly attended three species of Homoptera that inhabit the base of the host plant stems. These include an aphid (*Smynthuroides betae* Westwood), a membracid (*Oxyrhachis tuberculata* Walker), and an unidentifiable species of cottony scale (Margarodidae: Monophlebinae).

The cottony scale was the most abundant of the three Homoptera species in association with the host ant (Fig. 33), comprising 90% of total individuals and 25% infested host plant stems (n=50). Even so, this was probably a slight underestimation owing to the difficulty of sampling without damaging the host plant. Other plant species near the host plant were also infested with the cottony scale. During November and December, the cottony scale was about 2 mm long, and by April it was about 5 mm long. *C. natalensis* attended and stroke these Homoptera with their antennae. On being disturbed or exposed to sunshine, the ants quickly picked up the homopterans and moved them to deeper chambers, as they did when attending the butterfly larva.

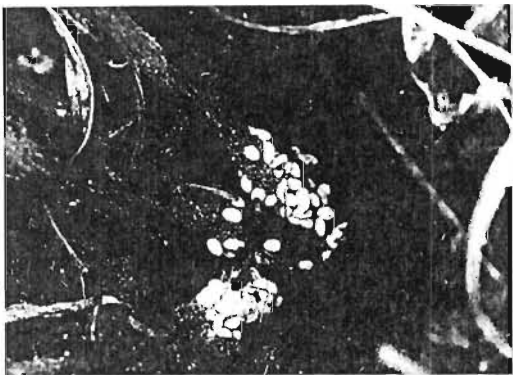


Figure 33. The host ant attending cottony scales at the base of host plant.

Brood

During October 1998, 20 ant nests were carefully examined after they had been exposed to grassland fire. There were only a few or no ants near the surface, with most individuals being deeper down. Only some nests supported many adult ants and pupae (Fig. 34). There were two different sizes of ant pupae, with very few ant larvae being present at this time. Interestingly, no larva of the butterfly were found in nests that contained ant brood.



Figure 34. The host ant, *Camponotus natalensis*, with its pupae.



Figure 35. Host ant swarming with winged individuals (drones) at soil crevices.

On 1 January 1999, one nest (#17) had more than 100 individual workers and soldiers swarming together with winged individuals (drones and one queen) along several long crevices (about 10cm long and 1-2 cm wide) in the nest (Fig. 35). There was however, no ant brood. On 8 January, the ants were still swarming, but by 13 January the long crevices had been sealed. On 4 March the nest was uninhabited. A further nest under host plant #1 on 28 February, was swarming with winged individuals and brood (larvae and pupae), including one queen (already with loose wings). The brood and alates were still present on 21 March 1999, although the pupae were then small in size.

3.6 Discussion

Habitat comparison

With four colonies now known, it was possible to make some initial comparisons and ascertain similarities between them. All sites are within the high-rainfall Moist Mistbelt (Camp, 1999). *Orachrysops ariadne* only occurs on the south-facing slopes, where the host plant occurs. Such south-facing slopes are moister than those facing north, owing to different slope exposures, as indicated by the morning dew that lasts until noon on the southerly slopes. Although the butterfly is clearly very particular with regards to the south-facing slope, it is not entirely clear what exact and direct benefit it receives. It may be that microclimates caused by different slope exposures play an important role for both development rates of the immature stages, and timing of plant senescence (Weiss *et al.*, 1988).

Thermal conditions influence activity patterns and growth of insects in all life stages (Weiss *et al.*, 1988). These thermal conditions, besides influencing the butterfly itself, also have an effect on the host ant. In the case of the butterfly *Maculinea arion* (Linn.) in Britain, even the host ant is affected, with only a slight change in microclimate making survival precarious. *M. arion* has a complex life cycle, with the larvae spending up to 11 months in the nest of host ant *Myrmica sabuleti* (Thomas, 1995). *M. sabuleti* in Britain was only able to support *M. arion* on south-facing slopes, where the warmer microclimate supported grass tufts grazed down to less than 3 cm tall (Thomas, 1995). However, in the Dordogne, France, where summer temperatures are about 3°C hotter than in Britain, the butterfly and the host ant only occur in swards more than 20 cm tall

and on all aspects of land except south-facing slopes (Thomas, 1995).

The south-facing slopes in the southern hemisphere receive less solar radiation than the north-facing slopes (Samways, 1990). Also, the tall vegetation that *O. ariadne* prefers, provides more shade than the short vegetation, and reduces soil exposure to the sun. This mirrors the *M. arion* situation in the Dordogne, where hotter conditions prevail. The ground temperature in long grass can be 10°C cooler than in short grass, and 20°C cooler than on bare ground at midday. It seems then that the south-facing slope, with long grass cover, has a more moderate and stable microclimate compared to the north-facing slope with its greater fluctuations in temperature and longer exposure time. These more stable conditions are preferred by both the larva and the host ant.

On one other south-facing slope site, Leopard Bush, where the host plant occurred but not the host ant, there was no butterfly. This may further emphasize that both the host plant and the host ant must occur together for the butterfly to survive. This in itself is an extremely limiting factor for the geographical distribution of the butterflies. Indeed, most butterfly species have a much more limited distribution than their host plant, or the combination of host plant and host ant, and such geographical limitations should be taken into account in addition to other microhabitat factors (Pringle, 1997).

A further point is that the upright host plant variety *Indigofera woodii* var. *laxa* is utilized, and not the prostrate variety *I. woodii* var. *woodii*. This further narrows the possibilities of more colonies. Searching for eggshells remains on the host plant was shown earlier to be very helpful in mapping the local distribution of the breeding sites, and can be used to locate and confirm new colonies (see details in Chap. 2). Locating the south-facing slope with host plant, as well as host ant, could enhance the possibility of finding further colonies, as was done in this study to locate the Stirling site.

Although this assessment of the ecological requirements cannot be regarded as scientifically rigorous (simply because the butterfly is so rare and provides few replicates), it is nevertheless useful for locating and identifying the potential colonies in practice. To assist in this search, databases on the host plant and host ant are being compiled, and, using fine-scale GIS mapping, prediction of potential sites is being undertaken in co-operation with KwaZulu-Natal Wildlife (A. J. Armstrong, pers. comm.). Predictions on the habitat and habitat requirements of this species are necessary for developing a conservation strategy and action plan (O'Dwyer and Attiwill, 1999).

A first priority in butterfly conservation is to maintain, and manage where necessary, the intact habitats, as is being done for the Brenton blue butterfly *Orachrysops niobe* (Trimen) (Steenkamp and Stein, 1999). Of concern in this regard is that the extent and rate of land transformation and habitat modification in KwaZulu-Natal is greater than in other parts of southern Africa (Scott-Shaw, 1999). Indeed, at least 92% of the KwaZulu-Natal Mistbelt has been transformed (Armstrong *et al.*, 1998), with only 1% of the Mistbelt grassland remaining (A. J. Armstrong pers. comm). *O. ariadne* is an inhabitant of this remaining small percentage, and it may be that the loss of the colony Balgowan in 1945 (Henning and Henning, 1989) was a casualty of the 99% loss of Mistbelt grassland. Similarly, a second site a few kilometers from The Start also disappeared with land transformation (C. Quickerberg, pers. comm.). It is possible that the fragmentation of the Mistbelt area is resulting in isolation of populations and perhaps reducing some remnant populations to below a minimum viable population size (Armstrong *et al.*, 1998). However, it is not known whether this is in fact the case for *O. ariadne*. Clearly, it is critical for long-term conservation of this species to locate any, as yet unknown, colonies within the threatened Mistbelt grassland.

O. ariadne is rare as a result of a combination of limited habitat, habitat loss and habitat degradation. These factors are synergistic, and make this a distinctly threatened species. Now that the habitat and resources for the survival of this butterfly have been determined, it narrows further searches to specific locations where the right habitat, host plant and host ant coexist. This is an ongoing and urgent task, as the Mistbelt grassland has largely gone. As *O. ariadne* is a specialist, location of the right habitat will be highly meaningful as this butterfly acting as an indicator of high-quality Mistbelt grassland habitat.

Position of eggs

Most of the eggs were positioned on the tip of the upright host plant (Table 7). Morning dews are heavy and frequent, especially on the south-facing slope, where they may last until noon. Eggs that are laid on the tip of the host plant are exposed to the warmth of sunshine, and the dew more readily evaporates. With risks of dew surface tension being a trap for the young, emerging larva, this is an important factor in the survival of the butterfly.

Several host plants that had eggs in 1997 or 1998 did not have any in 1998 or 1999.

Those host plants without eggs had become covered by dense grasses and forbs or had wilted. By contrast, the host plants with eggs remained exposed. The open condition of host plants possibly also makes them apparent and they thus receive more eggs (Dolek *et al.*, 1998). This may be important as the eggs are positioned prominently at the tip of host plant. Yet the new shoots are also damaged by the megaherbivores (such as bushbuck *Tragelaphus scriptus* or reedbuck *Redunca arundinum*), which is a distinct disadvantage to eggs being positioned in this sites.

Nectar plants

The spatial distribution and phenology of nectar plants may also influence the local distribution of butterflies (Schultz and Dlugosch, 1999). *Tephrosia polystachya* only occurred in small patches, but *Athrixia phylicoides* was widely distributed at The Start. *Hebenstretia dura* was the preferred nectar source, but was only found at Wahroonga and Stirling. *H. dura* occurs at forest edge (both natural and plantation), sometimes adjacent to small streams. *T. polystachya* has flowers at the beginning of the flight season of the butterfly, and *A. phylicoides* flowers later in the flight season, allowing switching of nectar plants. The utilization of nectar plants differed between colonies, and appeared to be largely fortuitous. The butterfly, although having certain preferences, was not severely restricted by nectar resources, which nevertheless must be present alongside the particular host plant and host ant.

Host ant

The details of the interaction between *O. ariadne* and its host ant are still not fully known. Nevertheless, this study has elucidated aspects of the ant's behavioural ecology that have a bearing on the conservation of the butterfly. The host ant's nest can be readily located after fire, principally by the form of the soil mounds. The excavated soils and entrance holes may also help to ascertain the presence of the host ant. Also, the presence of the ant can be determined using sugar-water baits (Fig. 25). Together these various signs may enhance location of the host ant, and help to understand the interaction between it and *O. ariadne*.

The pitfall trapping and sugar-water bait results indicated that the ant's foraging activity decreases gradually during May (Figs 30 & 31) as ambient temperatures drop (Figs 23 & 24). The peak of ant activity coincides with hatching of the butterfly larva,

which occurs in April. Indeed, the sugar-water results indicated maximum activity between 21h00 and 03h00. The presence of ant brood and alates, also reveals ant colony activity. At The Start, there were two sizes of pupae in October, with the smaller pupae possibly being workers and the larger ones drones. Swarming of alates indicates the imminence of nuptial flight, which was between January and March. In March, the wingless queen, larvae and smaller pupae possibly represented the newly-founded colony.

The fact that the host ant attended a sap-sucking cottony scale as well as feeding directly on the host plant by sucking liquid from the damaged stem, accords with other findings that ant taxa which tend butterfly larvae are the same as those that tend extrafloral nectaries and Homoptera (DeVries, 1991). Such complimentary feeding behaviour has also been described elsewhere (Buckley, 1987; Way, 1963). This was further emphasized by the fact that one butterfly larva was being stroked by ants while at the same time they fed from a damaged host plant stem.

Chapter 4. Comparative population structure and movement of a threatened and a common African species of *Orachrysops*

4.1 Abstract

The population structure and movement of the threatened *Orachrysops ariadne* and the common *O. subravus* were studied by mark-release-recapture method. A total of 290 *O. ariadne* individuals were marked over 48 days. Of these, 124 (42.8%) were recaptured at least once. Of 631 *O. subravus* individuals marked over 63 days, 311 (49.3%) were recaptured at least once. Both species exhibited protandry, with males on the wing one to two weeks prior to the female. The sex ratio of *O. ariadne* was heavily male-biased 5.6:1 (246 males to 44 females), while the sex ratio of *O. subravus* was 1.6:1 (387 males to 244 females). The Jolly-Seber model was used to estimate daily population numbers (N_i), survival rates (ϕ_i), recruitment rates (B_i), proportion of marked animals in the total population (α_i), and the number of marked animals at risk (M_i). *O. ariadne* is a remarkably rare animal, averaging only 10 individuals ha⁻¹ within the small colonies. Average residence times of male adults were generally similar in both species in the range of 5.36-5.44 days, and were slightly longer for male than for female *O. subravus* (by 4.09 days). *O. ariadne* is a strong and rapid back and forth flier, covering mean recapture distances of 157 m, almost twice that of *O. subravus*, principally in search of scarce nectar sources. The extreme rarity of *O. ariadne* is not so much to do with behaviour, survivorship or longevity, but rather the butterfly is limited in distribution by suitable habitat for both larva and adult. The habitat patches, which are naturally scarce, have become increasingly isolated through transformation of the surrounding landscape. Reduction of the barrier effects of agroforestry through creation of linkages between colonies is recommended and is now being implemented.

4.2 Introduction

The Karkloof blue butterfly *Orachrysops ariadne* is globally Red-listed as 'Vulnerable' (Henning and Henning, 1995). It is endemic to KwaZulu-Natal Province, South Africa, and is currently known from only four sites within Mistbelt grassland (Acocks, 1988). The Grizzled blue butterfly *O. subravus*, in contrast, is a common species, and is widespread from the Eastern Cape Province to the KwaZulu-Natal midlands and inhabits the grassland biome (Pringle *et al.*, 1994).

Mark-release-recapture (MRR) has been widely employed to estimate absolute population parameters in mobile animals (Arnold, 1983; Southwood, 1978). This method estimates absolute abundance of population by using ratios of marked to unmarked individuals. This technique is also particularly useful for monitoring movements of butterflies (Scott, 1975; Warren, 1987). In addition, it can also be used to estimate population structure, including composition by age and sex (Wright, 1951 *cf.* Watt *et al.* 1977).

The method must be considered carefully in the case of a threatened species such as *O. ariadne*, as the method can have adverse effects on the subject individuals, from the direct impact of handling adult butterflies to indirect damage to the habitat by trampling by the observer (Morton, 1982; Murphy *et al.*, 1986; Murphy, 1988).

The four extant colonies of *O. ariadne* vary in size from 1 ha to 10 ha. These colonies are situated far apart (from 2 km to 100 km), and are surrounded by commercial forestry and cultivated farmland. With the limitation of number of suitable sites for MRR of *O. ariadne*, it is not possible to undertake a study of the species as a large-scale, replicated experiment. Nevertheless, after initial appraisal of the largest site, Wahroonga, indications were that this large colony could sustain a MRR study. *O. subravus* also occurred at the site, although the two species have different microhabitat preferences. This co-occurrence afforded the opportunity of comparing the adult population parameters to test whether any differences in the adult population parameters between this rare *O. ariadne* and the common *O. subravus* (Murphy *et al.*, 1986).

An understanding the spatial arrangement of suitable habitats and of movement patterns of threatened species in the fragmented landscape, helps us make more informed decisions on their conservation (Shreeve, 1995). The aim of this study is to

gather information of population structure and movement patterns of the rare *O. ariadne*, and to compare results with the common *O. subravus* at the Wahroonga site to improve conservation efforts for *O. ariadne*.

4.3 Study sites

The farm Wahroonga (29°36'S, 30°07'E) was selected for a MRR study based on preliminary observations in 1998. Wahroonga is a fine example of Mistbelt grassland, and with its unique and rare floral diversity, it was registered as a Natural Heritage Site (36 ha) in 1990. The average rainfall is about 900 mm, and elevation ranges from 1320 m to 1440 m a.s.l.

Surrounding Wahroonga are pastures and timber plantations. *O. ariadne* inhabits about 10 ha of tall grassland on the southwest-facing slope, adjacent to pastures (Fig. 36). *O. subravus* inhabits a further 10 ha of shorter grassland on the north- and west-facing slopes, adjacent to timber plantations (Fig. 36). These two sites were separated by a strip of Mistbelt forest. The grassland/plantation boundary and a further side adjacent to buildings were burned every year as firebreaks. The rest of the grassland was burned on a rotational basis (two to four years), but the extent of the burn is not known.

4.4 Materials and Methods

Study animals

Both *O. ariadne* and *O. subravus* are univoltine (one brood per year). As well as being segregated in space, these two species fly at different times of the year. The flight period of *O. ariadne* is March-April, and the female uses the host plant *Indigofera woodii* H. Bol. var. *laxa* for oviposition. *O. ariadne* is ant-dependent, with the young larva being associated with *Camponotus natalensis* (F. Smith) (Lu and Samways, 2001). At Wahroonga, *O. subravus* is on wing from late August-November, and the female uses both *I. woodii* var. *woodii* and *I. tristis* E. May for oviposition. The life cycle of *O. subravus* is not known, but an ant, *Camponotus* sp., is associated with its larva (S.-S. Lu, personal observation).

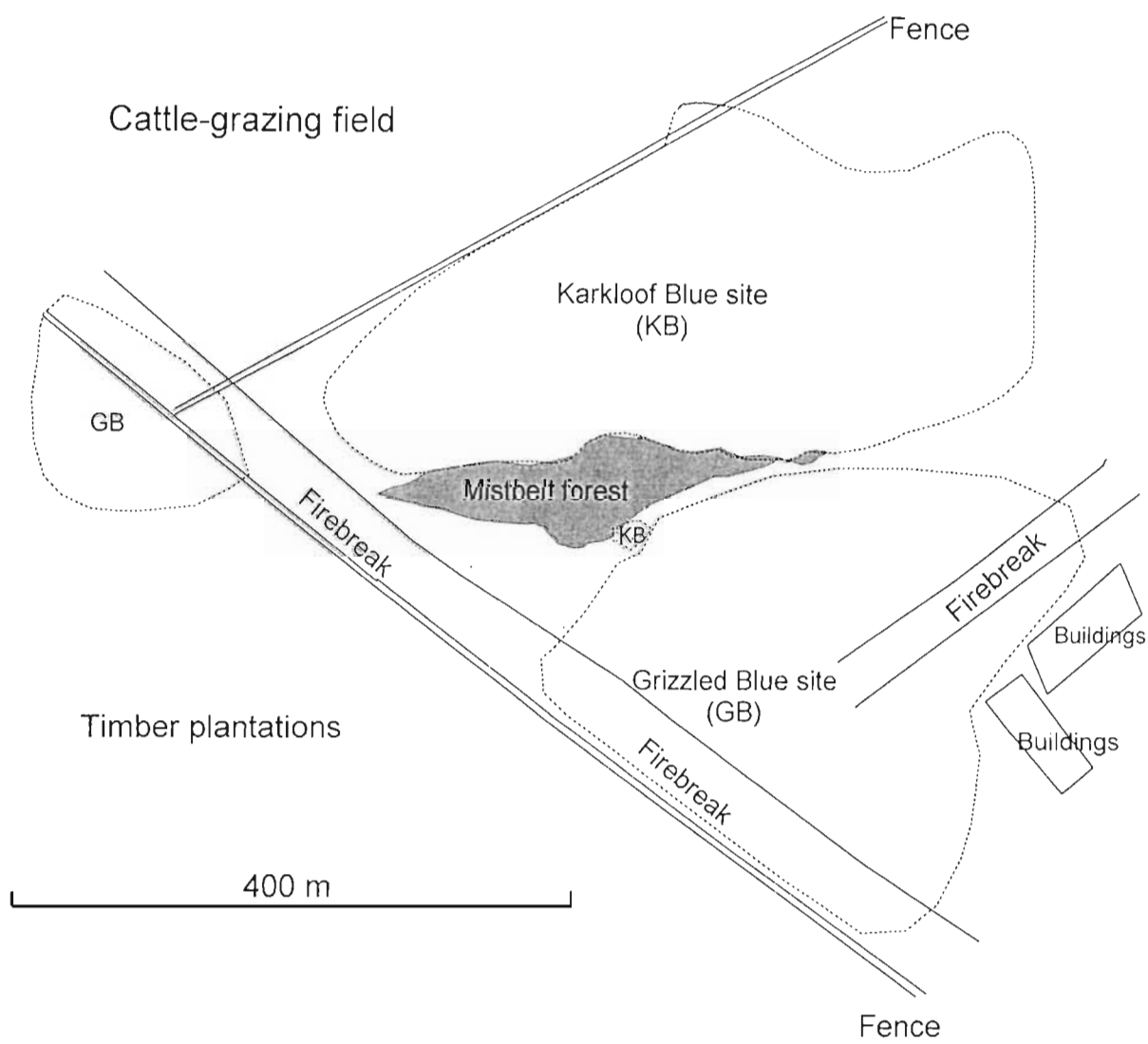


Figure 36. Study site at the Wahroonga farm.

Methods

Both species' flight is not easy to follow, with frequent changes of direction (Pringle *et al.*, 1994), making them difficult to sample. To increase the chances of capture, preliminary observations were made at Wahroonga in 1998 to determine the flight area and flight paths of both species. Owing to the dense vegetation and difficult topography, *O. ariadne* was sampled opportunistically over the whole site. As *O. subravus* was observed mostly in the regularly-burned firebreaks, two of these firebreaks were selected as the main sampling locations for this species, with supplementary sampling nearby in the grassland.

At Wahroonga in 1999, the flight season of *O. ariadne* was from 4 March to 22 April, and *O. subravus* from 6 September to 7 November 1999. During these times, a MRR study was undertaken to determine each species' population size, sex ratio and adult movement patterns. Butterfly sampling was performed two to three times daily,

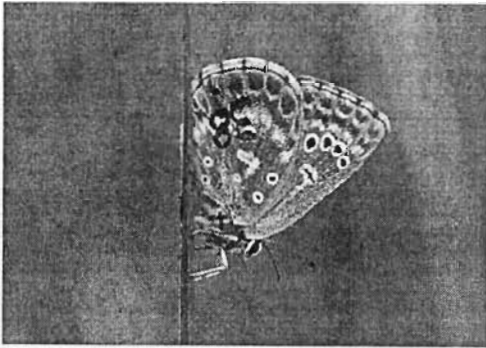


Figure 37. The hindwing of a marked individual of *O. ariadne*.

weather permitting. Each individual was caught, numbered on the hindwing (Fig. 37) using a permanent 'Zebra' felt-tip pen, and immediately released. Recaptures indicated that marks were recognizable as long as the wings did not sustain any later, major damage.

The following data were recorded for each capture: number of mark, sex, time, and position of capture. The degree of wing-wear was also recorded as follows: 0.5=very fresh, 1.0=fresh, 1.5=fresh-intermediate, 2.0=intermediate, 2.5=intermediate-worn, 3.0=worn, and 3.5=very worn (cf. Murphy *et al.*, 1986). The Fisher-Ford, Manly-Parr and Jolly-Seber models are three of the most widely applied multiple-marking models. The Fisher-Ford method requires more assumptions but few data, while the Manly-Parr method needs few assumptions but requires the sampling of a relatively high proportion of the population (Southwood, 1978). On several days, the daily recapture rate was lower than the minimum proportion suggested by Manly-Parr. For this reason, I chose the Jolly-Seber stochastic model for estimation of population parameters (Arnold, 1983).

Average residence rates (including losses due to emigration and deaths) were estimated from recapture decay plots (Watt *et al.*, 1977). The total number of brood was estimated using the method of Watt *et al.* (1977), i.e. $\sum N_i (1-\phi_i)$ the sum of daily population estimates was multiplied by the average daily lost-rate (1-average residence rate). Geographic information system (GIS) and a global positioning system (GPS) were used for mapping the position of each capture and recapture. Distances travelled (in metres) between each capture point were calculated as straight lines. The following mobility parameters were then calculated for each recaptured individual (Scott, 1975; Warren, 1987):

d_i : minimum distance in metres between capture i and $(i+1)$;

t_i : time in days between capture i and $(i+1)$;

D: sum of d_i 's for each individual (minimum distance moved);

D_{\max} : maximum D recorded in the population;

R: the distance in metres between the two farthest capture points (minimum range);

R_{\max} : maximum range recorded within each population;

T: the number of days between first and last capture.

The sample size of d_i and t_i is total number of recaptures, and the sample size of D, R and T is the number of individuals recaptured (Scott, 1975; Warren, 1987).

4.5 Results

Capture sex ratio and butterfly physical condition

Number of newly-marked individuals of *Orachrysops ariadne* and *O. subravus* on successive sampling days, their sex ratio, and physical condition are given in Tables 8 and 9.

A total of 290 *O. ariadne* were marked over 48 days between March and April, and 124 (42.8%) were recaptured at least once. The overall sex ratio for *O. ariadne* was unequal, being 246 males and 44 females (5.6:1) (Table 8). Early in the flight season, the captures consisted almost entirely of males. In contrast, during the last few days of the flight period, most individuals were females (Table 8). The wing-wear rating of the newly-marked individuals was mostly 'fresh' (47.2%) to 'fresh-intermediate' (19.7%), but tending to poor condition in the late flight season (Table 8).

Of 631 *O. subravus* marked between September and November, 311 (49.3%) were recaptured at least once. The overall sex ratio of *O. subravus* was 1.6:1 (387 males and 244 females) (Table 9). The sex ratios (male:female) of *O. subravus* in the

early half of flight season were male biased, but female biased towards the end of the flight period. The wing-wear rating of the newly-marked individuals was mostly ‘fresh’ (18.7%), ‘fresh-intermediate’ (31.1%) and ‘intermediate’ (31.2%), with tendency towards poor condition in the late flight season (Table 9).

Table 8. Number of newly-marked individuals of *Orachrysops ariadne* on successive sampling days, their sex ratio, and physical condition in relation to the progress of the 1999 flight season.

Date	Male	Female	Ratio	Condition*						
				0.5	1.0	1.5	2.0	2.5	3.0	3.5
March 7	3	0	100:0	0	1	2	0	0	0	0
11	11	0	100:0	0	7	4	0	0	0	0
14	9	2	82:18	2	4	3	1	1	0	0
17	13	0	100:0	0	11	1	0	1	0	0
20	18	0	100:0	4	7	4	1	1	1	0
23	23	2	92:8	3	15	5	2	0	0	0
26	26	1	96:4	8	10	4	4	1	0	0
29	35	2	95:5	8	13	5	7	1	3	0
April 1	29	4	88:12	6	15	4	5	1	2	0
4	29	4	88:12	1	17	6	7	2	0	0
6	23	9	72:28	1	13	9	6	1	1	1
8	8	4	67:33	2	6	2	1	0	0	1
12	4	3	57:43	0	3	3	1	0	0	0
14	4	6	40:60	0	6	2	1	1	0	0
16	9	3	75:25	0	8	1	1	2	0	0
20	2	3	40:60	0	1	2	2	0	0	0
22	0	1	0:100	0	0	0	0	0	1	0
Totals	246	44	85:15	35	137	57	39	12	8	2

* See text for details.

Table 9. Number of newly marked individuals of *Orachrysops subravus* on successive sampling days, their sex ratio, and physical condition in relation to the progress of the 1999 flight season.

Date	Male	Female	Ratio	Condition*						
				0.5	1.0	1.5	2.0	2.5	3.0	3.5
September 6	39	12	77:23	3	14	14	14	6	0	0
8	24	13	65:35	8	14	3	9	2	1	0
14	33	22	60:40	5	11	12	19	2	6	0
16	22	14	61:39	2	18	8	7	1	0	0
19	21	10	68:32	2	13	9	4	3	0	0
23	31	12	72:28	0	13	19	11	0	0	0
30	33	17	66:34	0	8	17	17	6	2	0
October 2	13	9	59:41	0	6	10	3	3	0	0
4	15	8	65:35	0	2	10	8	3	0	0
6	28	12	70:30	0	3	20	13	3	1	0
9	17	23	43:57	0	2	16	19	3	0	0
11	26	18	59:41	0	4	18	19	2	1	0
14	19	16	54:46	0	8	8	9	5	4	1
22	29	17	63:37	0	1	10	18	14	2	1
28	6	9	40:60	0	0	6	6	2	1	0
30	19	11	63:37	0	1	11	9	6	3	0
31	10	16	39:61	0	0	4	10	7	5	0
November 7	2	5	29:71	0	0	1	2	3	1	0
Totals	387	244	61:39	20	118	196	197	71	27	2

* See text for details.

Mating in *O. ariadne* was observed on two occasions and described in chapter 2. In both cases, the couple's wings were brilliant, possibly due to the fact that the individuals had recently emerged. Ten matings of *O. subravus* were recorded in 1998 (n=5) and 1999 (n=5). The wings of the mating pairs were in excellent condition, except those of one pair in the late flight season. The courtship activity involving marked butterflies of both species was recorded on six separate occasions (*O. ariadne*=1; *O. subravus*=5). One marked *O. subravus* female was mating on 15 September 1999, and again on 18 September 1999. On this second occasion, the female wing condition had become 'intermediate', while the male was 'fresh'.

Daily population size and total number of brood

Due to the low recapture rate of females (only four female individuals were recaptured) for *O. ariadne*, only males were used for the estimation of population parameters (Table 10) from Jolly-Seber model. The main flight season of *O. ariadne* was about one month, from mid-March to mid-April, when most of the adults (90%) were caught. Population estimates for males ranged from a low of 23 on 11 March to a high of 205 on 23 March, with an overall average population size of 92.3 (SD=58.4). Population estimates when lumping both sexes together gave an overall average population size of 111 (SD=68.5) individuals. The population peak on 1 April is consistent with the Jolly-Seber estimate of recruitment (B_i). Daily population size estimates for males (Fig. 38) has large standard errors owing to low recapture rate.

The population parameters of *O. subravus* males and females, estimated from Jolly-Seber model, are given in Tables 11 and 12. Daily population size estimates for males (Fig. 39) were more reliable than those of females (Fig. 40), as more males were captured. The main flight season of *O. subravus* was about two months, from early September to late October. Population estimates for males ranged from a high of 186 on 11 October to a low of 31 on 30 October, with an overall average population size of 82.9 (SD=44.1) individuals. Female population size ranged from a high of 124 on 19 September to a low of 9 on 30 September, with an overall average population size of 60.4 (SD=36.1) individuals. Population size estimates of both sexes together was 240.1 (SD=226.6) individuals.

Total number of brood was estimated at 624 male *O. ariadne*. The female *O. ariadne* could not be calculated due to the small number of recaptures. Total number of brood was estimated at 538 male and 424 female individuals of *O. subravus*.

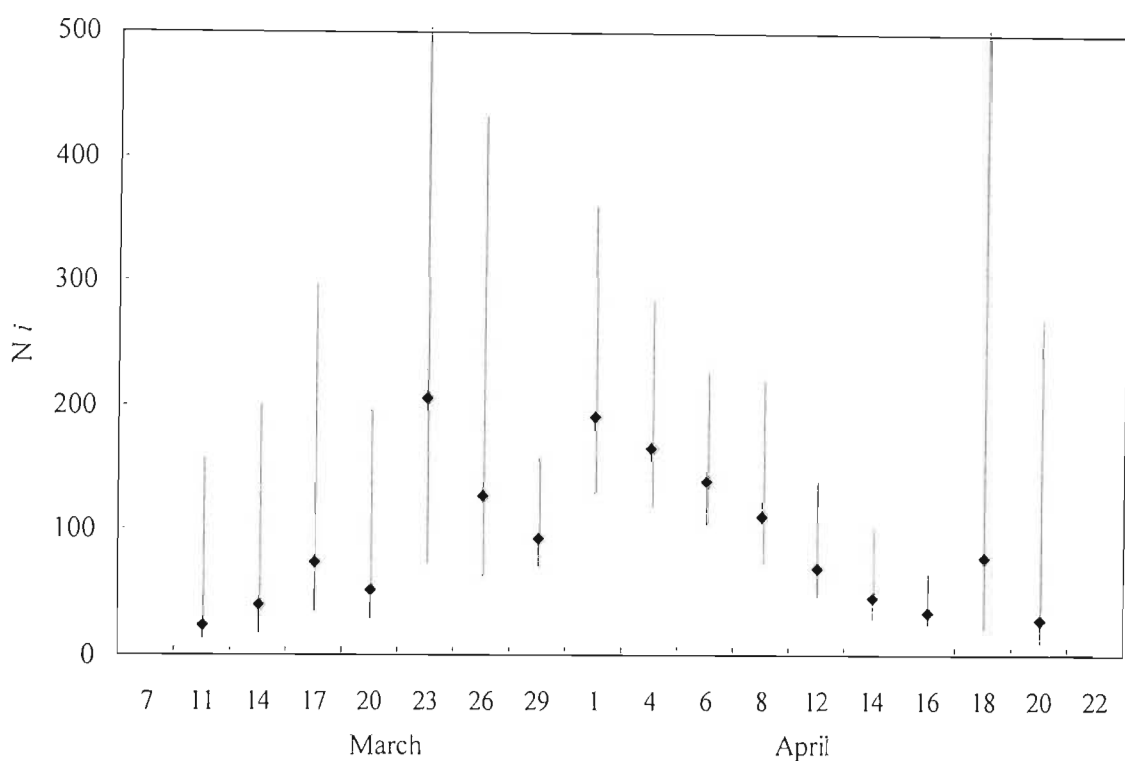


Figure 38. Population estimates (N_i) of male *Orachrysops ariadne* from the Jolly-Seber model. Lines cross to the upper range indicate that the variance is larger than the range of values illustrated.

Table 10. Population parameters estimated for male *Orachrysops ariadne* from the Jolly-Seber model. α_i = proportion of marked animals, M_i = total marked animals, N_i = total population estimate, ϕ_i = survival rates, B_i = no. of new animals.

Date (1999)	α_i	M_i	N_i	ϕ_i	B_i
March 7	0.000	0.0	(a)	1.200	(a)
11	0.154	3.6	23.4	0.499	28.4
14	0.182	7.3	40.1	1.596	10.3
17	0.350	26.0	74.3	0.333	27.2
20	0.250	13.0	52.0	0.763	165.4
23	0.115	23.7	205.1	0.364	52.8
26	0.133	17.0	127.5	0.617	14.2
29	0.286	26.5	92.8	1.032	94.7
April 1	0.333	63.5	190.5	0.707	30.6
4	0.400	66.1	165.2	0.806	5.8
6	0.540	75.0	138.9	0.759	5.8
8	0.667	73.7	110.5	0.686	-6.6
12	0.810	56.0	69.2	0.583	6.1
14	0.750	34.4	45.9	0.460	14.9
16	0.471	16.3	34.6	3.216	-29.1
18	1.000	79.0	79.0	0.266	7.0
20	0.750	21.0	28.0	(a)	(a)
22	1.000	(a)	(a)	(a)	(a)

(a): The Jolly-Seber method does not compute this value.

Table 11. Population parameters estimated for male *Orachrysops subravus* from the Jolly- Seber model. α_i = proportion of marked animals, M_i = total marked animals, N_i = total population estimate, ϕ_i = survival rates, B_i = no. of new animals.

Date (1999)	α_i	M_i	N_i	ϕ_i	B_i
September 6	0.000	0.0	(a)	2.313	(a)
8	0.400	27.8	69.4	0.773	38.7
14	0.298	22.2	74.6	0.753	12.9
16	0.476	25.0	52.5	1.333	44.7
19	0.400	34.7	86.7	0.841	45.5
23	0.295	30.0	101.5	0.454	31.1
30	0.233	15.0	64.5	1.217	10.2
October 2	0.480	28.0	58.3	0.875	11.9
4	0.516	28.9	55.9	1.008	30.8
6	0.378	26.1	69.0	2.596	20.9
9	0.541	70.3	130.1	0.880	80.3
11	0.366	68.0	185.9	0.250	-4.9
14	0.525	20.0	38.1	1.583	123.9
22	0.237	38.0	160.4	0.673	-29.9
28	0.500	33.0	66.0	0.157	20.2
30	0.208	6.4	30.9	(a)	(a)
31	0.444	(a)	(a)	(a)	(a)

(a): The Jolly-Seber method does not compute this value.

Table 12. Population parameters estimated for female *Orachrysops subravus* from the Jolly- Seber model. α_i = proportion of marked animals, M_i = total marked animals, N_i = total population estimate, ϕ_i = survival rates, B_i = no. of new animals.

Date (1999)	α_i	M_i	N_i	ϕ_i	B_i
September 6	0.000	0.0	(a)	1.667	(a)
8	0.235	20.0	85.0	0.385	73.2
14	0.120	12.7	106.0	0.536	-1.0
16	0.333	18.6	55.8	1.012	67.3
19	0.267	33.0	123.8	0.114	-0.7
23	0.375	5.0	13.3	0.067	8.1
30	0.111	1.0	9.0	0.753	24.3
October 2	0.412	12.8	31.1	0.404	11.4
4	0.385	9.2	23.9	0.465	12.9
6	0.333	8.0	24.0	0.518	57.5
9	0.148	10.4	70.0	0.914	27.5
11	0.333	30.5	91.5	0.371	16.0
14	0.360	18.0	50.0	0.395	69.8
22	0.150	13.4	89.5	1.052	-4.5
28	0.357	32.0	89.6	0.371	9.8
30	0.353	15.2	43.1	(a)	(a)
31	0.444	(a)	(a)	(a)	(a)

(a): The Jolly-Seber method does not compute this value.

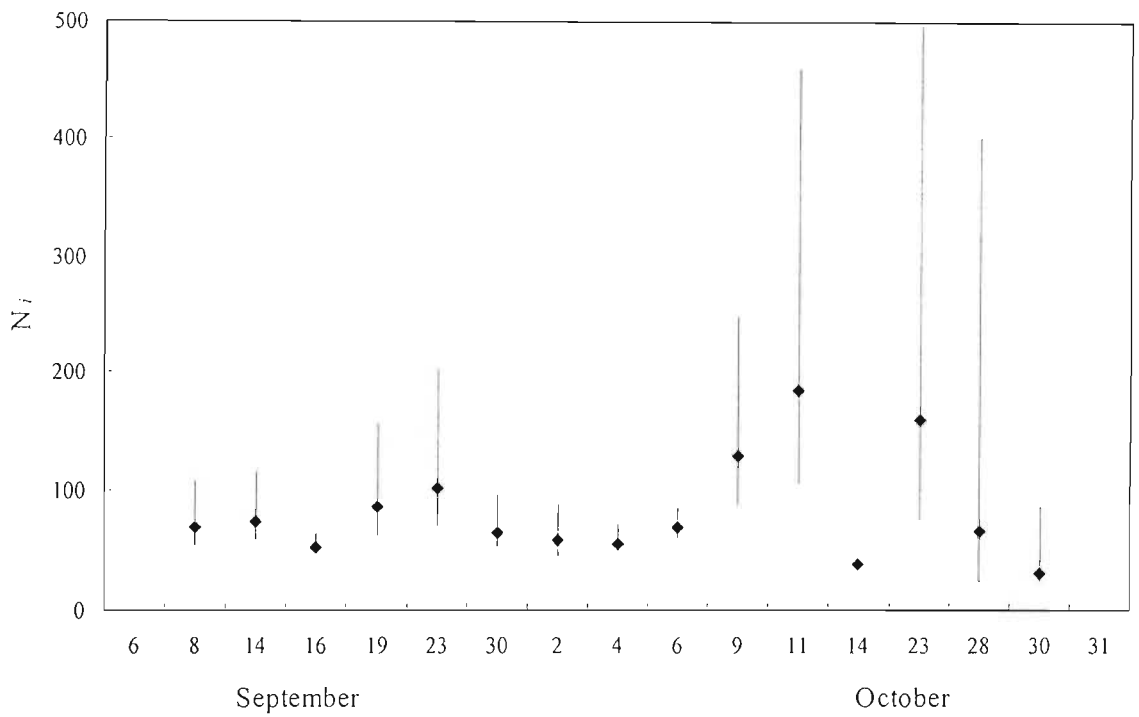


Figure 39. Population estimates (N_i) of male *Orachrysops subravus* from the Jolly-Seber model. Lines cross to the upper range indicate that the variance is larger than the range of values illustrated.

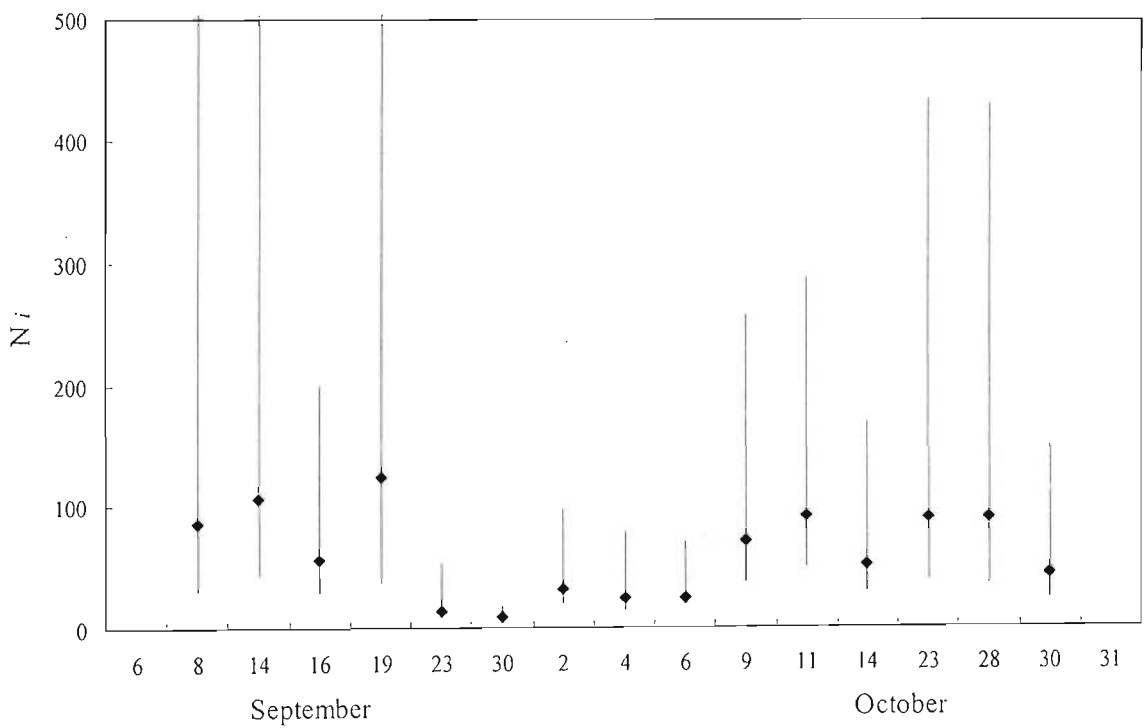


Figure 40. Population estimates (N_i) of female *Orachrysops subravus* from the Jolly-Seber model. Lines cross to the upper range indicate that the variance is larger than the range of values illustrated.

Recapture probabilities and residence

O. ariadne showed a bias towards males over females captured, both as a sex ratio and as a substantially higher rate of recaptures per individuals. This contrasted with *O. subravus* where differences in sex ratio and in number of recaptures were considerably lower. Most of the recaptured *O. ariadne* individuals were handled twice (49.6%) or even three times (26.8%) either on same day or on different days. The maximum number of times that a male *O. ariadne* individual was handled was seven times on three different days. In the case of *O. subravus*, most of the recaptured individuals were handled twice (45.5%) or three times (26.0%). The maximum number of times that a male *O. subravus* was handled was twelve on four different days, and for a female it was nine on two days. The maximum number of times that a male *O. subravus* individual was handled was ten on seven different days.

Recapture decay plots of male *O. ariadne* (Fig. 41), male *O. subravus* (Fig. 42) and female *O. subravus* (Fig. 43) showed that residence has a constant loss rate. Average residence times of adult male were generally similar in both species in the range of 5.36-5.44 days, and were slightly longer for male than for female *O. subravus* (by 4.09 days). The maximum longevity observed was 18 days for male *O. ariadne*, 18 days for male *O. subravus* and 19 days for female *O. subravus*. The daily activity of *O. ariadne* was from 10h00 or 11h00 to 14h00 or 15h00, and *O. subravus* was from 09h00 or 10h00 to 15h00 or 16h00.

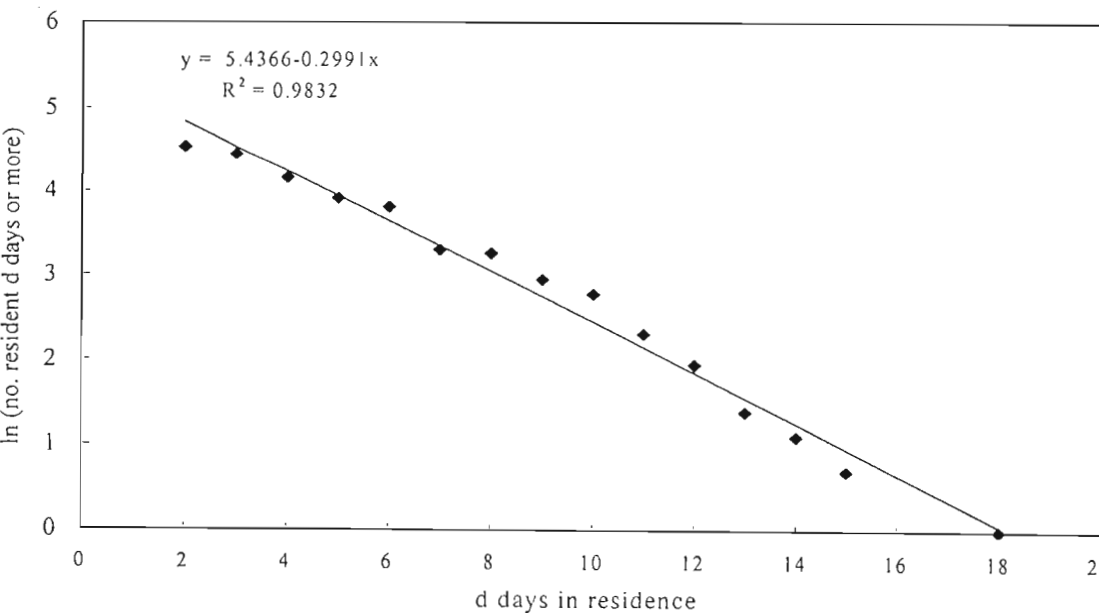


Figure 41. Recapture decay plots of male *Orachrysops ariadne*. d is the time between first and last captures of given individuals, in days.

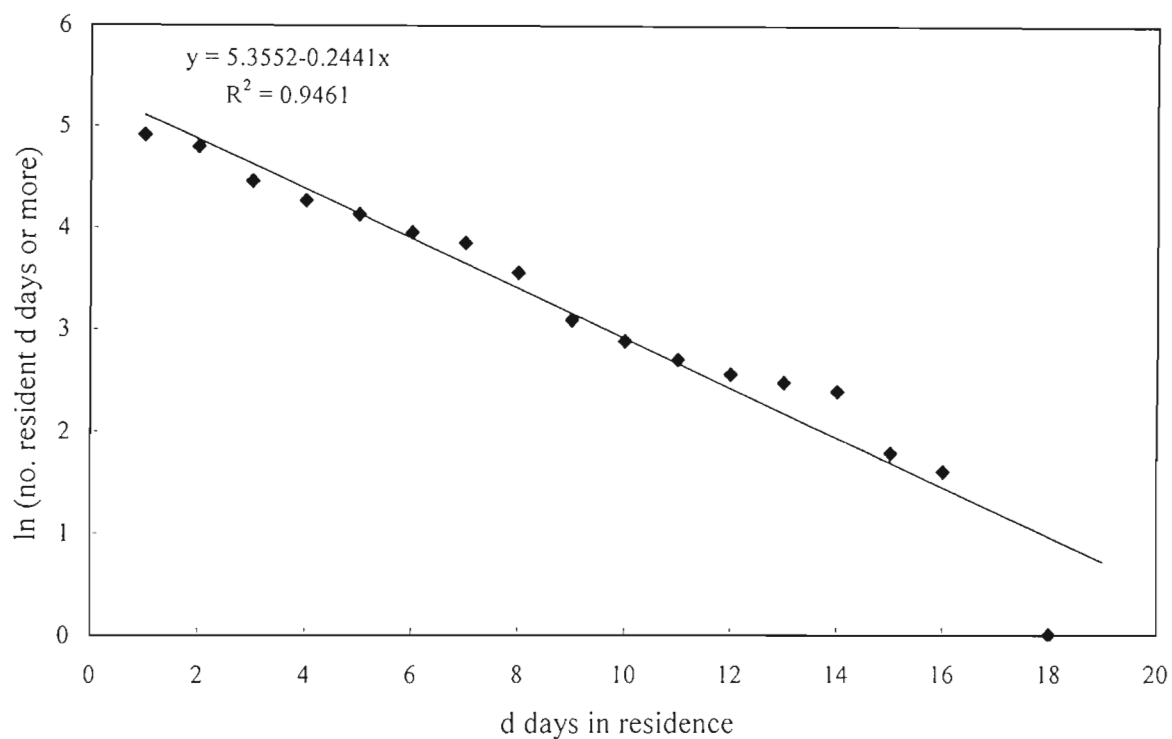


Figure 42. Recapture decay plots of male *Orachrysops subravus*. d is the time between first and last captures of given individuals, in days.

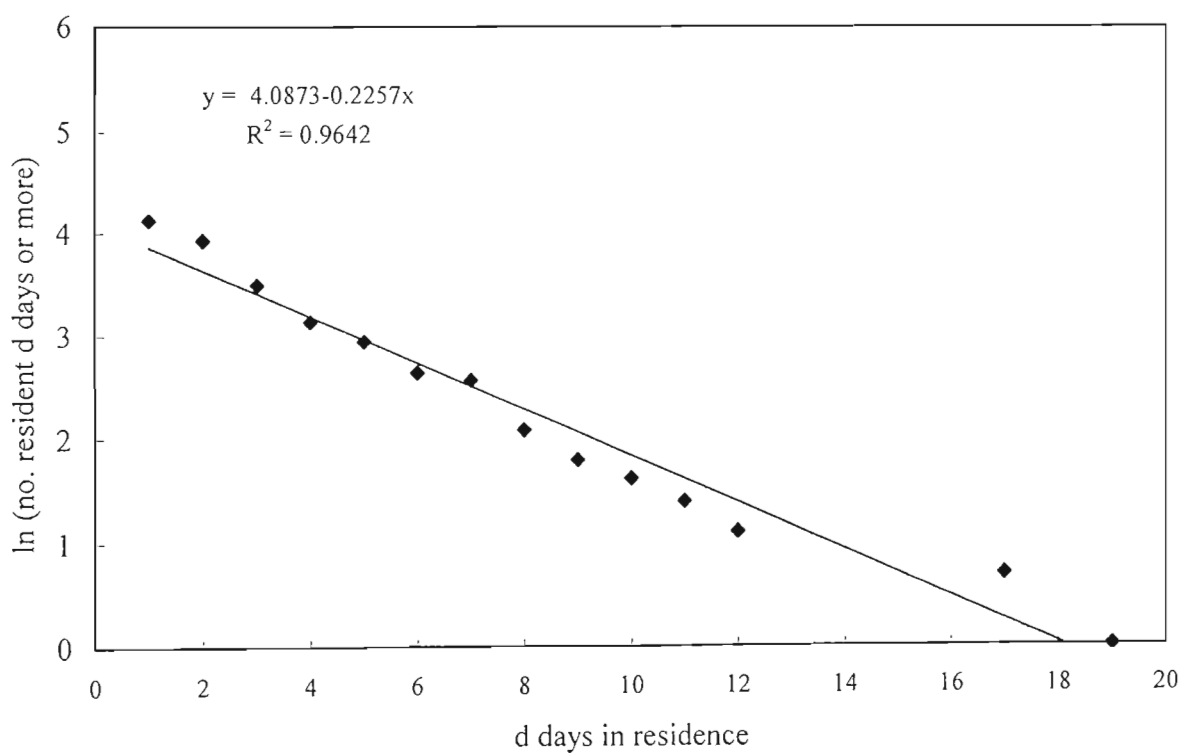


Figure 43. Recapture decay plots of female *Orachrysops subravus*. d is the time between first and last captures of given individuals, in days.

Adult movement pattern and parameters

The flight paths of male *O. ariadne* movement during the MRR study are shown in Fig. 44. The *O. ariadne* males patrolled widely back and forth within the whole colony (Fig. 44), where nectar sources are abundant. The presence of Mistbelt forest restricted the free movement of individuals. *O. ariadne* flew along the edge of the Mistbelt forest, and only crossed this barrier at narrow gaps at the end of the Mistbelt forest patch. Some of the male adults that were marked at the periphery of colony where recaptured well within the colony where the host plant was abundant.

Examples of flight patterns of *O. subravus* are given in Figs 45 and 46. Most of the adults flew within the colony, searching for host plants or nectar plants which were mainly along firebreaks where the nectar plants and host plants had recently emerged after fire. The firebreak, with its abundance of nectar plants, meant that *O. subravus* was frequently encountered. Furthermore, the *O. subravus* flight domain shifted from early-burned firebreak to late-burned areas where there was early- then late-emergence of nectar plants. The Mistbelt forest also restricted movement of *O. subravus* individuals, which flew only along the edge of the forest patch.

Adult movement parameters of *O. ariadne* and *O. subravus* are given in Tables 13 and 14. The maximum recorded movement range (R_{\max}) of male *O. ariadne* on the same day was 310 m and over more than one day was 320 m, which is nearly the maximum length of the site. The R_{\max} of male and female *O. subravus* on the same day was 350 m and 320 m respectively, but over more than one day it reached 680 m and 750 m respectively. But the mean range (R) of *O. subravus* (102-104 m) was significantly smaller than that of *O. ariadne* (177 m) ($t=6.01$, $p<0.0001$). Although the vast majority of *O. subravus* individuals were recaptured within the colony, a few individuals (0.05%) were recaptured in neighbouring areas by additional sampling efforts.

The mean flight distance (d_i) of male *O. ariadne* (157 m) was significantly greater than that of male *O. subravus* (81m) ($t=6.16$, $p<0.0001$) and female *O. subravus* (89m) ($t=4.1$, $p<0.0001$). The results indicate that *O. subravus* is more sedentary than *O. ariadne*. Although the minimum movement distance (D) for male *O. ariadne* was 780 m, which is smaller than for male *O. subravus* (1260 m) and for female *O. subravus* (1300 m). This may be partially explained by the fact that *O. subravus*

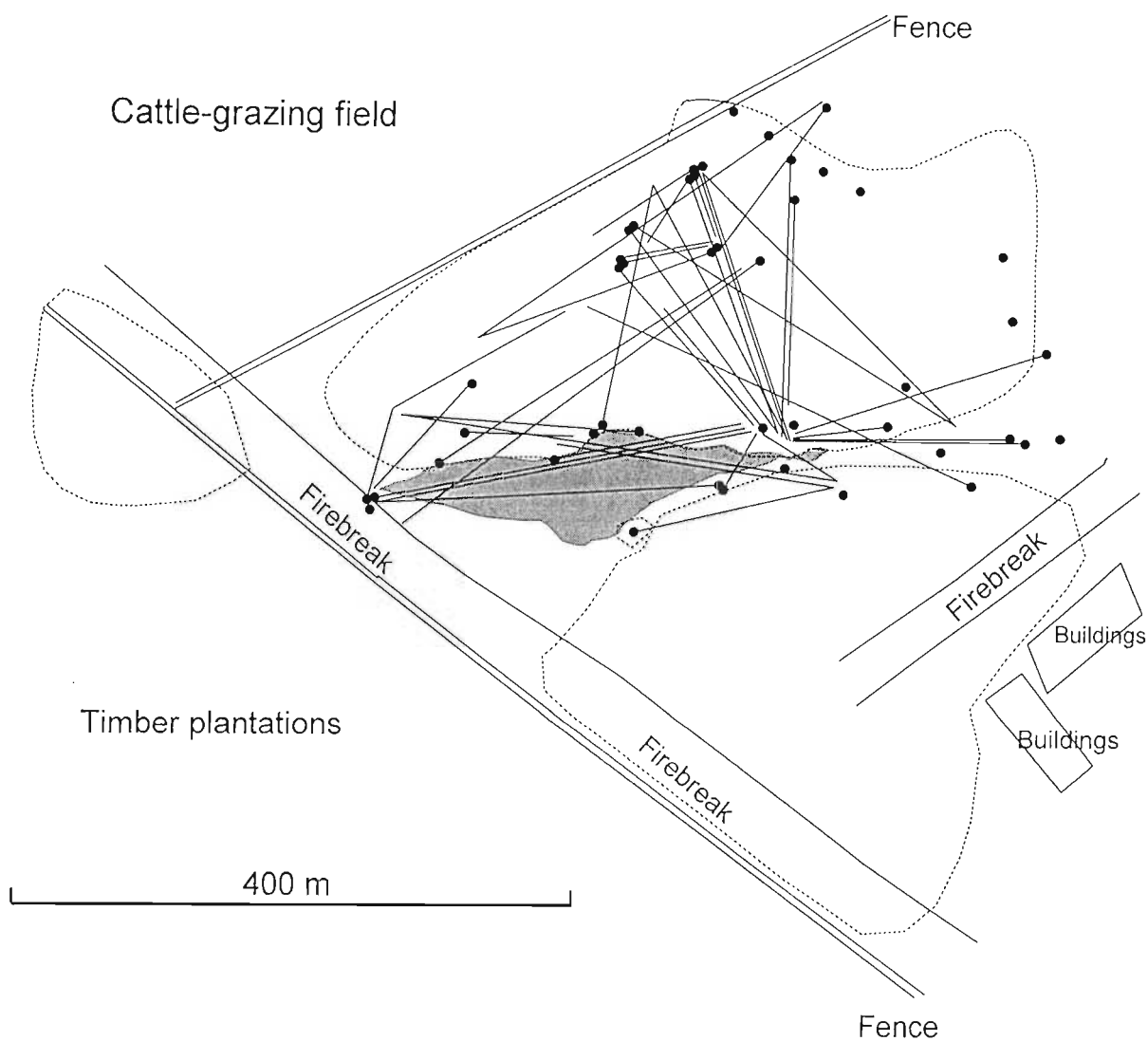


Figure 44. Male *Orachrysops ariadne* movement at Wahroonga in 1999. Dots indicate points of initial capture and lines indicate each movement recorded.

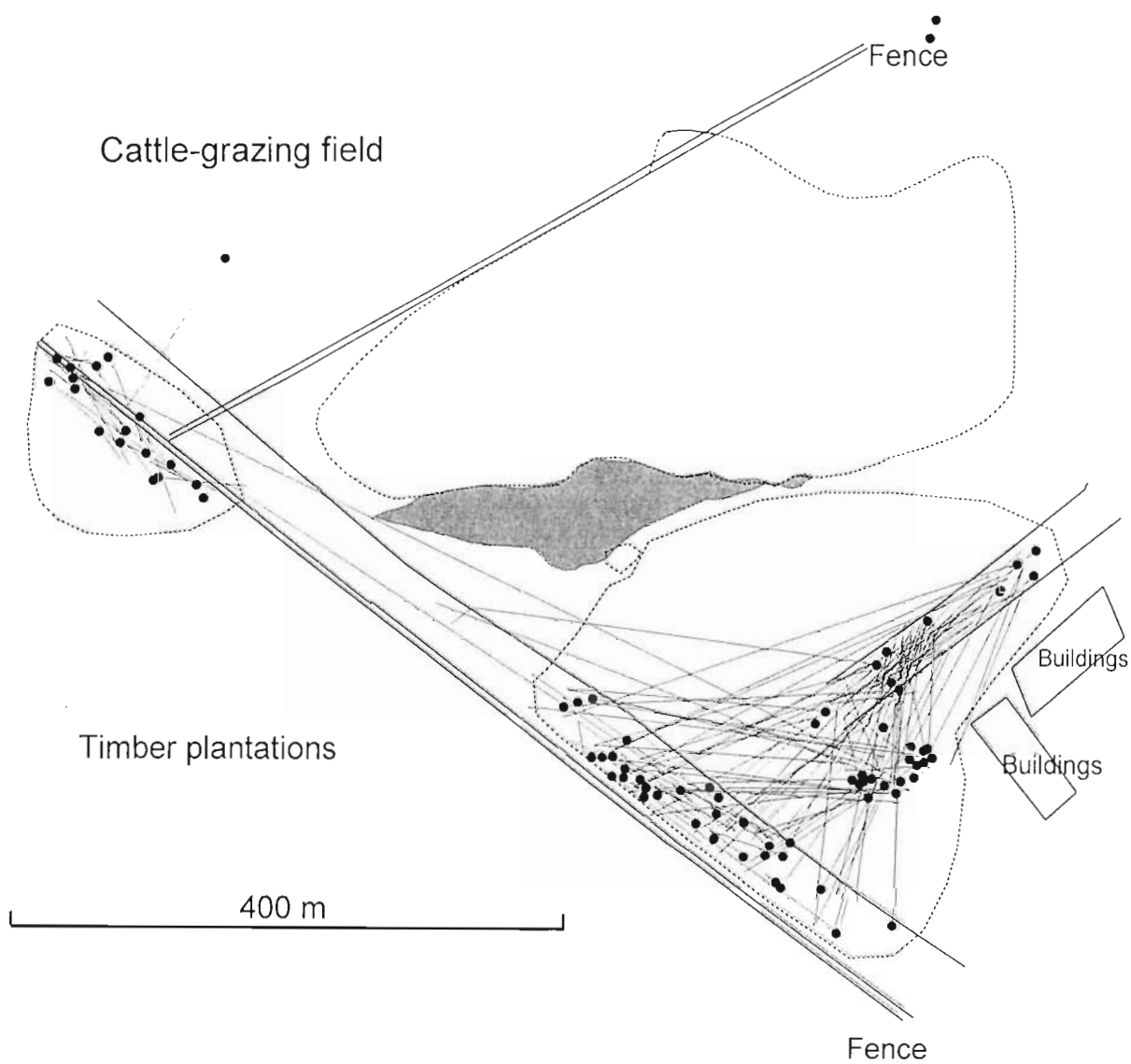


Figure 45. Male *Orachrysops subravus* movement at Wahrenonga in 1999. Dots indicate points of initial capture and lines indicate each movement recorded.

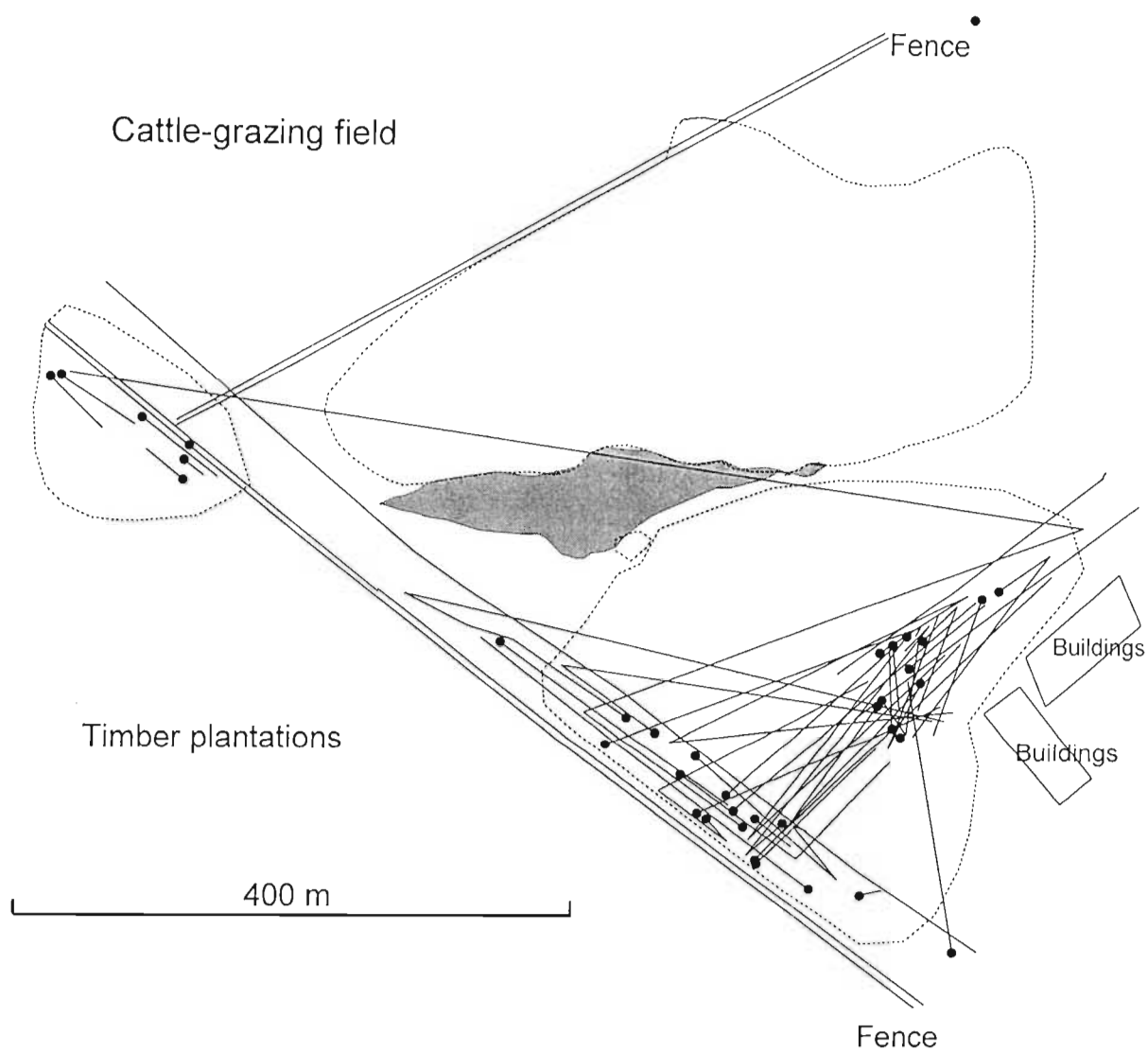


Figure 46. Female *Orachrysops subravus* movement at Wahrenonga in 1999. Dots indicate points of initial capture and lines indicate each movement recorded.

individuals were recaptured many times and thus appeared to have greater ranges and total distances than *O. ariadne* males which were recaptured infrequently (Gall, 1984).

Table 13. Adult *Orachrysops ariadne* movement parameters.

Movement parameter	Same day		More than one day	
	Males	Females	Males	Females
No. of individuals marked	246	44	246	44
No. of individuals recaptured	68	2	95	4
Total number of recaptures	98	2	148	5
Mean T (days)	0.085	--	5.67	5.25
Mean t_i (days)	0.074	--	3.69	4.2
R_{\max} (m)	310	200	320	200
Mean R (m)	159	--	177	--
Mean D (m)	164	--	219	--
Max D (m)	450	--	780	--
Mean d_i	147	--	157	--

Table 14. Adult *Orachrysops subravus* movement parameters.

Movement parameter	Same day		More than one day	
	Males	Females	Males	Females
No. of individuals marked	387	244	387	244
No. of individuals recaptured	156	85	137	61
Total number of recaptures	276	127	212	80
Mean T (days)	0.083	0.082	5.26	4.05
Mean t_i (days)	0.057	0.064	3.51	3.34
R_{\max} (m)	350	320	680	750
Mean R (m)	78	66	104	102
Mean D (m)	90	78	162	142
Max D (m)	580	620	1260	1300
Mean d_i	62	61	81	89

4.6 Discussion

The peak flight season of *Orachrysops ariadne* at Wahroonga was from mid-March to mid-April, while that of *O. subravus* was from early September to late October. The timing of flight period of *O. ariadne* is different from all other eight species in the genus which are on wing from September to December (Henning and Henning, 1994). It is not clear why this is the case. This unique attribute may have particular bearing on the conservation of *O. ariadne*.

Studies elsewhere have pointed out that despite similarities in population parameters, the conservation status of closely-related species may be very different (Murphy *et al.*, 1986). The results here on *Orachrysops* spp. support this view. Both *O. ariadne* and *O. subravus* show protandry, male-biased seasonal sex ratios even

late in the flight season, similar wing-wear rating progression, constant population loss rates, number of MRR handling times per butterfly individual, and similar longest recapture periods.

Both *O. ariadne* and *O. subravus* are protandrous, with the males appearing one to two weeks before the females. Furthermore, the sex ratio is heavily biased towards males in the case of *O. ariadne* (5.6:1), but less so in the case of *O. subravus* (1.6:1). These differences between the sexes relate to other features, with males being blue and conspicuous, while females are brown and inconspicuous. In addition, females are cryptic and fly low, searching host plants compared with the males' higher, patrolling flight. The different sex ratios between the two species is probably due to the differences in the way the two species respond to the plantscape. Female *O. ariadne* search for host plants among dense, tall vegetation, whereas female *O. subravus* occur in open, newly-burned firebreaks. The difference in flight areas made female *O. ariadne* less catchable, particularly in the dense high vegetation grassland in comparison with open, newly-burned firebreaks.

The overall average population size was 92.3 for male *O. ariadne*, 82.9 for male *O. subravus*, and 60.4 for female *O. subravus*. Population estimates, when lumping both sexes, indicated an overall average population size of 111 for *O. ariadne* and, 240.1 for *O. subravus*. These estimates are in accordance with the total number of captured individuals of *O. subravus* about more than twice that *O. ariadne*, although the total sampling period (flight season) was a half month longer for *O. subravus*.

We estimated the total brood to be 624 male *O. ariadne*, and 538 male and 424 female *O. subravus*. If a 1: 1 sex ratio is applied to the two species, the two species would be of similar abundance at the site. However, this speculation is not matched by field observations. In addition, there is no reason to assume a male-biased sampling error for *O. ariadne*. In terms of long-term genetic viability, the size of *O. ariadne* Wahroonga population (ca. 600 individuals) is only a marginally safe number, in an area of about 10 ha. Of even greater concern are the other two smaller sites, The Start and Stirling, each of which is only 1 ha. The population levels for these two, very small populations is cause for considerable concern in view of multiple impacts in and around the colonies.

Density of *O. ariadne* was about 20 individuals ha⁻¹ during the peak flight season, with a whole flight-season average of only 10 ha⁻¹ at Wahroonga. This is a similar

figure to that of another colony at The Start (1 ha in size) (Lu & Samways, 2001). The current population size and density estimates of *O. ariadne* suggest that the population levels are probably too low to sustain any loss of individuals. This emphasizes that great care must be exercised when using MRR studies on this threatened species with such small colonies. This point has been emphasized by Murphy (1988) and Mattoni *et al.* (2001) with regard to other threatened species.

Recapture decay plots (Fig. 41) showed that residence has a constant loss rate. It is not clear whether this population loss is due to death or to emigration (Warren, 1987). The wing-wear figures (Table 8) shows that most of the butterflies when first caught were in good condition. It is possible that only old adults emigrate, as Gall (1984) found for *Boloria acrocnema* (Nymphalidae), where old females were perhaps leaving their colony site.

The average residence times were generally similar in both species, and agree with other results on lycaenid butterflies (Arnold, 1983; Fischer, 1998; Scott, 1973). The longest recapture period was 18 days for male *O. ariadne* and the same for male *O. subravus*, while it was 19 days for female *O. subravus*. This suggests that the life span of *O. ariadne* and *O. subravus* can be at least this long. The life span of other butterflies may be underestimated owing to shorter sampling periods (Arnold, 1983). Repeated mating was confirmed to occur in *O. subravus*, but whether this occurs in *O. ariadne* is uncertain as only two sightings of mating pairs were made. The relatively long life span of both species suggests that multiple matings are probably more frequent than field observations indicate. The Karner blue butterfly (*Lycaeides melissa samuelis*) has a long daily active period (09h00 to 18h00 or 19h00), but lives for only a few days (Schweitzer, 1994). In contrast, the daily activities of *O. ariadne* (10h00 or 11h00 to 14h00 or 15h00) and *O. subravus* (09h00 or 10h00 to 15h00 or 16h00) are shorter possibly allowing them to live longer.

Threatened species are often assumed to be short-lived, comparatively sedentary, host-plant specialists, low in mobility and often are rare or local endemics (Arnold, 1983; Gall, 1984; Murphy *et al.*, 1986; Shreeve, 1995). In fact, many butterflies are capable of travelling long distances, although their activities are often restricted to relatively small areas (Arnold, 1983), while some specialist butterflies are more mobile than previously thought (Mousson *et al.*, 1999). A limitation of MRR is that the maximum movement distance detectable is the maximum linear dimension of the

survey area (Knutson *et al.*, 1999). It might be difficult to detect butterflies that move long distances because some individuals may move outside the study area, or individuals may disperse prior to being marked (Lawrence, 1994).

The distribution of both the host plant and the nectar plants may be the two limiting factors for the distribution of both species (Murphy *et al.*, 1984; Schultz and Dlugosch, 1999). *O. ariadne* is a rare species, and its only host plant *I. woodii* var. *laxa* is clearly very rare. In contrast, *O. subravus* is a more abundant and widespread species, and its host plant, both *I. woodii* var. *woodii* and *I. tristis* E. May, also has a wider distribution (Lu and Samways, 2001). This resource availability may explain why *O. ariadne* is rare, but *O. subravus* is common. Rare species may utilize resources which themselves occur at lower abundances or restricted areas than do those resources used by common species. Also, rare species may utilize a narrower range of resources than do common species (Gaston and Kunin, 1994).

The host plant of the larva and nectar sources for adult are uneven in distribution within the heterogenous habitat. Thus, plant resource distribution is critical in determining the population spatial structure and movement in butterflies (Arnold, 1983; Brommer and Fred, 1999). The differences in the movement patterns and mean distances (d_i) between *O. ariadne* (157 m) and *O. subravus* (81-89 m) are mainly explained in the distribution patterns of adult nectar plants and host plants. The more abundant and clumped distribution of nectar flowers for *O. subravus* results in more short-distance movements in and around nectar clumps, while *O. ariadne* flies longer distances in search of its scarce nectar flowers.

O. ariadne has a strong flight, back and forth within the colony. Furthermore, the life span is not as short as originally thought. These two factors make possible the opportunity for long-distance dispersal, but whether it occurs on a regular basis or not remains to be discovered. As the four known colonies are situated relatively far apart (about 2 km to 100 km), there is no evidence yet for inter-colony movement. There is however, a small piece of supporting evidence that suggests that some individuals do disperse widely. One male individual was recorded at Howick about 10 km from The Start Colony by C. Curtis (pers. comm).

As the results here illustrate that *O. subravus* flies beyond the core of the colony, *O. ariadne* may also do so on a regular basis. The problem remains however, that for *O. ariadne*, it must find suitable or potential habitats with only about 1% of good

quality Mistbelt grassland remaining (Lu and Samways, 2002). Even if suitable vacant habitat patches exist, the species still has to cross considerable unsuitable habitat, particularly plantation forestry and crop fields. This is a serious problem because butterfly flight paths can be deflected by tall, alien trees (Wood and Samways, 1991), and another lycaenid, the Adonis blue *Lysandra bellargus*, will not cross even 100 m gaps of agriculturally-improved grassland (Thomas, 1983).

The large-scale transformation of grassland threatens not only *O. ariadne*. Such transformation can also have an adverse effect on some widespread species (Leon-Cortes *et al.*, 2000), although this has not yet been quantified for the more common *O. subravus*. Rare species are especially vulnerable to habitat fragmentation, although it may be difficult to predict how some common species will respond to habitat loss (Summerville and Crist, 2001). Decline of common species in Africa is always possible as has been the case for common butterflies in Britain (Cowley *et al.*, 1999). Furthermore, it is essential not to be complacent, because the Rocky Mountain grasshopper *Melanoplus spretus* (Walsh), which was once a common rangeland pest became extinct within a few decades of anthropogenic landscape transformation (Lockwood and DeBrey, 1990).

Availability of suitable habitats and the spatial arrangement of such habitats in fragmented landscapes should be emphasised in the management of threatened species like *O. ariadne*. In particular, there needs to be better management of remaining fragments and better integration of such protected areas into the surrounding landscape (Thomas, 1995; Warren, 1993). It is not enough simply to protect and manage habitat in isolated reserves. The surrounding landscape, and beyond, also has an important role in the long-term survival of butterfly populations (Thomas and Hanski, 1997; Warren, 1993). Understanding the population structure and mobility of *O. ariadne* have become important dimensions for its conservation, especially in the highly fragmented landscape, as for *Lycaena helle* in Germany (Fischer *et al.*, 1999). Conservation priorities in fragmented landscape may therefore require the establishment of network of suitable habitats (Mousson *et al.*, 1999). Nevertheless, progress is being made, with habitat linkages being installed in this southern African landscape for butterflies and for other biodiversity (Pryke and Samways, 2001), and which form a conservation network connecting the nodes like the areas occupied by colonies of *O. ariadne*.

Chapter 5. Conservation management recommendations for the Karkloof blue butterfly *Orachrysops ariadne*

5.1 Abstract

The threatened Karkloof blue butterfly, *Orachrysops ariadne* (Butler), is endemic to KwaZulu-Natal province, South Africa. Two of the four sites from which it is known are registered as Natural Heritage Sites within the Mistbelt grassland. Even with such protection, there is no guarantee that the species will survive in the long-term without habitat management. The aim of such management is to optimize the habitat so that it best meets the needs of the butterfly. Effects of the current fire regime on the butterfly, host plant and ant host were evaluated. It is recommended that burning only take place after the larvae have hatched and gone underground with the ant host. Using a Global Positioning System and Geographic Information System, core, quality habitat characteristics were defined. In cooperation with the landowner at one site, alien invasive plants are being removed to increase the area of quality habitat. Availability of host plants limits number of the butterfly in the field. Guidelines are provided for propagation and introduction of the host plant to provide the butterfly with more oviposition sites.

5.2 Introduction

The Karkloof blue butterfly, *Orachrysops ariadne* (Butler) (Lycaenidae: Polyommatainae: Polyommataini) is Red-listed as Vulnerable (Henning and Henning, 1995). It is endemic to KwaZulu-Natal province, and is currently known from only four sites within Mistbelt grassland (Acocks, 1988). Two of the sites are registered as Natural Heritage Sites, a status that is based upon agreement of stakeholder and not upon legislation (South African Natural Heritage Programme, Department of Environmental Affairs). Even with such protection, there is no guarantee that the species will survive in the long-term, especially in the face of global climate changes (Samways, 1994).

The key to protecting and managing a rare or threatened species is to understand its life history and to apply appropriate habitat management (Primack, 1993). The

decline in many butterfly species, especially in reserves, illustrates the importance of adequate habitat management (Deutschlander and Bredenkamp, 1999; Sutherland, 1995; Thomas, 1991). Many of the rare butterfly species are not being conserved effectively under site protection systems, and a comprehensive conservation strategy for butterflies is needed (Warren, 1993).

Currently, there are no guidelines on how the habitat should be managed for conservation of *O. ariadne*. Fire is an important ecological factor and management tool in the grassland biome of South Africa, preventing succession to scrub or forest (Tainton and Mentis, 1984). Prescribed fire has therefore become common management practice for grassland, especially for many rare or threatened butterflies in many parts of the world (Deutschlander and Bredenkamp, 1999; Kwilosz and Knutson, 1999; Murata *et al.*, 1998; Schultz and Crone, 1998; Swengel, 1994; New *et al.*, 2000). However, the effects of the current fire regime on *O. ariadne* habitat are largely unknown.

With respect to a fire regime for *O. ariadne*, there are three important considerations. First, the known sites for the butterfly are close to commercial forestry areas, which has risk implications for both forestry and the butterfly. Second, fire is a major natural phenomenon in the areas where the butterfly occurs. Well-planned fire management is therefore essential to provide optimal habitat. Third, prior to the current high human impact, indigenous megaherbivores were probably abundant in the butterfly's habitat and would have had a major impact on habitat structure.

This study has limitations, including unknown historical factors and fire risks to the butterfly and to plantation trees. Also, the highly localized distribution of the butterfly prevents experimental replication. Within these restraints, the aim here was to evaluate the positive and negative effects of the current fire regime on the butterfly, host plant and host ant, to enable a more rational and effective overall fire regime. Additionally, the intention was to assess and monitor the potential threats to each colony so that a management programme could be devised that better meets the requirements of the butterfly, both currently and in the long-term.

5.3 Study sites

There are four known colonies of this butterfly: The Start (29°24'S, 30°17'E), Wahroonga (29°36'S, 30°07'E), Stirling (29°35'S, 30°08'E) and Nkandla (28°42'S, 31°08'E) (Fig. 1). Most studies here were carried out at the farms The Start and Wahroonga, because the other two colonies at Stirling and the locality Nkandla were only discovered or rediscovered during this study, in 1999. These sites lie within the Mistbelt area, where the average rainfall varies from 700 mm to 1300 mm per annum, and the average temperature is 13°C in July and 21°C in January.

The best known breeding colony is at The Start, at an elevation of ca. 1080 m a.s.l.. The forestry company SAPPI is custodian of this area, and in 1996, registered the site as a Natural Heritage Site (8.9 ha) to secure the future of the butterfly. At this site it inhabits only a 1 ha area of steep, dense grassland on a south-facing hillside. Pine plantations, maize fields and cattle pastures surround the butterfly colony.

The Wahroonga colony is situated in a fine example of Mistbelt grassland (owing to its unique and rare floral diversity), and was registered as Natural Heritage Site (36 ha) in 1990. The butterfly inhabits ca. 10 ha area of rank grassland on the south to southwest-facing slope, adjacent to a strip of Mistbelt forest. The elevation ranges from 1320 m to 1440 m a.s.l.. The surrounding land is used for *Pinus* and *Eucalyptus* plantations with cattle-grazing.

The farm Stirling, adjacent to Wahroonga, also lies within Mistbelt grassland, at an elevation of ca. 1460 m a.s.l.. The butterfly was first recorded here during this study on 25 March 1999. At this site the butterfly was flying on a south-facing, long-grass slope, adjacent to a stand of wattle (*Acacia mearnsii* De Wild). The site is surrounded by pastures.

The colony at Nkandla (28°42'S, 31°08'E), near Nkandla Forest Reserve, is at an elevation of 1100 m to 1200 m a.s.l.. This colony was first discovered in 1979, and was revisited on 10 April 1999. The butterfly was flying on a south to southwest-facing slope, where there were several small forest patches near gullies, and where the host plant was abundant.

5.4 Materials and Methods

Study animal

Details of the life history of the Karkloof blue butterfly were discussed in chapter 2. To summarize, the species is univoltine, and the flight period is March and April, with male emergence taking place two weeks earlier than that of the female. The butterfly is on wing when conditions are warm and sunny, mostly from 10h00 to 14h30. After mating, the females search for the host plant *Indigofera woodii* H. Bol. var. *laxa* H. Bol. for oviposition. Eggs hatch in 10 to 30 days. Larvae then crawl down to the ground, where they are tended by the host ant *Camponotus natalensis* (F. Smith). The larva pupates in the ant nest.

Methods

Information on fire events and management activities were obtained from landowners or recorded during this study. Impacts of fire regimes on the butterfly, host plant and host ant were noted.

Searches for eggs took place between March to June 1997-1999 at The Start, and at Wahroonga and Nkandla in 1999. When eggs were found, the supporting twig was marked, using plastic electrical tape as a tag, with a serial number. This tag lasted several months, avoided duplicative sampling and made records on each egg possible. The status of each egg was categorized into 'hatched', 'damaged' or 'disappeared'. The date of egg hatching was also noted.

Before the prescribed fire at The Start in 1999, a firebreak adjacent to the maize field and pine trees had been burned about one month previously. The temperature of the ground surface and 10 cm below the ground (which was estimated to be the depth underground that the larva sheltered) were measured using a portable, electronic thermometer with a long probe (DeltaTRAK[®]) at the burned firebreak and unburned butterfly area on 28-29 May 1999.

The phenology of the host plant and response of the host plant after fire regimes were recorded for the different colonies, especially that of the newly-emerged seedlings. Ten 1 m x 1 m plots were randomly selected near the host plants at The Start and at Wahroonga on 14-15 November 1998, and the newly-emerged seedlings were counted. Further searching for newly-emerged seedlings also took place at the

burned area of The Start in December 1998. A total of 200 seedlings in three plots of variable size (two plots had 50 host plants, one plot had 100 individuals) were individually marked with a numbered stick to monitor their survival. The seedlings were individually reexamined every 2-3 months for one year. In addition, the width of host plant's stem was measured between the burned (n=40) and unburned (n=40) plots in 1998.

Host ants *Camponotus natalensis* were collected using test-tube pitfall traps from April 1998 to January 1999 at three-monthly intervals (Details of methods, see Chap. 3 p. 38).

Assessing and monitoring the potential threats to the butterfly were carried out principally at The Start, where a detailed Geographic Information System (GIS) dataset run by SAPPI, was available. Using both GIS and a Global Positioning System (GPS) for mapping, the precise flight-paths, egg distribution and the host plant positions could be assessed. In addition, the precise distribution of the threatening invasive alien bramble (*Rubus cuneifolius* Pursh) was also mapped at The Start in 1998. Removal of bramble and other alien plants (e.g. pine, wattle *Acacia mearnsii* De Wild and bugweed *Solanum mauritianum* Scop.) were carefully monitored at this site in 1999.

An attempt was made to propagate the host plant, to improve availability of oviposition sites at The Start. Seed-pods were collected from the host plant at The Start and Wahroonga in May and June 1999. The seed-pods were kept for two months in the laboratory, and were exposed to direct sunshine to stimulate dehiscence. One hundred seeds were cold-treated in a refrigerator (about 4°C), and another 100 seeds were kept at room temperature (about 20°C) for a week. Finally, the seeds were exposed to sunshine (temperature reached a maximum of 45°C at noon) for two days. After treatment the seeds were planted out in pots in the nursery at Wahroonga.

In a further test, seeds were immersed in hot water (80-90°C) for three minutes, after which time the testa swelled and the apical meristem appeared. The seeds that did not germinate were repeatedly immersed in hot water, as above, until they germinated. The seedlings were then planted out in the greenhouse at Wahroonga. With regular watering over two months seedlings reached a height of 3-10 cm. In total, 200 seedlings were selected and transplanted to the field in December 1999, and examined during March 2000.

5.5 Results

Effects of fire regimes on the butterfly, host plant and host ant

Fire regimes

The known sites, except Nkandla, have prescribed rotational burns between late June and October (Table 15).

Table 15. Fire regime and potential threats at four *Orachrysops ariadne* sites.

Site	Fire regime	Potential threats	Habitat size (ha)
The Start	Rotational burn	Alien plants	1
Wahroonga	Rotational burn	Alien plants	10
Stirling	Rotational burn	Alien plant, cattle grazing	1-2
Nkandla	Unknown	Alien plants	5-10

There were no records of fire events in the distant past, with records for only the last few years. The south-facing slope at The Start is divided into two sections, each of which receives prescribed burning in alternate years, between 15 June and 31 July. This burning programme has been followed from at least since 1985 (D. Deppe, pers. comm.). The last burn prior to this study was on 2 July 1998, in the eastern section (adjacent to the road) (Fig. 47). The dry conditions in 1999, prevented burning because of the risk of a ‘hot burn’ that might have irreversibly damaged the vegetation (T. Melle, pers. comm.). A firebreak at the top of the site, adjacent to maize fields and pine plantation was burned annually at the end of May to circumvent runaway fires.

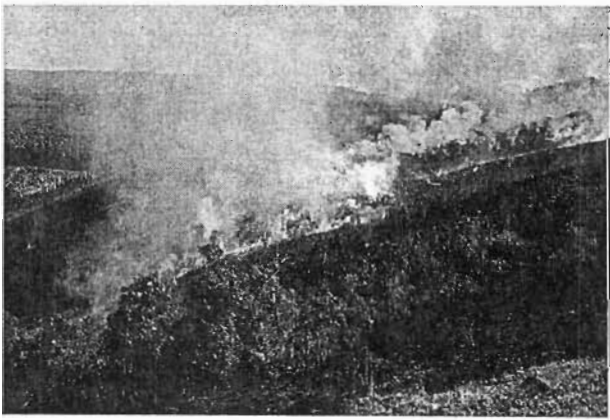


Figure 47. The fire regime at one of the sites, The Start on 2 July 1998.

The current management at Wahroonga caters primarily for the indigenous flora, with rotational spring burning (usually after the first rain) being practiced. The whole site was burned on 26 August 1998. Earlier fire records indicate that burns took place on 23 August 1996, 28 September 1992 and 9 September 1991, but the extent of these burns are not known. At Stirling, the most recent burning prior to this study was on 27 October 1998. Most of this farm was burned annually for summer cattle grazing, but

the specific area inhabited by *O. ariadne* was usually burned every second year. However, both at Wahroonga and Stirling there was no burning in 1999 as the risk of a ‘hot burn’ was too great. The burn history for the Nkandla site is unknown, but from observation of the stem bases of the host plants, it is clear that the site has not been burned for several years.

The butterfly

A total of 94 hatched eggs were recorded at The Start in 1997, 122 in 1998 and 90 in 1999. At Wahroonga, 170 hatched eggs were recorded in 1999. Almost all the eggs (97%, $n = 476$) hatched before the end of May (Fig. 48). At Nkandla, on the first visit on 18 May 1999, 90% (123/137) of the eggs had already hatched. At both Wahroonga and Stirling, the prescribed fires in 1998 burned all of the butterfly habitat. Nevertheless, the butterfly was on wing in 1999 at both sites, suggesting that the fire did no affect either the adult or the egg stages, probably because the fires were in the period June to October when the larvae were underground (Table 6, see in chapter 3).

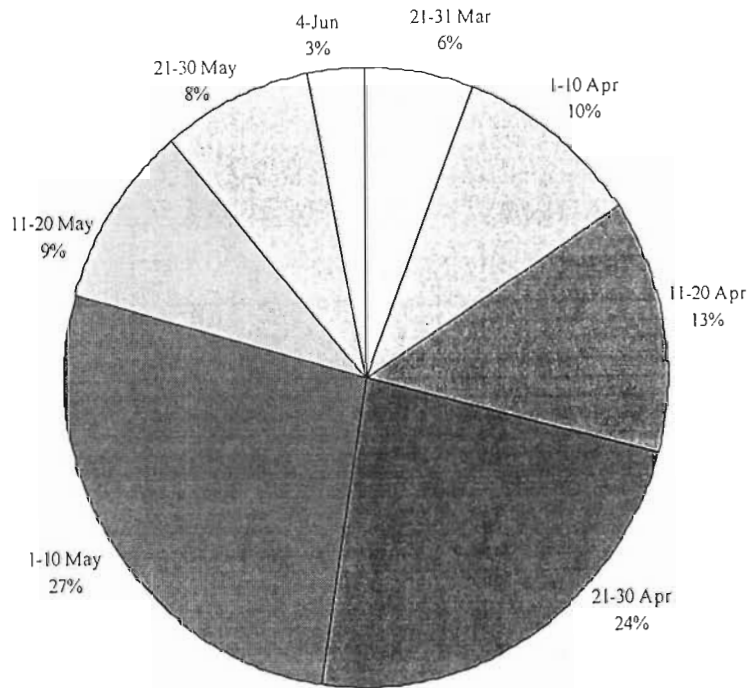


Figure 48. Percentage of total hatched eggs between late March and early June in 1997-1999.

The hatched larvae were underground for about eleven months, during which time the burned area was initially without vegetation cover and exposed to direct sunshine.

The temperature at the soil surface fluctuated greatly in the burned areas, with diurnal temperature differences reaching 30°C (Fig. 49). In the unburned areas with vegetation cover, the soil temperature difference was less, although still a range of 20°C (Fig. 49). Both in the burned and unburned areas, the temperature difference at a depth of 10 cm below the surface were minor, and in the unburned area there was only a 2°C diurnal temperature difference (Fig. 49).

The height of the vegetation affected micro-habitat temperature, with dense, tall grass being cooler and more stable than short grass. For example, on 4 August 1999, on the surface of burned, bare ground at noon was 30°C, 19°C at the base of unburned, short grass, but only 11.5°C at the base of unburned, dense, tall grass.

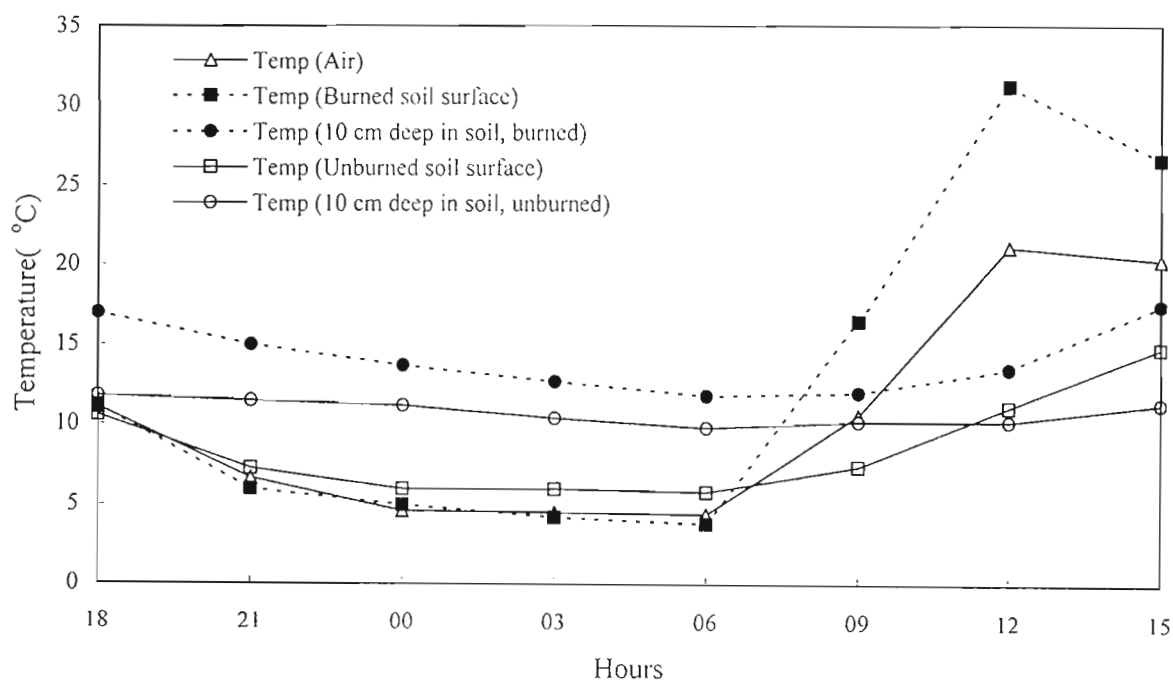


Figure 49. Diurnal air and soil temperature changes in burned firebreak and unburned grassland at The Start on 28-29 May 1999.

The host plant

The host plant *Indigofera woodii* var. *laxa* is an indigenous perennial legume, reaching a height of ca. 1m on moist, south or southwest-facing slopes. The phenological cycle of the host plant (Table 6) starts after complete removal of above-ground foliage after a burn (Fig. 50). The new stem then flushes from the rootstock and grows vigorously after the burn (Fig. 51), and with the onset of spring rains. The average width of the host plant stem is 4.58 ± 2.28 mm (n=40) on the unburned plot, 2.87 ± 1.04 mm (n=40) on the burned plot in 1997. There was a significant difference of average width of host plant stem between the burned and unburned plot ($t=4.3$, $p<0.0001$). Flowering is from December to April, with a peak between January and March. Seed pods are produced from March to May, with dehiscence during May and June (Table 6).

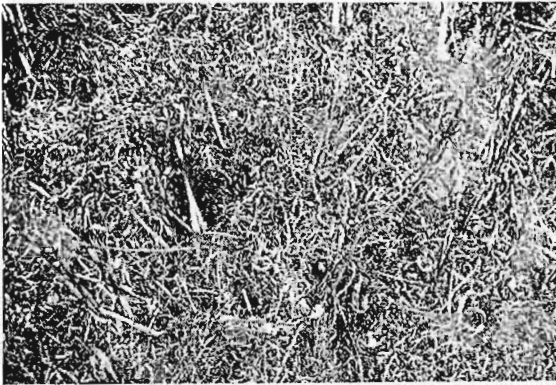


Figure 50. Above-ground host plant *Indigofera woodii* var. *laxa* foliage and stem die off after burning.



Figure 51. The new stem flushing from the old host plant *Indigofera woodii* var. *laxa* rootstock.

Seedlings emerged in September or October 1998 at The Start, and from October to December 1998 at Wahroonga and Stirling (Wahroonga was burned 1.5 months later than The Start). This means that in October, seedlings were 1-2 cm tall at The Start, but 0.5-1 cm at Wahroonga. The seedlings were very unevenly distributed both at The Start and Wahroonga. The density was $15.0 \pm 22.1/\text{m}^2$ (mean \pm SD, n = 10, range 0-74) at The Start, but only $4.4 \pm 3.4/\text{m}^2$ (mean \pm SD, n = 10, range 1-13) at Wahroonga in the middle of November, where seedlings were still busy emerging. The important point is that at Wahroonga seedlings were more variable in age and size than at The Start. The interval between burns was between two and four years at Wahroonga, but usually every other years at The Start.

In the burned area at The Start there were many new seedlings, unlike in the unburned area. Very few seedlings appeared in the unburned area before December, and those that did, appeared in the footprints or paths caused by trampling during the study, with few seedlings in dense vegetation and thick litter in the unburned area. Seedlings in footprints, however, were soon smothered by fast-growing grasses and forbs.

The survivorship of 200 young seedlings of *Indigofera woodii* var. *laxa* from three plots in the burned area of The Start showed a similar trend in three plots, varying between 40% and 60% survival after one year (Fig. 52). With arrival of the dry season in April, survival of the seedlings dropped, although once the seedlings were six months old, survivorship stabilized (Fig. 52).

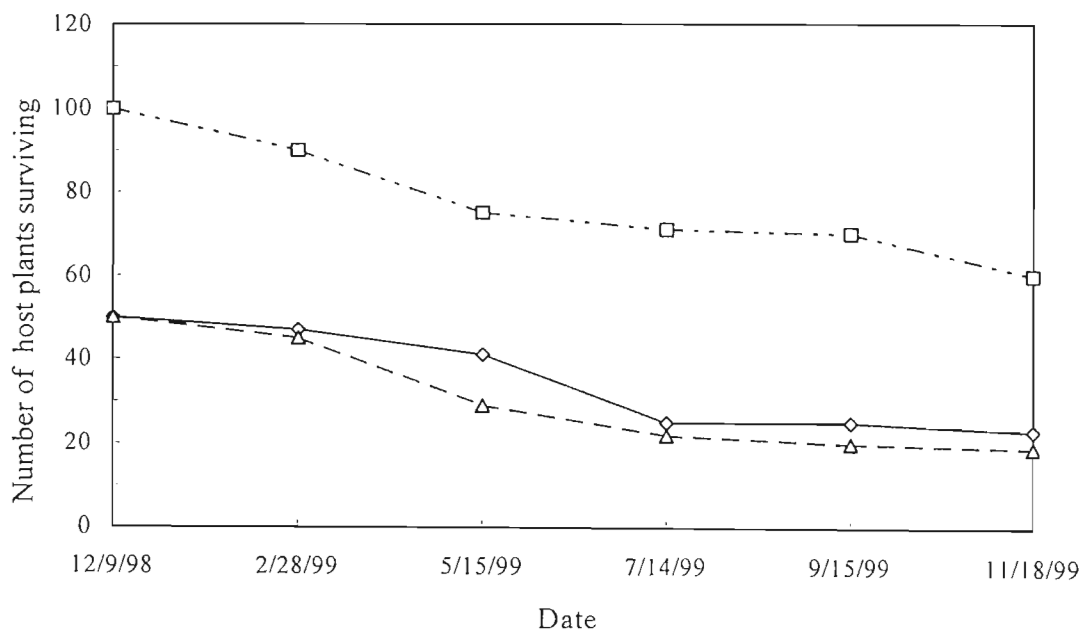


Figure 52. Survivorship of an initial 200 seedlings of *Indigofera woodii* var. *laxa* in three plots at The Start. One plot had 100 individuals, and two plots had 50 individuals each.

The host ant

Activity levels of the host ant was seasonal (Fig. 30). By the end of May, most of the butterfly larvae had hatched and the number of ants dropped, indicating that the

timing of the butterfly larvae hatching was coincident with peak activity of the host ants (Fig. 30, see details in Chap 3).

Habitat enhancement

Control of alien plants

The major threat to the butterfly colonies are alien plants (Table 15), especially in the case of the small (one hectare) site at The Start. At this site it is essential to remove bramble *Rubus cuneifolius*, especially on the north-facing slope next to the pine plantation, down to the bottom of the valley. The south-facing slope, where the butterfly is resident, has thick, but small, stands of bramble within and around the habitat. Some initial penetration of the habitat by wattle *Acacia mearnsii* and bugweed *Solanum mauritianum* individuals is an additional potential threat.

After GPS analysis (Fig. 53), and after discussion with the custodian of The Start, the bramble was removed on 6-7 January 1999 (Fig. 54). This was done under close supervision and careful knapsack application of 'Brushoff' herbicide (concentration 2.5 g/l) on bramble foliage with minimal disturbance to the habitat. One week after application the bramble had wilted (Fig. 55) and was dying, although some individuals survived the treatment.



Figure 54. Spraying 'Brushoff' herbicide for removal of bramble.



Figure 55. Wilted bramble after one week spraying of 'Brushoff' herbicide.

When The Start was registered as a Natural Heritage Site in 1996, some initial removal of alien plants was done. Pine saplings on the north-facing slope were also removed, to avoid future threats from shading by mature pine trees and from the destructive effects of later removal of large trees. This was clear when comparing

photographs of the habitat taken in 1997 and 1999. These show that the pine saplings on north-facing slope had grown to a height of about 2 m and had become threatening in only two years (Fig. 56). Removal of pine saplings (Fig. 57) was then undertaken on 13-17 August 1999, together with removal of wattle and bugweed.

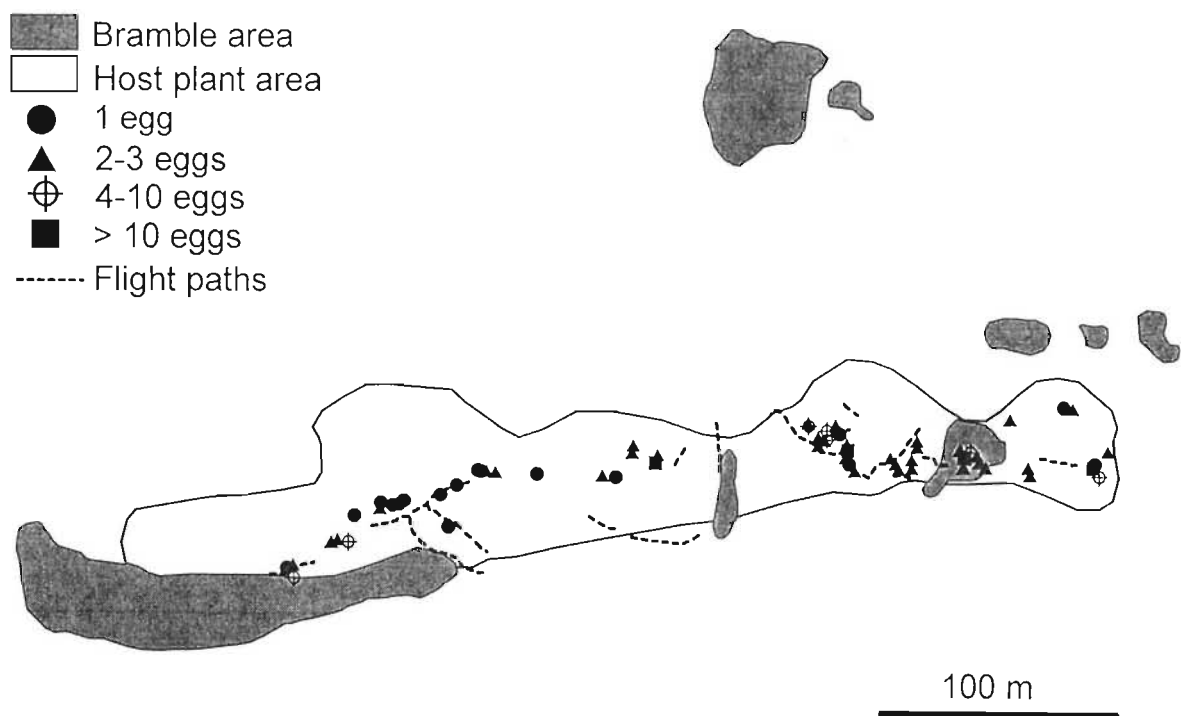


Figure 53. The GPS mapping of butterflies, eggs and host plants at the site The Start, with the potential threat from alien bramble.



Figure 56. Visible pine saplings on north-facing slope.



Figure 57. Removal of pine saplings.

The Wahroonga colony is much less threatened, being 10 ha in extent, and with a partial surrounding buffer of indigenous grassland and a strip of Mistbelt forest. However, this colony is threatened by a timber trail along one boundary, which is heavily invaded by bugweed. This situation needs close monitoring. Another threat at Wahroonga is an adjacent, heavily-grazed pasture. Although a fence separates the cattle from the butterfly colony, the grazed pasture nevertheless restricts the size of potential suitable habitat.

Most of the Stirling site is burned annually for cattle grazing, although the butterfly habitat was also burned on a rotational basis, with entry by cattle to this site only occurring in summer. There were 18 cows on the site during the 1999 survey, which caused some trampling of the habitat. The site was also limited by adjacent intensive grazing pastures, which stimulated invasion by bramble. As this colony was only discovered during this study in 1999, we have no records on historical aspects of the butterfly population that may serve to elucidate the effects of cattle at this site. It is essential to continue to monitor these grazing and trampling impacts.

At Nkandla, although the site is within a natural reserve, local people nevertheless graze cattle there. At this site, alien bramble has begun to seriously impact on the area.

Propagation of host plant

In November 1998, 50 young seedlings of the host plant (Fig. 58) were collected from the field at The Start, and transplanted onto the south-facing slope, adjacent to the butterfly colony. The plants grew very slowly and became smothered with grasses, resulting in only ten individuals surviving. This indicates that nurturing of established host plants is essential.

Only two and three seedlings, respectively, emerged from the seeds taken to the laboratory for one week of cold treatment and one week of room temperature treatment. These poor results led to further tests. Seeds were soaked in hot water (80-90°C) for 3-5 min. After the seed coat had swelled and fractured, and the apical meristem had emerged, the

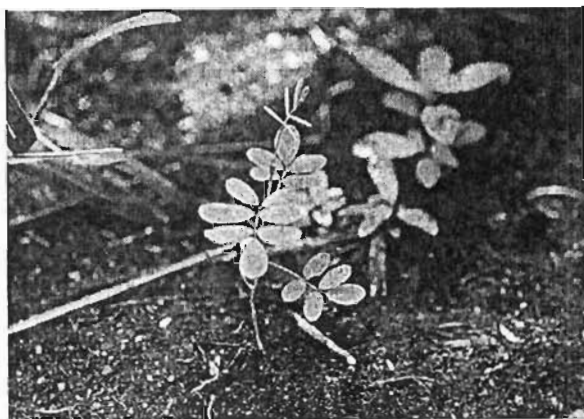


Figure 58. Young seedlings of the host plant

Indigofera woodii var. *laxa*.

seeds were planted in potting soils in the nursery. Seeds that did not germinate were soaked again, and all resultant seedlings were cared for in the nursery for 2-3 months, until the seedlings were 3-10 cm height. In total, 200 of these cultured seedlings were planted in the field at The Start, adjacent to the butterfly colony on the south-facing slope where there were no host plants in December 1999. They were watered regularly, but only 20 individuals survived until March 2000. At this time half of them were only about 5 cm tall, while the other half were 20-30 cm tall.

5.6 Discussion

Effects of fire regimes

Maintenance of fire regimes to avoid vegetation becoming overgrown and deterioration of the habitat is important for conservation of some butterflies (Deutschlander and Bredenkamp, 1999; Kwilosz and Knutson, 1999; Murata *et al.*, 1998; New *et al.*, 2000; Schultz and Crone, 1998; Swengel, 1994). Murata *et al.* (1998) concluded that routine fire-burning can have a positive effect on growth of host plant as well as of nectar plants for the rare lycaenid species *Shijimiaeoides divinus asonis* (Matsumura). Indeed, fire is a useful management tool, in general, for certain systems

when used under appropriate conditions (Bond and van Wilgen, 1996).

The timing of the fire regime has to consider the life cycle of the species in question (Sutherland, 1995). In this study, almost all the eggs had hatched before the end of May. This fact has important implications relative to the timing of current fire regimes. The prescribed fires were between late June and October at three colonies. Such timing does no direct damage to the egg stage or adults. However, fires at the time of egg laying (March to May) are likely to be highly adverse to the butterfly's survival (Henning *et al.*, 1997; Swanepoel, 1953). In addition to having no prescribed burning at this time, it is also necessary not to spray any herbicide for firebreaks at this period, when the host plant may suffer.

From the evidence at both Wahroonga and Stirling, the burns recorded here were over the whole colony area, yet both colonies continued to thrive. It seems that when the larva is underground it is able to survive fire. For invertebrate conservation, normally only a fraction of a site is burned at any one time (Kirby, 1992). In future years, it would probably be prudent not to burn the whole area annually, particularly since annual fires are probably unnatural at any one of these localities. In the interim, for management of these butterfly colonies, it is important to carefully record the fire events that take place and to assess the outcomes thereof (Hirons *et al.*, 1995; Kirby, 1992).

From present observations, the mature host plant appears to survive and respond to fire very well. After fire there is regrowth of young shoots, flowering and fruiting. Many seedlings appeared in the burned areas, but few in the unburned ones. It seems that fire stimulates, and may even be essential for seed germination. These observations need to be examined carefully because one anecdotal observation can have many different explanations (Swengel, 1994), and may even be misleading.

Fire can stimulate seed germination in many ways (Bond and van Wilgen, 1996; Brown, 1999), although it may not be obligatory for germination of host plant seed, since some seedlings appeared in unburned areas. The laboratory seed-germination test supports this contention, with seeds germinating in the absence of fire. Nevertheless, the seed coat became permeable after heat (both from flame and hot water) and fractured, enabling the seed to germinate (Brown, 1999). The seed can probably resist fire, as many seedlings emerged after the fire in the field. However, when the fires are too frequent it may be damaging to young seedlings (1-2 years old)

owing to their shallow root system.

Human and cattle footprints also stimulate seed germination. This mirrors the situation for *Maculinea rebeli* in the Palaearctic Region, where footprints of animals produce bare soil patches that stimulates growth of the gentian host plant (Dolek *et al.*, 1998). The trampling effect here is worth further investigating as an adjunct for management of the Karkloof blue. In particular, it is important to monitor the effects of grazing at the Stirling site. There are many successful examples of conservation grazing for grassland management, but mistakes have been also made (Oates, 1995). Caution is required when using any 'new' practice rather than locally 'traditional' management. In the cattle-grazed fields adjacent to the Wahroonga and Stirling sites, the impact was so high that the host plant had disappeared.

Current fire regimes appear to be suitable for the host ant, which is largely inactive during winter and early spring. Also, this ant is mostly nocturnal, which also, helps it to escape prescribed daytime fires. Knowledge of the life histories of the butterfly and host ant is essential for managing fire regimes (Sutherland, 1995). Indeed, current fire regimes do not directly harm the butterfly, host plant or host ant. This is important because butterflies are particularly vulnerable to fire during their main breeding season (Henning *et al.*, 1997).

In addition, the fire regime has some benefits for the habitat maintenance. First, fire reduces the fuel load, avoiding runaway fires, especially in view of the high frequency of lightning strikes in KwaZulu-Natal (Manry and Knight, 1986). Second, burning can also stimulate the regrowth of young shoots by removing old material and by adding nutrients to the soil (Curry, 1994), avoiding degradation of the vegetation. Third, grass litter may suppress seedling emergence by reducing seedling germination and/or by preventing shoot extensions (Bosy and Reader, 1995). Thus, burning creates opportunities for seed germination. Finally, in these high-frequency lightning areas, prescribed fires simulate natural fires, maintaining the original, natural condition (Samways, 1994).

Practical habitat maintenance

Control of alien plants

It is essential to continue to assess the current threats at each site (Hirons *et al.*, 1995). The most serious threat is from alien plants, which are continuing to expand,

outcompeting the indigenous flora. Such alien vegetation invasion is extremely difficult to reverse and very expensive to control on a large scale (Henning, *et al.*, 1997). Early detection and early action against invasives are critical, before weed populations grow exponentially (Hobbs and Humphries, 1994). Bramble is a particularly serious problem at The Start, where the *O. ariadne* colony is closely associated with timber plantations that act as source for these invasions (Macdonald and Jarman, 1985). The colony at The Start is confined to a narrow, south-facing, steep slope, adjacent to plantations. Being so small (1 ha), this colony is highly susceptible to disturbance and invasion. This colony has also been invaded by wattle and bugweed, that must also be controlled.

Bramble is one of the most widespread and serious alien invasives in KwaZulu-Natal (Bromilow, 1995). When ripe the fruits are readily eaten and dispersed by animals. In the adjacent areas, as well as on the north-facing slope, if bramble is not removed, it will become a serious threat for The Start colony. Currently bramble at The Start is controlled in January. However, it may be more effective if it is removed before fruiting, in October or November, thus decreasing seed dispersal. Also, when flowering in October, the white flower is easily visible from some distance, making detection of small plants easier.

It is important to carefully survey the current distribution and status of bramble at this colony and in adjacent areas using GPS (Nordmeyer *et al.*, 1996). This will enable detailed monitoring of control measures against bramble and at the same time determine whether the size of the colony increases with increased host plant area. Most importantly, this must be done in close association with the custodians of The Start, so that the scientific and practical components operate synergistically.

Some bramble plants inevitably escape herbicide treatment. Post-treatment assessments are therefore essential. Long-term monitoring of these alien plants, along with rapid control, will thus be necessary (Morrison, 1997). Based on mapping of the bramble, small patch treatment may be sufficient (Nordmeyer *et al.*, 1996). Because it would be cost-effective, it would minimize risks to the butterfly and decrease disturbance (Morrison, 1997), including impact on any not-target species. Furthermore, removal of other alien plants needs to be done simultaneously, as removal of bramble provides pioneering opportunities for the other aliens, especially wattle and bugweed.

Propagation of host plant

Resource availability is a critical factor in determining the distribution and abundance of species. Therefore augmenting the adult and larval resources, which play an important role in management of rare butterflies, can be important (Schultz and Dlugosch, 1999). Propagation of the host plant was attempted here that provided some guidelines for future initiatives.

Host plant *Indigofera woodii* var. *laxa* pods need to be collected and dried immediately before dehiscence, which is in May and June. If collection is too early the seed is not mature, and if it takes place too late, the seed pod will already have dehisced. The collected seed pods should be exposed to sunshine to stimulate release of the seeds. Collected seeds may be distributed directly into area unoccupied by the host plant, especially where there are open gaps, no alien invasives, and a reduced fuel load. It was found here that after hot-water treatment the seed germinated readily. This permits large-scale propagation in pots for transplantation to the field.

The results suggest however, that it is important to allow for the establishment of a good root system before transplantation. Transplanting young seedlings from the field directly is another possibility, but care should be taken to avoid damage to the original host plant population. Although only a few survivors may form the core of a new, self-perpetuating population (Zaremba and Pickering, 1994), this core may be sufficient to expand the butterfly colony. However, seedlings take time to mature, and the plant-butterfly-ant interactions may take time to establish (Howell and Jordan, 1991).

It is essential to time the planting of young seedlings carefully. This is best done on cool days during the rainy season, when the arduous task of watering is reduced. Occasional drought readily causes young seedlings to die, as in the case of *Lupinus perennis* L. (Zaremba and Pickering, 1994). After six months growth in the field the seedlings are sufficiently robust to resist drought. Finally, it is essential that the seedlings must be kept free from overgrowth by other plants.

Chapter 6. General Discussion

The Karkloof blue butterfly *Orachrysops ariadne*, is endemic to KwaZulu-Natal province, South Africa, with very few colonies present i.e. the species has the three rarity attributes (Rabinowitz, 1981): small geographical range, narrow habitat specificity and small local population size. Clearly, the species has a high conservation priority. Such rarity categorization can serve a useful framework for insect conservation biology (Samways, 1994).

The flight period of *O. ariadne* is from March to April, which is different from all the other species in the genus *Orachrysops*, which are on wing from September to December (Henning and Henning, 1994). This unique attribute may have particular bearing on the conservation of *O. ariadne*. Another important rider is that the butterfly has a high public profile. It is the logo of the Midlands Meander, and is constantly in the eye of the public.

The key to protecting and managing a rare or endangered species is to understand its natural history or autecology (Primack, 1993). The studies in this thesis address a wide range of aspects, including the Karkloof blue butterfly's life cycle, developmental stages, behaviour, geographical distribution, habitat requirements, population structure and so on. All these aspects contribute toward the conservation of the butterfly. It has proved to be a worthy, yet difficult subject for scientific study, both in the field and the laboratory, in particular because of its complex life history and habitat requirement. Nevertheless, the biological and ecological research has produced valuable information as background for conservation management of the butterfly.

The behaviour and life history of this butterfly were studied in detail in chapter 2, including the adult, egg and larval stage. This study confirmed that the oviposition and host plant is an upright variety *Indigofera woodii* var. *laxa*, and clearly different from the prostrate *I. woodii* var. *woodii*. In addition, eight species of nectar plants were recorded. The distribution of both the host plant and the nectar plants may be the two limiting factors in the geographical distribution of this butterfly (Murphy *et al.*, 1984; Schultz and Dlugosch, 1999). Therefore, understanding the host plant and nectar plants indicates why the butterfly is so limited in distribution, particularly with respect to its oviposition

and host plant which is very rare.

All species in the genus *Orachrysops* Vari 1986 are endemic to South Africa, and four of the 11 species are on the red-list (Henning, 1995). Currently, we have a very incomplete knowledge of the life history of species of *Orachrysops* species in general (Henning and Henning, 1994; Heath, 1997, Heath and Claassens, 2000), except for *O. ariadne* (Lu and Samways, 2001) and *O. niobe* (Williams, 1996). This study established that the butterfly larva is associated with the host ant *Camponotus natalensis* (F. Smith). Although the fine details of the interaction between the *O. ariadne* and its host ant are still not fully known, this nevertheless, is a major first step in solving the complex relationship between the larva and its host ant. This ant association has important conservation implications, as it means that conditions for complex myrmeophilous behaviour must also be considered. Furthermore, study on life history of *O. ariadne* serves as a model for studies on other species in the genus.

The study here confirmed that, it is particularly valuable to use eggshell remains for assessing egg population status. Eggshell remains have some benefits, particularly for this rare species with its short flying-period. Owing to the rapid, elusive flight of this butterfly over dense vegetation, the adult population and distribution level are difficult to assess. Clearly, the eggs are good surrogates and have great potential for plotting the local distribution of the breeding sites, and as well as for locating new colonies. In addition, annual egg-count monitoring has proved important for assessing population levels over time.

Chapter 3 compared the ecological conditions at the four known locations so as to make informed decisions regarding the butterfly's conservation. With four colonies now known, it was possible to make some initial comparisons and ascertain similarities. All sites are within the high-rainfall Moist Mistbelt area. *O. ariadne* only occurs on the south-facing slopes, where the host plant and host ant co-occurred. Predictions on the habitat and habitat requirements of this species are necessary for developing a conservation strategy and action plan.

This part of the study emphasized the necessity for searches for further sites for this habitat specialist, as the Mistbelt grassland has largely gone. With the knowledge gained from habitat comparisons at different sites, locating the

south-facing slope with host plant and host ant could enhance the possibility of finding further colonies, as was done in this study with location of the Stirling site. Amateur lepidopterists have also played an important role in providing data which contribute towards conservation of the species (Henning and Henning, 1989). Furthermore, using existing knowledge and cooperating with amateur lepidopterists and botanists, greatly enhances the possibility of locating new or potential sites.

Population structure and movement of *O. ariadne* were studied by the mark-release-recapture (MRR) method in chapter 4. The Jolly-Seber model was used to estimate population parameters. *O. ariadne* is a remarkably rare butterfly, averaging only 10 individuals ha^{-1} within a small colony. The current population size and density estimates of *O. ariadne* suggest that the population levels are probably too low to sustain any loss of individuals. This emphasizes that great care must be exercised when using MRR studies on this threatened species with such small colonies (Murphy, 1988; Mattoni *et al.*, 2001). Local movements and spatial distribution were also analysed. Knowledge of dispersal behaviour of this threatened species is of crucial importance for long-term population persistence (Mousson *et al.*, 1999).

It is remarkable how this species continues to survive at such low population levels, especially at The Start and Stirling, each of which is only about one hectare in size. In such small populations, even a minor change in any of the environmental factors could have adverse effects. In general, the small and isolated populations are easily subjected to debilitating effects of demographic instability, genetic deterioration and natural or anthropogenic catastrophes. But in the southern hemisphere, the remnant patches have had a long geological history. The potential problems such as inbreeding depression, demographic stochasticity, and adverse effect of environmental stochasticity and catastrophes, may have been overcome through time (Samways, 1995). Occasional gene flow may be enough to continue to overcome the risk of a genetic bottleneck. Prior to anthropogenic impact the habitat was likely to be relatively stable. The greatest risks were probably heavy rain and fire. The young larva avoids these by being present largely after the close of the rainy season and before winter fires.

Habitat management is the key to success in the conservation of endangered

butterflies (Primack, 1993). It is especially valuable to apply current research results to conservation management. The effects of current fire regime on the butterfly, host plant and ant host were evaluated in chapter 5. Fire is a useful management tool if applied in the right way under the correct and appropriate conditions. To minimize the possible detrimental effects of fire on Karkloof blue butterfly populations, managers should instigate appropriate fire management strategies. Prescribed burning should only take place after the larva has hatched and gone underground with the ant host. Alien invasive plants, which are a major threat, should also be removed so as to increase the area of quality habitat. Guidelines are also provided here for propagation and introduction of the host plant, so as to provide the butterfly with more oviposition sites.

The extent and rate of land transformation and habitat modification in KwaZulu-Natal is greater than in any other country or province in southern Africa. A large proportion of the grasslands in KwaZulu-Natal has been transformed by afforestation and cultivation. At least 92% of the Natal Mistbelt has been transformed (Armstrong *et al.*, 1998), only 1% Mistbelt grassland remains (Armstrong pers. comm). With continued habitat fragmentation, the biological diversity in KwaZulu-Natal is likely to continue decline. Preservation of native remnants, as well as management plans that recognize the key role of invertebrate, are essential for the long-term health of the remnant ecosystems.

As the Karkloof blue butterfly is so rare and inhabits such a threatened habitat type, the proposal here is that *O. ariadne* be considered as a 'flagship' species for indigenous and undisturbed grassland, at least where there is concordance between butterfly, host plant and host ant. Saving enough of the remaining Mistbelt grassland is a crucial issue, not just for the survival of this species, but also for the Mistbelt grassland community as a whole. In conclusion, if single species programmes are undertaken while considering the value of the habitat as a whole, then conservation of biodiversity can be achieved by focusing on the needs of this species (Pullin, 1997).

At present, two of the *O. ariadne* sites (The Start and Wahroonga) have been registered as Natural Heritage Sites. Such sites come about through agreement

between stakeholders and not upon legislation (South African Natural Heritage Programme, Department of Environmental Affairs). This programme is an important development for encouraging landowners to participate actively in conservation work. However, the owner of the Wahroonga site is currently looking for a buyer (Kunhardt, pers. comm.), so, the fate of this colony may be dependent on the attitude of the new owner. The Wahroonga site is also special as it is the habitat of at least four rare species of plants, making its value a reserve in general. Liaison with landowners and the local conservation authority (KwaZulu-Natal Wildlife), on how to maintain the extant colonies is the first priority, especially bearing in mind, that it is more feasible to keep the extant colonies intact than to restore degraded sites in the future.

Maintaining the current, extant Karkloof blue butterfly populations and encouraging practices that do not contribute to population declines in the Mistbelt area are important conservation priorities. In fragmented landscapes, emphasis is placed on the management of habitat fragments and the better integration of protected areas into the surrounding landscape (Warren, 1993). The problem for *O. ariadne* is that there is little suitable or potential habitat, with only about 1% of good quality Mistbelt grassland remaining (Lu and Samways, 2002). Even where suitable vacant habitat exists, it may be difficult for *O. ariadne* to cross unsuitable habitat, such as plantations or crop fields.

The large-scale deterioration of grassland and habitat fragmentation is threatening the survival of this species *O. ariadne*. Availability of suitable habitats and the spatial arrangement of such habitats in the fragmented landscape is crucial (Armstrong, 2002). It is not enough simply to protect and manage suitable habitat in isolated reserves, with the rest of the landscape playing an important role in the long-term survival of a geographically-restricted butterfly population (Thomas and Hanski, 1997; Warren, 1993). Understanding the population structure and mobility of *O. ariadne* is an important dimension for its conservation, especially in the highly fragmented landscape. How the extant colonies should be managed and how they are genetically connected in this fragmented landscape (Fischer *et al.*, 1999), is an important consideration for conservation of *O. ariadne*. As conservation priorities in fragmented landscape require the establishment of network of suitable habitats (Mousson *et*

al., 1999), it is now timely to find out whether there is sufficient gene flow between populations to overcome long-term bottlenecks, and to find new colonies .

Metapopulation theory is a powerful tool to predict the future of populations within a fragmented landscape (Neve *et al.*, 1996). About three-quarters of British butterflies and 60% of Finnish butterflies appear to have a metapopulation structure, and most threatened species that have been studied in detail conform to this pattern (Thomas and Hanski, 1997). The biased impression of population isolation may be caused by the lack of large-scale mark-release-recapture studies (Mousson *et al.*, 1999). However, with the limitation of number of suitable sites for mark-release-recapture of *O. ariadne*, it is not possible to undertake a study of this species as a large-scale. Nevertheless, a first study was made here which showed at least that the butterfly is a strong flier.

In conclusion, there is still some lack of knowledge for assurance that there will be long-term survival of the Karkloof blue butterfly. There is still much to learn, but this is no excuse for delaying any conservation action, conservation biology being a 'crisis discipline' (Soulé, 1985). With the existing data, we can now make some informed decisions as to its conservation management and gauge what information needs to be collected in the future. This involves participation by all: land owner, conservation agencies and researchers.

Chapter 7. Conclusions

The Karkloof blue butterfly *Orachrysops ariadne* (Butler) is endemic to KwaZulu-Natal, with only four colonies known. It has specialized habitat requirements and a late-season flight period. These aspects are significant for conservation planning for the species, which has high conservation priority. An important rider also, is the high public profile of the butterfly, which is the logo of Midlands Meander, and thus is constantly in the eye of the public.

This thesis addresses a wide range of aspects regarding Karkloof blue butterfly conservation. Research on the life history and behavioural ecology has produced information which has significant management implications. This study confirmed that the host ant for *O. ariadne* is *Camponotus natalensis* (F. Smith), and host plant is *Indigofera woodii* var. *laxa* H. Bol., an erect variety. Eggs are valuable for the ascertaining the species' local distribution, and for locating potential colonies. Yearly egg-counts can be used for population monitoring.

Prescribed fire should not take place until the larva has hatched and has entered an ant's nest. Therefore no burning must occur between the beginning of March and mid June. Late June to the end of October is the optimal timing for burning. The fire regime should be on a rotational basis, between two and four years, with whole-area burns at one time being avoided. It is essential to carefully record every management action undertaken and its consequences. Monitoring the threat of alien plants is essential, and early removal of them is crucial.

The Karkloof blue butterfly is a rare species that inhabits the threatened Mistbelt grassland ecosystem of KwaZulu-Natal. This study has emphasized the necessity for searches for further sites for this habitat specialist. This is an ongoing and urgent task, as the Mistbelt grassland has largely gone, with only 1% remaining. Saving enough of the remaining Mistbelt grassland is a vital issue, not just for the survival of the butterfly, but also for the Mistbelt grassland community as a whole.

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