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# Behaviour and Ecology of the Primary Parasitoids *Cotesia urabae* and *Dolichogenidia eucalypti* (Hymenoptera: Braconidae) and their Host *Uraba lugens* (Lepidoptera: Noctuidae).

By

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October 1989

TO MY PARENTS

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## Summary

There is a large complex of parasitoids associated with *Uraba lugens* Walker in South Australia. Field surveys from 1985-1987 have shown 11 primary parasitoids, 10 hyperparasitoids and one facultative hyperparasitoid within this parasitoid complex. Biological details about these parasitoids, including host stages attacked, phenology, pupal duration and adult longevity were documented. A comparative study of two koinobiont, solitary, larval, endoparasitoids within the complex, *Cotesia urabae* Austin and Allen and *Dolichogenidia eucalypti* Austin and Allen, was undertaken. Prior to this study neither species had been described and no details of their biology recorded.

Preliminary observations of *C. urabae* and *D. eucalypti* had shown overlap in their phenologies and host stages attacked. Therefore a sampling program of collecting pupal cocoons in the field was set up which revealed differing patterns of host synchronization between these two parasitoids. During the summer generation of *U. lugens* which lasts approximately four months, both species typically oviposited in 1$^{st}$ instar larvae (small larvae), and then both completed a second generation by ovipositing in 4-5$^{th}$ instar larvae (mid larvae). However *D. eucalypti* emerged slightly earlier than *C. urabae* during its first generation in summer and emerged much later than *C. urabae* during its second generation; when most unparasitized hosts had pupated. During the winter generation of *U. lugens,* which lasts approximately eight months, both species again oviposited in small larvae but only *C. urabae* completed a second generation, which it commenced by ovipositing in 6-7$^{th}$ instar larvae (large larvae). *D. eucalypti* had only one generation ovipositing in small larvae and again emerging late during pupation of unparasitized hosts in the field. Continuity of parasitoids between host generations was maintained via the survival of adult parasitoids although by delaying pupation at the end of each host generation the shorter lived *D. eucalypti* had to survive fewer weeks as an adult than *C. urabae*.

*C. urabae* and *D. eucalypti* developed in a wide range of host sizes in the field and this affected their eventual adult size. The relationship between adult parasitoid size (wet

weight) and remaining host dry weight was described by an asymptotic curve. Parasitoids reached maximal weight late in each host generation when host size exceeded a certain threshold. Although adult sizes overlapped *D. eucalypti* was typically smaller than *C. urabae* but carried a greater load of fully developed eggs (up to 600). The number of fully developed eggs was positively correlated with adult wet weight in both species of parasitoid.

To further investigate the phenologies of *C. urabae* and *D. eucalypti*, a replicated temperature experiment was set up, using small and mid larvae, to test the importance of initial host size and temperature on the rate of development of *C. urabae* and *D. eucalypti*. Egg-larval development of *C. urabae* took almost twice as long from small larvae as it did from mid larvae whereas the reverse was true for *D. eucalypti* . For *C. urabae* development from small hosts appeared constrained by the host's development until the latter reached a critical size. At the lowest temperature tested (15°C), *D. eucalypti* developing from small larvae showed a prolonged physiological delay before emerging to pupate. Day-degree requirements and developmental thresholds were calculated for both species of parasitoid at each host size.

Concomitantly, experiments on the effect of temperature on the development of *U. lugens* were undertaken. Temperature and sex both influenced the number of larval instars of *U. lugens,* which varied between 6-14. Males underwent fewer moults than females and both sexes moulted more frequently at lower temperatures. Males developed more rapidly as larvae but developed more slowly as pupae. Developmental thresholds and day-degree requirements were calculated for the egg, larval and pupal stages of *U. lugens*.

To test the predictive value of the calculated temperature thresholds and day-degree requirements of *U. lugens*, *D. eucalypti*, and *C. urabae* these values were run in a computer simulation, together with the daily max.-min. temperatures over 1985-1987 in the Adelaide region. This simulation showed good agreement between the observed and the predicted duration of all life stages in the field.

The phenologies and temperature relationships of *D. eucalypti* and *C. urabae* indicated an 'avoidance' of large hosts by *D. eucalypti* in the field. Thus the behavioural interactions between *D. eucalypti*, *C. urabae*, and the three sizes of host attacked in the field were investigated. Of these three sizes, small and mid larvae are gregarious, whilst large larvae are solitary. The behaviour of *D. eucalypti* differed from *C. urabae* between different host sizes, with *D. eucalypti* making more visits to small larvae, ovipositing less in mid larvae, and failing to oviposit in large larvae. Both species of parasitoid were most successful attacking small larvae which were handled differently to mid and large larvae. Groups of small larvae responded to parasitoids by dispersing whilst mid larvae moved closer together. Rearing and/or thrashing behaviour frequently occurred amongst larvae being attacked and continued after the parasitoid departed. The chance of mid and large larvae being parasitized was decreased by up to 50% if they were rearing or thrashing immediately prior to an encounter with a parasitoid.

Small, mid and large *U. lugens* were tested for age related differences in their defensive behaviour. This was done by stabbing them with a micropin so as to standardize the attack stimulus. Small larvae were never observed to thrash or to move their body laterally during rearing and were the least responsive to attack. Defensive responses increased and locomotory responses decreased with increasing host age. Larvae still showed increased levels of rearing, regurgitation, and thrashing in response to attack up to two hrs after parasitoid attack. Changing host defensive behaviour with age and the influence of these behaviours upon the outcome of an encounter between a host and its parasitoid is not only relevant to the species studied here but also to current theories on host preference, host acceptance and superparasitism in parasitoids.

# Declaration

The work presented in this thesis is my own unless otherwise acknowledged, and has not previously been published or submitted to any university for the award of any degree or diploma. This thesis may be made available for loan or photocopying provided that an acknowledgment is made in the instance of any reference to this work.

(Geoff. R. Allen)

October 1989

# Acknowledgments

I would like to thank the many people who contributed to the eventual completion of my thesis. Early in my field work, Roger Bungey, Janet Farr and David Morgan all assisted in locating potential field sites suitable for *U. lugens*, although *U. lugens* eventually turned out to be typically absent from everywhere but the most public parklands of Adelaide! Several visiting postdoctoral fellows, with whom I was fortunate enough to share a laboratory, William Kirk, Tony Smith and Ian Dadour all provided helpful discussions of ideas, as did Gerrit Pak (Wageningen) who inspired me with his lectures on parasitoid behaviour.

Several staff within the Waite: Margie Morris (statistical advice for Chapter 4), Trevor Hancock (experimental design for Chapter 5), Sandra Pattison (log-linear contingency table analysis; Chapters 6 and 7), Scott Newman (SAS programming), David Messent (meteorological data), and Andrew Dunbar (printing of photographs) all aided with their expertise in the listed areas. Within the department of Entomology Gary Taylor, Janet Walker, and Rachel Short all gave friendly advice on departmental 'protocol' and equipment. Amongst the staff of the department Roger Laughlin, Derek Maelzer and Peter Miles provided useful advice, whilst Peter Miles additionally helped by reading earlier drafts of Chapters 2 and 6. The friendship of my fellow postgraduates is dutifully acknowledged, especially that of Chandra, Greg Rippon and Phil Weinstein - who eventually let me beat him at tennis. During my studies I was supported by a Commonwealth Postgraduate Research Award.

Invaluable amongst those who helped were my supervisors Andy Austin and Mike Keller whose respective taxonomic and behavioural backgrounds contributed to the scope of my critical thought, and directly to the end product produced. Mike especially bore the brunt of my questions, ideas, and problems, and I thank him for his patience. Finally I would like to thank Tracey whose companionship, support, and encouragement has kept me 'alive' throughout the long process of research and completing a thesis.

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# Preface

Parasitoid biology is a growing area of research and this is reflected in the literature by many recent reviews on the subject (e.g. Waage and Greathead 1986, Price 1987, May and Hassell 1988). Areas of interest in parasitoid biology include sex allocation, guild structure, population regulation and host selection. Parasitoid host selection has received much attention due to its importance in host specificity and biological control. Since last decade host selection has been broadly divided into five steps: host habitat finding, host finding, host acceptance, host suitability, and host regulation (Vinson 1975). Parasitoids have evolved many morphological, physiological and behavioural adaptations which deal with each of these five steps. Most parasitoid research has been within the framework of biological control and thus addresses the question of population control of pests by using parasitoids. However when trying to understand evolutionary adaptations that have occurred in hosts and their parasitoids, studies of huge populations of hosts in a situation of monoculture and of introduced hosts or introduced parasitoids are of limited value (Askew and Shaw 1986). To readdress this inbalance I set out to study an endemic parasitoid complex and then later concentrate the study on the ecology and behaviour of the two Braconid species within this complex. By doing so I hoped to find differences between these two parasitoids that reflected how two 'similar' species (diifferent tribes within the subfamily Microgastrinae; Mason 1981) have adapted to deal with the same host.

The central focus of this dissertation is the behaviour and ecology of the parasitoids *Cotesia urabae* Austin and Allen and *Dolichogenidia eucalypti* Austin and Allen. Despite this, it necessarily investigates many relevant details about the host of these two parasitoids *Uraba lugens* Walker, which is currently responsible for large scale defoliation of eucalypt forests in south-western Western Australia.

I chose to study *U. lugens* as it is fairly abundant in the Adelaide region and because a preliminary literature survey revealed it was attacked by several parasitoids. However on the few occasions parasitoids were recorded in the literature concerning

*U. lugens,* which is reviewed in Chapter 1, they were often identified no further than family level and authors disagreed on their effectiveness in regulating populations. Thus, I began my research with weekly surveys of surviving populations of *U. lugens* in the field, and continued for two and a half years, to collect and record biological details about the parasitoid complex associated with *U. lugens* in South Australia. A large parasitoid complex was found and is presented in Chapter 2 which forms a framework or setting to the remainder of the thesis. A taxonomic key to the 22 parasitoid species in the complex and description of two new species *C. urabae* and *D. eucalypti* is provided in Appendix 1. This key was co-authored with Dr. A. D. Austin and is to be published in November 1989.

*C. urabae* and *D. eucalypti* were chosen for comparative study from the 22 parasitoid species in the complex because of their proposed phylogenetic relationship and because preliminary observations had shown overlap in their phenologies and host stages attacked. The phenologies of these two species are detailed in Chapter 3 and their size relationships detailed in Chapter 4, neither of which had previously been documented. Information gathered on the phenologies of *C. urabae* and *D. eucalypti* led to the experiments conducted in the next three chapters.

The first of these chapters examined the effect of host size and temperature on development of *C. urabae* and *D. eucalypti* and of temperature on development of *U. lugens*. The series of experiments in Chapter 5 were designed to quantify temperature thresholds and day-degree requirements for all three species, and to understand the variable pattern of moulting and growth of *U. lugens*. Chapter 6 and Chapter 7 are closely linked, and explore possible reasons for the differing phenologies and host sizes handled by *C. urabae* and *D. eucalypti* in the field. It is in these two chapters that all three species are also used in a wider context, as 'models', to investigate the relevance of differing parasitoid and host behaviours to current theories on host acceptance, host preference and superparasitism by parasitoids. In particular host acceptance, the process whereby a parasitoid accepts or rejects a host for oviposition after contact is made with it

(Weseloh 1974), is reassessed by accounting for the effect of host defensive behaviour on the outcome of an encounter.

This thesis was written with the intention that all six experimental chapters (Chapters 2 to 7) would stand alone as separate papers for future publication. Thus I have included a final chapter, Chapter 8, which integrates the results reported in the preceding chapters and concludes the thesis.

# Chapter 1. Introduction to *U. lugens* and its biology.

This chapter reviews the general biology of *U. lugens* which is the host of the parasitoids *C. urabae* and *D. eucalypti* that are the principal focus of this thesis. The review presents mostly qualitative rather than quantative information as very little had been experimentally quantified on *U. lugens* prior to this study. If any further details are found within the thesis the reader is referred to the appropriate chapter. Since the principal parasitoids studied in this thesis are new species their biology and taxonomic description is presented elsewhere.

## Taxonomy and distribution

Since first described in 1863, *Uraba lugens* Walker has undergone many taxonomic vicissitudes (Hampson 1900; Turner 1944; Cobbinah 1978) having previously been referred to under several names, viz. *Coesa viduella* Walker, *Toxoloma australe* Felder, *Selca obscura* Swin., *Nola metallopa* (Meyrick), *Modosa jucunda* Walker, *Nola lugens* Meyrick and *Roeselia lugens* Hampson (Campbell 1962; Turner 1944). Recently, Kitching (1984) reduced the family Nolidae, in which *U. lugens* was placed, to a subfamily, the Nolinae, within the Noctuidae. Turner (1944) was the last person to revise the Australian Nolinae and placed three species along with *U. lugens* in the same genus. However, Turner's revision was deficient in the light of modern taxonomic techniques so that the morphological limits to species and their geographic boundaries are still uncertain (pers. comm. T. Edwards).

*U. lugens* is found in all Australian states (Turner 1944) and the larvae are known to cause significant defoliation of eucalypts in Queensland (Brimblecombe 1962), N.S.W., Victoria (Campbell 1962; Harris 1972, 1974; Harris *et al.* 1977), Western Australia (Strelein 1988), Tasmania (Palzer 1981) and the Adelaide region (pers. comm. F.D. Morgan). Occasionally large areas of native forest have been defoliated by outbreaks of *U. lugens*. Four large outbreaks this century have each defoliated more than 30,000 ha of *Eucalyptus camaldulensis* Dehnh. in the Murray Valley region of

N.S.W. and Victoria (Campbell 1962; Harris 1974; Harris *et al.* 1977). An outbreak which began in 1983 in south-western Western Australia has defoliated 160,000 ha of jarrah-marri forest (*E. marginata* Sm,. and *E. calophylla* R. Br. ex Lindl., respectively) (Strelein 1988). As well as damage to native forests, *U. lugens* larvae are responsible for partial defoliation of eucalypt trees planted as ornamentals in parks and gardens in many towns and cities around Australia (Campbell 1969; McFarland 1978).

## Host plants

*U. lugens* has been recorded as attacking many species of *Eucalyptus* and certain near relatives such as *Angophora* and *Tristania* (Campbell 1962; Brimblecombe 1962; Campbell 1969; Harris 1974; Harris *et al.* 1977; Strelein 1988). Morgan and Cobbinah (1977) published a comprehensive list of host trees when they surveyed over 240 species of eucalypts and other near relatives, principally in the aboretum of the Waite Agricultural Research Institute, for oviposition, establishment, and survival of larvae to the end of the 4$^{th}$ instar. Although convenient, this arboretum was a very artificial environment compared to the Adelaide region as the latter has only a few species of endemic eucalypt (Boomsma 1972). The close proximity of over 240 species of eucalypt in the arboretum may have confused chemical cues used by *U. lugens* for oviposition. Nevertheless oviposition was recorded on 149 species of *Eucalyptus* and one species of *Angophora* but only a small proportion of these trees were suitable for larval survival. Based on survival of larvae to the end of the 4$^{th}$ instar, Morgan and Cobbinah (1977) classified the "host -suitability" of these trees into five categories: superior (≥75% survival, 7 spp.), satisfactory (≥50% survival, 6 spp.), poor (≥25% survival, 43 spp.), inferior (≤15% survival, 8 spp.) and unsuitable (no survival, 86 spp.). Differences in host suitability between species were explained by a variable threshold of antifeedants in tree foliage. For those trees deemed suitable, variability in larval survival was thought to be influenced by the level of available nutrients. Unfortunately, Morgan and Cobbinah's work ignored possible differences in the survival of larvae between winter and summer generations of *U. lugens*. It also dismissed natural enemies and weather as possible

factors that could have influenced their experimental results (see Chapter 2). Cobbinah *et al*. (1982) later reduced these five categories to three, as did Cobbinah (1983) and Farr (1985), who further studied the biochemistry of feeding, but all used differing interpretations of the original five categories.

## General biology

The eggs of *U. lugens* are laid in batches varying in number from 20 to over 500 (Morgan and Cobbinah 1977) with a mean of approximately 80 eggs (Campbell 1962; Harris 1972). Eggs are green when first laid and several batches are often laid on one leaf by more than one individual (Campbell 1962; Morgan and Cobbinah 1977). They are laid in parallel rows about one egg diameter apart with adjacent eggs in line. Eggs are dorsoventrally flattened, cylindrical, 0.5 mm in diameter and 0.25 mm high. Each egg has a transparent disc-shaped operculum through which embryonic development is visible.

In the eastern states and South Australia egg deposition is confined to the lower crown of trees with over 80% of egg batches less than 3m from the ground (Campbell 1962; Harris 1974; Morgan and Cobbinah 1977). In Western Australia oviposition by *U. lugens* is apparently independent of foliage height (Strelein 1988). Eggs are most frequently laid on undamaged, maturing or mature, blue-green to dark-green leaves, on either upper or lower leaf surfaces (Morgan and Cobbinah 1977). Female moths lay consecutive eggs across rows rather than within rows (see Appendix 2), which is contrary to the assumptions of Cobbinah (1978; 1983).

Egg hatching within a batch takes place over several days although high relative humidity may reduce this variability (Morgan and Cobbinah 1977). No post-eclosion feeding by larvae on empty egg shells takes place (McFarland 1978), but larvae may shelter near the old egg mass in unfavourable weather conditions (Campbell 1962). Upon hatching larvae are approximately 2 mm long and grow to 25 mm by the final instar. Overall body colour is cream to pale yellow, patterned with grey, black, brown and pink, with a dark brown head capsule (Froggatt 1900; Campbell 1962). Setae, up to

8 mm long in mature larvae, arise from tubercles distributed along the body (Southcott 1978). Each body segment has two ventral, four lateral and four dorsal tubercles, except for those bearing legs or prolegs (i.e. segments 4, 5, 6 and 10) which lack the ventral tubercles (Campbell 1962). Larvae have two types of setae, short stiff venomous spines, densest along the dorsal surface, and the more common flexible ciliated setae, covering most of the body (Southcott 1978; see Chapter 7). The dorsal surface of the prothoracic segment also carries many setae of both types.

Young larvae of are overtly gregarious and feed only on the epidermal, pallisade and spongy mesophyll tissue of a leaf, thereby avoiding the oil cells and vascular tissue (Harris *et al.* 1977). The resultant leaf damage exposes the branching vascular system, effectively skeletonizing the gum leaf, hence the common name of *U. lugens* : the gum leaf skeletonizer. During this gregarious phase, larvae spin silk across the leaf surface possibly to aid in the maintenance of aggregations (Cobbinah 1978). Gregariousness breaks down from the 5$^{th}$ instar onwards, when larvae begin to feed singly or in small groups (Campbell 1962; Harris 1974). Feeding behaviour changes at this stage, with larvae often feeding from the leaf edge on the whole leaf blade, rather than skeletonizing it. Damaged leaves eventually turn brown and die, but usually remain attached to branches for several months (Harris *et al.* 1977). After the 4$^{th}$ or 5$^{th}$ instar, head capsules are no longer shed at ecdysis but are attached by the prothoracic setae above the current head capsule (Campbell 1962; Harris 1974; McFarland 1978; see Chapters 2 and 5). This process continues at each moult so that prior to pupation they form a characteristic "head dress" of up to six superimposed capsules (Campbell 1962; Harris 1974). The significance of stacking head capsules is unknown but may represent an anti-predator adaptation (McFarland 1978).

There is a considerable overlap of instars within populations of *U. lugens* in the field (Campbell 1962; Harris 1974). Early instars do not move far from their egg mass, but disperse more widely within the tree after the breakdown of gregariousness. Intertree dispersal is rare, even when the food supply on a tree is exhausted,and then only over short distances (Campbell 1962; Harris 1975).

The majority of larvae pupate in the leaf litter at the base of trees but sometimes pupate on the bark (Brimblecombe 1962; Strelein 1988) or leaves of trees (Campbell 1962; Harris 1974). Pupation takes place in an ovoid ('boat-shaped') cocoon and incorporates material from the surroundings such as bark and leaf litter. The stacked larval head capsules and many setae are attached to the cocoon's external surface (McFarland 1978). Males are usually the first to pupate (Cobbinah 1978) and individuals can be sexed at the pupal stage by comparing external genitalia (Campbell 1962).

Adult moths are nocturnally active, grey-brown in colour and show considerable variation in body size. Males have pectinate antennae (Turner 1944), a body length of 8.5-11.5 mm and forewing length of 10.5-12.0 mm and females measure 10.0-13.0 mm and 12.0-15.5 mm, respectively (Cobbinah 1978). There is a strong positive linear relationship between pupal weight and the egg load of female *U. lugens* (Cobbinah 1983). Adults have vestigial mouthparts and do not feed during their short lifespan (Campbell 1962) of two to eight days (Cobbinah 1978). Females have a pre-oviposition period of 24-48 hrs (see Appendix 2) rather than 10 days as reported by Campbell (1962), Harris *et al.* (1977) and Soutchcott (1978). Campbell (1962) observed that female moths do not fly great distances from pupation sites. Morgan and Cobbinah (1977) supported this observation when they found male-biased sex ratios of about 10:1 at U.V. light traps positioned 200 m from host trees despite even sex ratios closer to host trees and in laboratory rearings. However these observations do not necessarily indicate female moths are poor fliers as reported by Harris (1974, 1975) who also infers they oviposit on the lowest available foliage after emergence.

## Phenology

Campbell (1969) proposed that there were two morphologically indistinguishable and biologically different morphs of *U. lugens* in N.S.W.. A list of preferred host trees, apparently based on damage levels and abundance in the field, was given by Campbell for these two morphs, and he separated them as follows:

Highland form: Found above 610 m (2000 ft), lays eggs side by side with no

intervening spaces, more than 200 eggs per egg mass, 13 larval instars.

Lowland form: Found on coast and inland, lays eggs adjacent to each other in parallel rows,with rows about one egg diameter apart, usually less than 100 eggs per egg mass, 11 larval instars.

Harris (1974) similarly differentiated *U. lugens* into 'highland' and 'lowland' forms in Victoria on the basis of differences between egg masses and phenologies, with the highland form being univoltine rather than bivoltine. Subsequently *U. lugens* has been reported as being univoltine in Tasmania (Elliott and de Little 1985) and in south-western Western Australia (Strelein 1988). However Morgan and Cobbinah (1977) suggest Campbell's morphs may be temperature induced differences rather than the result of genetic differences (see Chapter 5).

In South Australia, *U. lugens* has two discrete generations per year, no diapause, and the number of larval instars varies between 8 and 13 in each generation (Morgan and Cobbinah 1977; Cobbinah 1978). This variation in instar number complicates the use of width of head capsule as an accurate measure of instar (see Chapters 3 and 5), although Campbell (1962) and Cobbinah (1978) distinguished instars on the basis of head capsule width. In South Australia, egg hatching in the summer generation of *U. lugens* begins around December-January and adults emerge around March-April, while in the winter generation of *U. lugens* egg hatching begins around April-May and adults emerge around October-November (Morgan and Cobbinah 1977; McFarland 1978; Southcott 1978). The development of larvae in the field is completed in 60-85 days in summer and 130-180 days in winter, and the average number of instars in each generation is 11 (Morgan and Cobbinah 1977).

The temperature thresholds and day-degree requirements of the life stages of *U. lugens* have not previously been recorded (but see Chapter 5) although Morgan and Cobbinah (1977) report egg development to take 30 days at 15$^{o}$C and 15 days at 28$^{o}$C, and pupal development to take 26 days and 10 days, respectively. During the winter

generation of *U. lugens* egg development may take up to five weeks and pupal development up to 54 days (Campbell 1962).

### Management and control of *U. lugens*

Prior to this study, there was no quantitative information on the effect of natural enemies on populations of *U. lugens* (see Chapter 2). Published records of parasitoids and predators of *U. lugens* include Brimblecombe (1962) who reported five species of primary parasitoid and a predatory spider, *Philodromus* sp., Campbell (1962) who reported ten species of primary parasitoid and an egg predator *Microsmaris goannae* Hirst, and Harris (1974) who mentions several parasitoids and the predatory pentatomid *Oechalia schellenbergii* (Guerin-Méneville). Few of these parasitoids and predators were identified further than family level. Despite this paucity of information, Harris (1974) concluded *U. lugens* had few predators and parasitoids and that they contributed little to the regulation of populations of *U. lugens*. Campbell (1962) also doubted that parasitoids and predators provided effective population control, whilst Brimblecombe (1962) proposed that the occurrence of periodic outbreaks of *U. lugens* indicated "strongly effective natural enemies". Pathogens, including entomogenous fungi and a nuclear polyhedrosis virus also kill *U. lugens* (Campbell 1962; Harris 1974; Strelein 1988). Campbell (1962) considered entomogenous fungi were important during and after floods as a mortality factor of *U. lugens* in the Murray Valley region of N.S.W. because the resultant high humidities were favourable to the spread of pathogenic fungi.

Aside from natural enemies, various management practices have been examined to control *U. lugens* in commercial forests. Thinning stands of trees was attempted by Harris (1975) in an effort to restrict the direct movement of larvae from one crown to another and to reduce the number of oviposition sites. Although results of these thinning trials were variable, he found damage to be reduced in thinned plots. Controlled flooding of forests also decreases survival if flooding occurs when *U. lugens* is in stages close to the forest floor (eggs, early instars and pupae) (Campbell 1962; Harris 1972; Harris *et al.* 1977).

# Chapter 2. Biology of the parasitoids of *U. lugens* and survival of larvae of *U. lugens* in the field in South Australia.

*Abstract*

Twenty-two species were found in the parasitoid complex attacking *U. lugens* ; 11 primary parasitoids, 10 hyperparasitoids and one facultative hyperparasitoid. All immature stages of *U. lugens* were parasitized, with larval parasitoids killing hosts from the 3$^{rd}$ instar onwards. The majority of parasitoids were found in both the summer and winter generations of *U. lugens,* and at least four of the primary parasitoids had more than one generation per generation of their host. Parasitoids were collected on several species of *Eucalyptus*. The mean adult longevity of female parasitoids in the complex varied widely from 8-254 days. Of the hyperparasitoids, many were gregarious and many were polyphagous within the complex; all but one parasitized the pupae of primary parasitoids.

Larvae of *U. lugens* experienced relatively high mortality in early instars, followed by a more constant but smaller level of mortality in the later instars. The survival of uncaged larvae was patchy among trees and among groups of larvae on the one tree, but caging both greatly increased survival of larvae and reduced its variability. Parasitism accounted for only a small proportion of mortality of *U. lugens* observed in the field, although parasitism was very high in some individual groups. Hyperparasitism and the presence of alternate hosts for the many polyphagous primary parasitoids in the complex may affect the level of parasitism of *U. lugens*.

## Introduction

Several authors have published work on the biology of *Uraba lugens* Walker (Brimblecombe 1962; Campbell 1962; Harris 1972,1974; Harris *et al.* 1977; Cobbinah 1983; Strelein 1988), but almost nothing has been published on its parasitoids. Brimblecombe (1962) listed five species and Campbell (1962) 10 species of primary parasitoid attacking *U. lugens,* but most of these were not identified further than family

level. There is no information on the biology of these parasitoids and opinions differ on what may regulate populations of *U. lugens* in the field (Brimblecombe 1962; Campbell 1962; Harris 1972,1974; Harris *et al.* 1977; Strelein 1988).

Despite these differing opinions, the survival of *U. lugens* larvae in the field has not been quantified. Morgan and Cobbinah (1977) classified the suitability of over 240 species of *Eucalyptus* to *U. lugens*, as superior, satisfactory, poor, inferior or unsuitable based on the survival of *U. lugens* to the end of the 4$^{th}$ instar, when gregarious feeding breaks down. Cobbinah (1983) later published survival curves of *U. lugens* to the end of the 4$^{th}$ instar on eight species of *Eucalyptus*. In South Australia where these studies were carried out, *U. lugens* still has four to nine instars to complete before pupation, for which survival data has not been published (Morgan and Cobbinah 1977). Further it could be that survival patterns differ between its two generations per year. The winter generation commences in April-May and lasts approximately eight months and the summer generation commences in December-January and lasts approximately four months.

The aim of this section was to discover the size of the parasitoid complex of *U. lugens* in South Australia and to document significant biological details about each parasitoid, including interspecific associations, in which generation of *U. lugens* they occur, and the species of *Eucalyptus* on which they forage. Austin and Allen (1989) (see Appendix 1) published a key to the identification of these parasitoids and they list other published host records for them. To identify peaks in larval mortality and understand availability of suitable host sizes for the differing larval parasitoids, the survival of larvae in the field was quantified in both the winter and summer generations of *U. lugens*. Finally, the impact of natural enemies upon larvae of *U. lugens* was assessed by comparing the survival of caged and uncaged groups in the field.

## Materials and methods

Three field sites were selected in the Adelaide metropolitan region and were visited weekly from September 1985 to December 1987. Site 1 was 2 km north of

Adelaide G.P.O., site 2 was 2 km south, and site 3 was 7 km south. The first two sites were parklands containing a diversity of *Eucalyptus* species; the trees most commonly monitored (in decreasing frequency) were *E. leucoxylon* F. Muell., *E. camaldulensis* Dehnh., *E. sideroxylon* Cunn. ex Woolls and *E. cladocalyx* F. Muell.. The third site was an abandoned quarry dominated by *E. microcarpa* (Maiden) Maiden with a small patch of *E. leucoxylon*.

In December-January and April-May of each year (i.e. at the beginning of the summer and winter generation), trees at the three sites were surveyed for eggs and 1$^{st}$ instar larvae of *U. lugens*. From this preliminary survey, up to 40 trees with a resident population of *U. lugens* were selected at each site, and the population on each tree then monitored weekly for surviving larvae. When larvae were between the 1$^{st}$ and 3$^{rd}$ instar, the number surviving was not counted as their small size and gregarious behaviour made them impossible to count accurately. Any larvae that were moribund and any parasitoid cocoons found associated with larvae were collected each week, returned to the laboratory, and held under the laboratory conditions used for all rearing of 20$^{o}$C and a photoperiod of 12L:12D until emergence of parasitoids. Near the end of each generation of *U. lugens*, surviving larvae at each site were collected and returned to the laboratory to pupate. This helped establish identification of parasitoids that oviposited in larvae but emerged from pupae.

Levels of parasitism were quantified for egg parasitoids as it was easy to collect ecloded egg batches and check each egg for emergence holes cut through them by parasitoids. Some pupae of *U. lugens* were collected in the field, but pupae were difficult to locate because of their cryptic cocoon and varied pupation sites (Campbell 1962; Strelein 1988). To supplement the low number of pupae found in the field and to help identify pupal parasitoids, pupae that had been reared in the laboratory were glued by their pupal case to cardboard and pinned to the base of eucalypt trees in the field. These pupae were collected after two weeks and returned to the laboratory where parasitoids were allowed to emerge.

To establish when larvae were attacked by parasitoids and at what stage of development they were attacked, groups of larvae of *U. lugens* were regularly collected from other sites around Adelaide. These larvae were returned to the laboratory and held in cages with cut foliage until all parasitoids collected had either emerged or pupated. Two species of primary parasitoid, *Cotesia urabae* Austin and Allen and *Dolichogenidia eucalypti* Austin and Allen were the subject of more detailed studies to be presented in later chapters. The description of these two new species is given in Austin and Allen (1989) (see Appendix 1). Their pupal cocoons were regularly sought and collected at all sites and data on hyperparasitoids presented are therefore biased in favour of those emerging from *C. urabae* and *D. eucalypti.*

For primary parasitoids, pupal duration was recorded upon emergence of adults in the laboratory, whilst for hyperparasitoids, time elapsed since returning the host's cocoon from the field was recorded. Most female parasitoids were provided with honey and checked daily until they died. The remaining females were provided with honey and held with either host larvae or pupal cocoons in order to record oviposition behaviour, confirm primary parasitism and establish egg-larval durations. The sample sizes listed for pupal duration and longevity do not reflect the relative abundance of the various parasitoids.

During the 1986 winter generation of *U. lugens*, an experiment was set up in late April, to determine the possible causes of larval mortality in the field. Six egg batches of *U. lugens* were each subdivided into two groups of 50 eggs and each group glued to a leaf on 12 different *E. microcarpa* trees at field site 3. Six groups of eggs, one from each egg batch, were then enclosed in 30x20 cm fine mesh cloth sleeve cages, whilst the remaining six groups of eggs were left uncaged on each tree. In this way, any maternal effect on larval mortality was accounted for in the data subsequently obtained. After hatching, the surviving larvae and their instars in each uncaged group were checked weekly. Larvae in sleeve cages were checked every three weeks to minimise the disturbance to larvae caused by removal of the cages. Until they reached the 4$^{th}$ instar, larvae were counted by photographing each group with colour slide film and then

counting the larvae on each slide using a dissecting microscope. When pupation of *U. lugens* began in the field in mid-October, final counts of larvae were made, and all larvae were collected and returned to the laboratory.

The survival of uncaged larvae in the 1985-1986 summer generation of *U. lugens* at field site 3 was also recorded. There were 9669 larvae on 29 trees which had hatched from 78 egg batches in the initial population of this generation.

## Results

### *1. The parasitoid complex*

Twenty-two species were found in the parasitoid complex of *U. lugens* (Table 1); 11 primary parasitoids, 10 hyperparasitoids and one facultative hyperparasitoid. All except one of the primary parasitoids were solitary whilst most of the hyperparasitoids were gregarious.

The majority of primary parasitoids were found in both generations of *U. lugens* but many of the less common hyperparasitoids were not found in the winter generation (Table 2). Most parasitoids were found on more than one species of *Eucalyptus* and there did not appear to be any division within the complex according to the species of *Eucalyptus* on which parasitoids foraged. *C. urabae, D. eucalypti, Casinaria micra* Jerman and Gauld and *Euplectrus* sp. were sometimes found emerging from larvae from a single egg batch within one week. The longevity of adult female parasitoids varied between species but many of the hyperparasitoids lived in excess of two months (Table 3).

Hyperparasitoids were collected from the cocoons of all the primary hymenopteran parasitoids of larvae. Most hyperparasitoids had several species of host within the complex (Table 4). The cocoons of hyperparasitized species were all exposed on leaf surfaces, but *Euplectrus* sp., which had just one hyperparasitoid, had the smallest and least exposed pupae. Empty cocoons of *C. micra, C. urabae* and *D. eucalypti* which had been hyperparasitized were easily recognized because of the smaller exit holes cut in

**Table 1. Summary of relationships between *U. lugens* and its parasitoids and hyperparasitoids.**

| Species of parasitoid | Family | Primary parasitoid(P) or Hyperparasitoid (H) | Solitary (S) or Gregarious(G) | Stage of *U. lugens* attacked | Stage emerges from |
|---|---|---|---|---|---|
| *Trichogramma* sp. | Trichogrammatidae | P | S | egg | egg |
| *Cotesia urabae* | Braconidae | P | S | larva | larva |
| *Dolichogenidia eucalypti* | Braconidae | P | S | larva | larva |
| *Euplectrus* sp. | Eulophidae | P | S | larva | larva |
| *Casinaria micra* | Ichneumonidae | P | S | larva | larva |
| *Exorista flaviceps* | Tachinidae | P | S | larva | larva |
| *Eriborus* sp. | Ichneumonidae | P | S | larva | pupa |
| *Xanthopimpla rhopaloceros* | Ichneumonidae | P | S | pupa? | pupa |
| *Antrocephalus* sp. | Chalcididae | P | S | pupa | pupa |
| *Brachymeria* sp. 1 | Chalcididae | P | S | pupa | pupa |
| *Winthemia lateralis* | Tachinidae | P | S | ? | pupa? |
| *Eurytoma* sp. | Eulophidae | P ; H | G ; S | pupa ; - | pupa ; parasitoid cocoon |
| *Centrodora* sp. | Aphelinidae | H | G | - | parasitoid cocoon |
| *Brachymeria* sp. 2 | Chalcididae | H | S | - | parasitoid cocoon |
| *Elasmus australiensis* | Elasmidae | H | G | - | parasitoid cocoon |
| species indet. | Eulophidae | H | G | - | parasitoid cocoon |
| *Pediobus* sp. | Eulophidae | H | G | - | parasitoid cocoon |
| species indet. | Eupelmidae | H | S | - | parasitoid cocoon |
| *Anastatus* sp. | Eupelmidae | H | G | - | parasitoid cocoon |
| *Mesochorus* sp. | Ichneumonidae | H | S | - | parasitoid cocoon |
| *Paraphylax* sp. | Ichneumonidae | H | S | - | parasitoid cocoon |
| *Pteromalus* sp. | Pteromalidae | H | S | - | parasitoid cocoon |

**Table 2. Phenology and host plant associations of the parasitoid complex of *U. lugens* in the Adelaide metropolitan region from Sep. 1985 to Dec. 1987.**

A (+) indicates the parasitoid was found in that generation of *U. lugens* and an (*) indicates that the parasitoid was collected on that species of tree.

| Species of parasitoid | Generation of *U. lugens* | | Species of *Eucalyptus* that parasitoids were collected on | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Summer | Winter | *E. camaldulensis* | *E. leucoxylon* | *E. microcarpa* | *E. sideroxylon* | *E. cladocalyx* | Other species |
| *Trichogramma* sp. | + | - | * | * | * | - | - | *E. torquata* |
| *C. urabae* | + | + | * | * | * | * | * | - |
| *D. eucalypti* | + | + | * | * | * | * | - | *E. platypus* |
| *Euplectrus* sp. | + | + | * | * | * | * | - | - |
| *C. micra* | + | + | * | * | * | * | * | *E. erythrocorys* |
| *E. flaviceps* | + | + | * | * | * | - | - | - |
| *Eriborus* sp. | + | + | * | * | * | - | - | - |
| *X. rhopaloceros* | + | + | * | - | - | - | - | *E. fasciculosa* |
| *Antrocephalus* sp. | + | - | - | - | * | - | - | - |
| *Brachymeria* sp. 1 | - | + | * | - | - | - | - | - |
| *W. lateralis* | + | + | * | - | - | - | - | - |
| *Eurytoma* sp. | + | + | * | * | - | - | - | - |
| *Centrodora* sp. | + | - | - | - | - | * | - | - |
| *Brachymeria* sp. 2 | + | - | - | * | - | - | - | - |
| *E. australiensis* | + | + | * | * | * | * | * | - |
| Eulophidae (indet.) | + | - | - | * | - | - | - | - |
| *Pediobus* sp. | + | - | - | * | * | - | - | - |
| Eupelmidae (indet.) | + | - | - | * | - | - | - | - |
| *Anastatus* sp. | + | - | - | * | - | - | - | - |
| *Mesochorus* sp. | + | + | - | * | * | * | * | *E. erythrocorys* |
| *Paraphylax* sp. | + | + | * | * | - | - | - | - |
| *Pteromalus* sp. | + | + | * | * | * | - | - | - |

**Table 3. The longevity of adult female parasitoids in the parasitoid complex of *U. lugens* when provided with honey at 20°C and a photoperiod of 12L:12D.**

Numbers are means±SE and are ranked from shortest to longest lived. Longevity is measured in days. For species marked with an * the sex of the wasps was not recorded. The *Eurytoma* sp. is the same species in both records, but longevity differed between individuals that emerged singly and individuals that emerged in groups.

| Species of parasitoid | n | Longevity | Range |
|---|---|---|---|
| *Trichogramma* sp.* | 14 | 8± 0.2 | 7 - 9 |
| *C. micra* | 11 | 14± 1.1 | 10 - 17 |
| *E. flaviceps* | 1 | 19 | - |
| *D. eucalypti* | 20 | 20± 1.0 | 12 - 27 |
| *C. urabae* | 20 | 27± 0.8 | 18 - 31 |
| *Antrocephalus* sp. | 1 | 30 | - |
| *Centrodora* sp.* | 11 | 30± 2.8 | 19 - 45 |
| *Anastatus* sp. | 4 | 47± 7.9 | 30 - 66 |
| *Paraphylax* sp. | 5 | 58± 7.7 | 35 - 81 |
| *Mesochorus* sp. | 14 | 62± 3.4 | 41 - 92 |
| *Eriborus* sp. | 7 | 65± 2.6 | 58 - 79 |
| *Brachymeria* sp. 2 | 3 | 67±10.0 | 53 - 93 |
| *E. australiensis* | 39 | 69± 2.2 | 46 -102 |
| *Eurytoma* sp. (gregarious) | 19 | 77± 4.1 | 57 -115 |
| *Pteromalus* sp. | 7 | 86± 7.0 | 63 -110 |
| *Pediobus* sp. | 3 | 95± 1.5 | 92 - 97 |
| *Euplectrus* sp.* | 12 | 111± 7.1 | 76 -146 |
| *Eurytoma* sp. (solitary) | 4 | 165±10.6 | 142 -192 |
| *Brachymeria* sp. 1 | 3 | 254±11.0 | 214 -309 |

**Table 4. The host relationships and the maximum duration that each species of hyperparasitoid remained within its host's cocoon after the latter were collected from the field and returned to the laboratory.**

Durations are in days, and all cocoons were held at 20°C and a photoperiod of 12L:12D. An * indicates that the maximum cocoon duration of the hyperparasitoid on that species of host was not recorded. Durations marked with an (a) are for hyperparasitoids where the actual day of oviposition into the cocoon was known. The durations for *Mesochorus* sp., which oviposits into the parasitoid larva, are means±SE calculated from the day the host's cocoon was spun. For gregarious species, only one duration was recorded for each group of hyperparasitoids to emerge from a cocoon.

| Hyperparasitoid | Number of host species | Host and duration within host's cocoon | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *C. urabae* | n | *D. eucalypti* | n | *C. micra* | n | *Euplectrus* sp. | n |
| *Centrodora* sp. | 2 | 22 | 2 | 26 | 2 | - | - | - | - |
| *Brachymeria* sp. 2 | 2 | 26 | 1 | 26 | 5 | - | - | - | - |
| *E. australiensis* | 3 | 19 | 12 | 20 | 12 | 21a | 1 | - | - |
| Eulophidae (indet.) | 1 | - | - | * | - | - | - | - | - |
| *Eurytoma* sp. | 1 | 33 | 1 | - | - | - | - | - | - |
| *Pediobus* sp. | 2 | - | - | * | - | - | - | 24 | 3 |
| Eupelmidae (indet.) | 1 | 25 | 1 | - | - | - | - | - | - |
| *Anastatus* sp. | 2 | 37 | 3 | * | - | - | - | - | - |
| *Mesochorus* sp. | 3 | * | - | 13±0.5 | 8 | 15±0.5 | 6 | - | - |
| *Paraphylax* sp. | 2 | 15 | 4 | 20 | 7 | - | - | - | - |
| *Pteromalus* sp. | 3 | 25 | 7 | 24 | 1 | 33 | 6 | - | - |
| Number of hyperparasitoids | | 9 | | 9 | | 3 | | 1 | |

these cocoons by the emerging hyperparasitoid. Gregarious hyperparasitoids typically emerged from one exit hole.

Up to two individuals emerged from each host cocoon for the gregarious hyperparasitoids *Anastatus* sp., the unidentified eulophid and *Pediobus* sp.. Among these three species all emergences were female, except for *Pediobus* sp., when if two individuals emerged, at least one was female. The maximum number of hyperparasitoids that emerged from a cocoon was 18. This was for *Centrodora* sp., the smallest of the hyperparasitoids, for which at least nine individuals (both male and female) emerged from a host's cocoon.

The duration that hyperparasitoids remained within their host's cocoon was considerably longer than their host's pupal duration, with the exception of *Mesochorus* sp. (Table 4). The most common hyperparasitoid of *D. eucalypti* and *C. micra* was *Mesochorus* sp., whilst *Elasmus australiensis* Girault was the most common hyperparasitoid of *C. urabae*. Aside from these two hyperparasitoids *Pteromalus* sp., and to a lesser extent *Paraphylax* sp., were also frequently found in the field. Samples of cocoons of *C. urabae* and *D. eucalypti* collected in 1986 had levels of hyperparasitism between 12% and 91% for *C. urabae* and between 38% and 79% for *D. eucalypti*. Hyperparasitism of *D. eucalypti* was generally more frequent than *C. urabae*, due to the high level of parasitism by *Mesochorus* sp. (Chapter 3).

Any other significant details about parasitoid species in the complex are given below as arranged in Table 1. This list excludes species of parasitoid where no further information was collected. Standard errors are provided for all pupal durations and cocoon dimensions given in the text.

***Trichogramma*** **sp.** (Family Trichogrammatidae)

*Trichogramma* sp. was only found in the 1986 summer generation of *U. lugens* and then only in abundance at field site 3. At this site 96 egg batches of *U. lugens* totalling 13455 eggs across 38 trees were examined and 47 of the egg batches had *Trichogramma* sp. present. From 0.8% to 98.4% of eggs in a batch were parasitized and

the overall level of parasitism was 7.9%. Excluding egg batches with no parasitized eggs the level of parasitism was 15.2%.

All *Trichogramma* sp. collected, emerged after *U. lugens* had already ecloded from the unparasitized eggs in the batch. Parasitized eggs turned black and were easily distinguished from unparasitized eggs which were initially green and later turned brown. Parasitized eggs retained their black colour after emergence of *Trichogramma* sp. and a small hole was visible in the egg where the parasitoid emerged. *Trichogramma* sp. adults were the shortest lived of all the parasitoids collected (Table 3).

***Cotesia urabae*** Austin and Allen (Family Braconidae)

The cocoons of *C. urabae* were frequently seen in the field. The cocoon is spun alongside the host and attached to the leaf; it is sulphur-yellow green in colour, has a surrounding silk matrix and excluding this matrix measures 3.7 to 4.6 mm (4.2±0.03, n=53) in length and 1.5 to 1.9 mm (1.7±0.02, n=53) in width. The average projection of the matrix around the sides and above the cocoon is 0.3 to 2.6 mm (1.4±0.04, n=53). When the adult emerges it cuts and pushes off a cap at the end of the cocoon.

*C. urabae* attacks a wide range of host sizes in the field and is in turn attacked by nine species of hyperparasitoid. Pupal duration of *C. urabae* for males was 6-9 days (8.0±0.09, n=39), and for females 6-10 days (8.5±0.12 days, n=48). *C. urabae* has two generations in both the winter and summer generation of *U. lugens*. The adults of each generation of *C. urabae* do not overlap in the field (Chapter 3).

***Dolichogenidia eucalypti*** Austin and Allen (Family Braconidae)

The cocoons of *D. eucalypti* were also commonly seen in the field. The cocoon of this species is spun alongside the host and is attached to the leaf; it is white in colour and lacks a surrounding silk matrix. The cocoon measures 3.0 to 4.7 mm (4.0±0.04, n=72) in length and 1.2 to 1.9 mm (1.6±0.02 mm, n=72) in width. Like *C. urabae*, *D. eucalypti* cuts and pushes off a cap at the end of the cocoon to emerge.

*D. eucalypti* attacks early to intermediate larval instars and is parasitized by nine hyperparasitoids, *Mesochorus* sp. being the most numerous. Pupal durations are about one day longer than *C. urabae* being 8-11 days (9±0.07, n=93) for males and in the same

range (9.5±0.13, n=44) for females. *D. eucalypti* has one generation in the winter generation of *U. lugens* and two generations in the host's summer generation. In the second of its two generations in summer and during its one generation in winter *D. eucalypti* pupates about the same time that *U. lugens* is pupating in the field (Chapter 3).

***Euplectrus*** **sp. (Family Eulophidae)**

*Euplectrus* sp. was the most frequently collected of all the parasitoids in the complex. Unlike the other primary parasitoids, it is an ectoparasitoid ovipositing onto the surface of its host and after hatching moves to a position underneath the host where it commences to feed. It pupates under the thoracic and first few abdominal segments of the host, frequently with the ventral side uppermost, and does not spin an obvious cocoon. Externally, the host's dorsal and lateral surfaces remain intact but change colour to a dark brown. A continuous coil of *Euplectrus* sp. faeces is sometimes visible alongside the host. Pupal duration is 12-14 days (12.5±0.18, n=13) and the adults are among the longest lived parasitoids in the complex (Table 3).

Next to *Trichogramma* sp., *Euplectrus* sp. is the smallest of the primary parasitoids; the body size ranges from 1.3 to 2.2 mm (measured from head to tip of gaster) and appears strongly influenced by the size of host from which it emerges. The pupae of *Euplectrus* sp. were found beneath dead 3$^{rd}$ instar larvae onward, from January to March during the summer generation of *U. lugens*, and from early September to October during the winter generation. More than one generation of *Euplectrus* sp. seems probable within each generation of *U. lugens*. In the winter generation adults were often seen in the field visiting and ovipositing on larvae during August and September.

***Casinaria micra*** **Jerman and Gauld (Family Ichneumonidae)**

*C. micra* spins a whitish cocoon which is attached to the leaf surface beneath the region anterior to its host's abdominal prolegs. All that remains of the dead host, which is still attached to the leaf by its prolegs, is its exocuticle and head capsule which sit on the dorsal surface of the parasitoid's cocoon. The cocoon measures 4.6 to 7.5 mm

(5.8±0.06, n=75) in length and 1.9 to 3.3 mm (2.6±0.03, n=75) in width. The cocoon is marked with characteristic black spots and takes one to two hrs to spin at 20°C.

*C. micra* emerges to pupate from the mid to late larval instars of *U. lugens* from February to early-April in the summer generation of *U. lugens* and from September to mid-November in the winter generation. The pupal duration is identical 9-11 days (10.2±0.14 days, n=23) for females and males. The cocoon is oriented with the head region either anterior or posterior relative to the host. The adult parasitoid cuts a circular hole through the cocoon 1.3 to 1.7 mm (1.5±0.03, n=20) in diameter from which to emerge. *C. micra* has been observed ovipositing into 1st instar larvae in the field, whilst in the laboratory it successfully attacks and develops in several instars up to and including the 7th instar. Females are not long lived (Table 3). Field observations indicate that there are at least two generations of *C. micra* within each generation of *U. lugens*.

***Exorista flaviceps*** Macquart (Family Tachinidae)

*E. flaviceps* deposits large macrotype eggs (Askew 1971) externally on its hosts. Eggs of *E. flaviceps* were frequently seen on larvae towards the end of each generation of *U. lugens*, particularly at field site 3. *E. flaviceps* attacks late instars and up to four eggs are often present on the one larva. It emerges to pupate from the larva after consuming all the body tissue of its host. In the laboratory, larvae of *E. flaviceps* wandered for a short period before pupating, whilst in the field they presumably drop to the ground where they pupate. Pupal duration is 19-21 days (20.1±0.40, n=5).

***Eriborus*** sp. (Family Ichneumonidae)

*Eriborus* sp. was only ever found emerging from the pupae of mid to large larvae brought back from the field. These occurrences indicated that *Eriborus* sp. must oviposit into intermediate larval instars of *U. lugens*. The occurrence of *Eriborus* sp. appeared patchy, as it did not occur in many collections, but when present in a collection was common and the sex ratio female biased. *Eriborus* sp. emerged 22-24 days (23.0±0.58, n=3) after pupation of *U. lugens*. This duration is similar to the pupal duration of the host which is 18-24 days at 20°C.

***Antrocephalus*** **sp.** (Family Chalcididae)

Only one specimen of this species was found. It took 29 days to emerge from a cocoon of *U. lugens* discovered on the bark of a *E. microcarpa* tree.

***Brachymeria*** **sp. 1** (Family Chalcididae)

Females of this species were the longest lived of all the species in the parasitoid complex (Table 3). Adult *Brachymeria* sp. 1 emerged from *U. lugens* sometime between 30 and 42 days after oviposition into the pupae. The overall sex ratio of the emerging parasitoids was close to 1:1.

***Eurytoma*** **sp.** (Family Eulophidae)

*Eurytoma* sp. was recorded as a primary parasitoid of the pupae of *U. lugens* and a hyperparasitoid of the pupae of *C. urabae*. As a primary parasitoid between 1 and 17 individuals emerged from a host pupa, whilst as a hyperparasitoid just one individual emerged from a host pupa. All solitary parasitoids were female. The sex ratio of gregarious parasitoids was dominated by females, typically with only one male emerging in each group. *Eurytoma* sp. took at least 24 days (n=10) to emerge from the pupae of *U. lugens*. It successfully parasitized and developed in laboratory-reared pupae of *U. lugens*.

***Elasmus australiensis*** **Girault** (Family Elasmidae)

*E. australiensis* was very commonly collected from the field. From one to five individuals emerged from the cocoons of *D. eucalypti*, *C. urabae* and *C. micra*. Males never constituted more than half the progeny and in some hosts progeny were all female. In the laboratory, females oviposited and successfully developed in the cocoon of *C. micra* and took up to 30 min. from ovipositor penetration to ovipositor withdrawal.

***Mesochorus*** **sp.** (Family Ichneumonidae)

*Mesochorus* sp. was the most commonly collected hyperparasitoid of *D. eucalypti* and *C. micra*, but only one specimen was ever collected from *C. urabae*. *Mesochorus* sp. oviposited into the larvae of *D. eucalypti*, *C. urabae* and *C. micra* through the exocuticle of the larvae of *U. lugens* and emerged from the pupae of these species. Males were commonly reared among collections made of *Mesochorus* sp.. In contrast to

the other hyperparasitoids, *Mesochorus* sp. emerged from its host's pupal cocoon only a few days later than the pupal duration of its host (Table 4).

*2. Survival of* U. lugens *larvae in the field*

A high mortality of larvae occurred early in the 1985-1986 summer generation of *U. lugens* (Fig. 1). By week six, gregariousness had begun to break down and only 15% of the original larval population was still present on trees. This population was reduced to just 8% by week eight when larvae began to pupate. A wide range of instars was present in the field at any one time as estimated by the number and size of head capsules stacked by larvae (Fig. 2). The survival of larvae was patchy between trees (Fig. 3), with over 50% of larvae surviving on 3% of trees and no larvae surviving on 30% of trees by week eight. Little of the larval mortality could be attributed to parasitoids, which killed less than 5% of all larvae, although on some trees larvae experienced much higher rates of parasitism (about 50%). *Trichogramma* sp. killed 7.9% of the original egg population of this generation. The mortality due to parasitoids at the other two field sites monitored was greater than at site 3 but still did not account for more than half of the total larval mortality observed.

The 1986 winter generation of *U. lugens* also suffered high mortality of uncaged larvae, with just 14% of larvae surviving by the onset of pupation in week 21 (Fig. 4). As with the summer generation, survival was patchy between trees; 51% of larvae in one group were surviving at week 21 whereas no larvae were surviving in another group at week three. The survival of caged larvae was much higher than uncaged larvae and was less variable between trees except at week 21. This was due to the starvation of larvae in one cage, which had eaten all the remaining leaves within the cage between the sampling dates. The figures for caged larvae relate to five groups of larvae, as a sixth cage was vandalized during week four in the field.

Even though all egg batches hatched within several days of each other in the caging experiment, up to three successive instars were still present in the field at any one time from the $2^{nd}$ instar onwards (Fig. 5). The smaller number of larvae enabled easier

**Fig. 1.** The number of larvae of *U. lugens* that were found during the 1985-1986 summer generation of *U. lugens* across 29 *E. microcarpa* trees at field site 3 from the 1st January 1986 (i.e. when the majority of larvae hatched). The number of larvae is expressed as a percentage of the total number of larvae (n=9669) that hatched and is plotted on a log scale. The arrow indicates the first pupation in the field.

**Fig. 2.** The proportion of larvae of *U. lugens* found in each instar during the 1985-1986 summer generation. Details and arrow as for Fig. 1.

100
10
1
.1
.01

% of larval population on trees

0 2 4 6 8 10 12 14 16

Number of weeks since hatching

100
80
60
40
20
0

% of larval population each week

3 4 5 6 7 8 9 10 11

5 6 7 8 9 10 11 12 13 14 15

Number of weeks since hatching

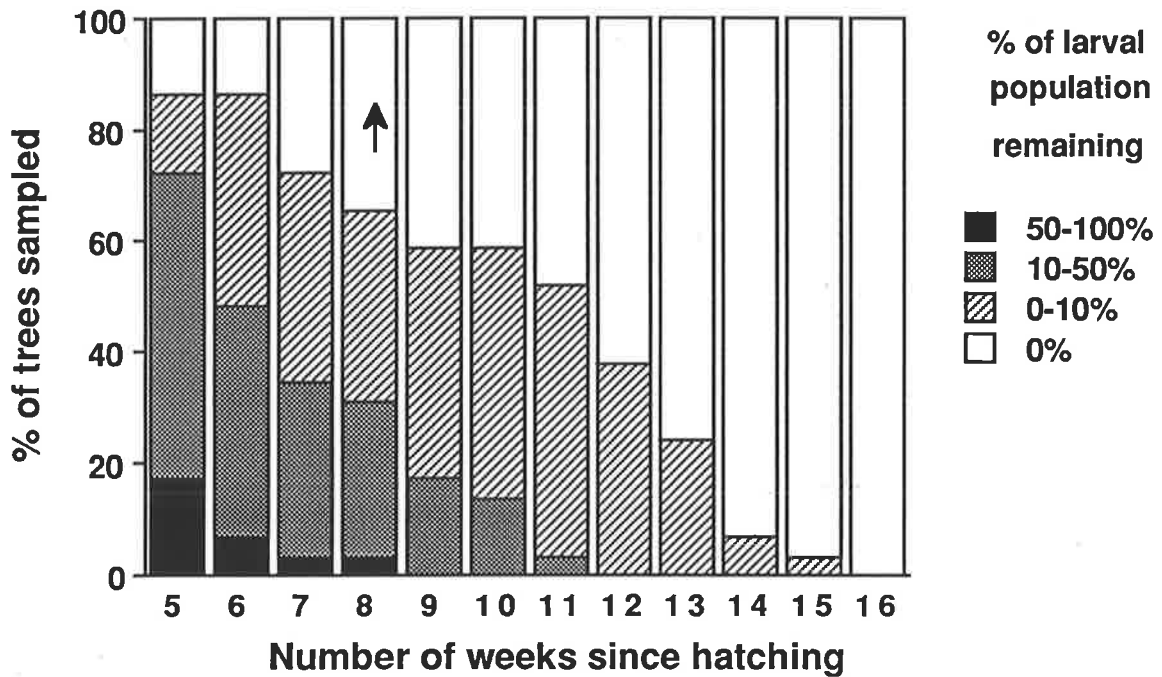
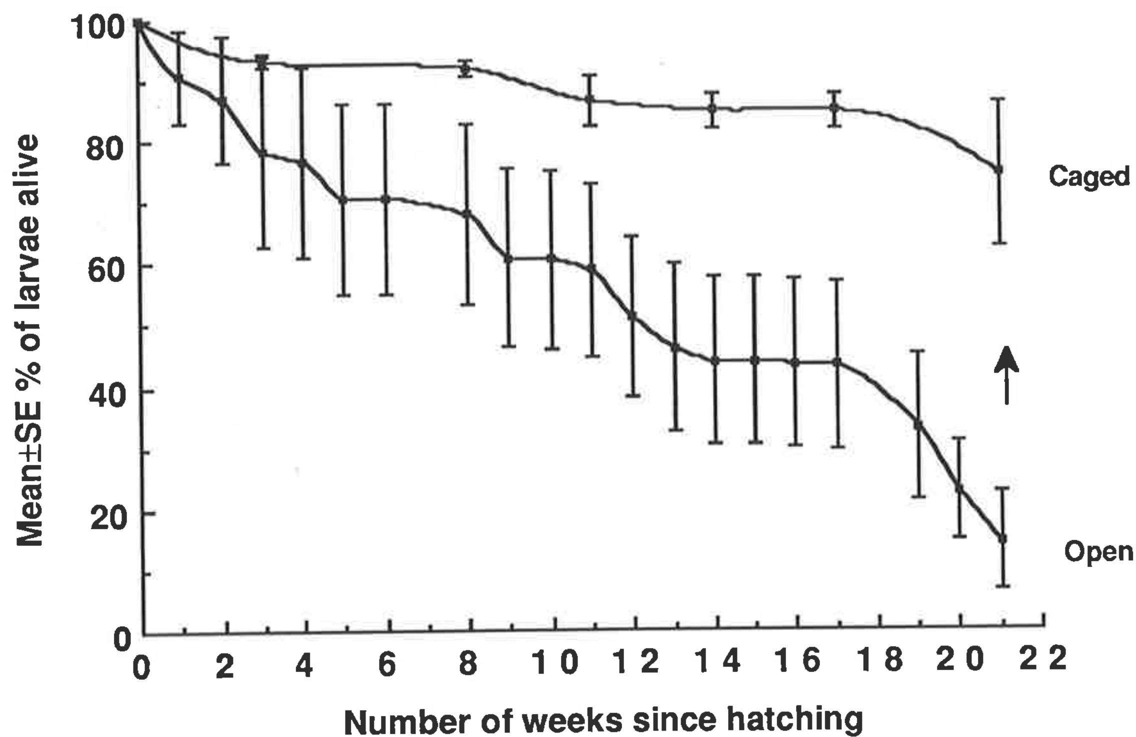
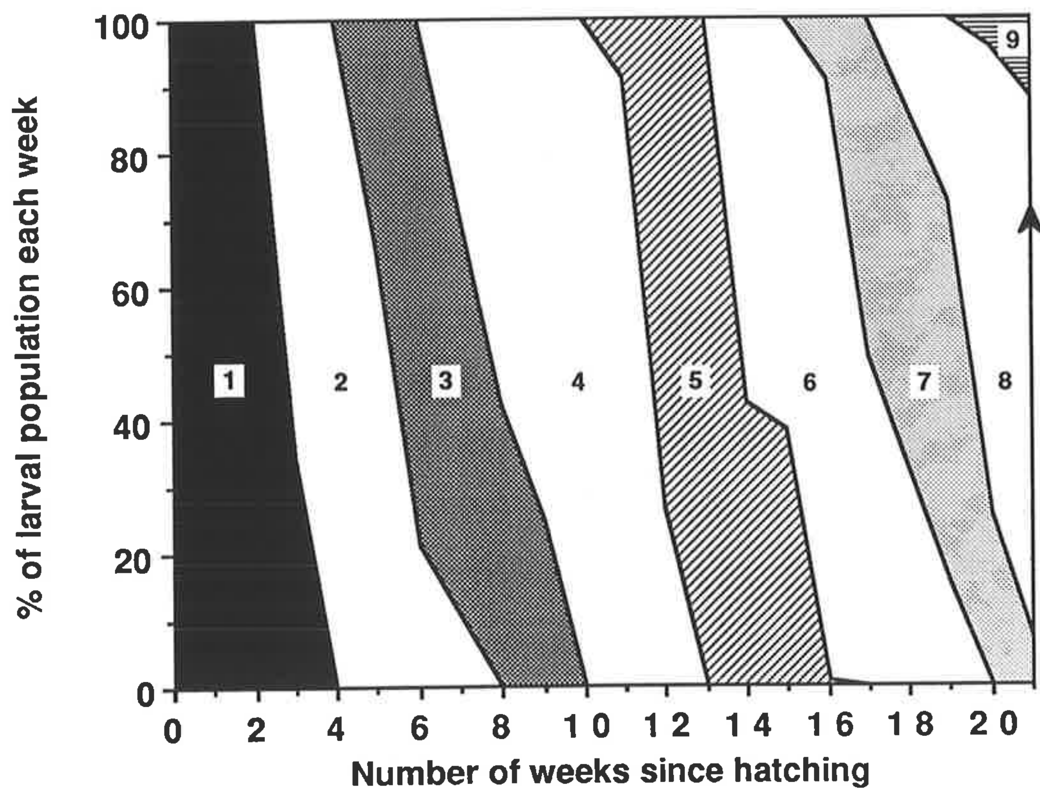



**Fig. 3.** The percentage of the larval population of *U. lugens* remaining on 29 different *E. microcarpa* trees during the 1985-1986 summer generation. Details and arrow as for Fig. 1.

**Fig. 4.** The mean ±SE percentage of larvae remaining on *E. microcarpa* trees at field site 3 during the 1986 winter generation of *U. lugens,* when larvae were either enclosed in a fine gauze sleeve cage (caged) or left uncaged (open). Six populations of uncaged larvae (initially numbering 50, 50, 50, 49, 48 and 47) and five populations of caged larvae (initially numbering 50, 50, 50, 49 and 49) were monitored. Arrow as for Fig. 1.

**Fig. 5.** The proportion of larvae of *U. lugens* in each instar of the uncaged population of larvae in Fig. 4 during the 1986 winter generation. Data for the caged population was similar but was not included as they were not checked every week. Arrow as for Fig. 1.


Mean±SE % of larvae alive
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22
Number of weeks since hatching
Caged
Open


% of larval population each week
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20
Number of weeks since hatching
1
2
3
4
5
6
7
8
9

estimation of instar than in the summer generation. By week three, when larvae were still in their 1st instar, some groups had moved to new leaves to feed. On cold, windy days the gregarious 1st and 2nd instar larvae often formed tight aggregations and did not feed. Similar aggregations were formed by gregarious larvae just prior to moulting. Gregariousness began to break down around the 5th and early 6th instar during the winter generation of *U. lugens*.

The first head capsule stacked by larvae (McFarland 1978) in the winter generation was the shed 5th instar head capsule stacked by the 6th instar. This differed from the summer generation, when capsule stacking commenced at the 5th instar. However the body size of larvae at the time of first head capsule stacking appeared similar in summer and winter generations. Stacked head capsules, particularly the first head capsule stacked, were commonly lost by larvae in the field. Estimates of the instar at which pupation took place ranged beween 8-13 for larvae in the summer and winter generation.

Although *C. urabae* and *Euplectrus* sp. were seen attacking uncaged larvae in the winter generation, only 10% of larval mortality was due to parasitoids (all *Euplectrus* sp.). Chrysopid larvae and salticid spiders were seen feeding on larvae and the numerous ants seen on trees were suspected of taking larvae. Nevertheless, as with mortality in the summer generation, not all the mortality experienced by uncaged larvae could be explained. In both the winter and summer generations of *U. lugens*, pathogens including *Beauvaria bassiana* (Balsamo) and an unidentified microsporidian, were occasionally found in larvae collected in the field. Pathogens became manifest only when larvae were brought back to the laboratory possibly because of higher humidities than experienced in the field.

## Discussion

The parasitoid complex of *U. lugens* included primary parasitoids of all sub-adult stages. One feature of this complex was the high proportion of polyphagous parasitoids. Nine of the 12 hyperparasitoids attacked at least two of the primary parasitoids in the

complex. Other published host records for *Xanthopimpla rhopaloceros* Krieger, *C. micra, E. flaviceps* and *Winthemia lateralis* (Macquart) (see Austin and Allen 1989; Appendix 1) show that each of these primary parasitoids has three or more hosts. Furthermore, many of the chalcidoid pupal parasitoids may also be polyphagous, as indicated by the broad host ranges for related taxa (Bouček 1988).

Despite this apparent lack of specificity many of the parasitoids may be specific within the "niche" in which they forage if they forage only in one type of habitat or only on closely related species of host plant (Vinson 1981, 1984; Lawton 1986). For example, *C. micra* may restrict foraging to *Eucalyptus* woodlands where Jerman and Gauld (1988) record its collection. The possibility that parasitoids in the *U. lugens* complex restricting foraging to one species of *Eucalyptus* is unlikely, as most parasitoids were collected on several eucalypt species.

Another feature of the parasitoid complex was the large number of parasitoid species it contained. One force which may shape the pattern of resource partitioning in a parasitoid complex is the degree of interspecific parasitoid competition; another may be random colonization processes (Askew and Shaw 1986). The presence of many polyphagous species in the complex may help reduce interspecific competition and so permit the large size of the complex observed. Large parasitoid complexes are often associated with hosts that feed on trees, which are more architecturally complex (Hawkins and Lawton 1987, Hawkins 1988) and apparent (Feeny 1976) than shrubs or monocotyledons. Additionally, larger parasitoid complexes may appear more probable in habitats and hosts in and with which the parasitoids evolved, than in extraneous habitats or with introduced hosts.

Larvae of *U. lugens* experience fairly high early-instar mortality (particularly the summer generation), followed by a steady but smaller mortality through the remaining instars. The level of pupal mortality was not assessed, although six parasitoids were recorded killing the pupae of *U. lugens*. Comparisons of larval mortality between the summer and winter generations of *U. lugens* were complicated by the much longer duration of the early instars in the field during the winter months.

Patchiness in survival of larvae between different trees and even between different groups on the same tree was noticeable in all five generations of *U. lugens* monitored. Caging populations of larvae greatly increased overall survival and decreased the fluctuations in mortality between larvae on different trees. The higher mortality of uncaged populations could not be accounted for by parasitoids. The higher survival of caged populations may have been due in part to the protection the cages provided from wind, rain and, to a lesser extent, low temperatures experienced during the winter months.

Predation on larvae of *U. lugens* also could explain both the difference in survival between caged and uncaged populations and the patchiness of mortality in the field. However, because populations were not continually observed, predation was only occasionally witnessed and its extent could not be quantified. Besides the invertebrate predators observed during this study, other known invertebrate predators of *U. lugens* include a predatory mite, *Microsmaris goannae* Hirst which attacks eggs (Campbell 1962), the crab spider *Philodromus* sp. (Brimblecombe 1962) and the pentatomid *Oechalia schellenbergii* (Guerin-Méneville) (Harris 1974). No avian predators of *U. lugens* were observed which is perhaps not surprising since the larvae of *U. lugens* exhibit many of the characteristics of unpalatable lepidopteran larvae. These include egg clustering, larval gregariousness (Sillén-Tullberg 1988), failure to conceal themselves, failure to restrict foraging times, and failure to remove damaged foliage (Heinrich 1979). Furthermore the long hairs and envenomating setae of the larvae (Southcott 1978) may provide protection from predation by birds, with the exception of cuckoos (Family Cuculidae) the gut contents of which are frequently recorded to include larval lepidopteran hairs and barbs (Lea and Gray 1935; McKeown 1944; Rose 1973).

Three to four larval instars of *U. lugens* commonly were present in the field at any one time. This degree of developmental variation has also been recorded by others (Campbell 1962; Harris 1974; Strelein 1988). For primary parasitoids which forage for larvae of specific sizes or ages, this range of instars increases the time interval when hosts suitable for oviposition are present. The synchrony of generations of *U. lugens*

may be assisted by the variation in the number of instars before pupation if this promotes synchrony of adult emergence. The number of instars ranged between 8 and 13 in the observed winter and summer generations.

*Trichogramma* sp., *Euplectrus* sp., *D. eucalypti* and *C. urabae* are all capable of killing hosts before the end of the 4th instar in the field. This contradicts the unpublished data cited by Morgan and Cobbinah (1977), from which they conclude that mortality from parasitoids was only significant in the later instars, and also Cobbinah (1978) who believed parasitism was only significant beyond the 8th instar. Cobbinah's (1983) generalisation that higher degrees of parasitism occur on host trees more suitable for the growth of *U. lugens* seems unfounded, since high levels of parasitism were found in this study on satisfactory host trees (i.e. *E. leucoxylon* ), poor host trees (i.e. *E. sideroxylon* and *E. cladocalyx* ) and trees which previously had no record of oviposition by *U. lugens* (i.e. *E. microcarpa* ) (see Morgan and Cobbinah 1977).

Hyperparasitism may limit the effectiveness of the primary parasitoids of *U. lugens*, particularly of *C. micra, D. eucalypti, C. urabae* and possibly *Euplectrus* sp., which spin exposed cocoons in the field. The extent to which the polyphagous parasitoids attack hosts outside the complex probably also influences the patchiness and level of parasitism seen in the field. However, some of the primary parasitoids, including *C. urabae* and *D. eucalypti*, appear to be host specific (Chapter 3). Further studies on the parasitoid complex of *U. lugens* over many more generations than observed here are needed, but the size of the parasitoid complex is obviously greater than previously thought and its potential to influence the mortality of *U. lugens* in the field cannot be overlooked.

# Chapter 3. The phenologies of *C. urabae, D. eucalypti* and their host *U. lugens* in the Adelaide region.

*Abstract*

A field study was undertaken to determine the phenologies of the solitary larval endoparasitoids *C. urabae* and *D. eucalypti* in relation to that of their bivoltine host *U. lugens*. *C. urabae* had two generations within both the summer and the winter generation of *U. lugens,* and *D. eucalypti* had two generations in the summer but only one generation in the winter. *D. eucalypti* parasitized a narrower range of host sizes in the field. Both parasitoids attacked recently hatched (typically 1$^{st}$ instar) or 'small hosts' at the beginning of each host generation. In summer *D. eucalypti* was the first to emerge from hosts, and along with *C. urabae*, emerged from hosts with a mode of 0.85-1.05 mm in head capsule width and 0.9-1.5 mg in dry weight (mid hosts). In winter, *C. urabae* emerged from hosts with a mode of 1.15 mm in head capsule width and 2.7 mg in dry weight (large hosts). Both species in summer and *C. urabae* in winter then proceed to parasitize hosts of around these sizes to commence second parasitoid generations. *D. eucalypti* in its second generation in summer and in its first generation in winter, typically delayed emergence from the host until most unparasitized hosts had pupated. Both species of parasitoid overwintered within the larval stage of their host. Levels of parasitism appeared to be low and dropped between first and second generations within each host generation. It was concluded that *C. urabae* and *D. eucalypti* displayed continuity of generations and a high level of synchronization with *U. lugens* in the Adelaide region.

## Introduction

The developmental synchrony between a parasitoid and its host is critical in allowing the continuity of parasitoid generations (Lawrence 1986). This is especially so for host specific parasitoids where parallel evolution between host and parasitoid may result in many adaptations by parasitoids for maintaining synchrony (Matthews 1974).

The precision of this synchrony is governed by environmental factors to which host and parasitoid respond, either independently or dependently, and by physiological interactions between them (Fisher 1971). Poor synchrony may well indicate adaptation is not complete or that parasitoids may have alternative host species (Fisher 1971).

Most species of parasitoid have a particular stage of host they attack and often a particular age or size within that host stage. Hosts that diapause or overwinter in a non-susceptible stage and hosts that possess discrete generations and long periods between stages susceptible to parasitoids may present problems of synchrony for parasitoids. For example, the larval endoparasitoid *Cotesia melanoscelus* (Ratzeberg) diapauses as a 3rd instar in its cocoon whilst its host *Lymantria dispar* L. overwinters as an egg (Weseloh 1978). *Apanteles fumiferanae* (*s.l.*) Viereck has a facultative dormancy within *Choristoneura fumiferana* (Clem.) when this host diapauses during winter (Nealis 1988).

Some parasitoids are able to attack more than one size or stage of host and consequently complete two or more generations within each host generation. By doing this, parasitoids increase the interval over which they can utilize hosts, and hence, can respond more rapidly to changes in host availability (Porter 1983). For example *C. melanoscelus* (Weseloh 1976) and *Cotesia euphydryidis* Muesebeck (Stamp 1984) each attack two differing stages of their univoltine hosts, whilst *Apanteles bignellii* (*s.l.*) Marshall attacks three differing stages of its host *Euphydryas aurinia* (Rottemburg) (Porter 1983).

Development of parasitoids frequently spans several host instars and some parasitoids are able to regulate the length of their host's final stadia, induce supernumerary moults in hosts or delay host development across several instars, compared to unparasitized hosts (Jones 1986; Slansky 1986). Lengthening of host development and induction of supernumerary moults has been recorded for *Autographa californica* (Speyer) when parasitized by *Cotesia yakutatensis* (Ashm.) (Madar and Miller 1983), while supernumerary moults by *Manduca sexta* (L.) occur when it is parasitized by *Cotesia congregata* (Say) (Beckage and Riddiford 1978). Parasitoids that prolong the final host stage include *Apanteles kariyai* (*s.l.*) Watanabe (Sato *et al.* 1983), *Cotesia*

*marginiventris* (Cresson) (Ashley 1983), *A. bignellii* (Porter 1983), and *C. euphydryidis* which lengthens the final host stage by up to eight weeks in the field (Stamp 1984). Synchrony can also be modified by physiological changes in the parasitoid, as in *A. bignellii*, which has a protracted summer dormancy as an adult inside its cocoon (Porter 1983).

The aim of this section was to determine the phenologies of *Cotesia urabae* Austin and Allen and *Dolichogenidia eucalypti* Austin and Allen in the Adelaide region and to relate them to that of their host *Uraba lugens* Walker. *U. lugens* has two discrete generations per year in South Australia. These are the winter generation commencing in April-May which lasts approximately eight months, and the summer generation commencing in December-January which lasts approximately four months (Morgan and Cobbinah 1977). By understanding their respective phenologies, it was hoped that the host stages attacked by *C. urabae* and *D. eucalypti* and the level of synchronization between them and their host in the field could be determined. The level of synchronization may then help determine the host specificity of *C. urabae* and *D. eucalypti*, by indicating whether alternative hosts are necessary to maintain continuity between generations of these two species of parasitoid.

## Materials and methods

### *1. Field monitoring*

Field monitoring was carried out at the three sites described in Chapter 2 and at other sites indicated below. Populations of *U. lugens* found at each site were checked weekly for cocoons of *C. urabae* and *D. eucalypti* and for the host larvae from which they had emerged. Dates of oviposition, egg hatching, and pupation of *U. lugens* were also recorded.

The host larva found with each cocoon was returned to the laboratory and its head capsule width measured with an ocular micrometer. Any stacked head capsules (McFarland 1978) on the larva were removed and the larva dried in an oven at 80±2°C

for 48 hrs. After this time the dry weight of each larva was recorded to the nearest µg on a Beckman LM-600 microbalance.

The instar of each host was recorded for only three groups of larvae as it could not be accurately determined in others. One group was in the field during winter 1986 at site 3 on *Eucalyptus microcarpa* (Maiden) Maiden, another in a constant temperature room at 20°C and 12L:12D on cut foliage of *Eucalyptus leucoxylon* F. Muell., and a third on a potted *Eucalyptus camaldulensis* Dehnh. held in the laboratory under ambient conditions during winter 1986. Larvae in these groups were dusted with fluorescent dye after each moult and checked for loss of dye to determine a moult. Counting the number of stacked head capsules to determine instar was not considered accurate because the instar at which head capsule stacking began varied, and because larvae often lost one or more stacked head capsules in the field (Chapter 2). Furthermore parasitism may alter the head capsule width of larvae which reduces the accuracy of this measure for instar determination (Vinson and Barras 1970; Iwantsch and Smilowitz 1975; Surgeoner and Waller 1975; Bartell and Pass 1978; Ashley 1983).

A total of four generations of *U. lugens* were monitored between September 1985 and May 1987. Only generations of *U. lugens* and not of parasitoids are hereafter referred to as summer and winter. The details of sampling varied among generations:

(i) 1985 winter generation. Sampling began part way through the host generation but before *C. urabae* and *D. eucalypti* began to pupate. All cocoons were collected and returned to the laboratory where they were held at 20°C and 12L:12D until parasitoid emergence.

(ii) 1985-1986 summer generation. Sampling was as for winter 1985 except at site 3 where leaves on which cocoons were found were tagged and then cocoons visited weekly until parasitoid emergence. Cocoons that had been hyperparasitized were identified by their exit holes; in contrast, *C. urabae* and *D. eucalypti* cut a cap at the end of the cocoon at emergence (Chapter 2).

(iii) 1986 winter generation. During this generation monitoring of trees was expanded to regularly include trees bordering roads near the Waite Institute (site 4;

6 km S.E. of Adelaide G.P.O.) and bordering roads 0.5 to 1.5 km south of site 2 (site 5). This nearly doubled the number of trees surveyed with populations of *U. lugens*. As in the previous winter generation, all cocoons were collected and returned to the laboratory for rearing.

(iv) 1986-1987 summer generation. Rather than continuously monitor populations of *U. lugens,* larvae were sampled on just two occasions during this generation. Sampling was done by collecting larvae in week 3 and again in week 9 of 1987. In the first sample, larvae were collected from just four trees at each site (sites 1-5), with 208-399 larvae being collected per site. Trees sampled at site 3 were all *E. microcarpa*, at sites 2, 5, and one at site 1 were *E. camaldulensis* whilst the remainder at site 1, along with those at site 4, were *E. leucoxylon*. Larvae collected were mostly 3$^{rd}$ instar and were feeding gregariously. The number collected varied between 25 and 164 per tree. The populations of larvae on each of the 20 trees sampled were held separately in 20 x 20 cm cages, fed on cut *E. leucoxylon* foliage, and kept in an insectary exposed to ambient outdoor conditions. By week 9 insufficient populations of *U. lugens* were left at sites 1 and 4 for a second collection, so two new sites, one nearby site 4 (site 6: *E. leucoxylon* ) and a second adjacent to site 5 (site 7: *E. leucoxylon*) were added to the samples. The number of larvae collected at these second five sites varied from 157-265. Larvae were collected by pooling them from many trees at each site and held in cages as for the first collection. In both samples, cages were checked daily for parasitoid cocoons and foliage was replaced regularly.

*2. Placement of parasitized and unparasitized larvae in the field*

A further approach to interpreting the phenology of *C. urabae* and *D. eucalypti* was to monitor parasitized or unparasitized larvae placed in the field on predetermined dates for pupation of their parasitoids. This was done in the 1986 winter generation and repeated in subsequent generations to estimate when larvae were first parasitized in the field.

In the 1986 winter generation, batches of laboratory reared 1$^{st}$ (head capsule width 0.21-0.24 mm), 2$^{nd}$ (0.32-0.35 mm), and 3$^{rd}$ (0.38-0.42 mm) instar larvae were held with either female *D. eucalypti* or female *C. urabae* for 24 hrs and then placed in cages on trees in the field at site 3. Cages were 29 x 8 cm and covered with fine mesh cloth to exclude any parasitoids or predators. Three instars were selected as they each may be conceivably encountered by *C. urabae* and *D. eucalypti* foraging in the field in winter (but especially 1$^{st}$ instars), due to varying eclosion dates of host larvae. The number of larvae placed in field cages was between 20-55, whilst a further 5-21 larvae in each treatment were kept in the laboratory at 20$^{o}$C and 12L:12D to confirm parasitism of each batch. Cages were checked weekly and any cocoons and their dead hosts returned to the laboratory for measuring. Then in week 42 which was two weeks after the first cocoon was found in a cage (thereby approximating pupal duration (see Chapter 5)) laboratory-reared larvae of a size similar to those present in the field were exposed to parasitoids as before, and placed on potted *E. camaldulensis* trees held in a large (2 x 3 x 8 m) outdoor screen cage at the Waite Institute, rather than in sleeve cages at site 3. This site was protected from the vandalism experienced at site 3 and a large cage enabled easier access to larvae for observation. Placing groups of larvae parasitized on predetermined dates into this field cage was repeated with 4-5$^{th}$ (0.63-0.83 mm) instars in week 7 during the 1986-1987 summer generation and again with 1$^{st}$ instars in week 20 and larger larvae in week 42 during the 1987 winter generation. Larvae placed in field cages in week 42 during the 1986 and 1987 winter generation had their stacked head capsules painted to mark their size, when parasitized, for subsequent measurements.

Finally unparasitized laboratory reared 4-5$^{th}$ (0.63-0.83 mm) instars or trap hosts (van Driesche 1983) were also marked with paint on their stacked head capsules and placed on a tree at field site 2 in week 8 of the 1986-1987 summer generation. These larvae were collected after two weeks (being easily identified by their painted head capsules), and reared in 20 x 20 cm cages as for the 1986-1987 summer generation until either parasitoids emerged or larvae pupated. This method was repeated with larger larvae at site 2 in week 42 during the 1987 winter generation.

*3. Analysis of data*

Since understanding the phenologies of *C. urabae* and *D. eucalypti* was the principal aim of the study, parasitoid abundance, parasitism and hyperparasitism were not accurately quantified. Abundance in this work refers to the number of trees on which parasitoids were eventually found relative to the total number of trees which had resident populations of *U. lugens* when the first cocoon of either species was found. Similarly, percent parasitism is defined as the number of cocoons found relative to the number of larvae on all the trees, when the first cocoon was found. Levels of hyperparasitism were determined at site 3 in the 1985-1986 summer generation by leaving cocoons in the field until parasitoid emergence. The technique of sampling cocoons by returning them to the laboratory possibly affected hyperparasitism due to the shortened time that cocoons were in the field. Despite these shortcomings, levels are quoted more as a guide to rather than an actual measure of abundance, parasitism and hyperparasitism of *C. urabae* and *D. eucalypti* in the field. Time during the year was divided into 52 weeks to facilitate comparisons among years.

## Results

*1. Phenology of* U. lugens

The phenology of *U. lugens* was very similar from 1985 to 1987 (Fig.1). The greatest difference between appearances or disappearances of a life stage in the field between years was just two weeks. All life stages were present in the field for at least one month, but the larval stage was longest within each generation. There was a gap of two to four weeks when no larvae were present between generations, but for most of the population this gap was even wider.

Larvae underwent 8-13 instars before pupation in the field (Chapter 2) but the instar of a larva may not be accurately determined by measuring head capsule width. Head capsule width for a given instar varied greatly when individuals were reared under different conditions (Table 1). The head capsule width of each instar (excluding the first

**Fig. 1.** Phenology of *U. lugens* from September 1985 to December 1987 inclusive. Figures are for: **(a)** 1985, **(b)** 1986, and **(c)** 1987. On the x-axis weeks are numbered consecutively from January to December as 1 to 52 and the corresponding month of the year is given below that. A black square indicates that the life stage was present in the field during that week. The larvae of the summer generation of *U. lugens* continue until about week 15 and those of the winter generation span most of the remaining year. The arrow in 1985 indicates when sampling began.

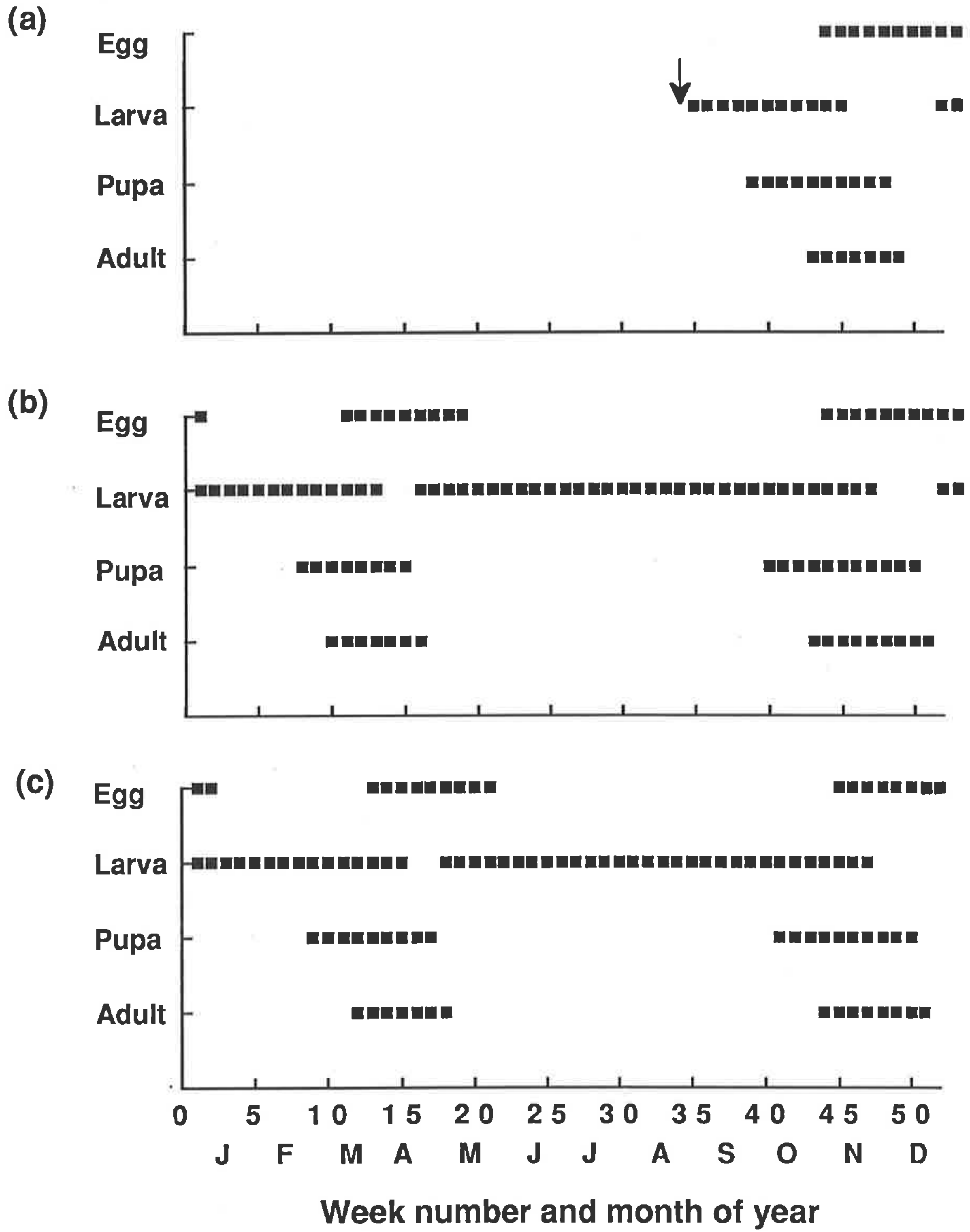

(a)
Egg
Larva
Pupa
Adult
(b)
Egg
Larva
Pupa
Adult
(c)
Egg
Larva
Pupa
Adult
0
5
10
15
20
25
30
35
40
45
50
J F M A M J J A S O N D
Week number and month of year

**Table 1. Head capsule width of instars of *U. lugens* when reared under differing conditions.**

All head capsule widths are presented as mean±SE and the sample size listed underneath. Campbell's data was field collected in the Murray Valley region of N.S.W. but the generation of *U. lugens* was unspecified. Winter 1986 (in lab.) refers to larvae that were held under unregulated laboratory conditions during winter 1986.

| Instar of *U. lugens* | Head capsule width (mm) | | | |
|---|---|---|---|---|
| | Campbell (1962) | 20°C (temp. room) | Winter 1986 (field) | Winter 1986 (in lab.) |
| 1 | 0.23±0.002 (58) | 0.24±0.002 (12) | 0.23±0.002 (8) | 0.23±0.002 (13) |
| 2 | 0.29±0.003 (52) | 0.31±0.002 (12) | 0.29±0.004 (17) | 0.28±0.002 (41) |
| 3 | 0.35±0.002 (70) | 0.43±0.003 (12) | 0.35±0.002 (41) | 0.39±0.003 (40) |
| 4 | 0.46±0.002 (66) | 0.68±0.009 (12) | 0.40±0.005 (34) | 0.56±0.004 (42) |
| 5 | 0.55±0.002 (57) | 0.98±0.015 (12) | 0.53±0.008 (11) | 0.78±0.007 (46) |
| 6 | 0.67±0.008 (81) | 1.37±0.036 (12) | 0.71±0.013 (4) | 1.07±0.008 (26) |
| 7 | 0.88±0.014 (72) | 1.83±0.024 (12) | 0.99±0.008 (3) | 1.47±0.019 (23) |
| 8 | 1.08±0.006 (53) | 2.38±0.035 (12) | 1.34±0.038 (2) | 1.82±0.026 (16) |
| 9 | 1.46±0.012 (38) | 2.66±0.027 (6) | - | 2.30±0.028 (14) |
| 10 | 1.89±0.011 (37) | - | - | - |
| 11 | 2.37±0.012 (50) | - | - | - |

instar) for the winter generation was less than that of larvae reared in an unregulated laboratory over winter, and the latter was less than that for larvae reared at 20$^{o}$C. These results suggest temperature may affect the head capsule width of a given instar (see Chapter 5) and thus instar size. This negates the usefulness of instar as a relative measure of host size, especially between generations of *U. lugens*. In the summer generation, larvae experience much higher temperatures in the Adelaide region than does the winter generation (Table 2) which may therefore affect the size of each instar.

2. *Phenologies of* C. urabae *and* D. eucalypti

The pattern of pupation of *C. urabae* and *D. eucalypti* differed within and between the two host generations (Fig. 2). Parasitoid pupae were not present throughout the whole year, but spanned up to 15 weeks in the summer and up to 16 weeks in the winter generation. In both summer and winter generations, *D. eucalypti* pupae were recovered beyond the time that unparasitized larvae remained in the field.

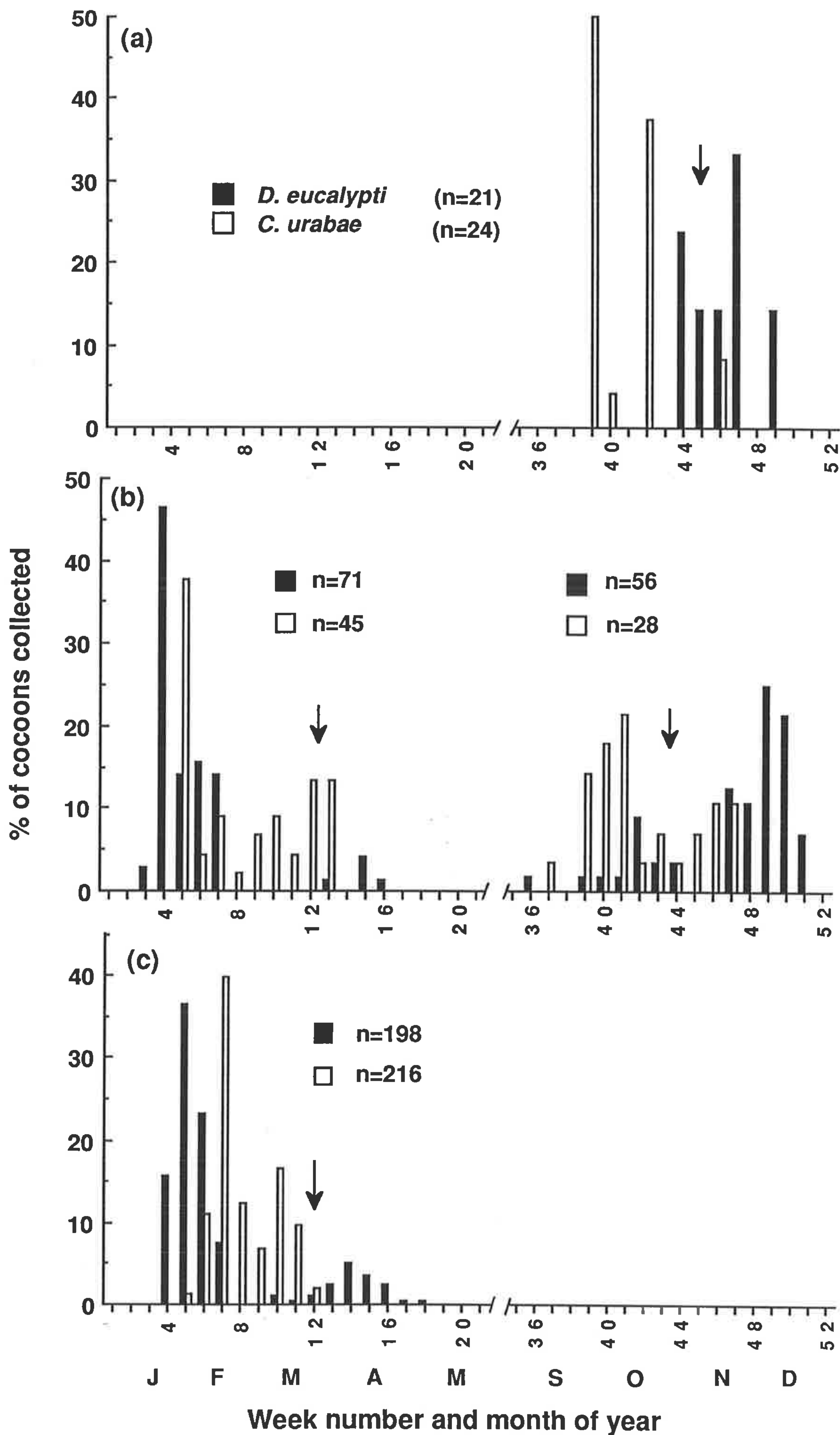
In the winter generation of 1985, *C. urabae* pupated from weeks 39-46 with a peak in week 39, and of 1986 it pupated from weeks 37-47 with a broader peak over weeks 39-41. *D. eucalypti* pupated from weeks 44-49 and from weeks 36-51, respectively. Appearances of cocoons of *D. eucalypti* from weeks 36-41 in 1986 were represented by just one cocoon in each of these 'early' weeks. Overall for both winter generations the majority of *C. urabae* had pupated before most *D. eucalypti* had begun to pupate.

In contrast to the pattern of cocoon appearances in the winter generation, *D. eucalypti* was clearly the first parasitoid to begin pupation in the summer generation. Cocoons of *D. eucalypti* appeared from weeks 3-7 and 13-16 in 1986 and from weeks 4-7 and 10-18 in 1987. As in winter, cocoons of *D. eucalypti* continued to appear well after 90% of hosts had pupated. There was no gap in pupation of *C. urabae* from weeks 5-13 in 1986 nor from weeks 5-12 in 1987. In the 1985-1986 summer generation there were two apparent peaks of *C. urabae* pupation; one in week 5 and a smaller peak in weeks 12-13. In the 1986-1987 summer two similar peaks also occurred, but it was

**Table 2. Average daily air temperatures recorded over 1925-1987 at the Waite Institute, Adelaide. (Waite Biennial Report 1987).**

| Month | Temperature ($^{o}C$) | | |
|---|---|---|---|
| | Maximum | Minimum | Mean |
| January | 27.8 | 16.3 | 22.0 |
| February | 27.7 | 16.5 | 22.1 |
| March | 25.6 | 15.4 | 20.5 |
| April | 21.5 | 13.0 | 17.3 |
| May | 17.8 | 10.7 | 14.2 |
| June | 15.1 | 8.6 | 11.9 |
| July | 14.1 | 7.8 | 11.0 |
| August | 15.2 | 8.2 | 11.7 |
| September | 17.6 | 9.4 | 13.5 |
| October | 20.3 | 11.0 | 15.6 |
| November | 23.4 | 12.9 | 18.1 |
| December | 25.8 | 14.7 | 20.3 |

**Fig. 2.** The temporal pattern of pupation of *C. urabae* and *D. eucalypti* in each host generation as determined by weekly sampling for cocoons in the field. Figures are for: **(a)** 1985, **(b)** 1986, and **(c)** 1987. The total number of cocoons collected in each host generation is listed above every host generation. The arrow indicates when more than 90% of host larvae had pupated in the field.


(a)
D. eucalypti (n=21)
C. urabae (n=24)
(b)
n=71
n=45
n=56
n=28
(c)
n=198
n=216
% of cocoons collected
J F M A M S O N D
Week number and month of year

clear that by collecting larvae in week 3 and again in week 9, that these two peaks were from two discrete parasitoid generations. This was also found to be so for *D. eucalypti* where two distinct periods of pupation were observed. All *C. urabae* had pupated from the first collection by week 9 and all *D. eucalypti* had pupated by week 7 which meant that the pupae found in the second collection of week 9 were from larvae that had been parasitized sometime between weeks 3-9. The most probable source of parasitization would be from female parasitoids emerging from cocoons during the intervening weeks (3-9) between the two collections.

Unfortunately field sightings of adult parasitoids were very infrequent and only in the winter generation. Two *C. urabae*, one male in week 43 of 1985 and one female in week 40 of 1986 were seen, but neither sighting was unusual since adults were emerging from pupae at these times. However the female was seen preening alongside a group of larvae that were displaying thrashing behaviour; a host behaviour that typically follows parasitoid attack (Chapter 6). Two *D. eucalypti* were observed, one female in week 21 of 1986, and one female in week 19 of 1987. Both females were seen ovipositing into 1st instar larvae in the field. Attempts at pan trapping adults in the field during 1986 using titanium white and yellow pan traps (see Kirk 1984 for trap details) collected only one female *D. eucalypti* in week 22 of 1986.

The size of host from which parasitoids emerged differed between generations of *U. lugens* (Figs 3 and 4). Host size data for the two winter generations was pooled following log-likelihood contingency table analysis or Fisher's exact test. These showed no difference in the frequencies of head capsule widths ($X^2_2$=4.57; 0.25>p>0.10 for *C. urabae,* and $X^2_2$ =0.2961; 0.9>p>0.75 for *D. eucalypti* ) or dry weights ($X^2_2$= 5.77; 0.10>p>0.05 and tail probability=1.0 respectively) between the two winter generations observed. The host size from which the first generation of *C. urabae* emerge in winter was larger than that in summer, with a mode of 1.15 mm for head capsule width and 2.7 mg for dry weight. The mode for the first generation of *D. eucalypti* in winter was very large (1.75 mm and 5.1-5.7 mg) and of a similar size to that which *D. eucalypti* was emerging from during its second generation in summer. The smallest host sizes from

**Fig. 3.** The distribution of final host sizes, as determined by head capsule width, from which *C. urabae* and *D. eucalypti* emerged to pupate in the field. Figures are for different generations of *U. lugens* : **(a)** pooled 1985 winter and 1986 winter generations, **(b)** 1985-1986 summer generation, and **(c)** 1986-1987 summer generation. The number of hosts measured for both species of parasitoid is listed above each figure. The labels on the x-axis refer to the mipoint of each size interval, eg. 1.15mm = 1.10-1.19 mm inclusive.

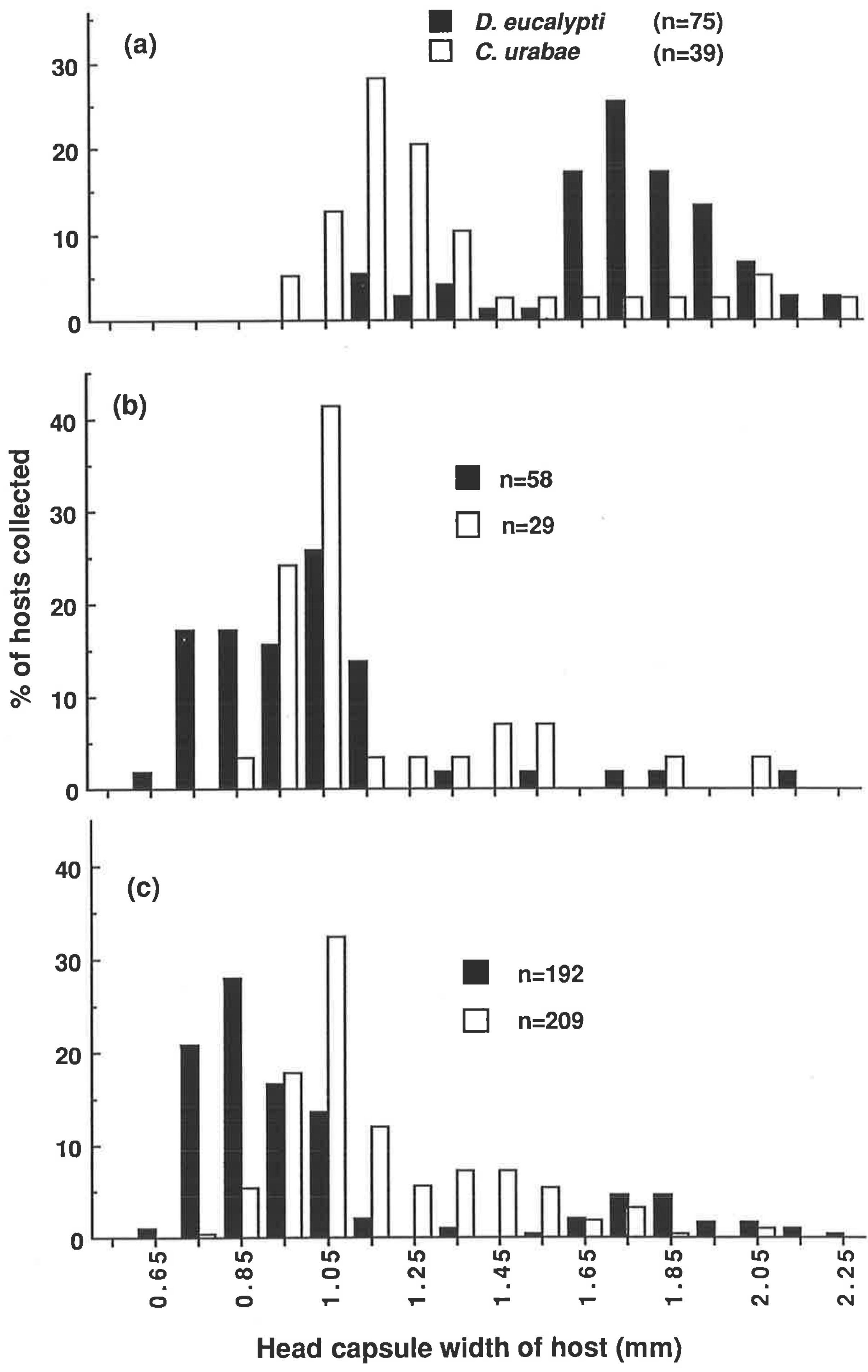
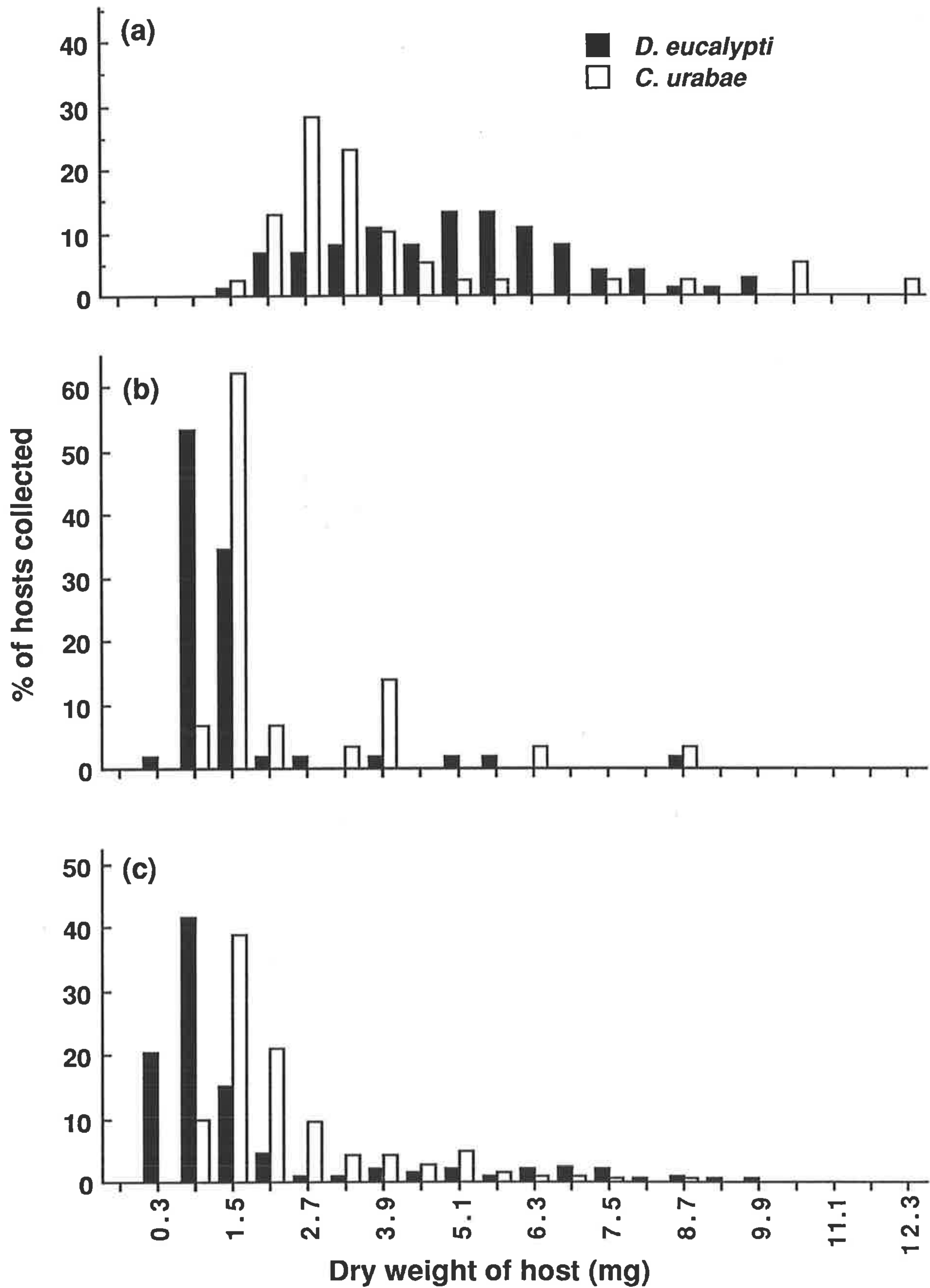

(a)
D. eucalypti (n=75)
C. urabae (n=39)
0
10
20
30
(b)
n=58
n=29
0
10
20
30
40
% of hosts collected
(c)
n=192
n=209
0
10
20
30
40
0.65
0.85
1.05
1.25
1.45
1.65
1.85
2.05
2.25
Head capsule width of host (mm)

**Fig. 4.** The distribution of final host sizes, as determined by dry weight of host, from which *C. urabae* and *D. eucalypti* emerged to pupate in the field. Labels and sample sizes as for Fig. 3. The labels on the x-axis refer to the midpoint of each size interval, eg. 1.5 = 1.3-1.8 mg inclusive.


(a)
D. eucalypti
C. urabae
40
30
20
10
0
(b)
60
50
40
30
20
10
0
% of hosts collected
(c)
50
40
30
20
10
0
0.3
1.5
2.7
3.9
5.1
6.3
7.5
8.7
9.9
11.1
12.3
Dry weight of host (mg)

which both species emerged was in summer, with *D. eucalypti* emerging from hosts as small as 0.65 mm and 0.5 mg and *C. urabae* from hosts as small as 0.78 mm and 0.9 mg.

The placement in the field of parasitized larvae of the size from which first generation *C. urabae* were emerging in winter, indicated that only *C. urabae* completed a second generation in its host during winter (Table 3). *D. eucalypti* did not successfully parasitize this host size in these and other experiments (Chapter 6). Furthermore *D. eucalypti* is unlikely to be present in the field as an adult when hosts of this size are available. This is because placement of parasitized 1$^{st}$ instars in the field showed that *D. eucalypti* pupated and emerged 5-11 weeks later than *C. urabae* in winter. In summer, when early instars were parasitized and placed in the field, it was *D. eucalypti* rather than *C. urabae* that first pupated (Table 3). Earlier pupation of *D. eucalypti* also occurred in the subsamples of the 1986 winter groups reared at 20$^{o}$C, except for hosts of 0.38-0.42 mm in head capsule width for which no parasitism by *D. eucalypti* was recorded. The feasibility of second generations of both *C. urabae* and *D. eucalypti* in summer was successfully demonstrated by placing hosts parasitized, when their head capsules were 0.63-0.83 mm wide, into the field. Again, as with parasitized early instars in winter, *D. eucalypti* emerged from hosts much later than *C. urabae* in this second generation. Further confirmation of this second generation for *C. urabae* was provided through rearing *C. urabae* from trap hosts of this size, which were only 'exposed' to parasitoids for a two week period in the field from weeks 8-10 of 1987 (Table 3). Trap hosts placed in the field in winter 1987 failed to attract any parasitism.

Not every tree had populations of larvae that were parasitized by *D. eucalypti* or *C. urabae* (Fig. 5). Cocoons of *C. urabae* and *D. eucalypti* were found on a similar proportion of trees in each generation and often together on the one tree. Only a small proportion of the larval population that was present at the time the first parasitoid pupa appeared was eventually killed by *D. eucalypti* and *C. urabae*. The percentage killed was 1.1 and 0.9% for *C. urabae* and 1.9 and 1.3% for *D. eucalypti* in the 1985 winter generation and 1985-1986 summer generation, respectively. In the 1986-1987 summer

**Table 3. Appearances of cocoons of *C. urabae* and *D. eucalypti* from larvae of *U. lugens* when parasitized in the laboratory and then placed in the field.**

Week number of year is given between 1 and 52. Number parasitized refers to number of cocoons recovered from the batch of larvae. A 1 alongside week of oviposition refers to larvae that were unparasitized when placed in the field. A ? for number parasitized is because all larvae died before parasitoid emergence due to vandalism of field cages.

| Generation of *U. lugens* | Host size parasitized: Range of head capsule widths (mm) | Number parasitized | Week of oviposition | Weeks over which cocoons were spun | Host size killed by parasitoid: Mean±SE head capsule width (mm) | Host size killed by parasitoid: Range of head capsule widths |
|---|---|---|---|---|---|---|
| | | | *Cotesia urabae* | | | |
| Summer 1986-87 | 0.63-0.83 | 21 | 7 | 9-11 | 1.28±0.039 | 1.05-1.63 |
| " | " | 16 | 8[1] | 10-12 | 1.35±0.057 | 1.08-1.63 |
| Winter 1986 | 0.21-0.24 | 10 | 25 | 41-43 | 0.99±0.029 | 0.88-1.15 |
| " | 0.32-0.35 | 32 | 25 | 40-42 | 1.03±0.018 | 0.88-1.25 |
| " | 0.38-0.42 | 11 | 25 | 40-41 | 1.11±0.020 | 1.03-1.23 |
| " | 1.90 | 1 | 42 | 46 | 2.03 | - |
| Winter 1987 | 0.21-0.24 | 9 | 20 | 39-40 | 1.10±0.026 | 0.96-1.18 |
| " | 1.35 | 1 | 42 | 45 | 1.65 | - |
| | | | *Dolichogenidia eucalypti* | | | |
| Summer 1986-87 | 0.63-0.83 | 7 | 7 | 13-19 | 1.84±0.066 | 1.63-2.03 |
| Winter 1986 | 0.21-0.24 | ? | 25 | >43 | - | - |
| " | 0.32-0.35 | ? | 25 | >43 | - | - |
| " | 0.38-0.42 | ? | 25 | >43 | - | - |
| Winter 1987 | 0.21-0.24 | 53 | 20 | 45-51 | 1.64±0.016 | 1.45-2.05 |

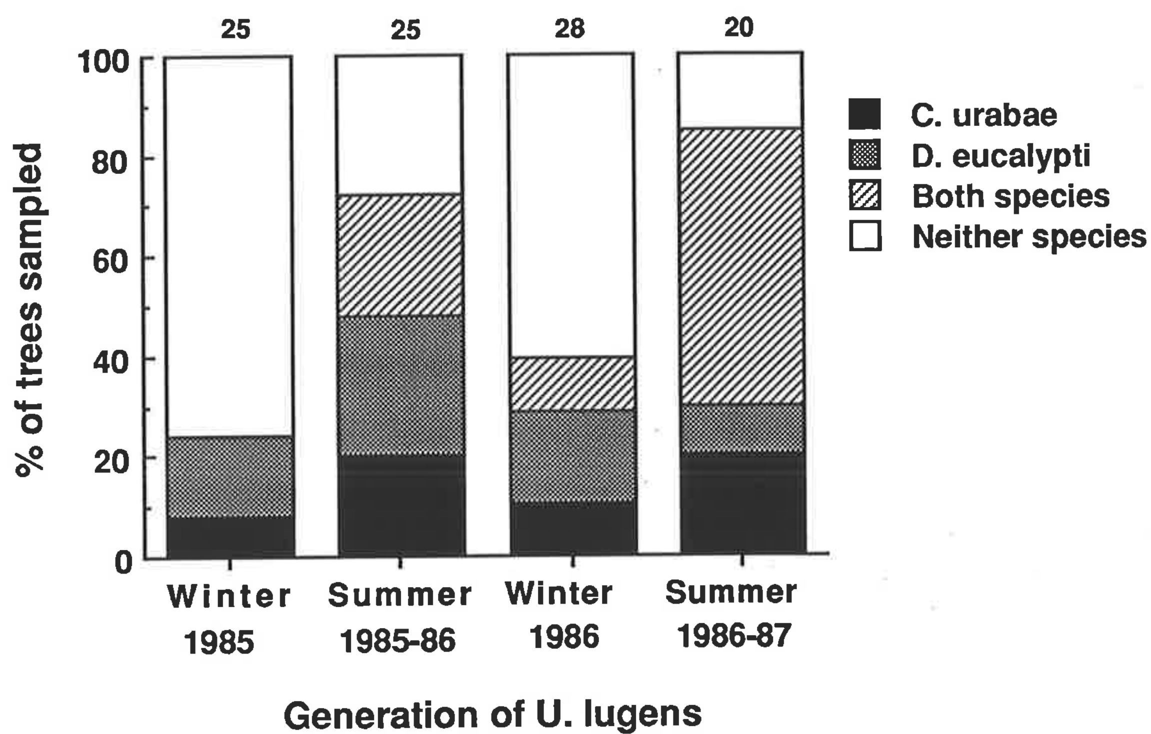



**Fig. 5.** Proportion of the total number of trees with host populations of *U. lugens* when the first cocoon of either parasitoid species appeared, that subsequently had cocoons of *C. urabae* or *D. eucalypti* collected upon them. Numbers above bars refer to the total number of trees sampled in that host generation.

generation the percentage of larvae collected in week 3 parasitized by *C. urabae* was 10.0% (range between sites 7.5-19.0%) and by *D. eucalypti* was 11.5% (0.0-33.2%). Parasitism was lower in the second collection (week 9) with 6.8% (3.2-10.4%) and 3.2% (0.5-7.5%) being parasitized, respectively. A decline in parasitism in the second generation occurred at each of the three sites sampled. A drop in parasitism between parasitoid generations was also evident in the 1985-1986 summer generation (Fig. 1b).

One factor limiting the success of both parasitoids may be hyperparasitism, which killed 0.0-90.4% of *C. urabae* and 9.5-78.6% of *D. eucalypti* for cocoons returned to the laboratory (Table 4). Of the 11 species of hyperparasitoid of *C. urabae* and *D. eucalypti*, *Mesochorus* sp. oviposited into parasitoid larvae rather than pupae (Chapter 2) and emerged from 15.4% of *D. eucalypti* cocoons spun in the laboratory in the 1986-1987 summer generation. Predation upon pupae may further decrease the adult parasitoid population since 25% of pupae of *C. urabae* and 3% of pupae of *D. eucalypti* were eaten by predators in summer 1985-1986 when pupae were 'left in field'. The remnants of the cocoons of these pupae that had been eaten, remained attached to the leaf and ants which were seen feeding on these pupae were suspected of killing them. A small proportion of parasitoid pupae in this generation was lost and may also have been eaten by predators. The pooled sex ratio of adult parasitoids that did emerge from cocoons across all four host generations was male: female 1:1.06 (n = 233) for *C. urabae* and 1:1.2 (n = 181) for *D. eucalypti*.

## Discussion

The phenologies of *C. urabae* and *D. eucalypti* differed not only within, but also between, the two generations of *U. lugens* per year. During the summer generation of *U. lugens*, two generations of *C. urabae* and two generations of *D. eucalypti* occurred and their first generations emerged from overlapping host sizes. Both species emerged from much larger hosts in their second generation in summer but *D. eucalypti* remained within its host for up to three weeks beyond the duration that unparasitized larvae remained in the field. In the winter generation of *U. lugens* only *C. urabae* completed

**Table 4. Hyperparasitism of cocoons of *C. urabae* and *D. eucalypti* collected over four generations of *U. lugens*.**

| Generation of *U. lugens* | Sampling method of cocoons | % of cocoons hyperparasitized | | | |
|---|---|---|---|---|---|
| | | n | *C. urabae* | n | *D. eucalypti* |
| Winter 1985 | returned to lab. | 24 | 0.0 | 21 | 9.5 |
| Summer 1985-86 | " | 21 | 90.4 | 23 | 65.2 |
| " | left in field | 24 | 16.7 | 34 | 38.3 |
| Winter 1986 | returned to lab. | 16 | 12.5 | 14 | 78.6 |
| Summer 1986-87 | returned to lab. | 20 | 50.0 | 86 | 38.4 |
| " | spun in lab. | 178 | 0.6 | 253 | 15.4 |

two generations, with its first generation emerging from larger hosts than in summer and its second generation from hosts that were approaching pupation. *D. eucalypti* completed just one generation in winter and remained within its host for a long period, with some parasitoids emerging as late as week 51 during 1986. Both species overwintered within their host which continued to feed and slowly develop over the winter months.

The means of host synchronization by the two parasitoids differed, with *C. urabae* maintaining continuity of generations by handling and ovipositing in a wider range of host sizes than *D. eucalypti*. The host sizes parasitized in the field are referred to from hereon in this study as (1) 'small', for larvae (typically 1$^{st}$ instars) parasitized at the beginning of each host generation by both species, (2) 'mid', for larvae around the size from which the first generation of *C. urabae* and especially *D. eucalypti* emerge in summer, and (3) 'large' for larvae around the size from which the first generation of *C. urabae* emerge in winter. The instar of these hosts was around 4-5$^{th}$ instar for mid hosts and 6-7$^{th}$ instar for large hosts, although caution must be exercised when interpreting instars of *U. lugens* (Chapters 3 and 5). *D. eucalypti* by emerging earlier in summer and very much later in winter 'avoids' having to handle large hosts. Large hosts, unlike small and mid hosts, do not feed gregariously. The skewed distribution of cocoon appearances for *D. eucalypti*, early in the winter generation of 1986, was due to a few individuals pupating from weeks 36-41 and does not contradict the proposed phenologies. Such a distribution may be explained by heterogeneity in the development of individual larvae in the host population. The size of these hosts was no smaller than those from which *D. eucalypti* was emerging beyond week 41 of 1986. Hence the hosts of these parasitoids may have been early pupating individuals in the *U. lugens* population with their development possibly being enhanced by more favourable microclimates or nutritional resources.

By prolonging the duration that it remained within its host in winter and in the second generation in summer, *D. eucalypti* bridged the period when suitable hosts were not available for oviposition. Whether this involves passive or active host regulation by *D. eucalypti* is unclear. The variability in number of instars and in head capsule width of

each instar of *U. lugens* further complicates interpretation (see Chapter 5 for further discussion). Recorded methods of host regulation in Microgastrinae include induction of supernumary moults (Beckage and Riddiford 1978), lengthening of host development across several instars (Madar and Miller 1983), and prolonging the final host stage (Ashley 1983; Sato *et al.* 1983; Stamp 1984). Hosts parasitized by *C. urabae* and *D. eucalypti* in the field and laboratory showed no obvious morphological or behavioural differences that may distinguish them from unparasitized hosts.

*C. urabae* must also survive the periods at the end of each host generation when no suitable hosts are available for oviposition, and because it does not delay pupation, must survive most of these periods in the adult stage. When provided with honey, virgin female *C. urabae* survived an average of 27±0.8 days at 20°C (compared to 20±1.0 days for *D. eucalypti* ) and survived even longer at lower temperatures (Chapters 2 and 5). Provided adequate food is available, *C. urabae* seem capable of surviving as adults over these two 'vulnerable' periods in its phenology. The longevity of both species of parasitoid is, however, probably insufficient for adults of the first generation (except for *D. eucalypti* in winter) to survive sufficiently long to parasitize small hosts in the subsequent host generation. This problem necessitates a further parasitoid generation in each host generation to maintain synchrony.

An advantage of having at least two parasitoid generations and a wider host range is that parasitoids can respond more rapidly to changes in host availability (Porter 1983). For *C. urabae* and *D. eucalypti* small hosts are the most numerous, with host availability declining as host size increases. By the time first generation parasitoids in summer and in winter are foraging for mid and for large hosts, respectively, the number of surviving *U. lugens* has dropped by over 50% (Chapter 2). Furthermore, the larval parasitoids *Casinaria micra* Jerman and Gauld, *Euplectrus* sp., and the larval-pupal parasitoid *Eriborus* sp. may already be present within apparently available hosts (Chapter 2). The intrinsic competitive ability of *C. urabae, D. eucalypti* and these other parasitoids is unknown and may depend on their temporal pattern of oviposition. Many 1st instar larvae of solitary species within the genus *Apanteles* (in which *C. urabae* and

*D. eucalypti* were originally placed (Mason 1981)) are known to engage in physical combat to kill competitors (Harvey and Partridge 1987). However competition within a host may not arise if *C. urabae* and *D. eucalypti* display active host discrimination against multiparasitism.

Reduced host availability, host mortality after parasitization, and competition with other parasitoid species may all contribute to the drop in parasitism observed between the first and second generations of *C. urabae* and *D. eucalypti* within a single host generation. Another reason for this drop may be differing host suitability for parasitoid development between host sizes of *U. lugens*. Successful emergence of parasitoids when differing host instars are parasitized, is often highest when early instars are parasitized and is probably due to age related changes in internal defences (Lewis and Vinson 1971; Vinson and Iwantsch 1980). Finally, developmental changes in host morphology or in host defensive behaviour may also result in lower parasitism levels in larger host larvae (Cornell *et al.* 1987; Hofsvang and Hågvar 1986; Weseloh 1976; Chapters 6 and 7).

Overall the observed levels of parasitism by *C. urabae* and *D. eucalypti* were low, although host mortality before parasitoid pupation may reduce these levels. Such mortality may explain the differences observed in parasitism between the sampling techniques of summer 1986-1987 and those before this host generation with larval survival probably being higher in cages than in the field. If *C. urabae* and *D. eucalypti* overwintered as pupae, or had a prolonged dormancy within the cocoon their effectiveness would be likely to be even further reduced by even higher levels of cocoon hyperparasitism and predation. Other Microgastrinae have been recorded to suffer high levels of mortality when they remain as pupae in the field for prolonged periods. These include *Cotesia rubecula* (Marshall) (Nealis 1985), *C. melanoscelus* (Crossman 1922; Weseloh 1983) and *A. fumiferanae* (Elliott *et al.* 1986). In these cases hyperparasitoids, birds, and possibly small mammals are reported as killing many pupae.

More information about adult behaviour of *C. urabae* and *D. eucalypti* would improve interpretation of the phenologies of these two species. Understanding the

pattern of adult activity may be best achieved through selective positioning of malaise traps which are efficient collectors of Hymenoptera (Reardon *et al.* 1977) rather than pan trapping. Neither suitable sites or resources were available to attempt this during the present study. Observations on parasitoid mating and dispersal would also be beneficial. A concurrent sampling technique involving the monitoring of adult parasitoid activity, using trap hosts, and the sampling of larvae for parasitoids throughout each host generation, would help to better quantify mortality and abundance of *C. urabae* and *D. eucalypti* in the field.

Nevertheless it was evident that synchronization of *C. urabae* and *D. eucalypti* with *U. lugens* strongly supports co-evolution between each species of parasitoid and their host. The degree to which both species of parasitoid responded when developing within the host, either dependently or independently of environmental conditions, remains to be determined (Chapter 5). However, evidence that *D. eucalypti* appeared to be exerting physiological host regulation upon *U. lugens* during and/or at the end of each host generation was observed in the field. Both parasitoids had vulnerable periods (especially *C. urabae* ) at the end of each host generation, when they must survive as adults until suitable hosts become available. Unfavourable weather conditions during these periods may jeopardize the continuity of parasitoid generations. Despite this, neither *C. urabae* nor *D. eucalypti* appear to utilize alternative hosts to maintain their synchrony with *U. lugens* in the Adelaide region.

# Chapter 4. The size of adult *C. urabae* and *D. eucalypti* in relation to their host *U. lugens*.

*Abstract*

*C. urabae* and *D. eucalypti* oviposit in and emerge from a wide range of host sizes of *U. lugens* and it was therefore investigated whether parasitoid size fluctuated along with host size in the field. A curvilinear relationship was established, over both host generations, between the adult parasitoid weight of both *C. urabae* and *D. eucalypti* and remaining dry weight of *U. lugens* after parasitoid emergence. This relationship was described by the curve for negative exponential growth. A threshold size of host existed for both sexes and both species of parasitoid above which parasitoids reached maximal weights (as described by the asymptote). Males were typically smaller than females in both species of parasitoid. Maximal weights were smaller for *D. eucalypti* (2.35-3.01 mg) than for *C. urabae* (3.13-3.90 mg). Fecundity was positively correlated to body weight with large *C. urabae* carrying up to 400 eggs and *D. eucalypti* up to 600 eggs. There was a temporal pattern to parasitoid size with the smallest adults of both species of parasitoid emerging from hosts early in the summer generation of *U. lugens*. Larger adults developed later during a host generation and their increased size may improve survival and recruitment of parasitoids in the following host generation.

## Introduction

A host can be regarded as a container that provides all the nutrients for growth and development of a parasitoid (Vinson 1975). Those host stages which do not feed or are permanently paralyzed by their host at oviposition are essentially fixed at the time of oviposition in their nutritional resources (Vinson and Barbosa 1987). However a more complex developmental interaction between host and parasitoid occurs in koinobiont parasitoids where the host continues to grow after oviposition (Askew and Shaw 1986). The host of a koinobiont apparently develops until an adequate nutritional level is reached upon which the parasitoid may complete its larval development (Vinson and Barbosa

1987). Parasitoids which oviposit in 'small' hosts, although not able to 'immediately' complete development, are at an advantage in that such hosts are generally more abundant, have fewer or less effective defenses, and are less likely to have a parasitoid competitor already established within them (Slansky 1986).

Relative differences in host size have also attracted attention with respect to sex-ratio allocation (Charnov *et al.* 1981). The relationship between host size and the sex of the emerging parasitoid has been examined in many parasitoid species, where, if any bias is found, it is generally in favour of sons emerging from small hosts and daughters emerging from large hosts (King 1987). Less frequently examined, particularly in solitary species of parasitoid, has been the relationship between host size and parasitoid size. Beckage and Templeton (1985) described a linear increase in the size of *Hyposoter exiguae* (Viereck) with increasing host size as did King (1988) with *Spalangia cameroni* Perkins, whilst Charnov *et al.* (1981) and Nealis *et al.* (1984) also showed a positive relationship for the parasitoids *Lariophagus distinguendus* (Foerster) and *Cotesia rubecula* Marshall, respectively. Some of the advantages of larger size in parasitoid females may include increased fecundity, longevity, and ability to obtain hosts, whereas in males they may include increased sperm production, longevity, and ability to compete for mates (King 1987; van den Assem *et al.* 1989).

*Cotesia urabae* Austin and Allen and *Dolichogenidia eucalypti* Austin and Allen are two koinobiont, solitary, larval, endoparasitoids which oviposit in and emerge from a wide range of host sizes of *Uraba lugens* Walker in the field (Chapter 3). Preliminary field observations had shown variable cocoon sizes between and within these two parasitoid species. Thus it was decided to quantify these differences by comparing parasitoid sizes with host sizes in the field, as well as recording the possible size related parameters of quantity of host remaining after parasitoid emergence and position along the host from which the parasitoid emerged. How fecundity was affected by parasitoid size was subsequently quantified in the laboratory. By examining adult sizes in the field rather than in the laboratory, I hoped to directly relate the observed variations in parasitoid size to the phenologies and ecology of *C. urabae* and *D. eucalypti*.

## Materials and methods

Four generations of *U. lugens* were monitored weekly in the Adelaide region from September 1985 to May 1987 for cocoons of *C. urabae* and *D. eucalypti* (see Chapter 3 for details). The host larva found with each cocoon was returned to the laboratory and the exit position through the host's body wall was recorded. Exits that occurred through the wall of a body segment were labelled from 1-13 (from first thoracic to last abdominal segment) and exits that occurred through an intersegmental membrane were labelled from 1.5-12.5 respectively. The head capsule width and remaining dry weight of each host was also recorded (Chapter 3).

In the 1986 winter and 1986-1987 summer generations of *U. lugens* each parasitoid cocoon collected was weighed prior to parasitoid emergence and again 24-48 hrs after parasitoid emergence on a Beckman LM-600 microbalance to the nearest µg. The weight of each parasitoid was calculated as the difference between the two weights. Weight was used to measure parasitoid size as the distribution of hind tibial lengths was discrete and too narrow when compared to parasitoid weight. The linear relationship between tibial length and female parasitoid weight is given in Appendix 3.

Preliminary dissections of female *C. urabae* and *D. eucalypti* showed that egg load varied between females of both species. Further dissections also indicated that egg load increased rapidly during the first 24 hrs after emergence. Thus to measure egg load, rearing conditions and sampling times were standardized and the weight of each female recorded. All females of *C. urabae* and of *D. eucalypti* were reared from *U. lugens* that had been parasitized and subsequently held at 20°C and 12L:12D. Hosts were fed throughout on cut *Eucalyptus leucoxylon* F. Muell. foliage until parasitoids emerged to pupate (see Appendix 2 for details of laboratory rearing of *C. urabae, D. eucalypti* and *U. lugens* ). Parasitoid cocoons were collected daily and weighed within two days of being spun. All parasitoid cocoons were held at 20°C, 12L:12D and 75% relative humidity. Females that emerged from cocoons were either killed immediately upon eclosion or else left unfed until death. Thus two measures of egg load were recorded ;

egg load at time of emergence and egg load at time of death. The weight of each female dissected was calculated as before. Females were dissected in physiological saline to remove their ovaries and oviducts which were then stained with methylene blue. Each ovary and associated oviduct was placed on a glass slide, 'squashed' with a coverslip, and the number of fully developed eggs counted. The relationship of the latter to a female's potential lifetime fecundity is unknown.

*1. Analysis of data*

The dry weight of a host remaining after parasitoid emergence was compared to the head capsule width of that host for the 1986 winter and 1986-1987 summer generation of *U. lugens*. This enabled comparison of what quantity of host was left by each species of parasitoid after it had fulfilled its nutritional requirements. Multiple linear regression was used to describe this relationship with the sex of the parasitoid only added to the equation when it improved the fit of the model (Genstat 1987).

Parasitoid exit position was described by the non-linear model $y = a + b/x$ where y is the exit position (1-13), x is the host head capsule width, and a and b are constants. The relationship between adult parasitoid weight and the host's remaining dry weight after parasitoid emergence was described by the asymptotic equation for negative exponential growth $y = a(1 - e^{-bx})$ where y is the adult parasitoid weight, x is the remaining dry weight of the host, a is a constant describing the asymptote, and b is a constant describing the gradient approaching the asymptote. Both equations were fitted using the Marquardt iterative method via the NLIN procedure of SAS (1985).

Egg load was plotted against female body weight for each species of parasitoid and for each time interval sampled.

## Results

The exit position of *C. urabae* and *D. eucalypti* larvae became closer to the last abdominal segment of the host as host size increased (Fig. 1). Observations showed that just before emergence, parasitoid larvae oriented themselves along the host's axis and

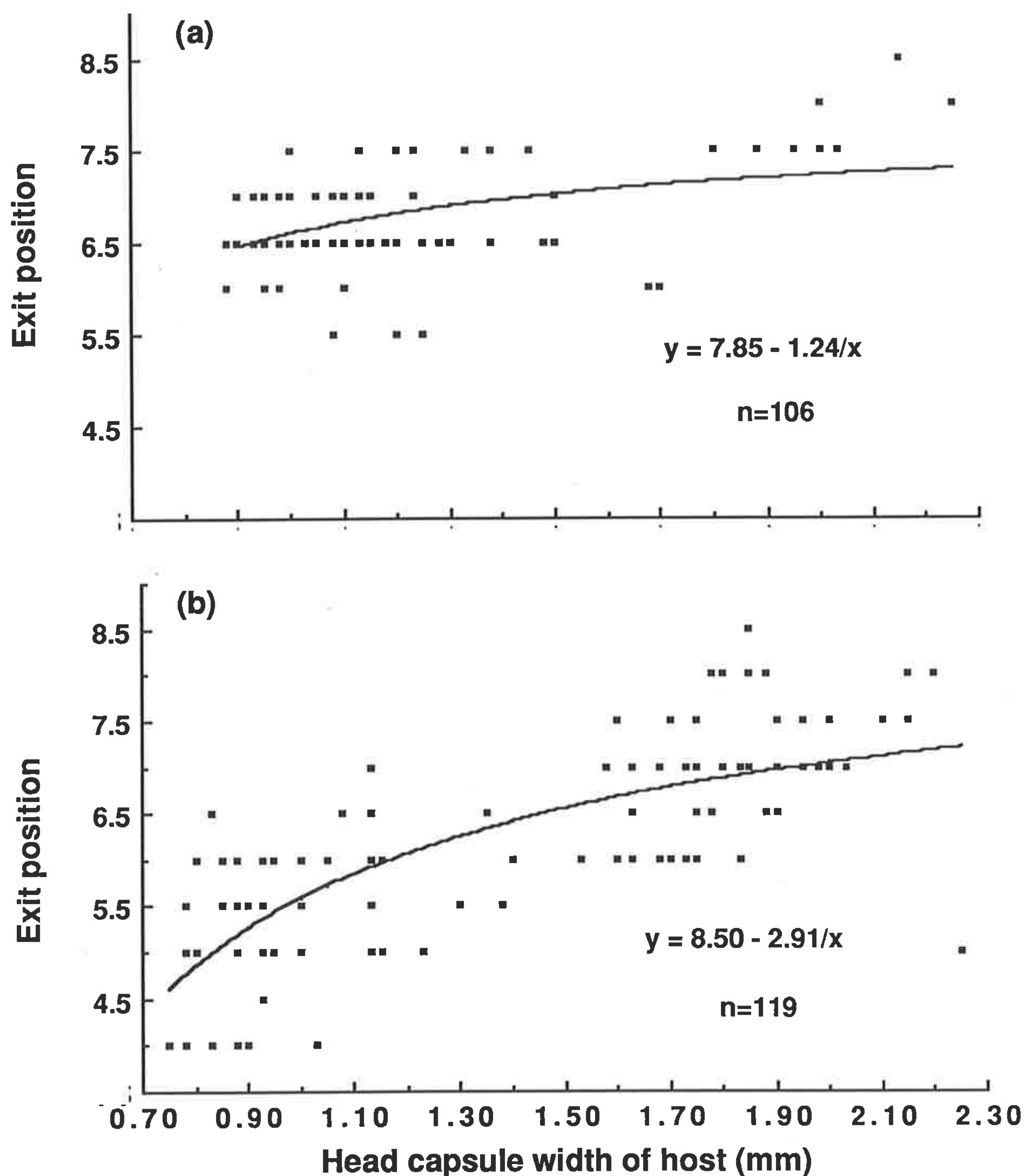



**Fig. 1.** Exit position of larvae of **(a)** *C. urabae*, and **(b)** *D. eucalypti* along their host's body length for different sizes (head capsule widths) of host. Exit position was recorded as the body segment of the host from 1-13 (first thoracic to last abdominal segment) through which the parasitoid larva emerged. Parasitoid larvae that emerged through an intersegmental membrane were recorded as 1.5-12.5 respectively. Results of tests for significance of the equations fitted for (a) $F=22363.9$; $p<0.001$,and for (b) $F=10140.4$; $p<0.001$.

then positioned themselves within the last few abdominal segments of the host. Exit position therefore reflected the length of a parasitoid larva within a host when measured from the last abdominal segment of that host. The exit position of both parasitoids was not just via the intersegmental membrane but occurred also through the sclerotized segmental plates. The range of exit positions was greater for *D. eucalypti* (4.0-8.5) than for *C. urabae* (5.5-8.5).

When *C. urabae* and *D. eucalypti* emerged from a host of the same head capsule width it was *C. urabae* that left behind more dry weight within the host (Table 1). This relationship was consistent between hosts of the 1986 winter and 1986-1987 summer generations of *U. lugens*. In the 1986-1987 summer generation, when sample size was much larger, females were leaving more of the host's dry weight behind than males, although this was not as significant ($t_{295} = 4.03$) as the difference between species ($t_{295} = 6.91$).

Adult parasitoid weight was found to be dependent upon the host from which it emerged, although once a critical host size was reached this relationship no longer held (Table 2). This critical host size, beyond which adults were capable of attaining maximal weights, was smaller for *D. eucalypti* than for *C. urabae*. *C. urabae* reached larger maximal weights than *D. eucalypti*, and females of both species reached larger maximal weights than males. Typically, the first adults to emerge during a host generation were small since they were emerging from sub-optimal size hosts, but subsequent adults to emerge then increased in weight as host size increased over time. Eventually adults reached maximal weights later in a host 's generation when the host threshold for maximal size was surpassed. Thus, the smallest adult parasitoids during a year occurred within the summer generation and were the adults of the first generation of both *C. urabae* and of *D. eucalypti*. In this parasitoid generation the smallest male was 1.65 mg for *C. urabae* and 0.98 mg for *D. eucalypti*, and the smallest female was 2.08 mg and 1.55 mg, respectively. Parasitoid sizes subsequently increased over two times for *C. urabae* and over two and a half times for *D. eucalypti* in the summer generation,

**Table 1. The relationship between host head capsule width and the cube root of the remaining dry weight of the host from which either *C. urabae* or *D. eucalypti* had emerged to pupate.**

Pooled data was analysed for each host generation and the model used was that for simple linear regression of y = a(x) + b. Head capsule width (mm) and dry weight (mg) were both multiplied by 100 before analysis and are the x and y values in the equation respectively.

| Species of parasitoid | Sex | Gradient (a) | Intercept (b) | n |
|---|---|---|---|---|
| 1986 winter generation of *U. lugens* ; n=50, F=92.12: p<0.001, $r^2$=0.80 | | | | |
| *C. urabae* | f+m | 0.03752 | 2.244 | 11 |
| *D. eucalypti* | " | 0.03752 | 1.441 | 39 |
| 1986-1987 summer generation of *U. lugens* ; n=299, F=1428.1: p<0.001, $r^2$=0.94 | | | | |
| *C. urabae* | f | 0.04023 | 1.482 | 102 |
| " | m | 0.04023 | 1.316 | 88 |
| *D. eucalypti* | f | 0.04023 | 1.187 | 56 |
| " | m | 0.04023 | 1.021 | 53 |

**Table 2. The relationship between the remaining dry weight of a host from which a parasitoid emerged and the weight of the subsequent adult parasitoid during the 1986 winter and 1986-1987 summer generation of *U. lugens*.** The non-linear regression model used was that for negative exponential growth of $y = a(1 - e^{-bx})$ where y= weight of adult parasitoid (mg x100), x=remaining dry weight of the host (mg x100), a=a constant describing the asymptote (ie. the maximal weight of the parasitoids), and b=a constant describing the gradient approaching the asymptote.

| Species of parasitoid | Sex | n | a | 95% confidence interval | b | 95%confidence interval | $F_{(2, n-2)}$ | Probability |
|---|---|---|---|---|---|---|---|---|
| | | | | 1986 winter generation of *U. lugens* | | | | |
| *C. urabae* | f | 6 | 366.8 | 284.9-448.6 | 0.0108 | -0.0199-0.0415 | 160.2 | <0.001 |
| " | m | 5 | 312.7 | 273.5-352.0 | 0.0073 | 0.0031-0.0115 | 635.1 | <0.001 |
| *D. eucalypti* | f | 21 | 275.1 | 255.7-294.5 | 0.0073 | 0.0031-0.0113 | 1005.7 | <0.001 |
| " | m | 18 | 240.5 | 216.6-264.4 | 0.0057 | 0.0030-0.0085 | 659.1 | <0.001 |
| | | | | 1986-1987 summer generation of *U. lugens* | | | | |
| *C. urabae* | f | 100 | 389.5 | 378.7-400.3 | 0.0087 | 0.0079-0.0095 | 9762.7 | <0.001 |
| " | m | 85 | 315.8 | 299.2-332.5 | 0.0096 | 0.0082-0.0110 | 3797.0 | <0.001 |
| *D. eucalypti* | f | 56 | 301.2 | 283.4-319.0 | 0.0109 | 0.0091-0.0128 | 1256.9 | <0.001 |
| " | m | 52 | 235.4 | 223.3-247.5 | 0.0153 | 0.0129-0.0177 | 1328.9 | <0.001 |

whereas during the winter generation this overall increase was only about one and a half times for both species of parasitoid.

Even though *C. urabae* achieved larger maximal weights than *D. eucalypti*, it was *D. eucalypti* that carried a greater number of fully developed eggs upon emergence and upon death. Egg load in both species nearly doubled between the time of emergence and time of death. A positive correlation between female weight and egg load at the time of emergence and especially at time of death was apparent for *C. urabae* and for *D. eucalypti* (Fig. 2). Neither species has a significant preoviposition period, both being capable of successful oviposition at least within several hours of emergence (Appendix 2). Eggs of both species were elongate and of approximately the same maximum width (13 µm) but the length of *C. urabae* eggs was greater (226±3.0 (SE), 213-238 µm; n=12) than those of *D. eucalypti* (200±2.3, 188-213 µm; n=12).

## Discussion

Despite *C. urabae* generally being the larger parasitoid, for hosts of the same head capsule width, it was *D. eucalypti* that left less dry weight of its host behind upon emergence. This may be due to host regulation whereby foliage consumption or head capsule width was affected by parasitism, or by *C. urabae* emerging later during the growth of that instar. Changes in the levels of foliage consumption by parasitized lepidopteran larvae have been widely reported (Vinson and Iwantsch 1980). Supporting evidence that host's of *C. urabae* were larger relative to head capsule width than hosts of *D. eucalypti* was provided by the analyses of exit position of the two parasitoids. Within hosts of the same head capsule width it was *C. urabae* that emerged closer to its host's last abdominal segment indicating body length to be greater in hosts parasitized by *C. urabae* than in hosts parasitized by *D. eucalypti*. Laboratory experiments upon host growth and development following parasitization by *C. urabae* and *D. eucalypti* would help determine at what stage of growth within an instar that parasitoids emerged and whether any significant host regulation was taking place.

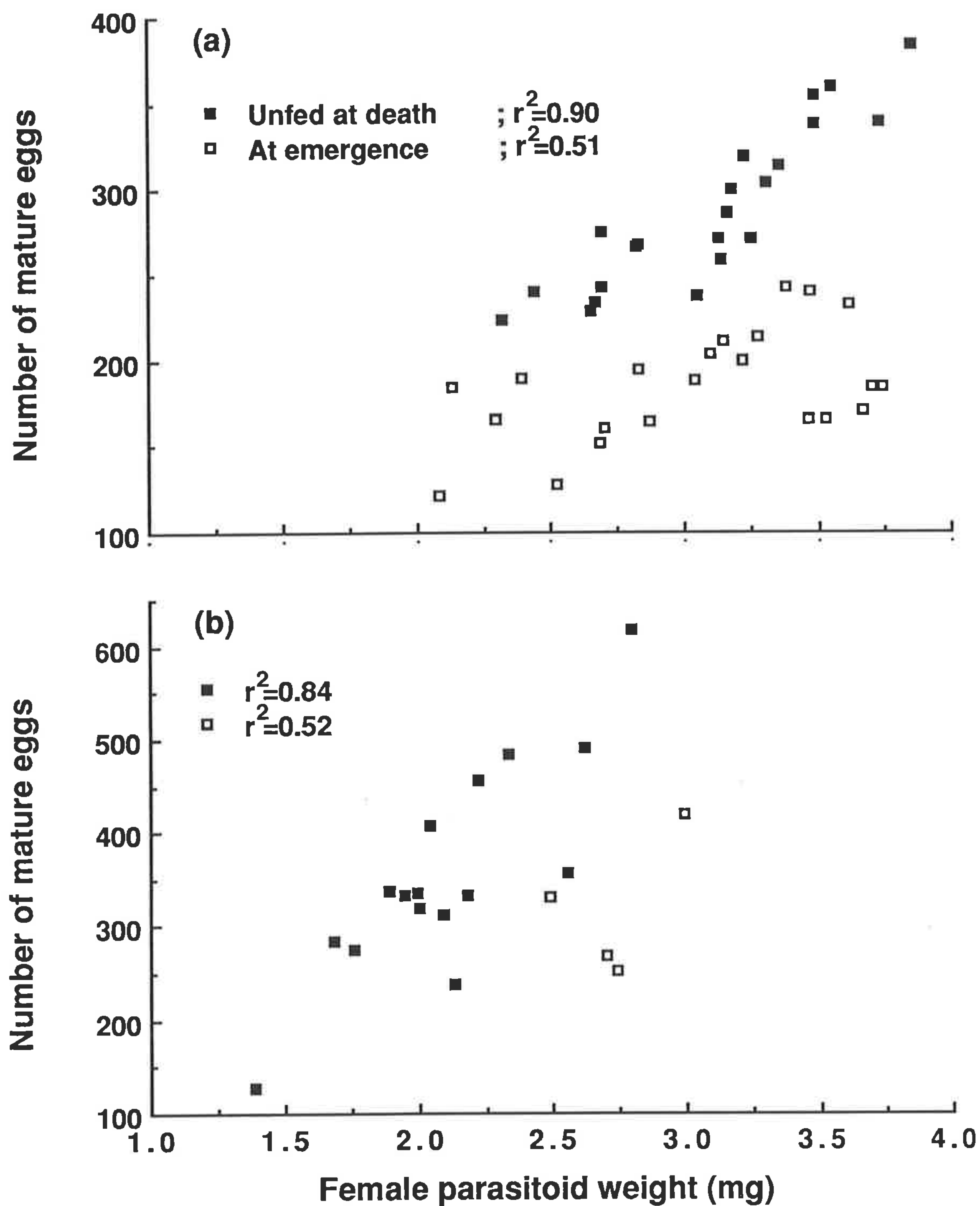



**Fig. 2.** The relationship between female parasitoid weight and the number of fully developed eggs held by a female at the time of emergence from the pupal cocoon, and upon death when left unfed at 20°C and 75% relative humidity, for **(a)** *C. urabae*, and **(b)** *D. eucalypti*.

The asymptotic relationship between host and parasitoid weight indicates an upper limit to parasitoid size for *C. urabae* and *D. eucalypti*. This contrasts with the simple linear relationship between parasitoid and host weight expressed by Beckage and Templeton (1985) for *H. exiguae*, Gunasena *et al.* (1989) for *Campoletis sonorensis* (Cameron), and King (1988) for *S. cameroni*. For hosts whose size exceeds that of the parasitoid a linear relationship would suggest no upper limit to parasitoid size. Although an equation was not fitted to the data of Charnov *et al.* (1981) on weights of *L. distinguendus* and its host their data clearly fits an asymptotic curve similar to that of *C. urabae* and *D. eucalypti*.

Lower limits to the weight range of koinobiont parasitoids that attack the same host may influence the phenology and biology of such parasitoids. *D. eucalypti* is able to successfully emerge from smaller hosts than *C. urabae* and is therefore capable of emerging earlier during its hosts larval development as was observed during the summer generation of *U. lugens* (Chapter 3). Early adult eclosion may be an advantage to *D. eucalypti* by enabling it to subsequently oviposit in hosts that are less likely to have other parasitoid competitors, including *C. urabae*, established within them (Chapter 2). Conversely the 'small' size of these early-emerging females may be a disadvantage if it affects their ability to overcome host defensive behaviour during oviposition (Chapters 6 and 7).

For both species of parasitoid males were smaller than females. If the growth rate of the host limits parasitoid development, then males may complete larval development before females by virtue of their smaller size. Early male emergence may be an adaptation of males to gain access to females for mating. However the high level of heterogeneity in size of *U. lugens* in the field (Chapters 2 and 3) may override the precision of a system based entirely on host size, and thus the possibility of differing rates of parasitoid development should also be examined (Chapter 5).

One character positively correlated with increasing parasitoid size in both *C. urabae* and *D. eucalypti* was egg load. Nealis *et al.* (1984) found a similar relationship between egg load and parasitoid size with *C. rubecula*. The increases

observed in egg load after eclosion have been recorded in other microgastrines including *C. rubecula* and *Apanteles fumiferanae* (*s.l.*) Viereck (Nealis *et al.* 1984; Nealis and Fraser 1988). Differences in techniques, coupled with changing egg loads over time, complicate direct comparisons of egg loads between microgastrines but both *C. urabae* and *D. eucalypti* appear to have relatively high fecundities. High fecundities are common in ichneumonids that attack early host developmental stages, particularly those that experience high mortality early in their development (Price 1975). Similarly *C. urabae* and *D. eucalypti* attack early host instars that experience high larval mortality (Chapter 2) and the positive relationship between parasitoid size and egg load would further suggest egg load to be important to the fitness of both species of parasitoid.

Longevity may be another correlate of parasitoid size although this relationship does not hold for every species of parasitoid (King 1988; van den Assem *et al.* 1989). For *C. urabae* and *D. eucalypti* increased longevity would benefit those adults which must survive the several weeks between host generations when no suitable hosts are available (Chapter 3). It is these adults that are emerging from large hosts, and are therefore of maximal size, which may increase their survival. Those adults which survive this period have many hosts available to them at the beginning of a new host generation and their high fecundity would help maximize the number of hosts attacked thereby spreading the high risk of parasitoid mortality from host death during the host's early instars (Chapter 2).

There may be many other costs and benefits associated with intraspecific variation in parasitoid size in the field. Where size variation has a temporal pattern, as with *C. urabae* and *D. eucalypti*, it is of particular relevance. However it is important to be aware of these variations when interpreting successful host synchronization and parasitoid survival in the field.

# Chapter 5. Temperature and the development of *U. lugens, C. urabae and D. eucalypti.*

*Abstract*

The relationship between temperature and development of *U. lugens*, and between temperature, host size and development of *C. urabae* and *D. eucalypti* was investigated using constant temperatures. For development of *U. lugens*, eggs required 449 day-degrees (DD) above 6.5°C, larvae required 453 DD above 13.2°C for females and 453 DD above 11.5°C for males, and pupae required 218 DD above 9.0°C for females and 228 DD above 9.3°C for males. *U. lugens* underwent anything from 6-14 larval instars before pupation which affected the allometry of head capsules. Females had more instars than males, and both sexes had more instars at lower temperatures. Female pupae of *U. lugens* were heavier than males, but pupal weight was not significantly affected by temperature.

Egg-larval development of *C. urabae* after oviposition in small hosts required 348 DD above 11.5°C for females and 363 DD above 11.2°C for males; whilst egg-larval development after oviposition in mid hosts was much faster requiring 349 DD above 6.5°C for females and 317 DD above 6.5°C for males. Pupal development of *C. urabae* required 89 DD above 9.7°C for females and 89 DD above 9.3°C for males. Egg-larval development of *D. eucalypti* after oviposition in small hosts was quicker than that of *C. urabae* from small hosts, and required 342 DD above 7.9°C. Pupal development of *D. eucalypti* was slightly longer than that of *C. urabae* requiring 86 DD above 10.7°C. When *D. eucalypti* were reared at 15°C, some individuals pupated when predicted by day-degree summation while others delayed emergence from the host by a further 120-182 days indicating a physiological delay in development. Weight of adult parasitoids increased with decreasing temperature for *C. urabae* but not for *D. eucalypti*. The longevity of adult *C. urabae* was greater than that of adult *D. eucalypti*, with honey increasing longevity by up to 15-28 times above that of unfed parasitoids.

Simulation of the phenologies of *U. lugens*, *C. urabae* and *D. eucalypti* in the Adelaide region using thermal constants showed good fit between field observed and predicted durations of life stages, and confirmed the presence of a physiological delay in emergence of *D. eucalypti* in winter and during its second generation in summer.

## Introduction

The growth and development of insects is profoundly affected by temperature where differing life stages may show differing temperature responses (Gordon 1984). For a koinobiont endoparasitoid, egg-larval development takes place within the host, and thus the host determines the temperature it experiences. Such relationships require physiological synchrony to maintain parasitoid success (Smilowitz and Iwantsch 1973). This is especially so for parasitoids with a narrow host range that attack hosts with discrete generations.

*Uraba lugens* Walker has two discrete generations per year in the Adelaide region; the summer generation hatches in December-January and lasts approximately four months whilst the winter generation hatches in April-May and lasts approximately eight months (Morgan and Cobbinah 1977). In other areas along the east coast of Australia *U. lugens* is also bivoltine (Campbell 1962, Harris 1974), whereas in highland Victoria (above 610 m) (Harris 1974), Tasmania (Elliot and deLittle 1985) and south-western Western Australia (Strelein 1988) it is univoltine. Campbell (1969) proposed that *U. lugens* had two morphologically indistinguishable yet biologically different morphs; the highland form which had 13 larval instars and a different pattern of egg deposition from the lowland form which had just 11 larval instars. However, in South Australia *U. lugens* has 8-13 larval instars which led Morgan and Cobbinah (1977) to conclude that Campbell's morphs may be temperature induced rather than the result of genetic differences. Changes in the head capsule width of an instar when *U. lugens* is reared under differing conditions (Chapter 3) and seasonal variation in the instar that first commences head capsule stacking (Chapter 2) have also been proposed to be affected by temperature.

*U. lugens* is the host of a large parasitoid complex in the Adelaide region including the solitary, koinobiont, larval, endoparasitoids *Cotesia urabae* Austin and Allen and *Dolichogenidia eucalypti* Austin and Allen (Chapter 2). The phenologies of these two species differs within and between the two generations of *U. lugens* per year (Chapter 3). Both parasitoids commence each host generation by typically parasitizing 1$^{st}$ instar (small) hosts but then differ in their phenologies according to which host generation and what host size is next parasitized. *C. urabae* completes two generations during each generation of *U. lugens*. In summer it oviposits in small and mid (4$^{th}$-5$^{th}$ instar; mode head capsule width around 0.85-1.05 mm) hosts whilst in winter it attacks small and large (6$^{th}$-7$^{th}$ instar; mode head capsule width around 1.15 mm) hosts. *D. eucalypti* also completes two generations during the summer generation of *U. lugens* when it oviposits in small and mid hosts, but in the winter generation of *U. lugens* it attacks small hosts and only completes one generation. In its single winter generation and second summer generation *D. eucalypti* apparently delays emergence from the host thereby reducing the time required for adult survival between host generations.

The relationship between temperature and development of *U. lugens*, and between temperature, host size and development of *C. urabae* and *D. eucalypti* is central to the many of the unanswered questions concerning *U. lugens* and the phenology of *C. urabae* and *D. eucalypti*. For *U. lugens* these questions include: how many instars does *U. lugens* have, does the development of the sexes differ in response to temperature, and is head capsule width of an instar affected by temperature ? For *C. urabae* and *D. eucalypti* these questions include: does egg-larval development differ according to host size parasitized, does development differ between sexes, is adult size affected by rearing temperature, and does adult longevity of these two species differ ? To answer these questions a series of experiments was set up, using a range of replicated constant temperatures, to quantify the rate of development of the various life stages of *U. lugens, C. urabae* and *D. eucalypti*. Egg-larval development of *C. urabae* and *D. eucalypti* was measured from hosts parasitized when small and when mid size as these two sizes are both parasitized by these species in the field. Thermal requirements for the

completion of all life stages were estimated based on simple linear regression (Campbell *et al.* 1974) and day-degree summation was used to simulate the phenology of all three species in the field. This model was checked against field collected data from September 1985 to December 1987 (Chapter 3) to verify the accuracy of all thermal estimates.

## Materials and methods

Four separate experiments were undertaken on the developmental biology of *U. lugens, D. eucalypti,* and *C. urabae.* These experiments were set up to examine the relationship between temperature and:

(a) the preimaginal development of *D. eucalypti* and *C. urabae* (experiment 1),

(b) the larval development of *U. lugens* (experiment 1),

(c) the egg development of *U. lugens* (experiment 2),

(d) the pupal development of *U. lugens* (experiment 3),

(e) the adult longevity of *D. eucalypti* and *C. urabae* (experiment 4).

*1. Experiment 1: Larval development of* U. lugens *and preimaginal development of* D. eucalypti *and* C. urabae *from small and mid hosts*

*a. Experimental protocol*

Small hosts used in this experiment were hatched within six days of each other from 18 egg batches of *U. lugens* held at 20°C. To control for any maternal bias, all the egg batches were subdivided among all treatments before hatching and then glued with "Selleys Kwik Grip ®" to 18 leaves of a potted *Eucalyptus camaldulensis* Dehnh. tree. Mid hosts used in this experiment were reared at 22.5°C on potted *E. camaldulensis.* A subsample of 46 mid hosts was drawn before parasitization. Their head capsule widths were 0.60±0.245(SE) mm and their dry weights were 0.70±0.07(SE) mg, which was around the minimum size of mid hosts that was attacked in the field (Chapter 3). Only mated female *D. eucalypti* and *C. urabae* were selected for use in this experiment and were held individually in plastic vials with honey at 12°C and 12L:12D prior to use. Parasitoid age was between 3-9 days for *D. eucalypti* and between 6-18 days for

*C. urabae*. All parasitoids had emerged from large hosts and were consequently around maximal size (Chapter 4).

Prior to the commencement of the experiment, each of the 18 *E. camaldulensis* leaves with approximately 60 small hosts upon them were caged within 5x7 cm gauze leaf cages. Mid hosts feeding upon the potted *E. camaldulensis* were randomly subdivided to 18 8.5x10 cm gauze stem cages (48 per cage) containing cut *Eucalyptus leucoxylon* F. Muell. foliage. Hosts were allowed to feed in these cages for 12 hrs to promote feeding damage and frass production, both of which may be used as host finding cues by parasitoids (Vinson 1975). After 12 hrs cages were streaked with honey, and two female parasitoids placed in each mid host cage and one placed in each small host cage. Six of the 18 cages for each host size had no parasitoids added to them and were used to document the development of *U. lugens*. Parasitoids were observed until at least one host was stung within each cage. Cages were then left at 20°C, checked after 12 hrs for any dead parasitoids which were replaced, and after 24 hrs all parasitoids were removed.

To control for differences between parasitoids, and for variation in levels of parasitism between cages, all hosts from each cage were equally subdivided (42 per cage for small hosts and 48 per cage for mid hosts) to rearing cages (20x20 cm), which were provided with cut *E. leucoxylon* foliage. In turn, rearing cages were allocated to one of six temperature chambers (2x15°, 2x20° and 2x25°C, of accuracy±1°C) at 12L:12D. Thirty-six rearing cages were used and distributed so that each temperature chamber had the following cages: unparasitized small hosts, unparasitized mid hosts, *C. urabae* parasitized small hosts, *D. eucalypti* parasitized small hosts, *C. urabae* parasitized mid hosts, and *D. eucalypti* parasitized mid hosts.

Foliage was changed in rearing cages every 4, 7, and 10 days at 25°, 20°, and 15°C respectively. Foliage was supplemented within these times if leaf quality deteriorated or if more than 75% of foliage was eaten. When foliage was replaced all exuviae and cast head capsules on the cage floor were collected and counted, and the

latter measured. The position of rearing cages was rotated at each foliage change to control for any microclimatic differences within a chamber.

Rearing cages containing parasitized larvae were checked daily to record when parasitoid larvae emerged from their host to pupate and later for adult eclosion. Adult parasitoid weight was measured by weighing cocoons before and after eclosion (see Chapter 4). The head capsule width and dry weight of each host killed by a parasitoid was measured as in Chapter 3. Rearing cages containing unparasitized larvae were checked daily for pupation and subsequent adult eclosion. Each pupa of *U. lugens* was removed from its cocoon, sexed and weighed. The larval head capsules attached to the cocoon (McFarland 1978) were also removed, counted and measured.

*b. Analysis of data*

All larval durations of *U. lugens* and egg-larval and pupal durations of *D. eucalypti* and *C. urabae* were converted to developmental rates for each sex by taking their inverse and regressed against temperature. Unequal sample sizes between replicates, sexes, and temperatures necessitated factorial regression analysis of developmental rates via the GLM procedure of SAS (1985). In this analysis replicate effect was tested for with a combined interaction term for all main effects. If the mean square for this term was not significant it was subsequently used along with its associated degrees of freedom as the error mean square for calculating type I F values on main effects and interactions. This was a more logical and conservative test of statistical significance as it tested for intercage rather than intracage differences. The residual mean square as an error term gave unrealistic weight to the chance of finding significant F values because of the high number of degrees of freedom associated with it.

The frequency data for dry weight and head capsule width of hosts from which parasitoids emerged, for the size of head capsules collected on the cage floors, and for the number of instars of *U. lugens* were tested for sex, replicate, and temperature differences by log-linear modelling via the CATMOD procedure of SAS (1985). Data

were pooled where necessary to ensure no more than 20% of cells had effective sample sizes less than five.

The relationship between parasitoid weight and host dry weight from which parasitoids emerged was fitted to the negative exponential growth curve using the Marquardt iterative method via the NLIN procedure of SAS (1985) as in Chapter 4.

*2. Experiment 2 : Egg development of* U. lugens

*U. lugens* egg development was studied by randomly subdividing 20 freshly laid egg batches of *U. lugens* and holding a row (9-37 eggs per row) from each of these batches at 10°, 15°, 20°, 25°, and 30±1°C. Eggs were kept at 75% relative humidity by holding them above a solution of saturated sodium chloride (Winston and Bates 1962). The number of eggs hatching in each row was recorded daily until hatching was completed. Eggs that failed to develop (head capsule never became visible through the operculum) and eggs that developed but failed to hatch were also recorded.

The developmental time of each egg was converted to a developmental rate and the rates fitted against temperature to a non-linear curve (Stinner *et al.* 1974) by the NLIN procedure of SAS (1985). The model used was: $d = \dfrac{c}{1 + e^{k1 + k2(t)}}$

where d = developmental rate (1/days), t = temperature (°C), and c, k1 and k2 are constants. Following this, the rates were reanalyzed over the linear range (15-25°C) using factorial regression via the GLM procedure of SAS (1985) to test for differences amongst temperatures and egg batches. Differences in failure to develop and failure to hatch of eggs were similarly tested amongst egg batches and temperatures.

*3. Experiment 3: Pupal development of* U. lugens

To determine developmental rates of pupae, 338 individuals that pupated at 20°C were sequentially subdivided among four temperatures (15°, 20°, 25° and 30°C) at 12L:12D and 75% relative humidity. Pupae were checked each morning for adult eclosion and sex was recorded upon emergence.

Developmental times were converted to developmental rates and fitted to the non-linear curve described for egg development of *U. lugens*. Results were analyzed over the linear range (15-25°C) using factorial regression via the GLM procedure of SAS (1985) to test for differences amongst temperatures and between sexes.

*4. Experiment 4 : Longevity of adult* C. urabae *and* D. eucalypti

Parasitoids were allowed to emerge from cocoons at 20°C and immediately transferred to either 10°, 15°, 20° or 25°C temperature chambers at 12L:12D. Both fed and unfed parasitoids were held individually in 1.6x4 cm plastic vials at 75% relative humidity. In the unfed treatments, 10 virgin female *C. urabae* and 10 virgin female *D. eucalypti* were placed in each temperature chamber and checked every six hrs until death. In the fed treatments, 20 virgin males and 20 females of both species were provided with honey and checked daily until death.

Lifespans were converted to a 'rate of ageing' by taking their inverse and differences between temperatures, species, food levels, and sex analyzed by factorial regression analysis using the GLM procedure of SAS (1985).

*5. Simulation of phenologies*

The development of *U. lugens*, *D. eucalypti* and *C. urabae* in the Adelaide metropolitan region was simulated using day-degree summation and compared to data collected from field populations within this region from September 1985 to December 1987 (Chapter 3). Meteorological records of daily maxima and minima from the Waite Institute, which was within the sampling region, were used to run the simulation program. Threshold temperatures and the accumulated number of day-degrees required to complete each stage of development were taken from the regression analyses of the data from experiments 1-3 (Tables 1 and 2). An upper threshold for development of 35°C was used in all simulations. Accumulated day-degrees were calculated by a computer program written in SAS using the sine curve method of Allen (1976).

Field populations of *U. lugens* in 1986 and 1987 had an observed mode for egg hatching of the summer generation around the 1st January. Thus the 1st January was used as the starting point for accumulating day-degrees in these two years. Oviposition for the winter generation was calculated from two days after adult eclosion of the previous generation (Appendix 2). In 1985 egg hatching of *U. lugens* could not be used as a starting point because egg hatching occurred before sampling began. In this year the mode week for pupation of *U. lugens* was used, and the 18th October selected, to accumulate day-degrees prior to and after this date.

Development of parasitoids was simulated within each generation of *U. lugens* from the predicted egg hatching date of *U. lugens* and to one week either side of this date. This range was selected because the frequency of field sampling was weekly and because it helped account for the spread in egg hatching observed of egg batches in the field (Chapter 2), and laboratory (this Chapter). First generation adults within each host generation were calculated to commence oviposition on their day of eclosion (Appendix 2).

In 1986 and 1987 larvae were parasitized at a predetermined date and size in the laboratory and then placed in the field. The development of these parasitoids was compared to that predicted by day-degree summation and helped to further validate the experimentally derived temperature thresholds.

## Results

*1. Development of* U. lugens

*a. Egg development*

Eggs within a row hatched over several days ranging from 4-10 days at 10$^{o}$C to 1-4 days at 30$^{o}$C. The failure of eggs to develop did not significantly differ between temperatures ($F_{4,76}$=0.89, p=0.477), but was significantly affected by which egg batch they came from ($F_{19,76}$=3.58, p<0.0001). However temperature did significantly affect the levels of eggs that began to develop but failed to hatch ($F_{4,76}$= 69.59, p<0.0001) with these levels rising to over 67% at either temperature extreme (Fig. 1). The egg

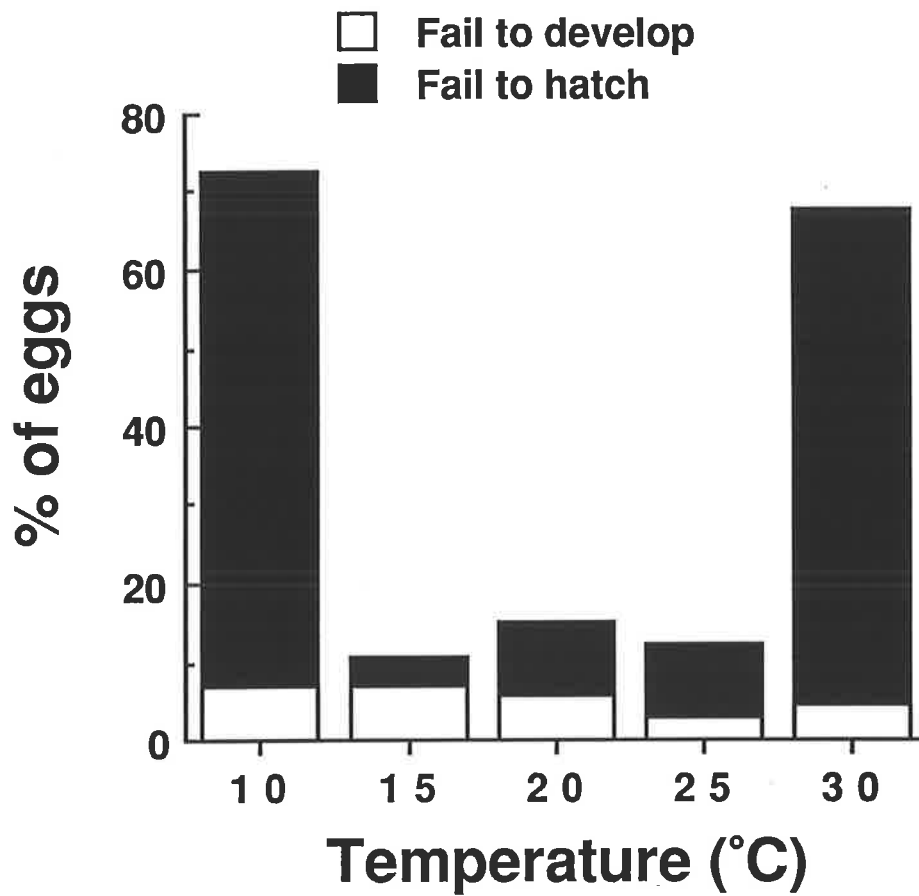



**Fig. 1.** The percentage of *U. lugens* eggs that failed to develop and developed but failed to hatch over five temperatures. Sample sizes at each temperature are 10°C : 381, 15°C : 326, 20°C : 335, 25°C : 350, and 30°C : 314.

batch from which an egg came was also a significant influence on whether eggs developed but failed to hatch ($F_{19,76}=2.00$, $p<0.025$).

The development of eggs of *U. lugens* over 10-30°C was non-linear and described by the following equation:

$$d = \frac{0.047}{1 + e^{3.447 - 0.202(t)}} \qquad (F_{3,1085}=68866.5,\ p<0.0001)$$

Developmental rates were linearly related to temperature over 15-25°C with a thermal constant of 449 day-degrees (DD) above a threshold of 6.5°C to complete development (Table 1).

*b. Larval development*

The number of larval instars of *U. lugens* varied with sex and temperature and ranged from 6-9 at 25°C to 10-14 at 15°C (Fig. 2). Between temperatures these difference were significant for males ($X^2_2=9.65$, $p<0.01$) but not for females ($X^2_2=2.52$, $p=0.284$). However within each temperature males underwent significantly fewer instars than females except at 15°C (25°C: $X^2_1=9.36$, $p<0.05$; 20°C: $X^2_1=7.55$, $p<0.025$; 15°C: $X^2_1=1.73$, $p=0.188$).

The number of head capsules shed by larvae before the commencement of head capsule stacking significantly changed with temperature ($X^2_{40}=86.08$, $p<0.0001$). At 15°C larvae dropped their first four head capsules whereas they only dropped their first three at 20°C and 25°C (Fig. 3). Hence 5$^{th}$ instar larvae developing at 15°C had no head capsules stacked whereas those developing at 20°C and 25°C had one head capsule stacked.

Tracing the sequence and width of head capsules attached to pupae of *U. lugens* showed that for larvae that underwent the same number of instars neither temperature nor sex significantly affected the width of an instar's head capsule. However, as the number of larval instars increased from 6-14 the mean head capsule width of each instar decreased (Fig. 4). The head capsule width of the ultimate instar could not be accurately

**Table 1. Relationship between temperature and rate of development of egg, larval, and pupal stages of *U. lugens*.**

Temperature range 15-25°C. Linear regression model: y=a+bx where: y= rate of development (1/days), x=temperature (°C), and a, b are constants. Temperature threshold for development=t (°C) and thermal requirement to complete development (day-degrees above t ) =k. Regression coefficients followed by the same letter are significantly different: a) $F_{1,6}=21.6$, $p<0.005$ ; b) $F_{1,333}=13.2$, $p<0.0005$ ; c) $F_{1,333}=92.7$, $p<0.0001$. Sexes pooled for use in phenology simulation with those individuals for which sex was not determined added to the data set.

| Life stage (sex) | n | b | a | $r^2$ | t (°C) | k (day-degrees) |
|---|---|---|---|---|---|---|
| Egg | 883 | 0.0022 | -0.015 | 0.96 | 6.5 | 449 |
| Larva (female) | 70 | 0.0022 | -0.029[a] | 0.83 | 13.2 | 453 |
| Larva (male) | 85 | 0.0022 | -0.025[a] | 0.83 | 11.5 | 453 |
| Larva (sexes pooled) | 158 | 0.0021 | -0.026 | 0.78 | 12.2 | 460 |
| Pupa (female) | 145 | 0.0046[b] | -0.042[c] | 0.96 | 9.0 | 218 |
| Pupa (male) | 107 | 0.0044[b] | -0.041[c] | 0.97 | 9.3 | 228 |
| Pupa (sexes pooled) | 252 | 0.0045 | -0.041 | 0.95 | 9.1 | 223 |

**Fig. 2.** Distributions of the number of larval instars for male and female *U. lugens* reared at three different temperatures: **(a)** 15°C, **(b)** 20°C, and **(c)** 25°C.

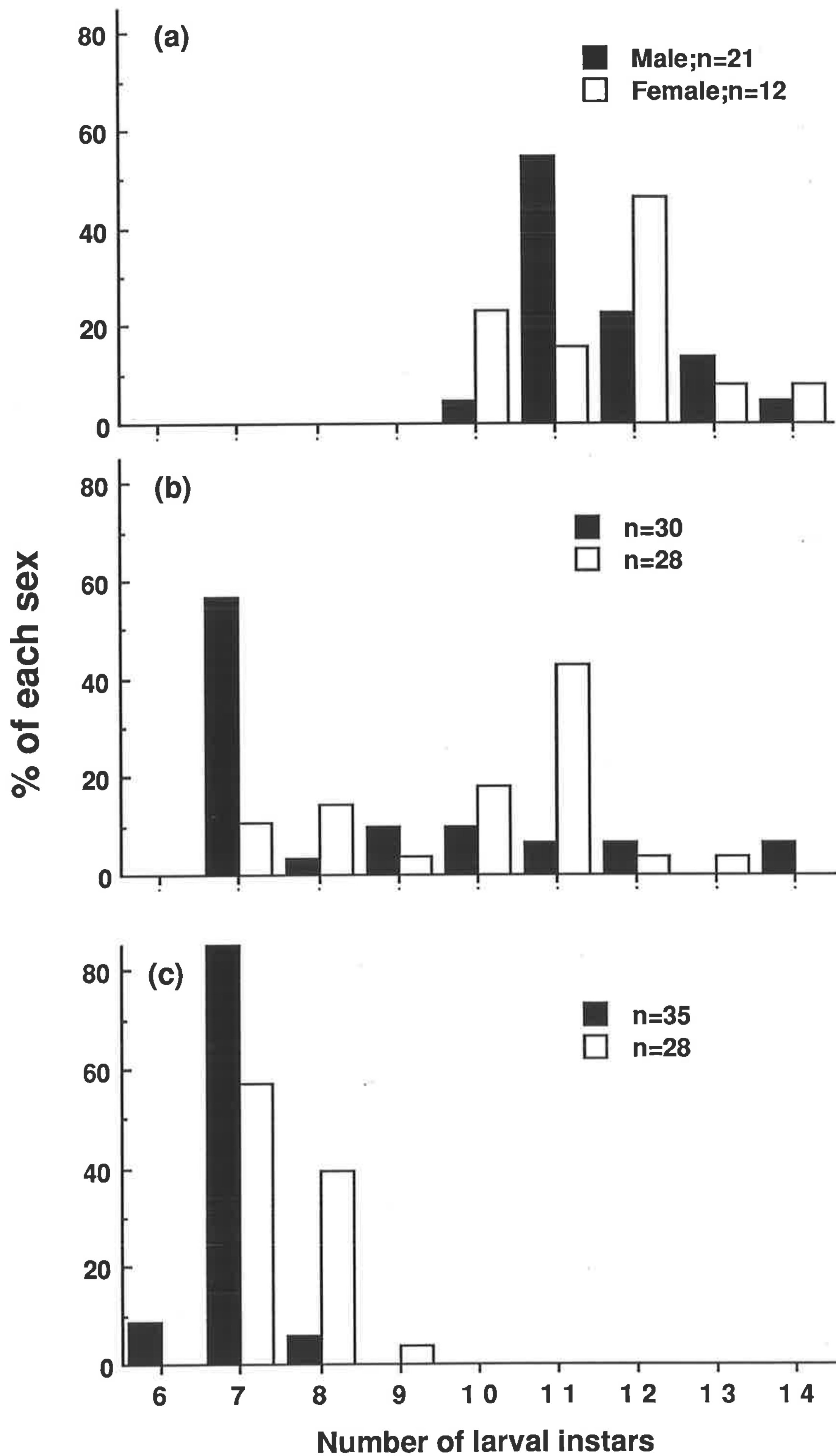
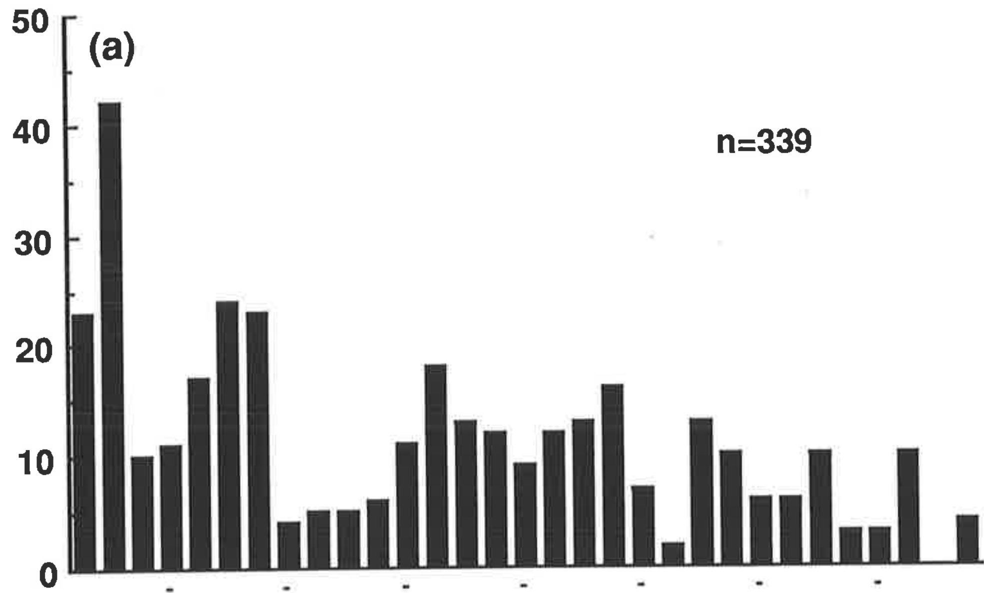
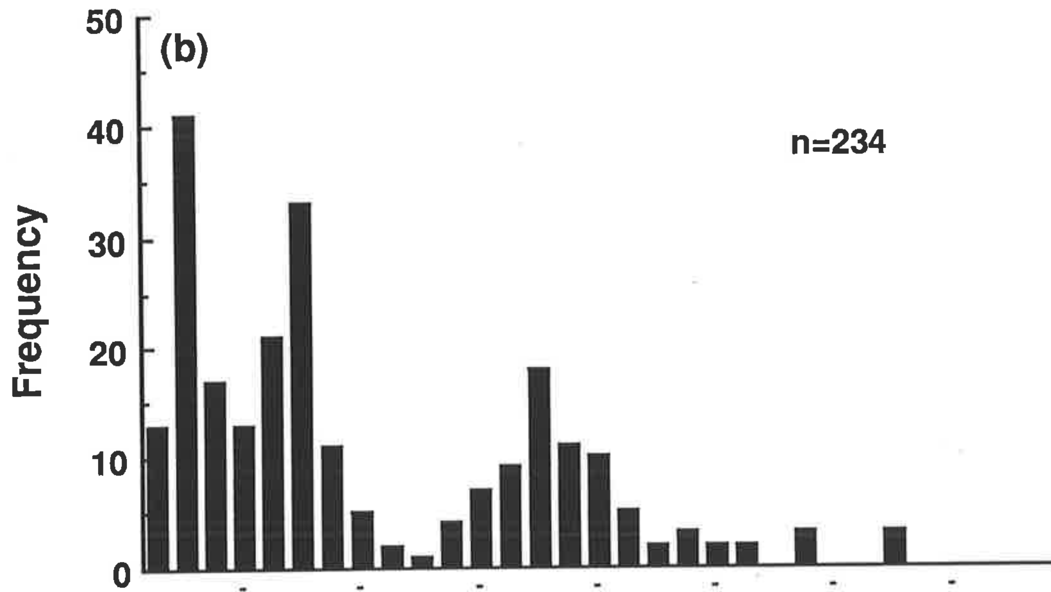

% of each sex
(a)
Male;n=21
Female;n=12
(b)
n=30
n=28
(c)
n=35
n=28
0
20
40
60
80
6
7
8
9
1 0
1 1
1 2
1 3
1 4
Number of larval instars

**Fig. 3.** The sizes of head capsules shed by larvae of *U. lugens* prior to the commencement of head capsule stacking when reared at three different temperatures: **(a)** 15ºC, **(b)** 20ºC, and **(c)** 25ºC. A total of 84 larvae were reared at each temperature.


50
40
30
20
10
0
(a)
n=339


Frequency
50
40
30
20
10
0
(b)
n=234

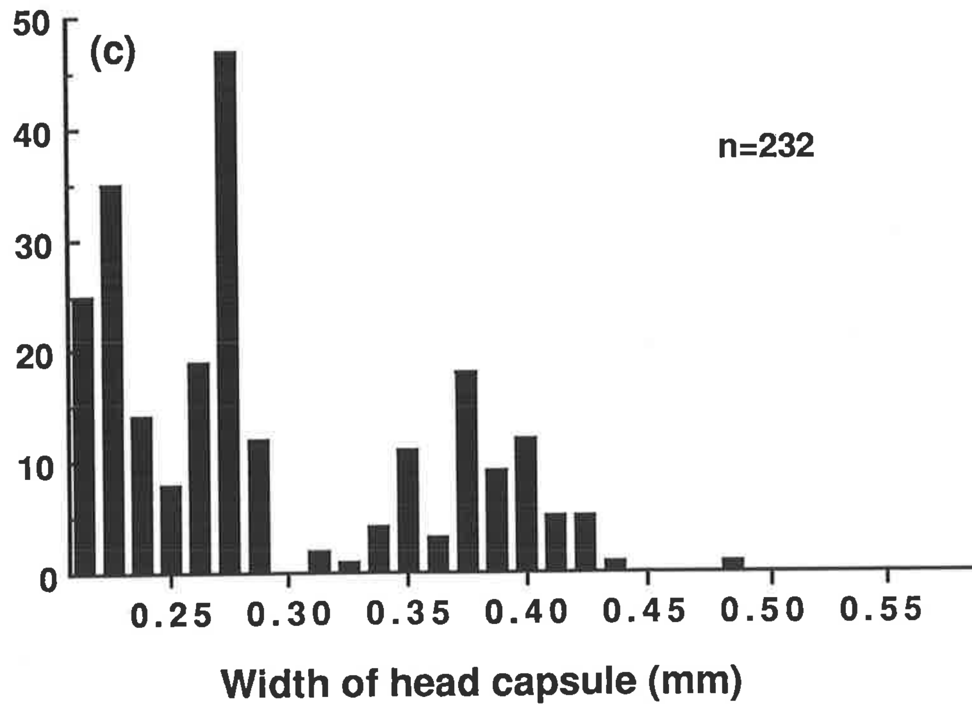
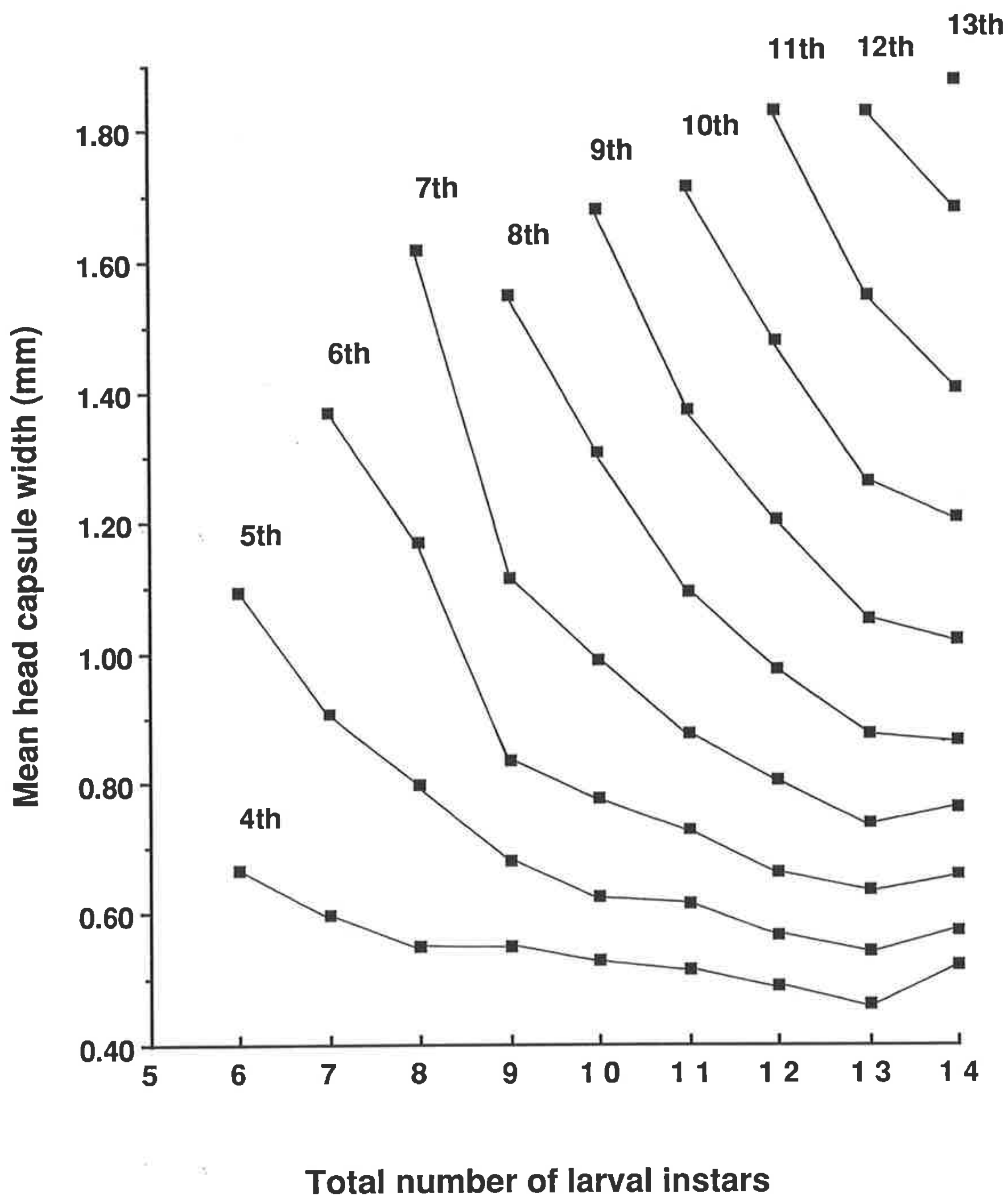

50
40
30
20
10
0
(c)
n=232
0.25
0.30
0.35
0.40
0.45
0.50
0.55
Width of head capsule (mm)

**Fig. 4.** The mean head capsule width of each instar of *U. lugens* for larvae that underwent from 6-14 larval instars before pupation across all three temperatures. Separate lines are plotted for each instar from 4th to 13th except for the first three instars, which are plotted in Fig. 3. Means calculated from 3, 67, 19, 4, 13, 27, 15, 5, and 3 individuals as number of larval instars increases from 6-14 respectively. All head capsule widths within an instar were significantly different at the 0.05 level except for the 12th instar : (4$^{th}$): $F_{8,103}$=11.2, (5$^{th}$): $F_{8,134}$=96.9, (6$^{th}$): $F_{7,143}$=294.0, (7$^{th}$): $F_{6,78}$=148.3, (8$^{th}$): $F_{5,62}$=34.4, (9$^{th}$): $F_{4,58}$=31.6, (10$^{th}$): $F_{3,46}$=39.6, (11$^{th}$): $F_{2,19}$=22.2, (12$^{th}$): $F_{1,5}$=5.1.



Mean head capsule width (mm)
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
Total number of larval instars
5
6
7
8
9
10
11
12
13
14
4th
5th
6th
7th
8th
9th
10th
11th
12th
13th

measured as it always split during pupation but that of the penultimate instar became significantly larger as the number of larval instars increased from 6-14.

The developmental rates of *U. lugens* larvae were significantly affected by temperature and sex (Table 1). Males pupated earlier than females larvae because of their lower temperature threshold of 11.5°C and fewer larval instars.

*c. Pupal development*

Successful pupal eclosion exceeded 88% over the 15-30°C range tested. Developmental rates over this range were non-linear and described by the equation:

$$d = \frac{0.097}{1 + e^{4.002-0.204(t)}} \quad (F_{3,195}=12745.6,\ p<0.0001)$$

Developmental rates were linearly related to temperature over 15-25°C with females having a thermal constant of 218 DD and a threshold of 9.0°C and males 228 DD and 9.3°C respectively to complete development (Table 1).

Weight of *U. lugens* pupae was not significantly affected by either the number of larval instars or temperature. However male pupae were significantly lighter (42.3±0.84 mg (SE); n=85) than females (68.6±1.84 mg (SE); n=70) : ($F_{2,6}=103.2$, $p<0.0001$). The cube root of the weight of pupae increased linearly with penultimate head capsule width ($r^2=0.62$, df=155) and also differed significantly between sexes ($F_{1,151}=115.88$, $p<0.0001$), indicating that penultimate head capsule width is a good predictor of pupal size.

2. *Development of* C. urabae *and* D. eucalypti

*a. Egg-larval development*

The rate of egg-larval development of *C. urabae* significantly differed with temperature ($F_{2,12}=190.0$, $p<0.0001$), host size parasitized ($F_{1,12}=205.9$, $p<0.0001$), and the interaction of host-size and sex of developing parasitoid ($F_{1,12}=5.90$, $p<0.05$) (Table 2). Although males of *C. urabae* developed faster than females it was host size parasitized that had the most significant affect on rate of egg-larval development. This

**Table 2. Relationship between temperature and rate of development of egg-larval, and pupal stages of *C. urabae* and *D. eucalypti*.**
Details as for Table 1.

| Life stage (sex) | Host-size | n | b | a | $r^2$ | t (°C) | k (day-degrees) |
|---|---|---|---|---|---|---|---|
| | | | | <u>*C. urabae*</u> | | | |
| Egg-larval (female) | small | 49 | 0.0029 | -0.033 | 0.86 | 11.5 | 348 |
| Egg-larval (male) | small | 74 | 0.0028 | -0.031 | 0.82 | 11.2 | 363 |
| Egg-larval (sexes pooled) | small | 130 | 0.0028 | -0.033 | 0.85 | 11.5 | 352 |
| Egg-larval (female) | mid | 74 | 0.0029 | -0.019 | 0.61 | 6.5 | 349 |
| Egg-larval (male) | mid | 55 | 0.0032 | -0.020 | 0.72 | 6.5 | 317 |
| Egg-larval (sexes pooled) | mid | 131 | 0.0030 | -0.019 | 0.65 | 6.5 | 335 |
| Pupal (female) | - | 120 | 0.0112 | -0.109 | 0.94 | 9.7 | 89 |
| Pupal (male) | - | 120 | 0.0112 | -0.105 | 0.94 | 9.3 | 89 |
| Pupal (sexes pooled) | - | 240 | 0.0112 | -0.107 | 0.93 | 9.5 | 89 |
| | | | | <u>*D. eucalypti*</u> | | | |
| Egg-larval (sexes pooled) | small | 113 | 0.0029 | -0.023 | 0.81 | 7.9 | 342 |
| Pupal (sexes pooled) | - | 232 | 0.0117 | -0.124 | 0.92 | 10.7 | 86 |

was reflected in the differences between temperature thresholds where *C. urabae* developing from small hosts had almost twice the temperature threshold of those developing from mid hosts.

The effect of host-size on egg-larval development was not successfully quantified for *D. eucalypti* as no parasitoids of this species emerged from mid hosts. Failure to emerge from mid hosts may have been due to one or more of the following: failure to successfully oviposit in mid hosts, failure to successfully develop in mid hosts, and high host mortality. The first of these was suggested from observations following parasitoid introduction, where the number of encounters between parasitoids and hosts was lower in these cages than in other 'treatments'. The last of these did occur after accidental introduction of a pathogen into mid host cages sometime after all *C. urabae* had emerged resulting in 21-46% of remaining larvae dying in cages with unparasitized and *D. eucalypti* parasitized larvae. Nevertheless the rate of egg-larval development of *D. eucalypti* from small hosts was found and was faster than that of *C. urabae* from small hosts (Table 2). Rates of egg-larval development of *D. eucalypti* from small hosts showed no significant difference ($F_{1,2}$=0.47, p=0.564) between sexes although a male-biased sex ratio of 10:1 hindered this comparison. At 15°C only six of the 10 parasitoids to emerge (emerging over days 49-56) were used in the analysis as the remaining four were clear outliers emerging at least 176 days after oviposition (Fig. 5). This delay exceeded the larval duration of the majority (over 90%) of the unparasitized population of *U. lugens* used in this experiment. Only 10% of small larvae were successfully parasitized at 15°C (parasitoid reached the pupal stage) whereas over 65% of small larvae were successfully parasitized at 20° and 25°C, despite equal subdivision of larvae among cages following oviposition. Dissection of the twenty larvae that died after day 130 at 15°C showed at least eight to have *D. eucalypti* larvae at varying stages of development within them. Thus the emergence pattern of *D. eucalypti* at 15°C appeared bimodal with some individuals emerging when predicted and others much later than predicted.

The size of host from which parasitoids emerged also varied with temperature, host size, and the species of parasitoid (Figs. 6 and 7). *D. eucalypti* consistently

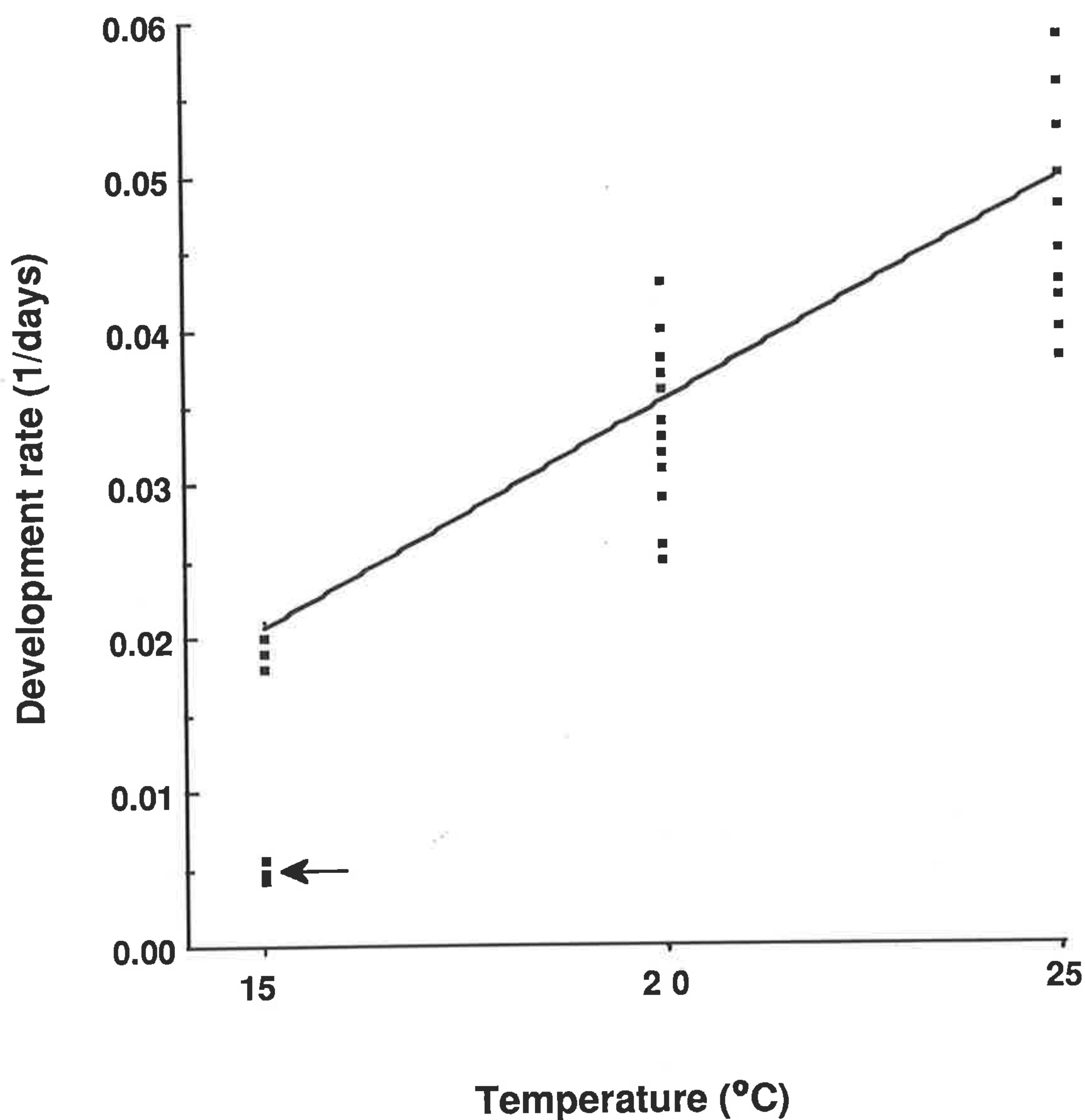



Fig. 5. The relationship between temperature and rate of egg-larval development of *D. eucalypti* when developing from small hosts. Where data points overlap only one point is plotted although the actual number of data points at 15°C is 10, at 20°C is 52 and at 25°C is 55. The equation describing the fitted line is given in Table 2. Outliers not included in the equation are evident at 15°C and are indicated by an arrow.

**Fig. 6.** Host head capsule width of *U. lugens* at the time of parasitoid emergence for *C. urabae* developing from small hosts, *C. urabae* developing from mid hosts, and *D. eucalypti* developing from small hosts when reared at **(a)** 15°C, **(b)** 20°C, and **(c)** 25°C.

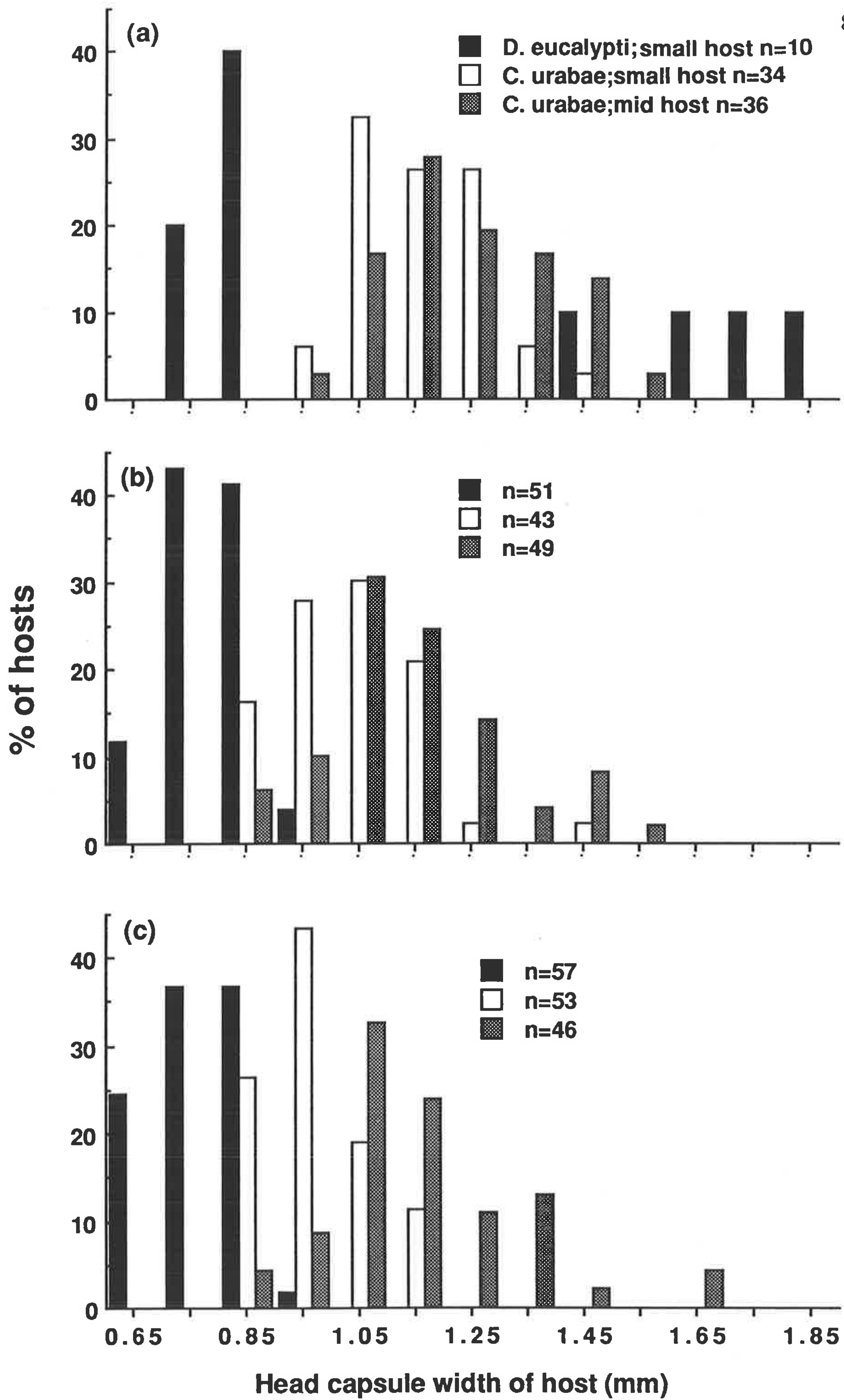
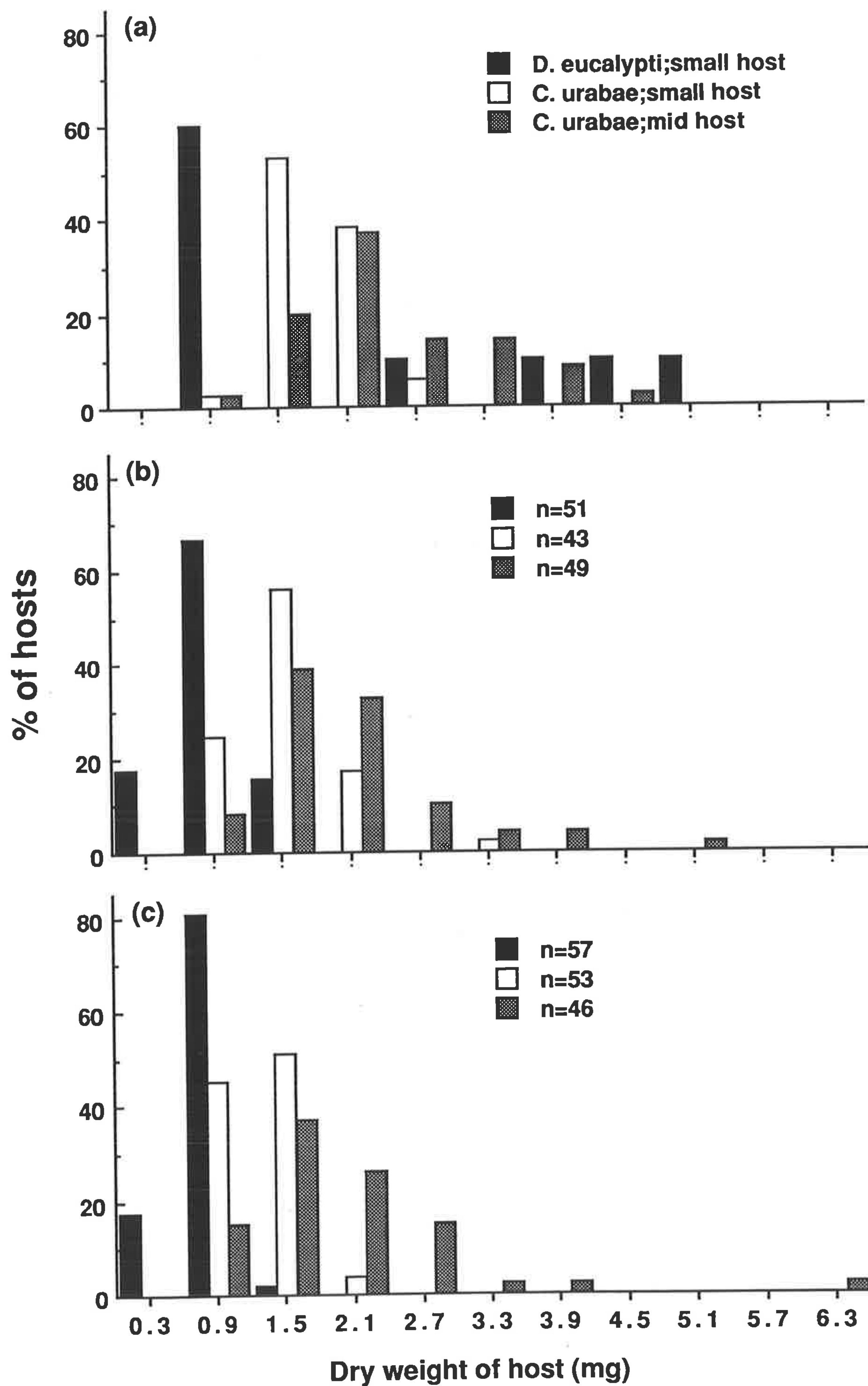

(a)
D. eucalypti;small host n=10
C. urabae;small host n=34
C. urabae;mid host n=36
(b)
n=51
n=43
n=49
(c)
n=57
n=53
n=46
% of hosts
0
10
20
30
40
0.65
0.85
1.05
1.25
1.45
1.65
1.85
Head capsule width of host (mm)

**Fig. 7.** Host dry weight at the time of parasitoid emergence for *C. urabae* developing from small hosts, *C. urabae* developing from mid hosts, and *D. eucalypti* developing from small hosts when reared at **(a)** 15°C, **(b)** 20°C, and **(c)** 25°C. Host numbers as for Fig. 6.


(a)
D. eucalypti;small host
C. urabae;small host
C. urabae;mid host
(b)
n=51
n=43
n=49
(c)
n=57
n=53
n=46
% of hosts
0
20
40
60
80
0.3
0.9
1.5
2.1
2.7
3.3
3.9
4.5
5.1
5.7
6.3
Dry weight of host (mg)

emerged from smaller hosts than did *C. urabae* when developing from small hosts across all three temperatures. Small hosts parasitized by *D. eucalypti* were killed at instars 4-6 (mode 5), 4-6 (5), and 6 or 12-13, whilst those parasitized by *C. eucalypti* were killed at instars 5-7 (5), 5-10 (6), and 8-10 (8) at 25°, 20° and 15°C respectively. Hosts killed by *C. urabae* that were parasitized when mid size were larger at parasitoid emergence than those that were parasitized when small size although there was overlap. Among all comparisons, the only statistically significant effect of temperatures was for *C. urabae* developing from small hosts for both dry weight ($X^2_4$=20.02, $p<0.005$) and head capsule width ($X^2_6$=21.39, $p<0.005$) (Figs 6 and 7).

*b. Pupal development*

Rate of pupal development was similar between the two parasitoids with *C. urabae* developing marginally faster than *D. eucalypti* (Table 2). The data for development of *C. urabae* pupae was pooled amongst those collected from small and mid host cages after testing showed no significant differences. The interval between sampling of one day for adult eclosion relative to pupal duration may have been too coarse to detect differences (if any) in development of pupae amongst small and mid hosts. The rates of development of male and female *C. urabae* pupae were not significantly different in gradient ($F_{2,6}$=1.26, $p$=0.349) but were in intercept ($F_{1,6}$=24.36, $p<0.0005$). For *D. eucalypti* pupal durations of further laboratory reared parasitoids were added to the data set to test for differences between sexes as no females emerged from pupae held at 10°C, and very few from the other temperatures during the experiment. Including this additional data the rates of development of male and female *D. eucalypti* pupae showed no significant differences between sexes ($F_{1,228}$=24.36, $p<0.0001$).

*c. Weight of adult parasitoids*

Changes in the temperature at which parasitoids developed influenced the final adult weight of *C. urabae* but had no impact on the final adult weight of *D. eucalypti*

(Table 3). Female *C. urabae* were typically larger than males and those that developed at lower temperatures reached larger maximal weights. Although maximal weights of *D. eucalypti* varied between temperatures, there was wide variation amongst individuals and no significant trend with changes in temperature.

*d. Longevity of adult* C. urabae *and* D. eucalypti

Honey and temperature significantly affected the longevity of *C. urabae* and *D. eucalypti* (Table 4). Honey increased the lifespan of female *C. urabae* by up to15x and of female *D. eucalypti* by up to 28x. *C. urabae* was significantly longer lived than *D. eucalypti* whether unfed ($F_{1,76}$=56.55, p<0.0001) or fed ($F_{1,312}$=81.45, p<0.0001). Males were shorter lived than females for both species, but these differences were not significant (*D. eucalypti* : $F_{1,156}$=1.67, p=0.199; *C. urabae:* $F_{1,156}$=3.76, p=0.054).

*3. Simulation of phenologies*

Simulation of life stages in the field from 1985-1987 showed a close agreement between observed and predicted durations of *U. lugens* and appearances of *C. urabae* and *D. eucalypti* cocoons (Figs. 8 (a)-(c)). Predicted durations for *U. lugens* fell within the observed duration of each life stage but overall totalled two weeks longer than observed in 1986 and one week shorter than observed in 1987. Predicted dates of pupation of *C. urabae* also fell within the observed pupal appearances and confirmed two generations of *C. urabae* in the summer and winter generations of *U. lugens*. A week's variation in the date of parasitization of *U. lugens* had the largest influence on the pupation date of *C. urabae* during its first generation in winter. This is because when parasitization is taking place in this generation, during April, daily temperatures are rapidly beginning to drop so that earlier parasitization enabled accumulation of many more day-degrees prior to the onset of colder winter conditions (Chapter 3). Day-degree summation for *D. eucalypti* showed good prediction of pupation from small hosts in summer but underestimated egg-larval duration and thus pupation from small hosts in winter (17-27% of observed). The equations for *D. eucalypti* developing from small

**Table 3. Relationship between remaining dry weight of a host from which a parasitoid emerged and weight of the subsequent adult parasitoid at 15°, 20° and 25°C.**

The non-linear regression model used was that for negative exponential growth of $y = a(1 - e^{-bx})$ where y=weight of adult parasitoid (mg x 100), x=remaining dry weight of the host (mg x 100), a= a constant describing the asymptote (ie. the maximal weight of the parasitoids), and b= a constant describing the gradient approaching the asymptote.

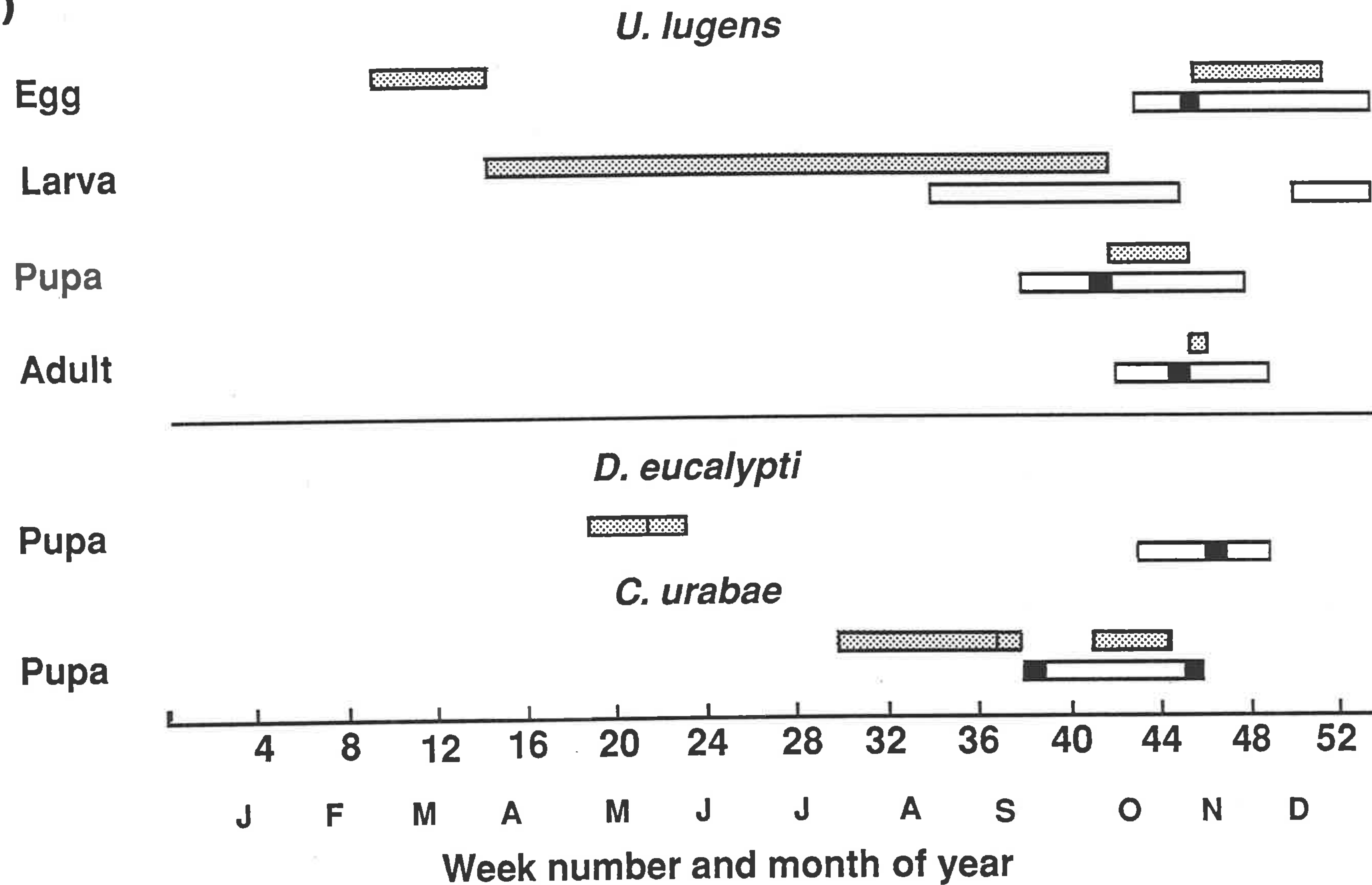
| Temperature (°C) | Sex | n | a | 95% confidence interval | b | 95% confidence interval | $F_{(2, n-2)}$ | Probability |
|---|---|---|---|---|---|---|---|---|
| | | | | | *C. urabae* | | | |
| 15 | m | 31 | 304.2 | 252.0-356.3 | 0.0097 | 0.0048-0.0145 | 608.4 | <0.001 |
| 15 | f | 23 | 621.1 | 308.3-933.9 | 0.0031 | 0.0008-0.0055 | 580.0 | <0.001 |
| 20 | m | 37 | 283.0 | 254.1-311.9 | 0.0136 | 0.0090-0.0183 | 1612.3 | <0.001 |
| 20 | f | 46 | 366.4 | 339.7-393.2 | 0.0110 | 0.0084-0.0136 | 2305.2 | <0.001 |
| 25 | m | 49 | 257.2 | 234.5-279.8 | 0.0136 | 0.0101-0.0171 | 1547.9 | <0.001 |
| 25 | f | 48 | 306.9 | 259.7-354.0 | 0.0113 | 0.0065-0.0161 | 802.8 | <0.001 |
| | | | | | *D. eucalypti* | | | |
| 15 | m | 9 | 213.3 | 132.6-293.9 | 0.0251 | -0.0570-0.1072 | 43.1 | <0.01 |
| 20 | m | 40 | 189.9 | 169.4-210.4 | 0.0223 | 0.0143-0.0303 | 1524.9 | <0.001 |
| 25 | m | 46 | 198.6 | 160.3-236.8 | 0.0153 | 0.0094-0.0212 | 1596.3 | <0.001 |

**Table 4. Temperature and longevity of fed and unfed adult *C. urabae* and *D. eucalypti*.**

Fed adults were provided with honey and unfed adults left without food and water. Longevity for unfed adults is in hours whilst for fed adults it is in days, and is given as a mean±SD.

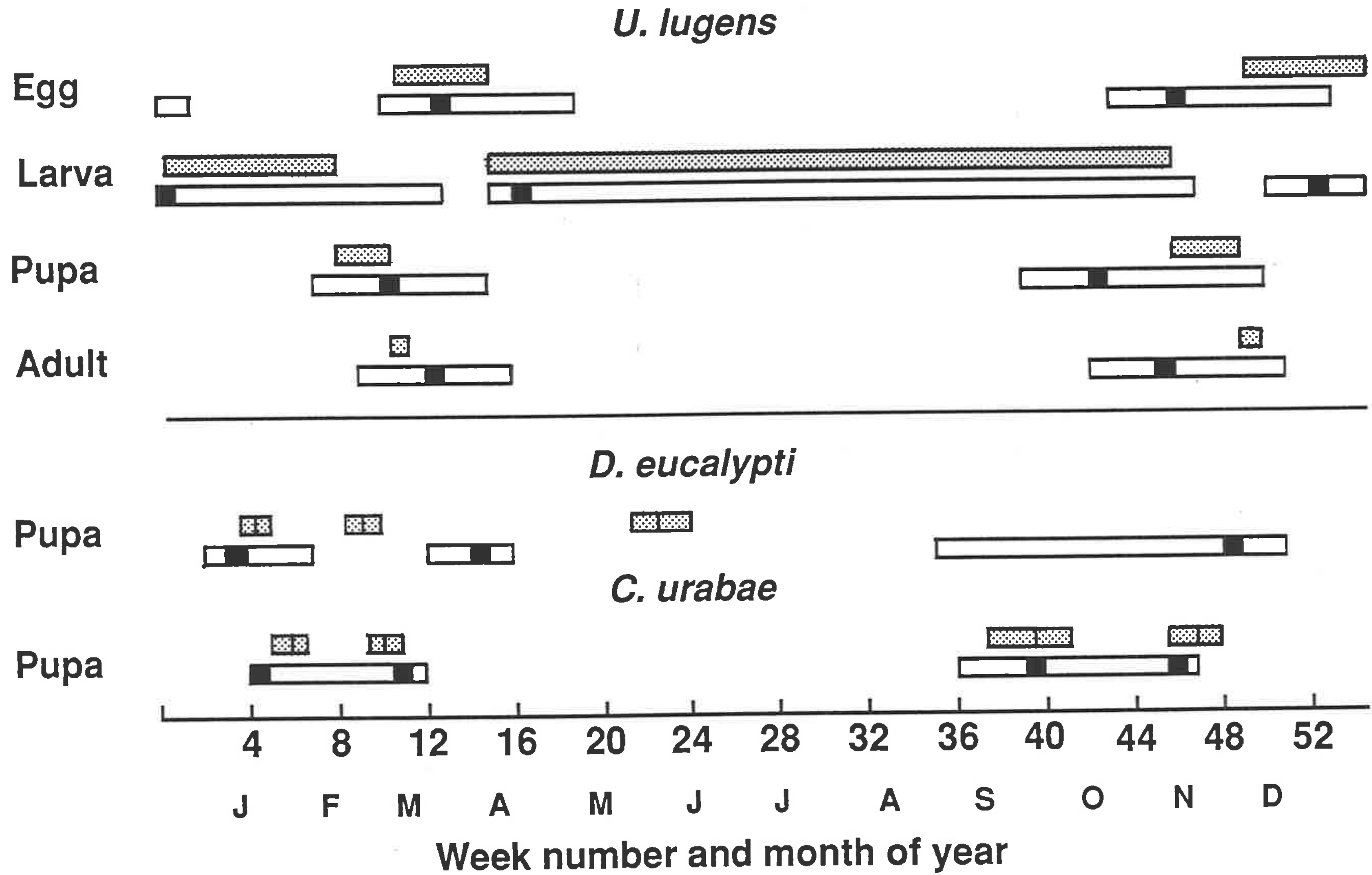
| Parasitoid species | Sex | Longevity | | | |
|---|---|---|---|---|---|
| | | 10°C | 15°C | 20°C | 25°C |
| | | **Unfed adults** | | | |
| *C. urabae* | female | 151.2±34.0 | 72.0±8.9 | 46.8±3.8 | 28.8±6.8 |
| *D. eucalypti* | female | 97.2±22.9 | 36.0±8.5 | 23.4±8.2 | 9.6±3.1 |
| | | **Fed adults** | | | |
| *C. urabae* | male | 55.1±13.6 | 33.6±6.4 | 24.7±4.7 | 19.0±2.3 |
| *C. urabae* | female | 59.0±12.6 | 35.8±5.8 | 27.1±3.6 | 19.0±1.9 |
| *D. eucalypti* | male | 46.6±6.8 | 39.0±3.7 | 16.4±4.9 | 11.1±4.8 |
| *D. eucalypti* | female | 42.7±6.2 | 39.0±3.4 | 19.8±4.5 | 11.4±3.7 |

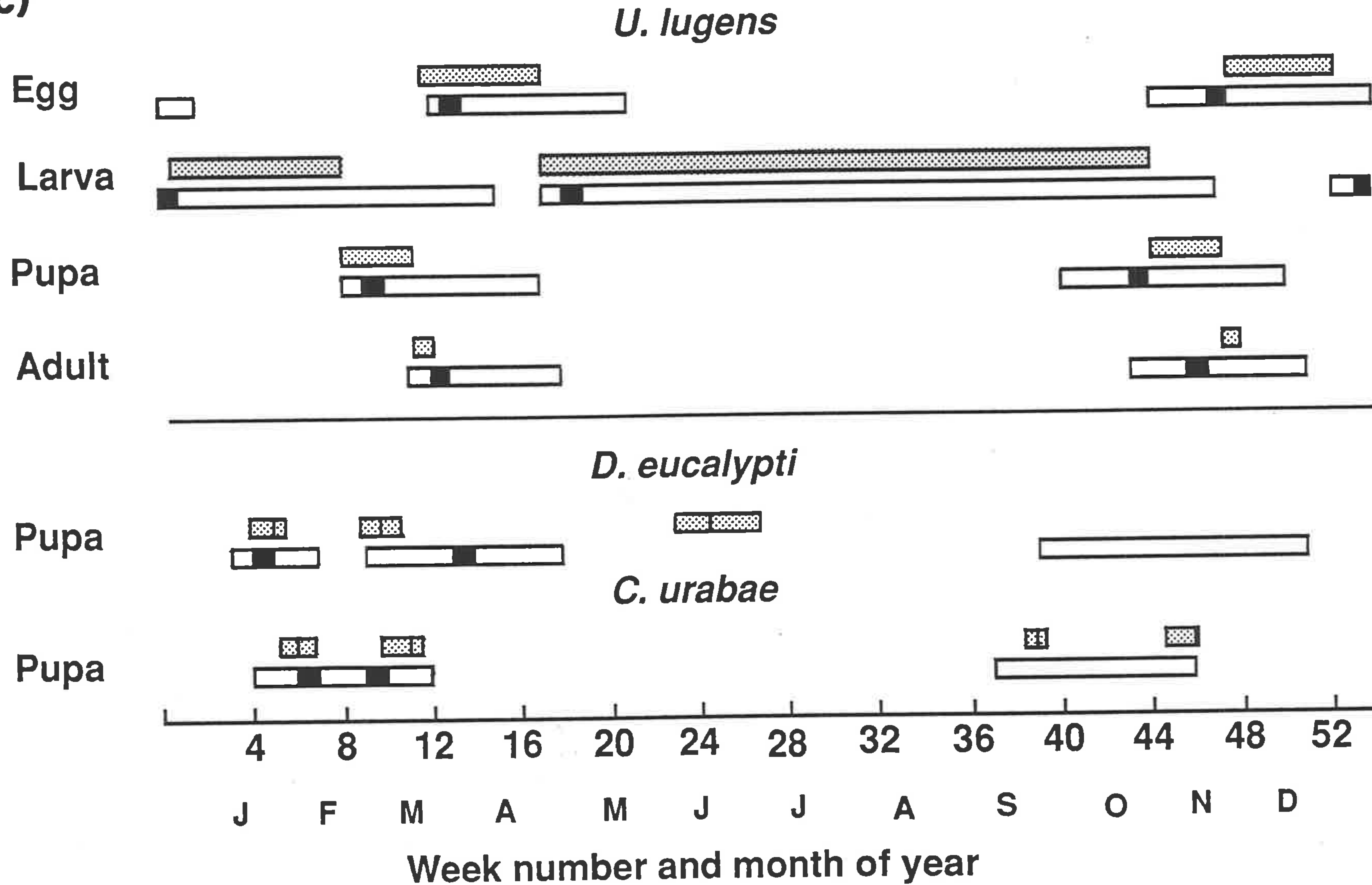
**Fig. 8.** Observed and predicted durations of the life stages of *U. lugens*, and observed and predicted appearances of cocoons of *C. urabae* and *D. eucalypti* for **(a)** 1985, **(b)** 1986, and **(c)** 1987. Observed durations and appearances are given in open bars with the peak week for beginning each life stage shaded in black. Peaks for egg hatching of *U. lugens* in 1985 and for pupation of *D. eucalypti* and *C. urabae* in late 1987 were not recorded. Predicted durations and appearances are in shaded bars and are based on accumulated day-degrees (see text for models and parameters). For parasitoids, the predicted pupal appearances represent the emergences from hosts parasitized a week to either side of a central date which was estimated by the predicted date of egg hatching for *U. lugens*. The line through these bars represents the day of pupation for parasitoids developing from hosts that were parasitized on this central date.



8(a)
U. lugens
Egg
Larva
Pupa
Adult
D. eucalypti
Pupa
C. urabae
Pupa
4
8
12
16
20
24
28
32
36
40
44
48
52
J
F
M
A
M
J
J
A
S
O
N
D
Week number and month of year

8(b)




8(c)
U. lugens
Egg
Larva
Pupa
Adult
D. eucalypti
Pupa
C. urabae
Pupa
4 8 12 16 20 24 28 32 36 40 44 48 52
J F M A M J J A S O N D
Week number and month of year

hosts also underestimated the egg-larval duration, when developing from mid hosts, during the second generation of *D. eucalypti* in summer (39-43% of observed). In contrast to *C. urabae,* it is evident from observed durations that the egg-larval development of *D. eucalypti* developing in mid hosts is longer than that when developing in small hosts.

Predicted egg-larval durations for parasitized *U. lugens* seeded to the field showed close fit to the observed durations except in the same instances mentioned above (Table 5). The best fit was for *C. urabae* developing from mid hosts. Interestingly, *C. urabae* developing from hosts of a size inbetween small and mid hosts (i.e. head capsule widths of 0.32-0.35 mm and 0.38-0.42 mm) had egg-larval durations that were inbetween those for *C. urabae* developing from small hosts and from mid hosts.

## Discussion

The number of larval instars in most, and perhaps all insects, is an indeterminate character, although many species apparently have a fixed number of larval instars (Nijhout 1981). Nijhout (1981) considers that it is principally the optimality of the conditions under which an insect is grown that determines the number of larval instars, with suboptimal conditions slowing growth and tending to achieve final size in a larger number of smaller steps. For *U. lugens* variability in the number of larval instars from 8-13 has been reported in the field (Morgan and Cobbinah 1977; Chapter 2), and from 6-14 has now been reported in the laboratory. Larvae held at lower temperatures and females have more larval instars. Thus, if the minimal number of instars is optimal (i.e. 6 instars) then development of *U. lugens* in the field indicates suboptimal conditions.

Temperatures are colder during the winter generation of *U. lugens* than in the summer generation, and so growth may take place through more instars. Winter generation larvae experience only a brief period of higher temperatures in May (mean daily temperature: 14.2°C), and then for three months are exposed to mean daily temperatures below 11.9°C, before temperature again rises and larval development can proceed more rapidly (see Chapter 3 for air temperatures). In contrast summer

**Table 5. Observed and predicted egg-larval durations of *C. urabae* and *D. eucalypti* from hosts parasitized in the laboratory and then placed in the field.**

Number parasitized refers to the number of cocoons recovered from the batch of larvae. A '?' for number parasitized is because all larvae died before parasitoid emergence due to vandalism of cages. An 'a' indicates predicted duration calculated by day-degree summation above the threshold for development from mid hosts, and a 'b' indicates predicted duration calculated by day-degree summation above the threshold for development from small hosts.

| Generation of *U. lugens* | Range of head capsule widths of hosts parasitized | Number parasitized | Date of oviposition | Duration of egg-larval stage (days) | |
|---|---|---|---|---|---|
| | | | | Observed duration | Predicted duration |
| | | | *C. urabae* | | |
| Summer 1986-87 | 0.63-0.83 | 21 | 10 Feb. | 17-29 | 23[a] |
| " | " | 16 | 15 Feb.[1] | 18-30 | 26[a] |
| Winter 1986 | 0.21-0.24 | 10 | 18 Jun. | 111-125 | 144[b] |
| " | 0.32-0.35 | 32 | 18 Jun. | 104-118 | 69[a] |
| " | 0.38-0.42 | 11 | 18 Jun. | 104-111 | 69[a] |
| " | 1.90 | 1 | 14 Oct. | 30 | 33[a] |
| Winter 1987 | 0.21-0.24 | 9 | 10 May | 137-145 | 155[b] |
| " | 1.35 | 1 | 11 Oct. | 25 | 28[a] |
| | | | *D. eucalypti* | | |
| Summer 1986-87 | 0.63-0.83 | 7 | 10 Feb. | 45-86 | 28[b] |
| Winter 1986 | 0.21-0.24 | ? | 18 Jun. | >125 | 85[b] |
| " | 0.32-0.35 | ? | 18 Jun. | >125 | 85[b] |
| " | 0.38-0.42 | ? | 18 Jun. | >125 | 85[b] |
| Winter 1987 | 0.21-0.24 | 53 | 10 May. | 181-218 | 71[b] |

generation larvae experience mean daily air temperatures between 20.3° and 22.1°C and therefore are capable of more rapid development. Support for developmental differences between summer and winter generations seems evident from differences in head capsule stacking behaviour. At 15°C, and in the winter generation, head capsule stacking began at the 6th instar whereas at 20°, 25°C and in the summer generation it began at the 5th instar.

Temperature rather than genetic differences may also explain the variation in instar number observed by Campbell (1969) between "highland" and "lowland" morphs of *U. lugens,* although it may not explain differences in the pattern of egg deposition (but see Appendix 2). It would be interesting to see if the univoltine-bivoltine pattern of *U. lugens* observed within its distribution (Turner 1944; Harris 1974; Elliott and deLittle 1985; Strelein 1988) can be explained by latitudinal and altitudinal temperature differences. This could be easily tested by finding the thermal requirements of *U. lugens* from different geographic regions, or less satisfactorily, by comparing the developmnental times of individuals from other regions with those predicted by the simulation equations developed for the Adelaide population.

The observed variation in larval populations of *U. lugens* in the field (Campbell 1962; Harris 1974; Strelein 1988; Chapter 2) may be accentuated by variable egg as well as larval development. This is especially so for the winter generation in Adelaide when egg development may benefit from the short periods of hot days that can occur from March to April in the Adelaide region. For instance in March 1985 there was a four day period when mean daily temperatures exceeded 30°C. Furthermore there appears to be an intrinsic variation in hatching even within an egg batch, but whether these differences are maternally inherited, as was indicated in the failure of eggs to develop and hatch, was not tested. Once hatched, larval development varies according to sex, microclimate, and possibly differing nutritional levels of foliage (Cobbinah 1978).

The egg-larval development of *C. urabae* is much faster than the larval development of its host and enables it to successfully complete two generations in both the summer and the winter generation of *U. lugens*. This ability to complete a second

generation is facilitated by the marked difference in temperature thresholds (cf. Tables 1 and 2) especially for egg-larval development of *C. urabae* when mid hosts are parasitized. Differences in developmental rates of parasitoids between host ages have been observed in other larval endoparasitoids, although developmental time do not always decrease as the host ages (Smilowitz and Iwantsch 1973; Beckage and Templeton 1985; Gunasena *et al.* 1989). Amongst the Microgastrinae, to which *C. urabae* and *D. eucalypti* belong, *Cotesia rubecula* Marshall (Nealis *et al.* 1984), *Cotesia congregata* (Say) (Beckage and Riddiford 1978), and *Cotesia glomerata* (L.) (Sato 1980) show decreasing developmental time with host age whereas *Cotesia yakutatensis* (Ashm.) is not affected by host age (Madar and Miller 1983). Regardless of the time of oviposition, some parasitoids remain as 1st instar larva within their host until the host reaches its final stage of development (Lawrence 1986). The egg-larval development of *C. urabae* appears constrained by small hosts, requiring hosts to reach a particular size or nutrient level (Slansky 1986), before completion of parasitoid development. That constraint is occurring is supported by the observations in Chapter 4 where adult weight of *C. urabae* was not maximal when emerging from hosts below a certain dry weight. Dissections of *U. lugens* larvae, when parasitized as small or mid hosts would help resolve whether and when *C. urabae* is constrained in its development from small hosts.

The much lower temperature threshold (7.9°C) of *D. eucalypti* compared to *C. urabae* (11.5°C) when developing from small hosts contributed to *D. eucalypti* pupating first in the summer generation of *U. lugens*. The development of *D. eucalypti* after oviposition in mid hosts could not be quantified but the phenology simulation indicates that development is longer than that in small hosts. Observations during laboratory rearing also showed a long egg-larval development time of *D. eucalypti* when larger hosts were parasitized (Allen pers. observ.). In contrast to *C. urabae,* increasing host size may be a 'trigger' for slower egg-larval development for *D. eucalypti*. However coupled with this, low temperatures (15°C) or factors associated with low temperatures, such as slow host development, also appear to be involved in 'triggering' physiological delay in *D. eucalypti*. This delay is most evident in the development of

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*D. eucalypti* in the winter generation of *U. lugens*, when similar low temperatures are experienced by parasitoid larvae early in their development. Again, as with *C. urabae*, it would be constructive to dissect hosts parasitized by *D. eucalypti* during the winter generation to observe their growth within the host.

Another correlate to field observations was the size of host from which parasitoids emerged at different temperatures. At 20° and 25°C the final host size was similar to those of hosts in the summer generation, whilst at 15°C it was similar to those of hosts in the winter generation (Chapter 3). That hosts prior to death in winter reach a larger size than they do in summer highlights the relative growth rates of hosts and parasitoids at lower temperatures. Larger host size at lower temperature is associated with increased size in *C. urabae*.

Pupal development of both parasitoids was relatively short in the field (around one week) as temperatures were typically high during pupation. Parasitiod pupae had low thermal requirements to complete development. If pupae were to be present in the field for long periods, this would increase their exposure to hyperparasitoids and possibly add to their already high levels of hyperparasitism in the field (Chapter 3).

Adequate longevity of adult parasitoids between host generations was shown to be dependent on parasitoids finding sufficient food for survival. Food sources for parasitoids can be a necessary prerequisite for parasitoid success in the field (Leius 1961). Between the two species, *C. urabae* is capable of surviving longer than *D. eucalypti*, over 10°-25°C, with or without food. Nevertheless the physiological delay in emergence of *D. eucalypti* from its host at the end of each host generation, to some extent compensates for the necessity to have 'long lived' adults to bridge the gap between generations of *U. lugens*.

The thermal requirements of *C. urabae* and *D. eucalypti* act to synchronize the development of these parasitoids with their host in the Adelaide region. To date *C. urabae* and *D. eucalypti* have only been collected in the Adelaide region (Austin and Allen 1989; Appendix 1), but if they are found over more of the geographic range of

*U. lugens* (Chapter 1), it would be interesting to test for thermal adaptation between regions. This may further contribute to understanding the variable development of *U. lugens* larvae and how their associated parasitoids overcome any such developmental differences.

# Chapter 6. Behavioural interactions between *U. lugens*, *C. urabae* and *D. eucalypti*.


*Abstract*

Behavioural interactions between *C. urabae, D. eucalypti* and their host *U. lugens* were observed at three host sizes over a 20 min. period. These sizes were 1st instar (small, gregarious), 4-5th instar (mid, gregarious) and 6-7th instar (large, solitary) larvae. The behaviour of *D. eucalypti* differed from *C. urabae* in that *D. eucalypti* used its fore and mid legs to hold small larvae before ovipositor insertion during 26.7% of visits, made more visits to patches of small larvae, infrequently proceeded through patches of mid larvae (1% of visits), made significantly less ovipositions in mid larvae, and failed to oviposit in large larvae. Small larvae were handled differently than mid or large larvae by both species of parasitoid. *C. urabae* and *D. eucalypti* made the most visits to patches of small larvae (33.0, 50.0 visits per 20 min. respectively), often made more than one oviposition during each visit to a patch of small larvae (34%, 48% of successful visits respectively) and always proceeded through the patch of small larvae. Small larvae responded to parasitoids by dispersing outward, increasing patch size by a factor of over 2.5 times, whilst mid larvae responded to parasitoids by moving inwards to form a denser group, decreasing patch size by a factor of up to 0.82 times. More mid larvae reared or thrashed after each parasitoid visit than small or large larvae and some larvae, particularly the mid size, continued to rear or thrash for up to two hours after parasitoid departure. Mid and large larvae occasionally injured parasitoids by biting held appendages. By rearing or thrashing immediately prior to an encounter with a parasitoid, mid and large larvae decreased by up to 50% their possibility of being parasitized. Variations in the success of *D. eucalypti* and *C. urabae* attacking hosts of different sizes reflected their phenologies in the field.

## Introduction

Animal defence can be divided into primary and secondary defence (Robinson 1969). Primary defences decrease the chance of an encounter with a predator (or parasitoid) and operate regardless of whether or not the predator is in the vicinity (Edmunds 1974). These include crypsis, aposematism, batesian mimicry and anachoresis. Secondary or direct defences operate when the prey (or host) is encountered and increase the prey's chance of survival. The secondary defensive behaviours of caterpillars include biting, regurgitation, thrashing, rearing and dropping on a silk thread (Awan 1985; Stamp 1986). Group defence may be classified as primary and/or secondary (Edmunds 1974).

Host acceptance is the process whereby a parasitoid accepts or rejects a host for oviposition after contacting it (Weseloh 1974). Host acceptance depends on the parasitoid detecting certain physical and chemical stimuli produced by the host (see Arthur 1981). Despite detecting these stimuli, host acceptance may still be interrupted by the aggressive behaviour of the host (Schmidt 1974). The outcome of an encounter is often assumed to be primarily due to the action of the parasitoid although some authors acknowledge that the behaviour of the host may be a factor (Noble and Graham 1966; Sullivan and Green 1950; Taylor 1988).

In this section I investigated the behavioural interactions between the parasitoids *Dolichogenidia eucalypti* Austin and Allen and *Cotesia urabae* Austin and Allen with their host *Uraba lugens* Walker. The specific aims were : (1) to compare how the defensive behaviours of different sizes of *U. lugens* influence host acceptance and the host-parasitoid interaction, and (2) to quantify and compare the oviposition behaviour of *C. urabae* and *D. eucalypti* when attacking different sizes of hosts. These two parasitoids attack three sizes of *U. lugens* in the field: small (typically 1$^{st}$ instar), mid (4-5$^{th}$ instar) and large (6-7$^{th}$ instar) (Chapter 3). This wide range of sizes enabled testing of how the interactions differ between the different developmental stages of host. *U. lugens* is gregarious until around the 5$^{th}$ instar (Campbell 1962; Morgan and

Cobbinah 1977) so that the effect of gregariousness on these defensive interactions was also quantified.

## Materials and methods

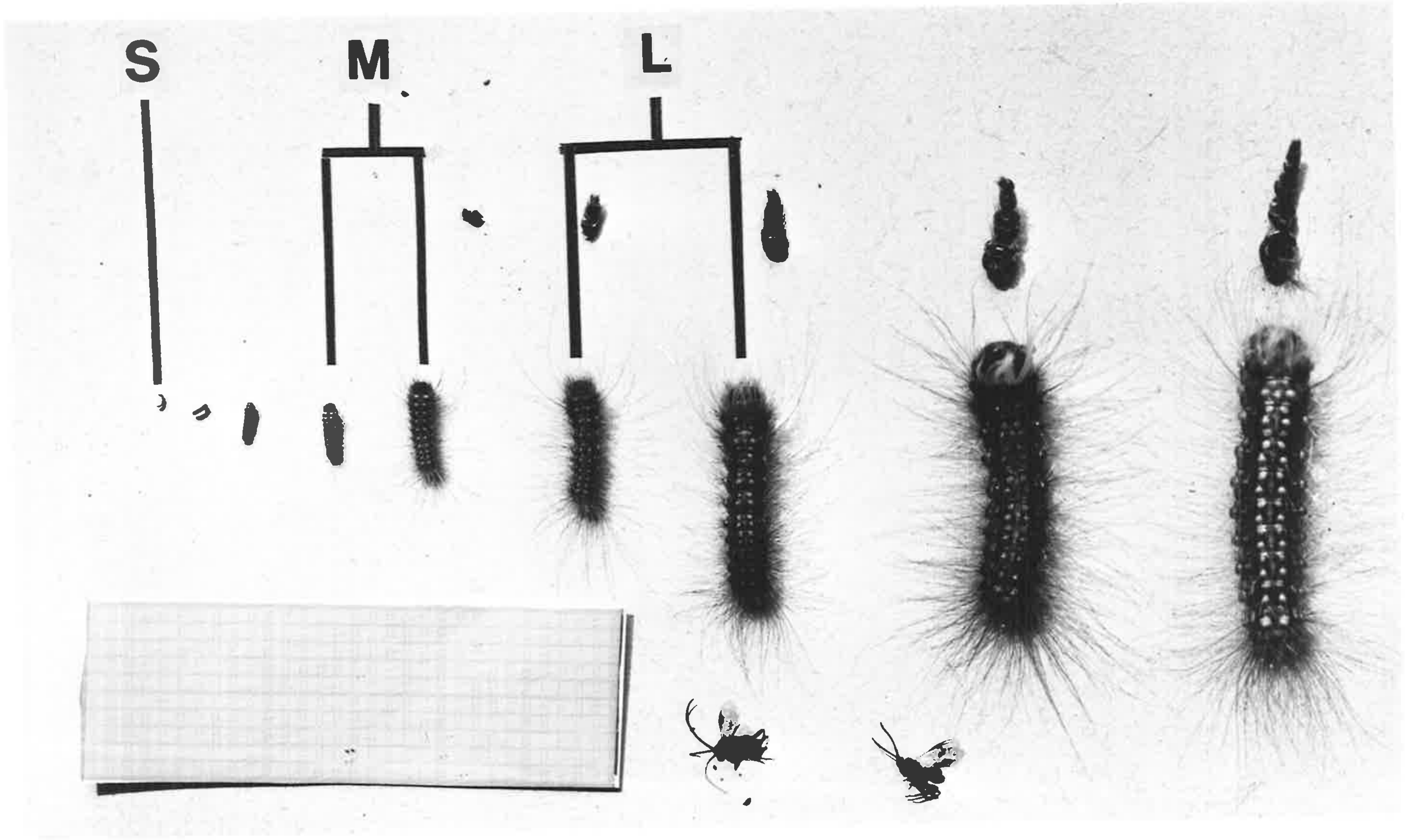
*1. Experimental protocol*

Thirty-six groups of approximately 60 *U. lugens* each, were reared on cut *Eucalyptus leucoxylon* F. Muell. foliage in 20x20 cm cages at 20ºC with a photoperiod of 12L:12D. At each of the three size classes tested (small, mid, large; Fig. 1), 12 groups were selected, and the numbers in each reduced to 40 larvae per group by removing excess larvae at random. Cut leaves, upon which larvae were feeding, along with the 40 larvae were placed in a 19x3 cm glass petri dish positioned under a colour video camera for observations. The groups of large larvae were accompanied by six leaves whilst the groups of small and mid larvae were each located entirely on one leaf.

Adult female *C. urabae* and *D. eucalypti* were collected as larvae in the field late in the host's summer generation, were reared at 20ºC, allowed to mate and then stored at 12ºC until use. Parasitoids for experiments were selected at random, their age varied from 1-19 days for *C. urabae* and from 4-22 days for *D. eucalypti*. Six *C. urabae* and six *D. eucalypti* were tested for each size class of *U. lugens*. Immediately prior to each experiment, each parasitoid was given three min. 'pre-exposure' in another petri dish with either small or mid *U. lugens* during which time they were allowed to oviposit in larvae. After 'pre-exposure' each parasitoid was released onto a leaf within the experimental petri dish containing *U. lugens*.

An event recorder program on a portable computer was used to record parasitoid oviposition and a voice tape recorder run to document both parasitoid and *U. lugens* behaviour. Recording began when the parasitoid first made contact with a *U. lugens* in the petri dish and continued for 20 min., at the completion of which the parasitoid was removed. The number of *U. lugens* rearing or thrashing was recorded at five min. intervals for two hrs after parasitoid departure. Subsequently all larvae were reared at 20°C until all parasites had emerged and the unparasitized *U. lugens* had pupated.

**Fig. 1.** The relative sizes of the first 9 instars of *U. lugens* reared at 20°C. Instars 1-9 from left to right and the stacked head capsules from above the head of each instar placed above that instar. A female *C. urabae* is bottom left and a female *D. eucalypti* is bottom right. Small (S), mid (M) and large (L) sizes of *U. lugens* used in this experiment are marked as indicated. Scale: 1 grid square = 0.5 mm.


S
M
L

The video, synchronized voice recordings, and event recorder data were used to produce time recordings of the following parasitoid behaviours associated with oviposition:

1) Visit. Time interval between patch entry and patch exit where a patch is defined as the group of small or mid larvae, whereas a single large larvae was considered equivalent to one patch.

2) Encounter. When a parasitoid contacted a *U. lugens* (for large larvae, encounter = visit).

Three types of encounter were noted:

(a) approach-retreat, parasitoid contacts a *U. lugens* but retreats;

(b) attack-fail, parasitoid contacts a *U. lugens*, sets upon it, but fails to insert ovipositor;

(c) attack-oviposit, parasitoid contacts a *U. lugens*, sets upon it, and inserts ovipositor (whether or not an egg was actually deposited was not determined).

The defensive behaviour of *U. lugens* was documented throughout each observation. The number of small *U. lugens* rearing or thrashing immediately after each visit by a parasitoid was voice recorded during the observation, but their size prevented any more detailed recordings of defensive behaviour. For mid and large *U. lugens*, slow motion video recordings were replayed to record the number rearing or thrashing immediately after each visit by a parasitoid, the position within the group of each *U. lugens* encountered, the frequency of encounter for each *U. lugens*, and whether larvae were rearing or thrashing prior to, during, and/or after, each encounter. For the gregarious sizes (small and mid) patch area prior to commencing the experiment and immediately after parasitoid departure was outlined on the television monitor. The area of each patch was then calculated using an "Apple Graphics Tablet ®" (Model no. A2M0029).

*2. Analysis of data*

Variations in the success of parasitoids in attacking *U. lugens* were examined, depending on the data, by two methods. The overall frequencies of approach-retreat, attack-fail and attack-oviposit were first tested between replicates (parasitoids), and then pooled to test between size classes and species of parasitoid, using log-linear contingency table analysis (LLCTA) (Genstat 1987). Number of visits and number of ovipositor insertions per parasitoid (hereafter termed 'ovipositions', see description of 'attack-oviposit' above) were compared between species of parasitoid and size classes of host using ANOVA and Student Newman-Keuls (SNK) ($P<0.05$) procedures of SAS (1985). The observation period was then subdivided into quarters to test for temporal variation in parasitoid behaviour. ANOVA and SNK procedures were used to compare the proportion of time spent in the patch, the number of visits and the number of ovipositions during each five min. interval of the 20 min. period.

To test whether the proportion of larvae exhibiting defensive behaviour changed with time, the total number of visits to each group by a parasitoid was equally subdivided into four successive periods or quarters. The number of larvae rearing or thrashing after the first visit of each quarter was then used to test temporal patterns in host defence. If the total number of visits was not divisible by four, the numbers either side of that quarter exhibiting defensive behaviour were added and divided by two. Division of the 20 min. observation time into periods was an unsatisfactory alternative to division of the number of visits because of differences between individual parasitoids in their total number of visits and in their temporal visiting pattern. ANOVA and SNK ($P<0.05$) tests were used to compare the mean number of larvae rearing or thrashing between quarters and to compare the mean duration of these behaviours after parasitoid departure. Changes in patch area between that prior to parasitoid entry and after parasitoid departure from the petri dish were analyzed using paired t-tests.

The influence of mid and large larval behaviour immediately prior to an encounter on the success of an attack by a parasitoid was examined by comparing the frequencies of attack-fail, approach-retreat, and attack-oviposit to larvae that were either exhibiting

rearing or thrashing behaviour, or not displaying these behaviours. Frequencies were pooled across replicates to overcome low expected cell frequencies and tested using LLCTA.

## Results

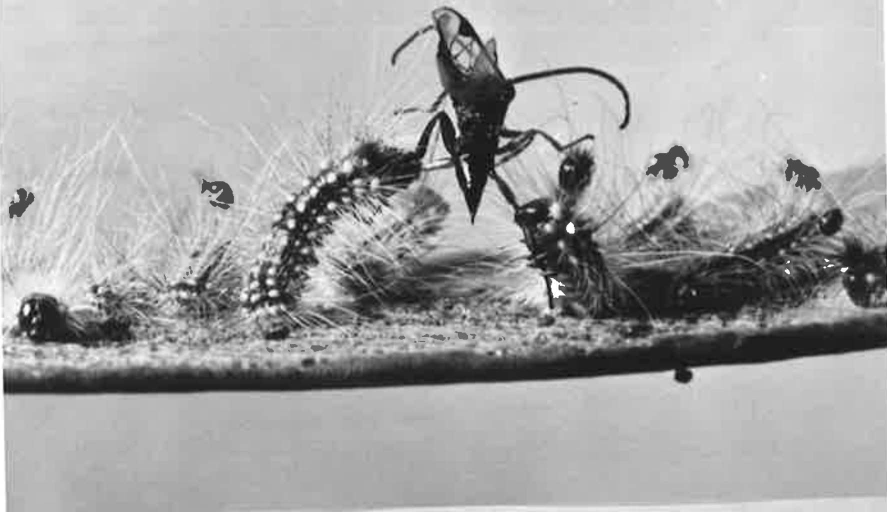
The behaviour of parasitoids attacking *U. lugens* differed depending on the size of host larvae attacked. Parasitoids had legs of sufficient length to raise their bodies above the level of rearing small larvae (Fig. 2(a)) and thus could proceed unhindered in any direction within the patch. In contrast, parasitoids could not walk through patches of mid *U. lugens* but instead ran over the top of them. This action increased the risk of contact with fluid regurgitated by larvae and of being bitten by them. Large larvae were approached from varying directions by parasitoids, but it was apparent that their longer setae hindered the parasitoids ability to reach the exocuticle for insertion of the ovipositor (Chapter 7). In preliminary tests mid and large larvae were occasionally seen to bite parasitoids, frequently not releasing their mandibles until the appendage had been severed (Fig. 2(b)).

Both species of parasitoid always proceeded to move through patches of small larvae after entering them. In contrast, *C. urabae* entered and continued to move through patches of mid larvae for just 21% of visits whilst *D. eucalypti* did so for only 1% of visits. In the remaining visits, parasitoids withdrew from the edge of the patch after an encounter. For those *C. urabae* seen to move through a patch of mid larvae 51% had at least one further encounter with a larva during that visit. This behaviour affected the distribution of parasitoid oviposition within patches of mid larvae. Thus every oviposition by *D. eucalypti* and 90% of ovipositions by *C. urabae* were with mid larvae on the edge of the patch.

Oviposition was very quick, lasting less than one second for both species of parasitoid. *C. urabae* typically moved around the patch and also oviposited with its wings raised whilst *D. eucalypti* typically kept its wings folded. Whilst walking through a patch of small larvae both species jabbed their ovipositor up and down like the needle

**Fig. 2.** Behaviour of *U. lugens* and parasitoids of *U. lugens* : **(a)** *C. urabae* proceeding through a patch of small larvae where many individuals are rearing as a result of several visits by this parasitoid, **(b)** *C. urabae* bitten and held by the mandibles of two mid *U. lugens* whilst proceeding through the patch, **(c)** *D. eucalypti* utilizing its legs to hold a small larva during oviposition (note larva dropping by a silk thread underneath the leaf and the closed wings during oviposition of *D. eucalypti* ).

A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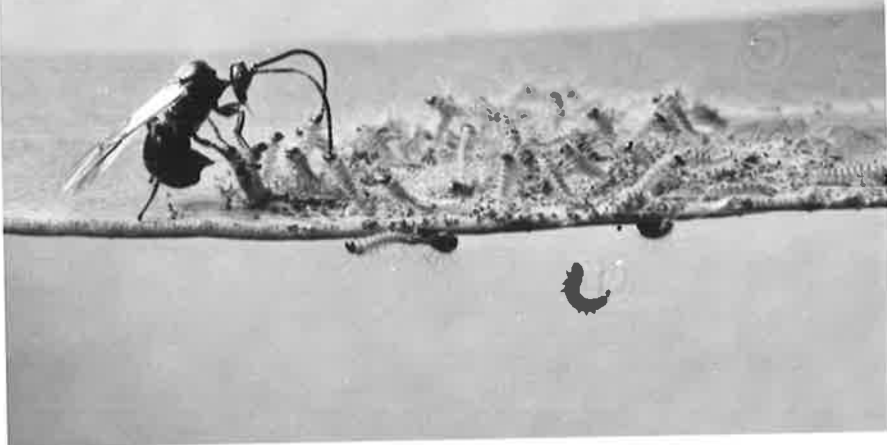

C

of a sewing machine (Fig. 2(a)). Such jabs frequently missed larvae and did not appear to be directed at specific individuals. Sometimes attacks were directed at small larvae by thrusting the ovipositor forward at an angle rather than from directly above. This frequently tended to displace the larva rather than penetrate its exocuticle. *D. eucalypti* often avoided such an outcome by using its front and/or mid pair of legs to hold small larvae when directing its ovipositor at a forward angle (Fig. 2(c)). Holding behaviour was employed on rearing and non-rearing larva and occurred at least once in 26.7% of all patch visits made by *D. eucalypti*.

The total number of visits made by parasitoids declined with increasing larval size for both species of parasitoid but the only statistically significant decline was for *D. eucalypti* between the small and the mid and large larvae (Fig. 3). *D. eucalypti* made more visits than *C. urabae* to patches of small larvae but significantly fewer to mid and to large larvae.

When the outcome of each visit was considered, *C. urabae* was more frequently successful in ovipositing into hosts than *D. eucalypti,* and the success of both species declined with increasing larval size (Fig. 4). The frequencies of approach-retreat, attack-fail and attack-oviposit for visits by both species of parasitoid on the three sizes of larvae were all significantly different from each other (overall $X^2_{10}$=625.3, P<0.0001). The frequency of approach-retreat was greatest for both species of parasitoid when they attacked mid larvae. If the visit proceeded beyond the approach-retreat stage, large larvae, though not gregarious, were apparently more difficult to oviposit into than either small or mid larvae. This was particularly true for *D. eucalypti* which failed to oviposit into large larvae. The greater size and longer setae of large larvae appeared to protect them from oviposition after a parasitoid had set upon it.

The mean number of ovipositions per parasitoid was significantly greatest when small larvae were attacked; oviposition in mid larvae for both species of parasitoid being next greatest (Table1). Multiple oviposition within a visit often occurred when parasitoids attacked the gregarious sizes of larvae. Multiple oviposition occurred within 34% of successful visits to a patch of small larvae for *D. eucalypti* and within 48% of

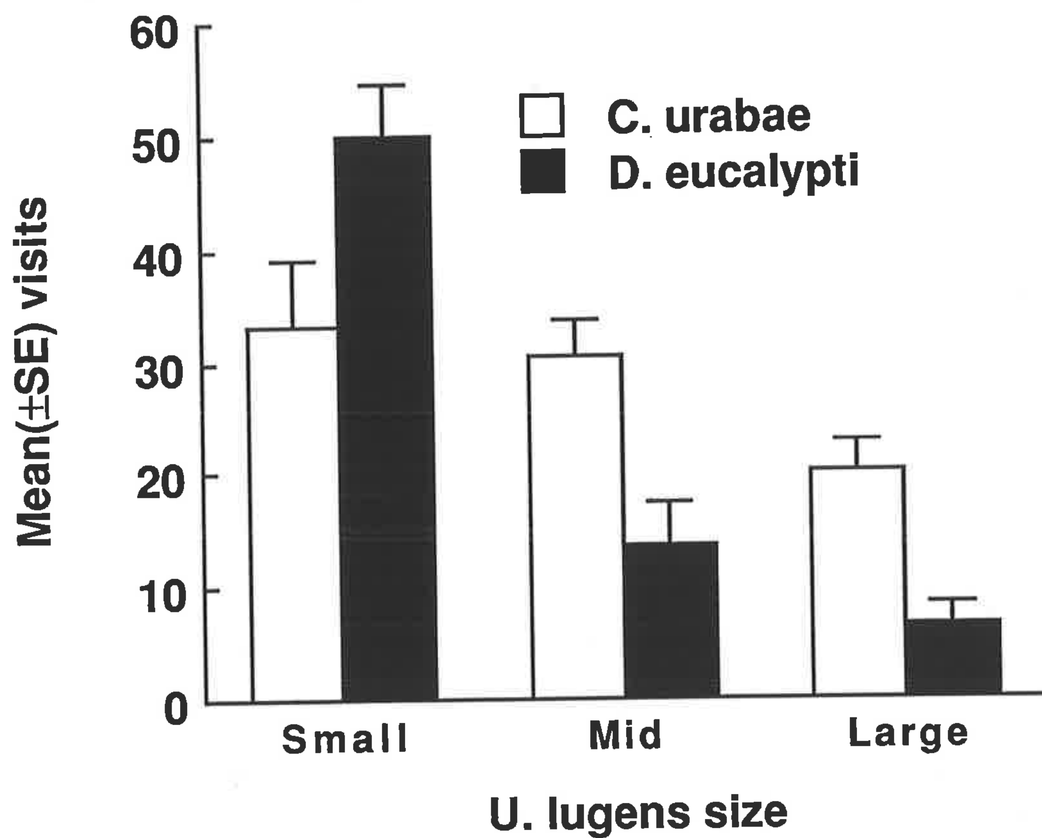



**Fig. 3.** The mean (±SE ; n=6) number of visits made by *C. urabae* and *D. eucalypti* to a patch of 40 small, 40 mid, and 40 large *U. lugens* over 20 min.

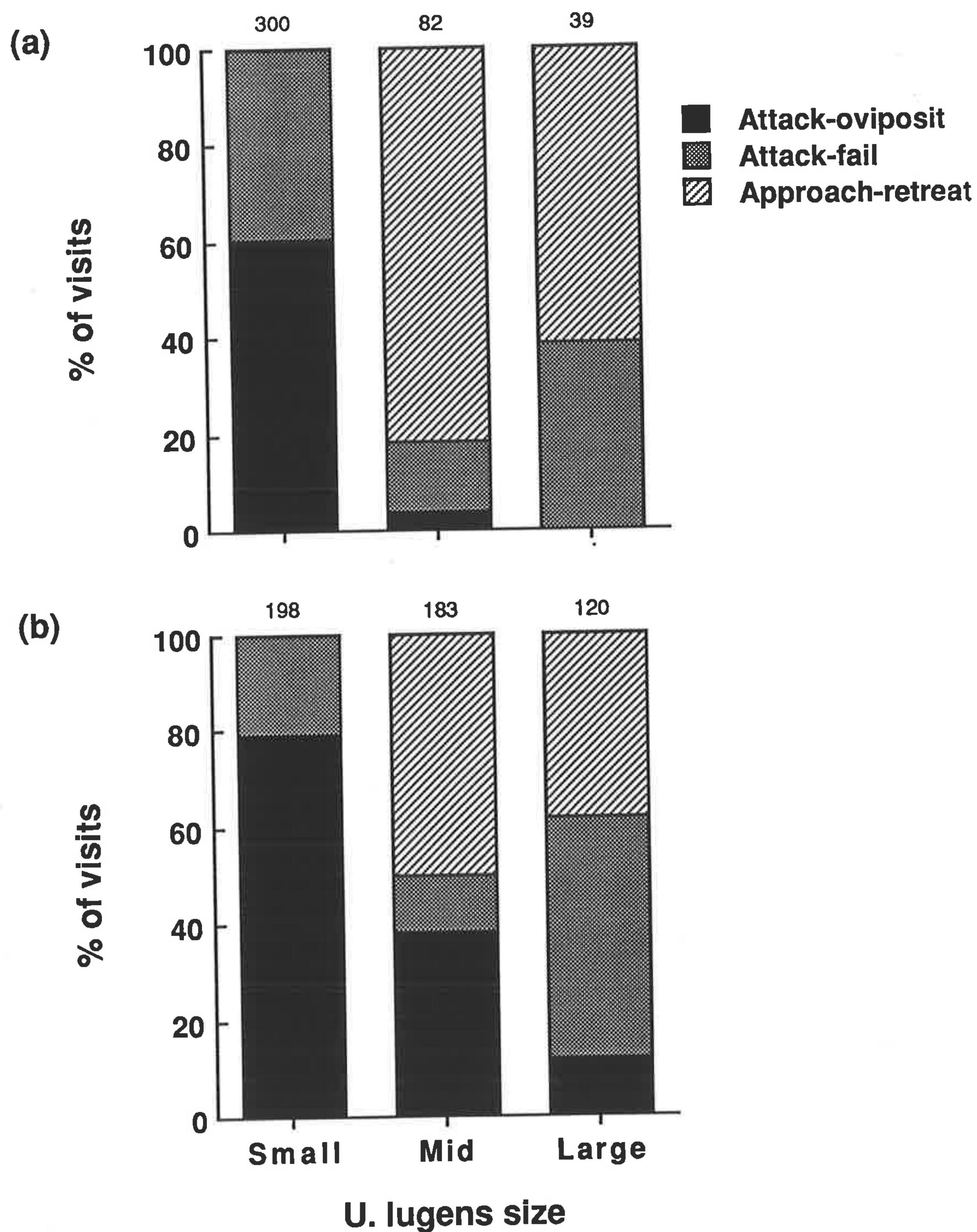



**Fig. 4.** The outcomes of all visits made to patches of 40 small, 40 mid, and 40 large *U. lugens* by **(a)** *D. eucalypti*, and **(b)** *C. urabae*. Data from six parasitoids were pooled for each bar. Numbers above bars are the total number of visits made.

**Table 1. The mean number of ovipositions per parasitoid and number of ovipositions per successful visit (visits where at least one oviposition occurred) for *C. urabae* and *D. eucalypti* attacking groups of 40 *U. lugens* of three different sizes.**

Values are mean ± standard error. Range in parentheses. Sample size is 6 for ovipositions per wasp. Differing letters alongside means within a species of wasp indicate significant differences ($P<0.05$) as determined by SNK tests.

| Species of parasitoid | Size of *U. lugens* | Mean no. of ovipositions per parasitoid | Total no. of ovipositions | Mean no. of ovipositions per successful visit | Number of successful visits |
|---|---|---|---|---|---|
| *C. urabae* | Small | 49.5±9.8[a] (22-84) | 297 | 1.89±0.11 (1-10) | 157 |
| " | Mid | 13.7±1.5[b] (8-17) | 82 | 1.17±0.06 (1-4) | 70 |
| " | Large | 2.3±0.8[b] (0-5) | 14 | 1.00 (1) | 14 |
| *D. eucalypti* | Small | 46.5±7.5[a] (32-80) | 279 | 1.54±0.07 (1-6) | 181 |
| " | Mid | 0.5±0.2[b] (0-1) | 3 | 1.00 (1) | 3 |
| " | Large | 0[b] | 0 | 0 | 0 |

successful visits to a patch of small larvae for *C. urabae*. Multiple oviposition during successful visits to mid larvae occurred during 14% of such visits by *C. urabae* but did not occur for *D. eucalypti*.

The frequency of superparasitism, particularly in small larvae, can only be inferred. High rearing mortality, especially of small larvae, made it impossible to determine the total numbers of larvae successfully parasitized and hence to compare the number of observed acts of oviposition with the number of parasites emerging. Furthermore ovipositor insertion may not necessarily lead to egg deposition so that multiple ovipositor insertion in a single larva may not necessarily indicate superparasitism. The number of ovipositor insertions exceeded the number of small larvae present in the petri dish for two *D. eucalypti* and four *C. urabae*, which seems to indicate a lack of discrimination before ovipositor insertion. For mid and large larvae it was possible to quantify information about each larva over the 20 min. period. With these sizes parasitoids frequently made more than one encounter with a larva particularly with the gregarious mid larvae (Fig. 5). This was indicated by *C. urabae* attacking mid and large larvae and *D. eucalypti* attacking mid and large larvae, which encountered only 106, 82, 45 and 30 individuals respectively although more encounters 205, 120, 82 and 34 respectively were made. Superparasitism of mid larvae by *C. urabae* may have occurred with five ovipositor insertions observed in one larva, three in another and two in seven of the sixty-nine larvae in which parasitoids inserted their ovipositors.

*U. lugens* showed both defence and escape (locomotory) behaviour during the 20 min. observation period. Locomotory behaviour significantly increased patch size for small larvae ($t=6.03$, $df=11$, $P<0.0001$) but significantly decreased patch size for mid larvae as mid larvae moved toward each other ($t=3.82$, $df=11$, $P<0.0001$) (Table 2). The change in patch area did not significantly differ in relation to the species of parasitoid. Small larvae frequently released their legs or prolegs from the leaf after several visits by a parasitoid. This accentuated the increase in patch size because parasitoids occasionally 'carried' small larvae which became temporarily impaled on their ovipositor. Random 'sewing machine' movements of the ovipositor became less

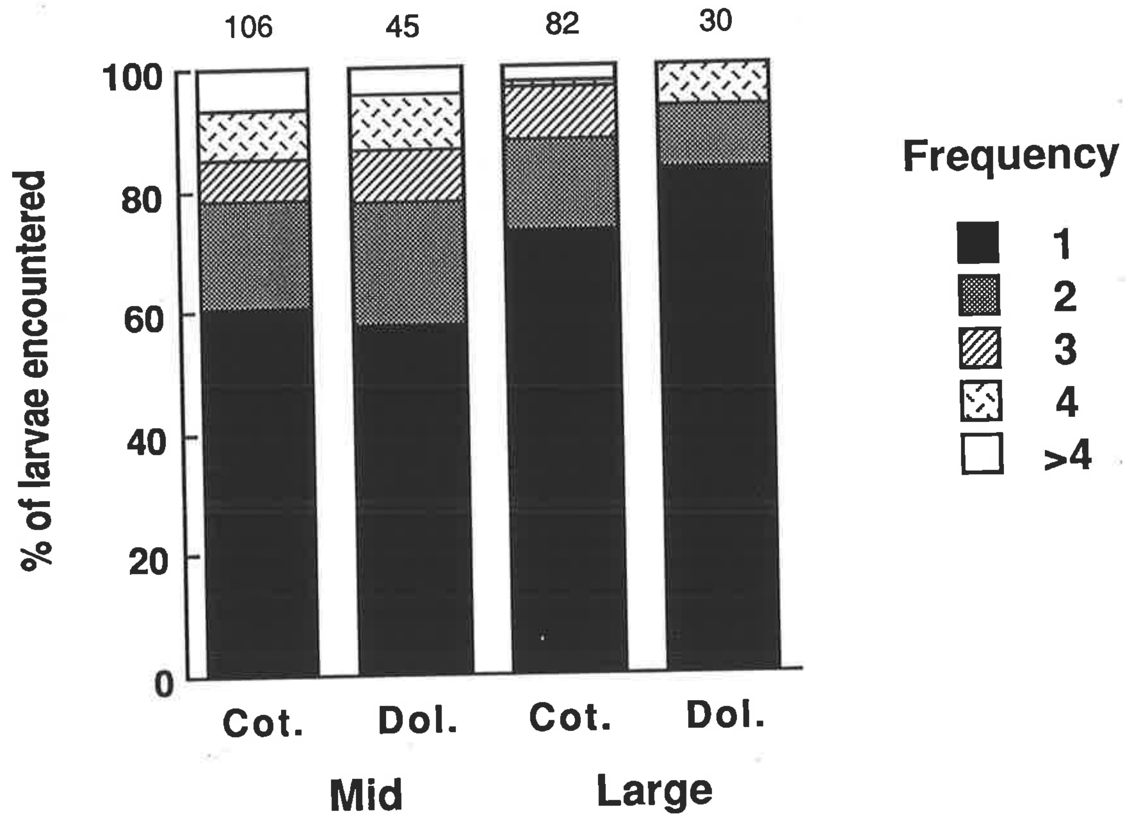



**Fig. 5.** The number of times each mid or large *U. lugens* was encountered by *C. urabae* (=Cot.) and by *D. eucalypti* (=Dol.). Data from six parasitoids were pooled for each bar. Numbers above bars are the total number of larvae encountered in that test (maxima 240).

**Table 2. The mean patch area and number of larvae in the patch centre prior to and after parasitoid departure for *C. urabae* and *D. eucalypti* attacking patches of 40 small and 40 mid *U. lugens*.**

Values are means ± standard error. Sample size is 6 for each mean.

| Species of parasitoid | Size of *U. lugens* | Mean patch area prior to parasitoid entry ($mm^2$) (P) | Mean patch area after parasitoid departure ($mm^2$) (A) | Mean ratio A/P | Mean no. of larvae in patch centre prior to parasitoid entry | Mean no. of larvae in patch centre after parasitoid departure |
|---|---|---|---|---|---|---|
| *C. urabae* | Small | 27± 4.0 | 69± 14.4 | 2.51 | - | - |
| " | Mid | 504± 74.1 | 407± 58.6 | 0.82 | 12.8±1.0 | 12.2±1.3 |
| *D. eucalypti* | Small | 35± 4.1 | 99± 14.2 | 2.83 | - | - |
| " | Mid | 663±123.1 | 587±115.8 | 0.89 | 9.3±1.1 | 8.2±1.0 |

effective and angled ovipositor thrusts frequently just displaced small larvae as dispersal increased and more larvae released their legs and prolegs. The decrease in patch area for mid larvae did not change the number of larvae in the centre of the patch.

The incidence of rearing or thrashing was low initially but reached a higher level after the first quarter of visits by parasitoids and remained at or above that level thereafter (Fig. 6). The majority of the 40 larvae, however, did not rear or thrash after each visit by a parasitoid although a greater number appeared to be doing so during, rather than after, each visit. Mid larvae exhibited the highest incidence of rearing or thrashing, particularly after visits by *C. urabae*. Small larvae were never observed to thrash (see Chapter 7).

Rearing or thrashing behaviour by all three sizes of larvae was continued by at least some larvae after the parasitoid departed the petri dish, but mid larvae continued to rear or thrash for the longest period of time. The mean±SE (range) number of minutes that rearing or thrashing continued after *C. urabae* departed was: small; 16±7 (0-35), mid; 108±7 (75-120), large; 56±16 (0-110) and after *D. eucalypti* departed was: small; 15±15 (0-90), mid; 68±21 (0-120) and large 30±19 (0-100). Small larvae typically maintained a rearing posture after parasitoid departure whilst sporadic thrashing was the more typical behaviour of mid and large larvae.

Rearing or thrashing by a larva did not necessarily prevent a subsequent encounter with a parasitoid, but the outcome of an encounter differed if a larva was rearing or thrashing just prior to that encounter (Fig. 7). Oviposition occurred less frequently in mid and large larvae that were rearing or thrashing immediately prior to an encounter with a parasitoid. These larvae typically elicited approach-retreat behaviour by parasitoids. However this trend was statistically significant only with *C. urabae* attacking mid larvae ($X^2_2$=21.23;P<0.001) ; small sample sizes occurred in the other tests. Upon contact by a parasitoid, over 95% of mid and large larvae responded defensively, mostly by rearing; after the parasitoid had left, levels of defensive behaviour remained high but were mostly expressed by thrashing. The behaviour of larvae prior to the encounter did not influence

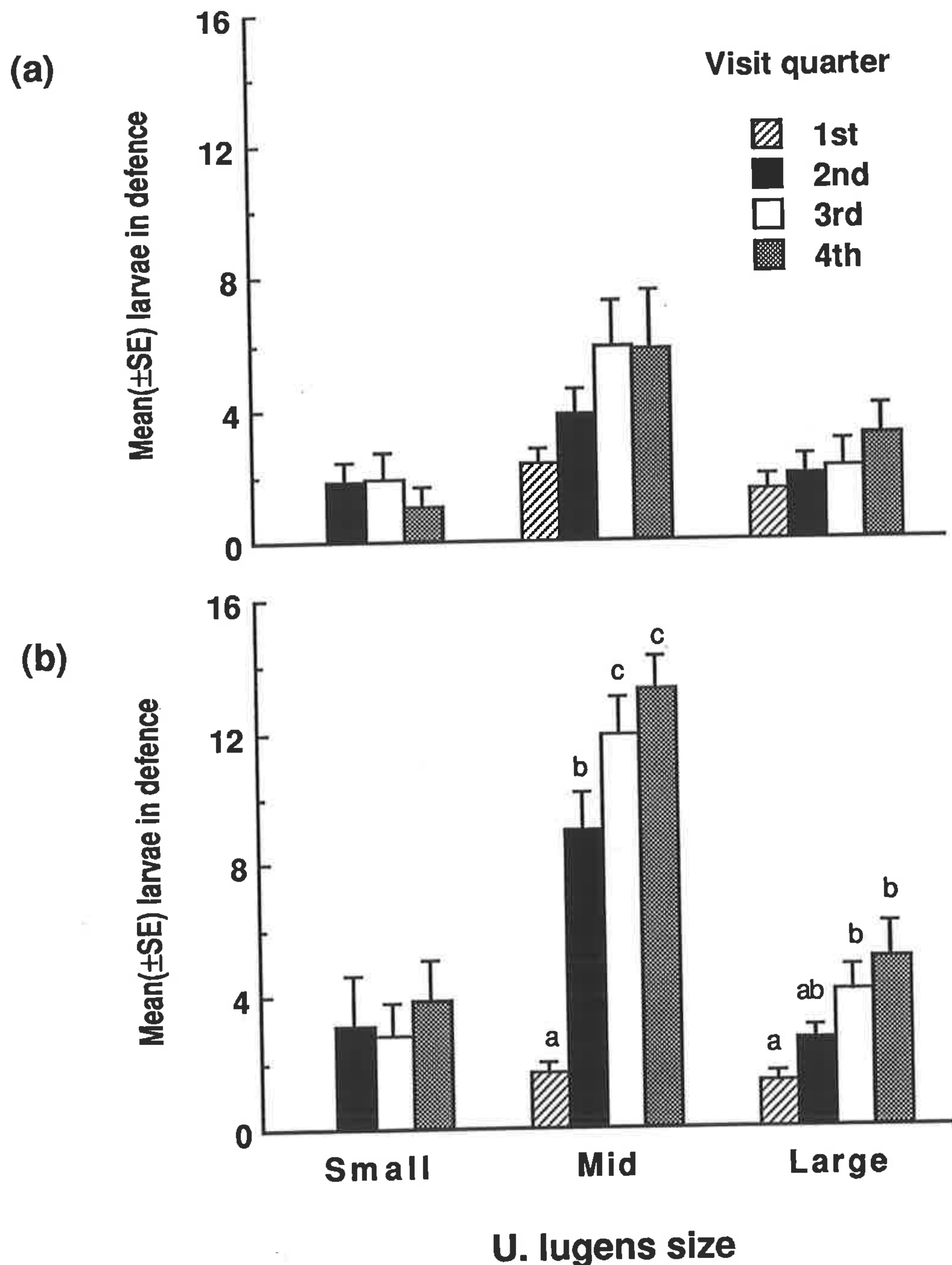



**Fig. 6.** The number of larvae (mean±SE) displaying defensive behaviour immediately following the visit by: **(a)** each *D. eucalypti*, and **(b)** each *C. urabae*, to a patch of *U. lugens* at the beginning of each quarter of the total number of visits made by that parasitoid. Each bar presents the mean of six observations. For each size of *U. lugens* differing letters above bars indicate significant differences ($P<0.05$) as determined by Student Newman-Keuls tests. Groups of bars without letters are not significantly different.

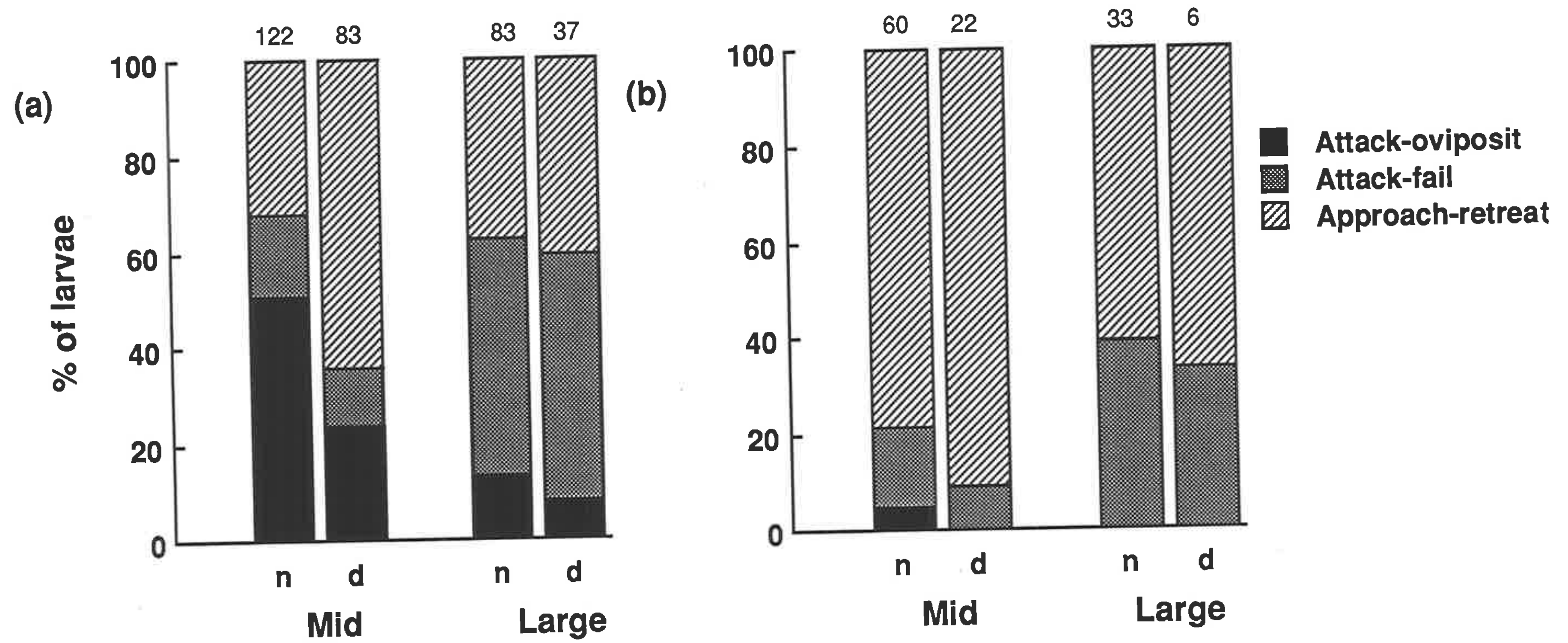



**Fig. 7.** The outcome of encounters between **(a)** *C. urabae,* **(b)** *D. eucalypti* and the mid or large larvae of *U. lugens* when, immediately prior to the encounter, the larvae were either : rearing or thrashing (d) or not rearing or thrashing (n). Data from six parasitoids were pooled for each bar. Figures above each bar indicate the number of encounters with larvae.

these latter behaviours. Insufficient video resolution did not enable documentation of the effect of defensive behaviour on the outcome of encounters with individual small larvae.

When the behaviour of parasitoids during the observation period was subdivided into five min. intervals, it was clear that most oviposition, visits and time in the patch occurred during the first five min. (Fig. 8). There was not a pronounced decline in the number of ovipositions per visit nor in the duration of each visit, hence total visits within each 20 min. observation period were comparable. Overall time spent within the patch was greatest for small larvae because parasitoids always proceeded to walk through these patches. The pattern of oviposition, visits and time over quarters was similar for each species of parasitoid, which meant that measures taken over the whole observation period were comparable between parasitoid species.

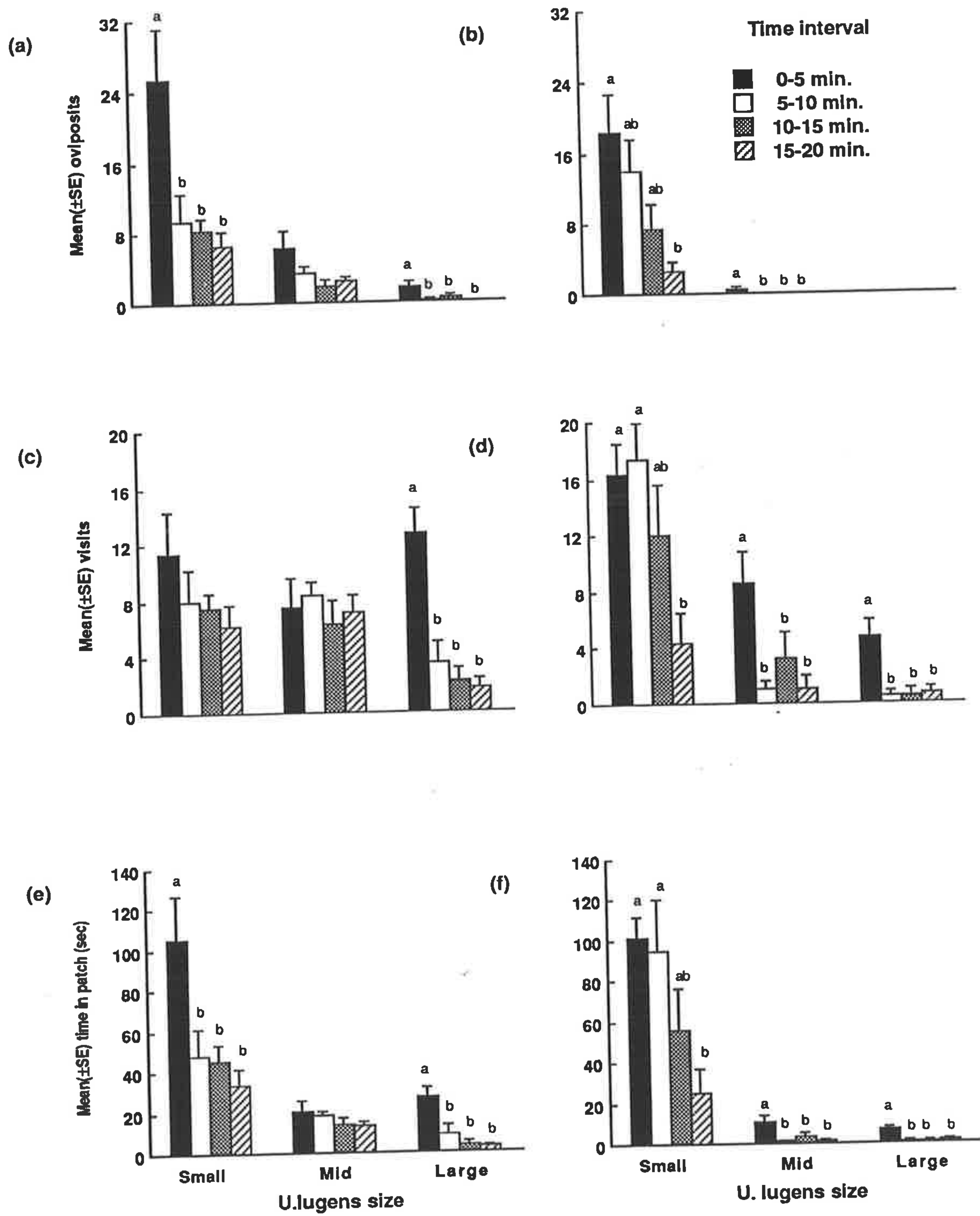
## Discussion

### *1. Gregariousness and the defensive behaviour of* U. lugens

Advantages of gregariousness in insects include aiding thermoregulation (Seymour 1974; Sullivan and Wellington 1953), facilitating feeding behaviour and helping overcome plant defences (Ghent 1960; Slansky and Panizzi 1987; Risebrow and Dixon 1987), and enhancing defence against predators (as indicated below). Gregariousness may assist defence by (1) aiding in early predator detection, (2) benefitting the individual by placing conspecifics in the path of predator attack, (3) confusing predators by diverting predator focus, (4) diluting the predator's effect, and (5) in some instances by endangering the predator (Pulliam and Caraco 1984). For aposematic insects gregariousness may also help reinforce the warning value of aposematic colouration (Cott 1940). Although defence may not necessarily be the primary function of the gregarious behaviour of the younger instars of *U. lugens,* it is constructive to look at how it may enhance the defence of *U. lugens* against *D. eucalypti* and *C. urabae.*

Predator detection may be enhanced by gregariousness in *U. lugens* because after the first visit by a parasitoid individuals not directly encountered by the parasitoid frequently began rearing, thrashing or walking. These individuals may detect and then

**Fig. 8.** The mean (±SE) number of ovipositions **(a-b)**, of visits **(c-d)**, and time spent in the patch **(e-f)**, for each 5 min. interval of the 20 min. observation for *C. urabae* **(a,c,e)**, and *D. eucalypti* **(b,d,f)** with the three sizes (small, mid, large) of *U. lugens*. Each figure is the mean of six observations. Letters above bars as for Fig. 6.



(a)
(b)
(c)
(d)
(e)
(f)
Mean(±SE) oviposits
Mean(±SE) visits
Mean(±SE) time in patch (sec)
Time interval
0-5 min.
5-10 min.
10-15 min.
15-20 min.
Small
Mid
Large
U.lugens size
U. lugens size

react to the defensive behaviour of attacked conspecifics via their close proximity to each other in the group and thus help reduce their vulnerability to parasitism. A similar behaviour has been demonstrated in aggregations of the marine gerrid *Halobates robustus* Barber (Treherne and Foster 1980; 1981; 1982). There was no evidence of detection of parasitoids by *U. lugens* prior to the first parasitoid visit although this has been reported for the solitary caterpillars *Barathra brassicae* L. (Noctuidae) and *Malacosoma pluviale* (Dyar) (Lasiocampidae) which respond to an approaching parasitoid's wing vibration frequency (Myers and Smith 1978; Tautz and Markl 1978). However, it was not possible to test *U. lugens* response to parasitoid flight in this experiment as parasitoids were unable to successfully fly within the petri dish.

Gregariousness seems of little benefit in enhancing the defensive actions of small larvae. Although regurgitation and biting were observed, mandibular size and volume of regurgitate limited their effectiveness. Outward dispersal of larvae seemed the most effective response to reducing the success of parasitoid oviposition. Dispersal diluted the parasitoid's effectiveness and possibly diverted the parasitoid's focus thereby countering the possible success had larvae remained in a tight group. Fujisaki (1975) concluded that the active breakup of colonies of 1st instar winter cherry bug (*Acanthocoris sordidus* Thunberg) in response to predator attack enhanced survival of this species. *A. sordidus* and small *U. lugens* do not disperse sufficiently to prevent subsequent reaggregation but for neither species could gregariousness be considered as enhancing defence.

For mid larvae there were probable benefits of gregariousness towards defence. Firstly individuals residing in the centre of the group were protected by their conspecifics because most encounters and ovipositions occurred with larvae on the group edge. Enhanced survival of insects located centrally within groups has also been demonstrated in the field with diprionid sawfly colonies, where larvae on the periphery of the colony show a higher percentage parasitism and more frequent predation from the pentatomid predator *Podisus modestus* (Dallas) (Tostowaryk 1971; 1972). For *U. lugens*, mid larvae in the centre of a group were protected by the tighter aggregation coupled with their longer setae which had become at least as long as the parasitoid's body length

(Chapter 7). Additionally moving over the top of a patch increased the likelihood of parasitoid injury from biting *U. lugens* and of regurgitate contacting parasitoids. Regurgitate appears to act as an irritant to the parasitoids and in at least some insects including grasshoppers (Eisner 1970) and sawflies (Morrow *et al.* 1976), it is protective against certain predators.

Individual mid *U. lugens*, by rearing or thrashing immediately prior to the encounter, were demonstrated to be able to decrease their chance of being parasitized, but whether the chance of an encounter was also decreased was not ascertained. This was because parasitoids approaching a group of mid *U. lugens* have a choice of larvae and the reason why any one larvae was attacked was unclear. Decreased encounters in response to defensive behaviour has been shown for the cereal aphid *Metopolophium dirhodum* (Wlk.) and its parasitoid *Aphidius rhopalosiphi* (DeStefani-Perez) (Gardner *et al.* 1984). For *U. lugens* the combined effect of many mid larvae displaying defensive behaviour may enhance any value of this defence.

Of the three sizes of larvae, large larvae although not gregarious, were encountered by parasitoids the least frequently and with least success. By this stage, however, the body size of *U. lugens* was much larger relative to the parasitoid (Chapter 7). Large *U. lugens* had 2-3 head capsules stacked above their head (Chapters 2, 5 and 7) and it was clear that these head capsules helped extend the 'area of defence' (Stamp 1986) to the *U. lugens* posterior during rearing behaviour.

There was no particular area surrounding a *U. lugens* body that was favoured for attack by *C. urabae* and *D. eucalypti* nor any clear mass orientation by *U. lugens* when in a group. Clausen (1940) cites many examples of tachinids favouring certain body regions of hosts because they are less defendable. Stamp (1982) mentions *Cotesia euphydryidis* Muesebeck frequently attacking solitary 2nd-3rd instars of *Euphydryas phaeton* Drury from behind. Similarly the rear of *U. lugens* appears the least defensible area and importantly the one region that remains stationary (fixed to the leaf) during defensive behaviours. This is important as attack to moving body regions may increase the chance of injury to the parasitoid.

Thus as host development progresses the behavioural responses of *U. lugens* to parasitoid attack change. Other insects also show changes with age in their responses to attack. *Heliothis punctiger* Wallengren when attacked by the predator *Oechalia schellenbergii* Guerin-Méneville changes the frequency of multiple defensive responses (Awan 1985), and the aphid *Myzus persicae* (Sulzer) also increases its overall level of defensive behaviour when attacked by the parasitoid *Ephredus cerasicola* Stary (Hofsvang and Hågvar 1986). Change of another kind is shown by *Hemileuca lucina* Hy. Edw. (Saturniidae) which is gregarious in early to mid larval instars and increases escape activity as it increases size (Cornell *et al.* 1987). Cornell *et al.* (1987) hypothesized that with *H. lucina* defence was more effective when gregarious and escape more effective when solitary. *H. lucina* differs in this from *U. lugens* as when *U. lugens* becomes solitary it appears individual defence is effective whilst escape occurs in early instars. This is an important difference that suggests a changing benefit of gregariousness to defence with age in *U. lugens*. For small larvae gregariousness is of little benefit to defence whilst for mid larvae which are larger relative to the parasitoid it clearly helps defence (Chapter 7). Obviously it would be constructive to test other possible reasons for gregariousness in order to understand more completely the evolutionary advantages of gregariousness in *U. lugens*.

2. *Experimental design,* U. lugens *defensive behaviour and superparasitism*

Unfortunately limitations were imposed by the experimental design on some potential host-parasitoid interactions. All encounters occurred with the leaf flush to the base of the petri dish so that larvae walking to the leaf edge could not escape by dropping on silk from the leaf. Furthermore no tests were done with the larvae feeding underneath the leaf (but see Chapter 7). In the field, *Eucalyptus* leaves are oriented at varying angles whilst *U. lugens* eggs and larvae occur on both the upright and underneath leaf surfaces (Campbell 1962; Morgan and Cobbinah 1977). Larvae if underneath the leaf could directly escape by silking thereby decreasing their possibility of encounter and parasitism. Silking may not necessarily ensure escape from parasitism as some species

of parasitoid, including *Diolcogaster facetosa* (Weed), descend down silk threads to attack larvae (Yeargan and Braman 1986). Neither species of parasitoid used in this study were observed to do so during previous research (Allen pers. observ.). For the parasitoids of *U. lugens* it may be more profitable to concentrate attacks on those larvae remaining on the leaf surface, especially when attacking groups.

A further possible type of encounter limited by the experimental design was for parasitoids to parasitize larvae feeding on a leaf from the opposite side of the leaf. The gregarious feeding by *U. lugens* causes skeletonizing of leaves, providing numerous holes through which a parasitoid could oviposit into the ventral surface of *U. lugens*. The leaf would also provide protection for the parasitoid from any defensive behaviour by the larvae. An analogy to this is *C. euphydryidis* which attacks larvae of *E. phaeton* through this species communal webbing (Stamp 1982). I have observed ovipositor probing from underneath the leaf by both species of parasitoid at *U.lugens* but have not confirmed parasitism resulting from this behaviour.

The duration that the defensive behaviour of *U. lugens* continued for up to two hrs after parasitoid departure may affect the frequency of superparasitism. Gardner *et al.* (1984) showed that the defensive behaviour of the aphid *M. dirhodum* stimulated by the first 'stabbing attack' of *A. rhopalosiphi* decreased the frequency of further encounters. Stamp (1982) recorded that *E. phaeton* maintained defensive behaviours for up to 14 min. after *C. euphydryidis* visited the web in the field and that the parasitoid moved away from the group as a result. Thus prolonged defensive behaviour may alter the frequency of encounters and decrease the possibility of further parasitization. On the other hand, some parasitoids such as *Cotesia plutellae* Kurdj. are attracted to moving hosts (Arthur 1981; Lloyd 1940). Just how the frequency of subsequent encounters would be affected by the prolonged rearing and thrashing of *U. lugens* was not directly investigated in the experiment described here and remains uncertain.

Although more than one ovipositor insertion was observed when either parasitoid attacked small larvae and when *C. urabae* attacked mid larvae true superparasitism (multiple deposition of eggs) was not confirmed. According to van Lenteren (1981)

parasitoids that attack clusters of strongly moving hosts tend to use internal markers since external markers may be inadvertently transferred to unparasitized hosts. *C. urabae* and *D. eucalypti* may use this means of host discrimination and thus discrimination would take place only after ovipositor insertion. However a complication of internal markers is that insufficient time may elapse between ovipositor insertions for the marker to take effect (van Lenteren 1981). The experimental design did not allow parasitoids to leave the petri dish which may encourage superparasitism if insufficient hosts are present and parasitoids are unable to leave the patch (van Lenteren 1981). Host numbers may have become limiting with small larvae but whether the petri dish provided sufficient area for the parasitoid to leave the patch (as perceived by the parasitoid) was unknown. Although prolonged defensive behaviour helps limit superparasitism (and parasitism) it does not exclude further encounters. This is important as encounters with defensive larvae would add to the total 'handling time' of foraging parasitoids.

*3. Host acceptance and the success of oviposition of* C. urabae *and* D. eucalypti

Host acceptance is the process whereby hosts are accepted or rejected for oviposition after contact has been made (Weseloh 1974). Hopper and King (1984) defined parasitoid preference as when the relative frequency of host types parasitized differs from the relative frequency of host types available. Preference may arise either because parasites find some hosts more easily than others or because, once found, some hosts are more likely to be parasitized than others (Hopper and King 1984). True preference testing (simultaneous choice) was not undertaken in this experiment as neither parasitoid had the simultaneous choice of all three sizes of *U. lugens* in the field. Nevertheless many factors that may influence host acceptance and host preference were examined in this experiment, some mostly determined by the parasitoid (i.e. frequency of visits) and some mostly by the host (i.e. type of defence). However the influence of the host's behaviour is often ignored when determining a parasitoid species levels of host acceptance and preference. It is also important to be aware that differences in the host

habitat and the host finding ability of parasitoids (Vinson 1975) will also influence parasitism success in the field.

*C. urabae* was most successful attacking small larvae and least successful attacking large larvae but initiated an equivalent number of visits to all three sizes tested. This parasitoid species oviposited successfully and developed in all three larval sizes, although the degree of parasitoid mortality from internal host defenses was not determined. Its ability to handle all three larval sizes reflects on its phenology where it encounters all three larval sizes in the field. During the summer generation of *U. lugens, C. urabae* oviposits into small larvae, emerges, then completes a second generation by ovipositing into mid larvae. During the winter generation of *U. lugens, C. urabae* again oviposits into small larvae, emerges later, then completes a second generation by ovipositing into large larvae (Chapter 3). Nevertheless the varying degree of success of oviposition in hosts of different size reflects less on its host preferences and degree of host acceptance than on the behavioural interactions of parasite and host during oviposition. The increasing setal length, body size and effectiveness of defensive behaviour largely determined the outcome of encounters with successive size classes of host.

*D. eucalypti* made fewer visits to mid and large larvae than to small larvae and did not succeed in ovipositing in large larvae. This result also parallels the phenology of this species in the field. During the winter generation of *U. lugens, D. eucalypti* has only one generation, ovipositing into small larvae and emerging to pupate during pupation of that generation of the host. During the summer generation of *U. lugens, D. eucalypti* has two complete generations, first ovipositing into small larvae, emerging, then ovipositing into mid larvae, and finally like the winter generation emerging when the host is pupating (Chapter 3). Unlike *C. urabae, D. eucalypti* does not parasitize large larvae and is absent as an adult at the time large larvae occur in the field. *D. eucalypti* is able to 'avoid' large larvae through a physiological delay to development whereby it remains within the host during the winter generation of *U. lugens* (Chapter 5). Once again as with *C. urabae*, the overall success of oviposition by this parasitoid was influenced by the size and effectiveness of defensive behaviour of *U. lugens*.

The poor success of *D. eucalypti* when attacking mid larvae is apparently a limiting factor in the phenology of the parasitoid in the field. None of the three ovipositor insertions observed during the experiment resulted in emergence of an adult parasitoid, and although these stung *U. lugens* could have died during rearing, internal host defences could well have killed the developing *D. eucalypti*. Hopper (1986) found a higher egg-larval mortality of *Microplitis croceipes* (Cresson) in older (5$^{th}$ instar) *Heliothis virescens* (F.) as did Lewis and Vinson (1971) for *Cardiochiles nigriceps* Viereck in the 4-5$^{th}$ instars of the same host. Mid larvae of *U. lugens* would thus seem likely to have more effective internal defenses than small larvae.

Even if *D. eucalypti* are presented with difficulties in relation to oviposition (and possibly development) in mid larvae there are at least three ways in which the parasitoid may enhance its percent parasitism in the field through adaptive behaviour. Firstly it may exploit the range of *U. lugens* sizes present in the field (Chapter 2) by selectively foraging for patches of *U. lugens* with smaller host sizes. Secondly *D. eucalypti* may attack some larvae from the opposite side of the leaf by inserting its ovipositor through the feeding holes. Finally *D. eucalypti* may exploit patches of *U. lugens* when the larvae are moulting (Chapter 2). *E. phaeton* is less responsive to attack when moulting (Stamp 1984) and this was also observed of *U. lugens* during preliminary experiments.

The varying success of *C. urabae* and *D. eucalypti* in attacking hosts of different sizes reflects their respective phenologies. *C. urabae* was the more 'general' and more successful of the two parasitoids with all three larval sizes tested and both were most successful attacking small larvae. Thus, although it is important to understand the preference and host acceptance behaviour of a parasitoid it is also equally important to account for the host's defensive behaviour during oviposition to truly elucidate a parasitoid's oviposition success.

# Chapter 7. Larval stage and response of *U. lugens* to attack.

*Abstract*

The instars of *U. lugens* were examined for morphological features and responses that may influence the success of *C. urabae* and *D. eucalypti* when attacking them. Long setae surrounding each instar's body gave *U. lugens* potential protection from the relatively short ovipositors of *C. urabae* and *D. eucalypti*. First instar (small), 4-5$^{th}$ instar (mid), and 6-7$^{th}$ instar (large) larvae of *U. lugens* were attacked with a micropin to examine their responses to attack by natural enemies. Defensive responses increased and locomotory responses decreased with each increment in developmental stage tested. Small larvae, unlike mid and large larvae, never displayed thrashing behaviour nor moved their body laterally during rearing. Large larvae most frequently reared in response to attack. Defensive responses decreased and locomotory responses increased in all sizes of larvae when attacks were repeated at 15 sec. intervals. Ten min. after *C. urabae* had been allowed to attack larvae for 20 min., all larvae but particularly small and mid larvae showed higher levels of rearing, regurgitation and thrashing in response to attack than they did prior to *C. urabae* attacking them. After two hrs this change in response to attack was maintained in small larvae, had dropped in mid larvae and had returned to, or else remained at, the level prior to *C. urabae* attacking them in large larvae.

## Introduction

The responses of larvae of *Uraba lugens* Walker to parasitoid attack include biting, rearing, thrashing, regurgitation, walking and dropping from the host plant on a silk thread (Chapter 6). Larvae of other species of Lepidoptera show some different types of response to *U. lugens* including tail wagging, rolling over (Awan 1985), eversion of an osmeterium (Eisner and Meinwald 1965; Evans 1984), retreating into a silk web (Fitzgerald 1980) and dropping from the host plant (Myers and Campbell 1976). Despite this considerable array of responses in lepidopteran larvae, their

cylindrical body and short thoracic legs impose constraint on manoeuvrability, and thus limit their potential defensive responses compared to many other insects (Stamp 1986). The body diameter and body length of each developmental stage may also affect responses to attack (Stamp 1986) with some lepidopteran larvae changing their frequencies or types of response with increasing developmental stage (Stamp 1986; Cornell *et al.* 1987).

Obvious changes that occur during larval development of Lepidoptera include changes in body size and setal lengths. Differing body size ratios between predator and prey correlate to predator success in many insects (Evans 1976; Stamp 1986), whilst long setae are known to hinder ant attack (Ayre and Hitchon 1968) and possibly obstruct ovipositor insertion of some parasitoids (Stamp 1982). Some species of tachinid only deposit eggs on certain regions of their host (Clausen 1940) whilst other parasitoids (Stamp 1982) and predators (Dixon 1958; Klingauf 1967) achieve most success if they attack their host from one particular direction.

Although experiments in the previous chapter did document some of the responses of *U. lugens* to parasitoid attack they failed to address many of the aforementioned possibilities. Hence it was decided to examine the body sizes and setal lengths of *U. lugens* and to test the response to attack of the three sizes of *U. lugens* used in Chapter 6. To test the latter, a series of experiments was set up to assess the influence of (a) developmental stage, (b) direction of attack, (c) larval position with respect to gravity, and (d) repeated attack on the response of *U. lugens* to attack. Finally, since an artificial stimulus was used to attack larvae in the above experiments, all larvae were again tested, this time after a parasitoid had attacked them, to see if their response to attack differed and if so for how long this difference lasted.

## Materials and methods

Three independent experiments were conducted to examine the defenses of *U. lugens*. The first recorded potential fixed defenses by measuring setal lengths and body dimensions of each instar of *U. lugens* and comparing the former to ovipositor

lengths of two of its primary parasitoids *Cotesia urabae* Austin and Allen and *Dolichogenidia eucalypti* Austin and Allen. The second recorded responses to attack of the three host sizes ( 1st instar (small), 4-5th instar (mid), and 6-7th instar (large)) typically attacked in the field by *C. urabae* and *D. eucalypti* (Chapter 3). The last, measured the effect of repeated attack on response of these three sizes of *U. lugens*.

All experiments were carried out at 20°C and all larvae used in experiments were reared in 20 x 20 cm cages at 20°C and 12L:12D on cut *Eucalyptus leucoxylon* F. Muell. foliage which was replaced at regular intervals. All statistical tests were carried out at the 0.05 significance level unless otherwise stated.

*1. Experiment 1: Setal and body dimensions of larvae of* U. lugens

A single egg batch of *U. lugens* with approximately 200 eggs was allowed to develop at 20°C and 12L:12 D. Upon hatching, larvae were reared as described above, and twelve 1st instar larvae were killed two days after hatching. Twelve larvae of each subsequent instar were killed two days after each moult until all larvae had either pupated or been killed.

The following measurements were taken of each larva: wet weight (excluding any stacked head capsules), head capsule width at its widest point, body length, body height (at first abdominal segment) and body width (at metathorax). The longest seta extending from the side, from the front, from the rear, and from three positions above the body of *U. lugens* was also measured. These three positions above the body were anterior to the first abdominal segment, between the first and ninth abdominal segments, and posterior to the ninth abdominal segment. Three measurements of height were taken as setal height visibly changed along the length of the body. Setal lengths were measured as distance projecting beyond the tubercle from which they arose and since setae were not always straight, were not measures of actual length, but of maximum distance projected from the body.

Ovipositor lengths of *C. urabae* (n = 33) and *D. eucalypti* (n = 45) extending beyond the hypopygium when viewed laterally in the 'at rest' position were measured as

was wet weight of both species of parasitoid (see Chapter 3). Ovipositor lengths were compared to setal lengths of *U. lugens* to indicate the degree of obstruction provided by setae to ovipositor insertion, and were regressed against the cube root of parasitoid wet weight for each species using the GLM procedure of SAS (1985).

2. *Experiment 2: Response of* U. lugens *to attack*

Six groups of up to 80 larvae of *U. lugens* were selected from each size class of larvae (small, mid and large). To test their responses to attack larvae were stabbed with a micropin which simulated the insertion of a parasitoid's ovipositor. Parasitoids were not used to attack larvae because their direction of attack and behaviour during attack was not consistent. Three hypotheses were tested in this experiment:

1) That direction which a larva was attacked from affected response to attack.
2) That position of a larva with respect to gravity (upright or underneath a horizontal leaf) affected response to attack.
3) That exposure of a larva to a parasitoid prior to attack affected subsequent response to attack.

Larvae were attacked from behind, from the side, and from the front, with contact being made on the dorsal surface of the tenth abdominal segment, on the lateral surface between the fifth and ninth abdominal segments, and on the head capsule respectively. The position of a larva to be attacked in a group (edge or centre) and its feeding behaviour prior to attack (feeding or not feeding) were recorded for each small and mid *U. lugens* attacked. Each group was attacked on four separate occasions. Only thirty-six randomly selected larvae in each group were attacked on each occasion, 12 of the 36 larvae from behind, 12 from the side, and 12 from the front. The first two of the four occasions were when the group was feeding on top of a horizontal leaf (upright) and when the group was feeding underneath a horizontal leaf. The order of these was reversed between the six groups in a size class, to eliminate experimental bias, and 30 min. was allowed to elapse between them. Each group was then transferred to a 19x3 cm glass petri dish and a single mated female *C. urabae* (1-7 days old) introduced into the

petri dish and allowed to attack larvae for 20 min., after which time the parasitoid was removed (see Chapter 6 for further details). Groups were then attacked with a micropin on two more occasions at ten min. after exposure to a parasitoid and two hrs after exposure to a parasitoid.

Responses to attack were recorded at two different times because larvae, particularly small larvae, often had a delay in their response. These two times were labelled 'immediate' response: response at instant of attack, and 'ultimate response': final response recorded within 30 sec. of attack. Responses to attack were assigned to three 'overall' categories:

A) *No response* : no movement by the larva observed,

B) *Locomotory response* : larva walked away and/or dropped from leaf surface on a silk thread,

C) *Defensive response* : larva displayed at least one of the following responses:

1) *Pulse* : body segments of larvae are raised in a wave along its length in either direction,
2) *Head curl* : head is tucked under thorax and thorax is arched upwards,
3) *Rear* : head and thoracic legs raised from leaf surface, resulting in anterior end of body reaching towards posterior end of body,
4) *Thrash* : thoracic legs lifted off leaf surface and body region anterior to abdominal,prolegs waved from side to side, pivoting from one or more pairs of prolegs still attached to leaf,
5) *Bite* : larva opens and closes mandibles at least once,
6) *Regurgitate* : a drop of fluid is produced at the mouthparts by the larva.

Head curling often preceded thrashing, as when thrashing, the head of the larva was usually tucked under the thorax. By tucking the head under the thorax during thrashing, any stacked head capsules projected parallel and closer to the leaf surface which increased the area in front of the larva being swept by thrashing.

Immediate response, ultimate response, and type of defensive response (when displayed) were tallied into frequencies for the four occasions that attacks were made to

each group of larvae. Differences between the six groups in each size class were examined by log-linear contingency table analysis (LLCTA) (Genstat 1987). If no significant difference between groups was found, all six groups were pooled for remaining analyses. Differences in immediate response, ultimate response, and type of defensive response (if displayed) were tested for using LLCTA between :

1) larvae attacked from different directions.
2) larvae attacked on top of versus larvae attacked underneath a horizontal leaf.
3) larvae attacked prior to exposure to a parasitoid, and larvae attacked 10 min. and two hrs after exposure to a parasitoid.

LLCTA was also used to test for any significant difference between immediate and ultimate response of larvae to attack. For all analyses if over 20% of expected cell size frequencies fell below 5.0, the cells were pooled to avoid bias in chi-square values. If pooling was insufficient to overcome this, then Fisher's exact test was used to test between frequencies in the reduced 2x2 contingency table (Zar 1974).

Finally, whether response to attack differed between the first half and last half of larvae tested in each group was analysed using LLCTA. This last analysis tested whether the responses to attack of other larvae in the group affected the response of those larvae yet to be attacked.

*3. Experiment 3: Response of* U. lugens *to repeated attack*

As in the previous experiment six groups of *U. lugens* in each small, mid, and large size class were used but only 10 larvae in each group were attacked. Each larva was attacked from behind with a micropin, six successive times, with a 15 sec. delay between attacks. Immediate response, ultimate response and type of defensive response were recorded for each attack as before.

To test whether groups within each size class were similar in response to attack, the six attacks per larva and 10 larvae per group were pooled for each size class and resultant tables analysed using LLCTA. To test for differences in responses of larvae to successive attacks, each of the six successive attacks to a larva were pooled within, and

then between groups in a size class, to provide 60 observations per attack and resultant tables analysed using LLCTA. The frequency of regurgitation, biting, and thrashing was also tested for differences between successive attacks within each size class using LLCTA.

## Results

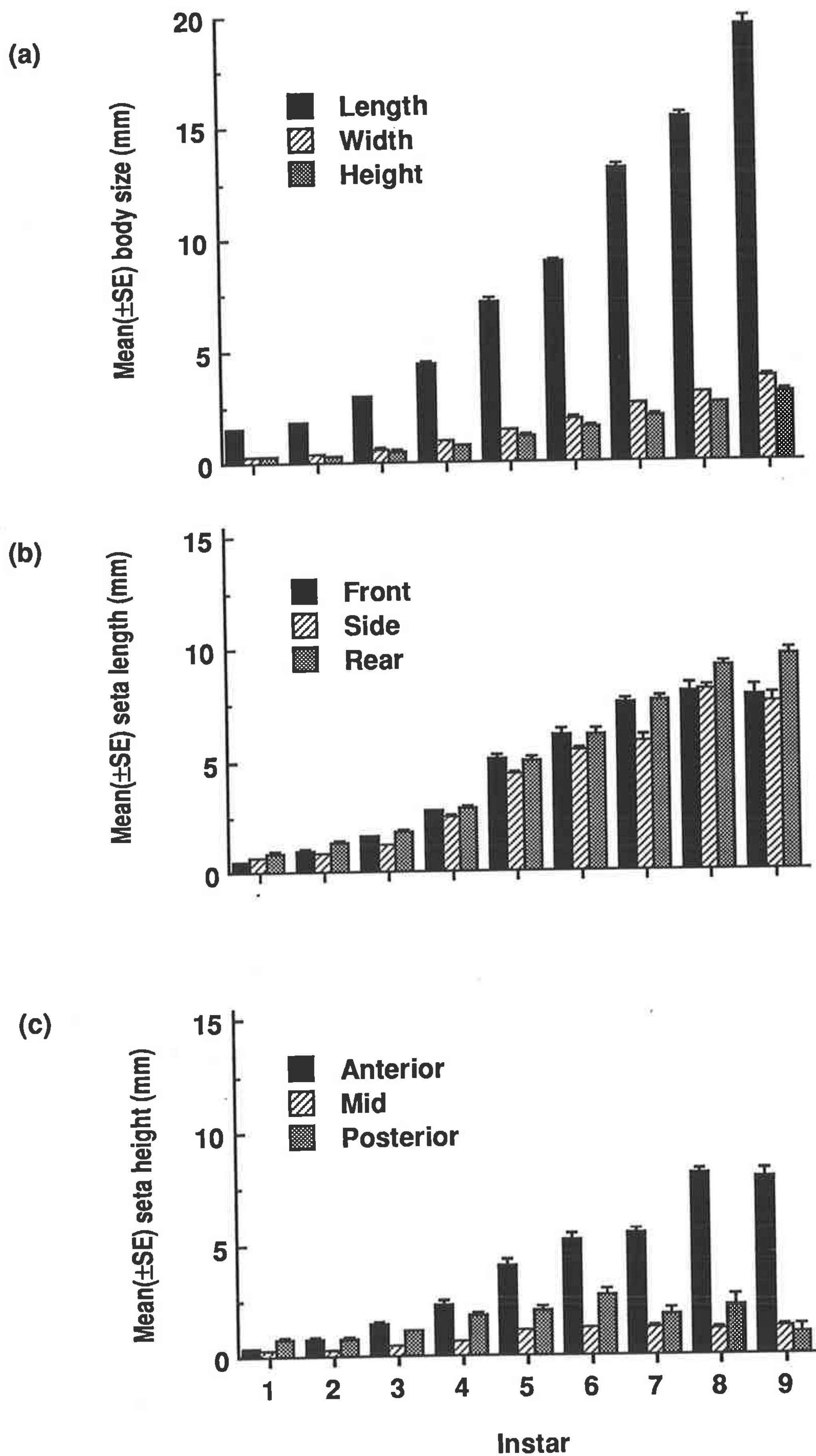
*1. Experiment 1: Setal and body dimensions of larvae of* U. lugens

Only instars 1-9 were measured as the surviving number of *U. lugens* dropped to six by the 9th instar. The mean±SE body weights (mg) and head capsule widths (mm) respectively of each instar were *1st* : 0.07±0.01, 0.24±0.002; *2nd* : 0.13±0.01, 0.31±0.002; *3rd* : 0.49±0.06, 0.43±0.003; *4th* : 1.69±0.26, 0.68±0.009; *5th* : 7.11±1.68, 0.98±0.015; *6th* : 15.14±4.36, 1.37±0.036; *7th* : 42.84±7.96, 1.83±0.024; *8th* : 85.23±13.10, 2.38±0.035; and *9th* : 150.83±28.98, 2.66±0.027.

Setae encompassed a large area beyond the body surface of *U. lugens* (Fig. 1). Setal lengths to the front, side, and rear of the body added approximately half the body length in each direction. The only body region where stiff envenomating spines, rather than flexible ciliated setae (Southcott 1978), were longest was above abdominal segments 1-9. This region also had the shortest setae of all regions measured. An unusual trend was that setal heights above the mid and posterior regions decreased relative to head capsule width with each rise in instar number. For setal height to the posterior this decrease was not because setae were shorter but because they were more flexible and therefore did not stand as upright as those to the anterior of *U. lugens*.

All body regions at every instar had setae longer than the ovipositor lengths of *C. urabae* and *D. eucalypti*. Ovipositor length of *C. urabae* ranged from 0.14 to 0.19 mm whilst ovipositor length of *D. eucalypti* ranged from 0.25 to 0.30 mm. Ovipositor lengths were positively correlated with the cube root of body weight for *D. eucalypti* ($r^2 = 0.66$, $p < 0.001$) but not for *C. urabae* ($r^2 = 0.005$, $p = 0.69$). Body weights of *C. urabae* were from 2.31 to 4.07 mg and of *D. eucalypti* were from 1.39 to 3.36 mg and were exceeded by *U. lugens* body weight by the 4-5th instar. Body length of

**Fig. 1.** Mean±SE measurements of each larval instar of *U. lugens* for: **(a)** body dimensions, **(b)** the longest seta surrounding the body from three directions, and **(c)** the highest seta above three regions of a larva's body. (n = 12; except instar 9 when n = 6).


(a)
20
15
10
5
0
Mean(±SE) body size (mm)
Length
Width
Height
(b)
15
10
5
0
Mean(±SE) seta length (mm)
Front
Side
Rear
(c)
15
10
5
0
Mean(±SE) seta height (mm)
Anterior
Mid
Posterior
1
2
3
4
5
6
7
8
9
Instar

*U. lugens* exceeded that of *C. urabae* and *D. eucalypti* (around 3 mm ; Austin and Allen 1989) by the $4^{th}$ instar.

*2. Experiment 2. Response of* U. lugens *to attack*

*a. Response to attack*

The immediate and ultimate response to attack did not differ between groups of larvae within a size class nor between the three different directions of attack. This was concluded from LLCTA on the 24 differing occasions for which responses were recorded (i.e. response: immediate or ultimate (n=2) x number of size classes (n=3) x number of occasions that attacks were made to each group (n=4)). Groups were only significantly different for four of the 24 analyses and direction only significantly different for three of the 24 analyses. Thus groups and directions were pooled for all further analyses of overall response to attack.

Position only significantly influenced the ultimate response of small larvae to attack (Fig. 2: Table 1). When underneath a leaf, small larvae displayed more locomotory responses than when on top of a leaf. This increase in locomotory response was due in part to larvae that dropped from the leaf on silk. Larvae only dropped on silk when located underneath a leaf and the percentage of locomotory response accounted for by dropping on silk was 10.5%, 4% and 0% for small, mid, and large larvae respectively.

The frequency of defensive responses increased with increasing larval size for both immediate and ultimate responses (Fig. 2). For mid and large larvae defensive responses accounted for over 85% of all responses whilst small larvae frequently exhibited 'no response' to attack. Each larval size showed similar differences between immediate and ultimate response to attack, in that ultimate response was more frequently locomotory than immediate response (Fig. 2). This difference was most pronounced and significant for small larvae, but was also significant for mid larvae, except in the two hr test, and for large larvae in the upright and 10 min. tests.

**Fig. 2.** Frequency of defensive, locomotory and no response recorded as immediate (i) and ultimate (u) response to attack with a micropin to small, mid and large larvae of *U. lugens*. Figures are for larvae: **(a)** when on top of a horizontal leaf, **(b)** when underneath a horizontal leaf, **(c)** 10 min. after parasitoid exposure, and **(d)** two hrs after parasitoid exposure. Sample size is 216 for each bar. Symbols above bars refer to the outcome of LLCTA or Fisher's exact test between immediate and ultimate response for each pair of bars: **=P<0.005, *=P<0.05, ns= not significant at the 0.05 significance level.

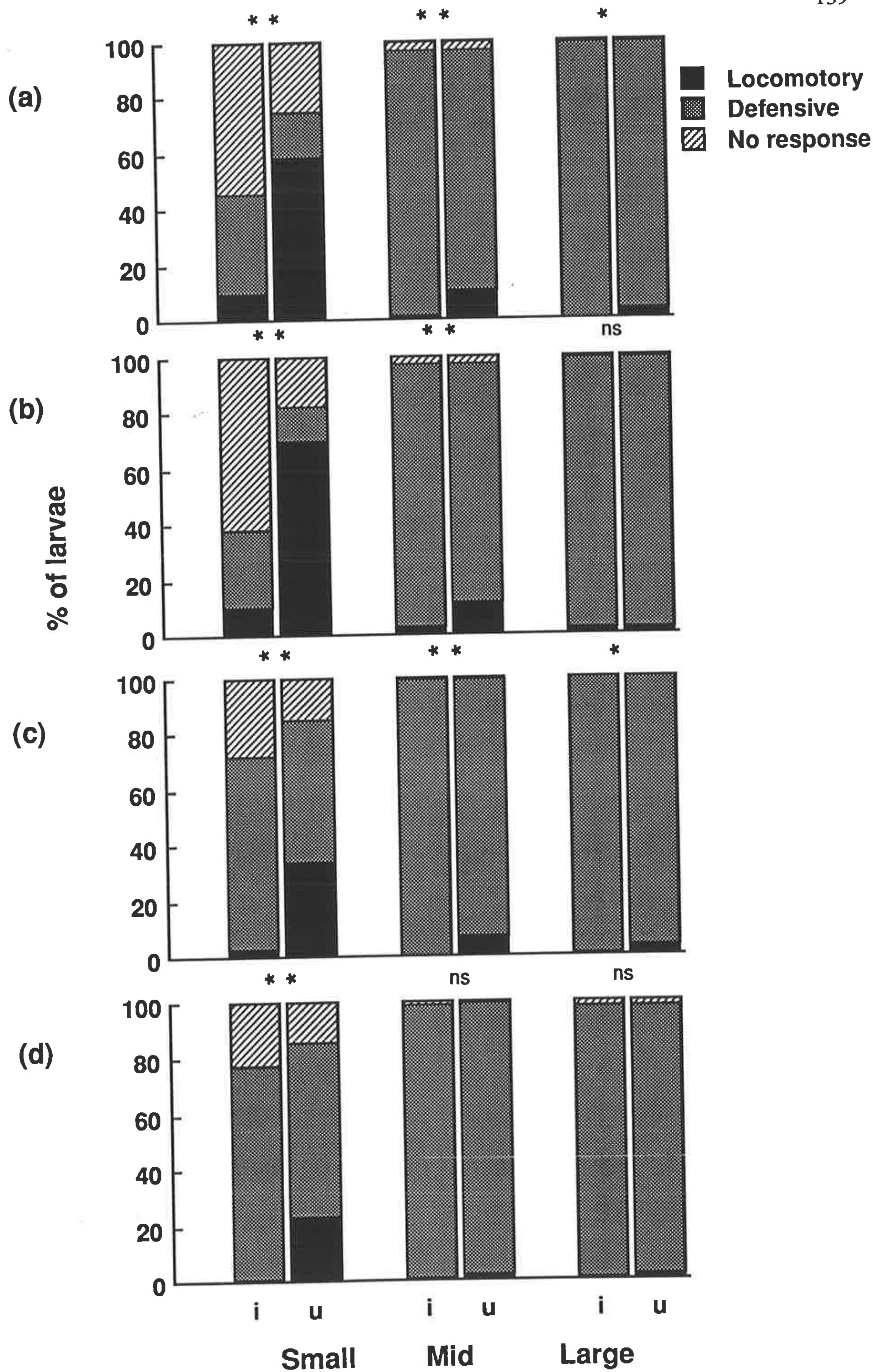

(a)
(b)
(c)
(d)
% of larvae
100
80
60
40
20
0
* *
*
ns
Locomotory
Defensive
No response
i
u
Small
Mid
Large

**Table 1. Outcome of LLCTA and Fisher's exact tests on the frequencies of defensive, locomotory, and 'no response' between: the four differing occasions that attacks were made on larvae (a-d of Fig. 2), and small, mid, and large larvae of *U. lugens* for the upright, 10 min. and two hr tests. Tables are for (a) the immediate response to attack, and (b) the ultimate response to attack.**

Frequencies and symbols as for Fig. 2, except **a-d** of Fig. 2 which correspond to Upr, Und, Ten, and 2hr respectively.

| (a) | Small | | | | Mid | | | | Large | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr |
| Small Upr | - | ns | ** | ** | ** | - | - | - | ** | - | - | - |
| " Ten | | | - | ns | | | ** | - | | | ** | - |
| " 2hr | | | | - | | | | ** | | | | ** |
| Mid Upr | | | | | - | ns | ** | ns | * | - | - | - |
| " Ten | | | | | | | - | ns | | | ns | - |
| " 2hr | | | | | | | | - | | | | ns |
| Large Upr | | | | | | | | | - | ns | ns | ns |
| " Ten | | | | | | | | | | | - | ns |
| " 2hr | | | | | | | | | | | | - |

| (b) | Small | | | | Mid | | | | Large | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr |
| Small Upr | - | * | ** | ** | ** | - | - | - | ** | - | - | - |
| " Ten | | | - | * | | | ** | - | | | ** | - |
| " 2hr | | | | - | | | | ** | | | | ** |
| Mid Upr | | | | | - | ns | * | ** | * | - | - | - |
| " Ten | | | | | | | - | * | | | ns | - |
| " 2hr | | | | | | | | - | | | | ns |
| Large Upr | | | | | | | | | - | ns | ns | ns |
| " Ten | | | | | | | | | | | - | ns |
| " 2hr | | | | | | | | | | | | - |

After exposure to a parasitoid the proportion of responses to attack that were defensive increased for small and mid larvae (Fig. 2: Table 1). The level of defensive response did not change for large larvae as they were already high prior to exposure to a parasitoid. Two hrs after exposure to a parasitoid the level of defensive response by small and mid larvae was still maintained (immediate), or even increased (ultimate), relative to the level 10 min. after exposure.

In summary with increasing larval size defensive responses increased and locomotory responses decreased when larvae were attacked. After exposure to a parasitoid, small and mid larvae showed an increase in level of defensive response to attack. Direction of attack did not influence overall response to attack but position with respect to gravity of small larvae influenced the level of locomotory response shown to attack.

*b. Type of defensive response*

Rearing was the most variable defensive response observed. Variation occurred in angle the body reared to, direction of rear, number of prolegs attached to the leaf, and number of rears per attack. Small larvae only reared in one fixed plane (straight up and down) whereas mid and large larvae frequently turned the anterior of their body toward the direction of attack.

More than one type of defensive response was often displayed by larvae when attacked. Thrashing only followed on from pulsing, rearing and head curling. Regurgitation and biting were always associated with pulsing, rearing or head curling. Consequently for analyses, immediate defensive response were first divided into just three 'types'; pulsing, rearing and head curling, and only later subdivided into thrashing, biting and regurgitation by examining what other defensive responses.were associated with or followed on from these three responses.

The position of a larva with respect to gravity did not influence its type of defensive response (Figs 3, 4: Tables 2, 3, 4). However direction of attack did influence type of defensive response shown by a larva (Fig. 3; Table 2). Head curling was unique

**Fig. 3.** Subdivision of immediate defensive response for small, mid and large larvae of *U. lugens* into pulse, rear and head curl when attacked with a micropin from the front (F), from the side (S) and from behind (B). Figures **(a)**-**(d)** as for Fig. 2. Numbers above bars are sample sizes.

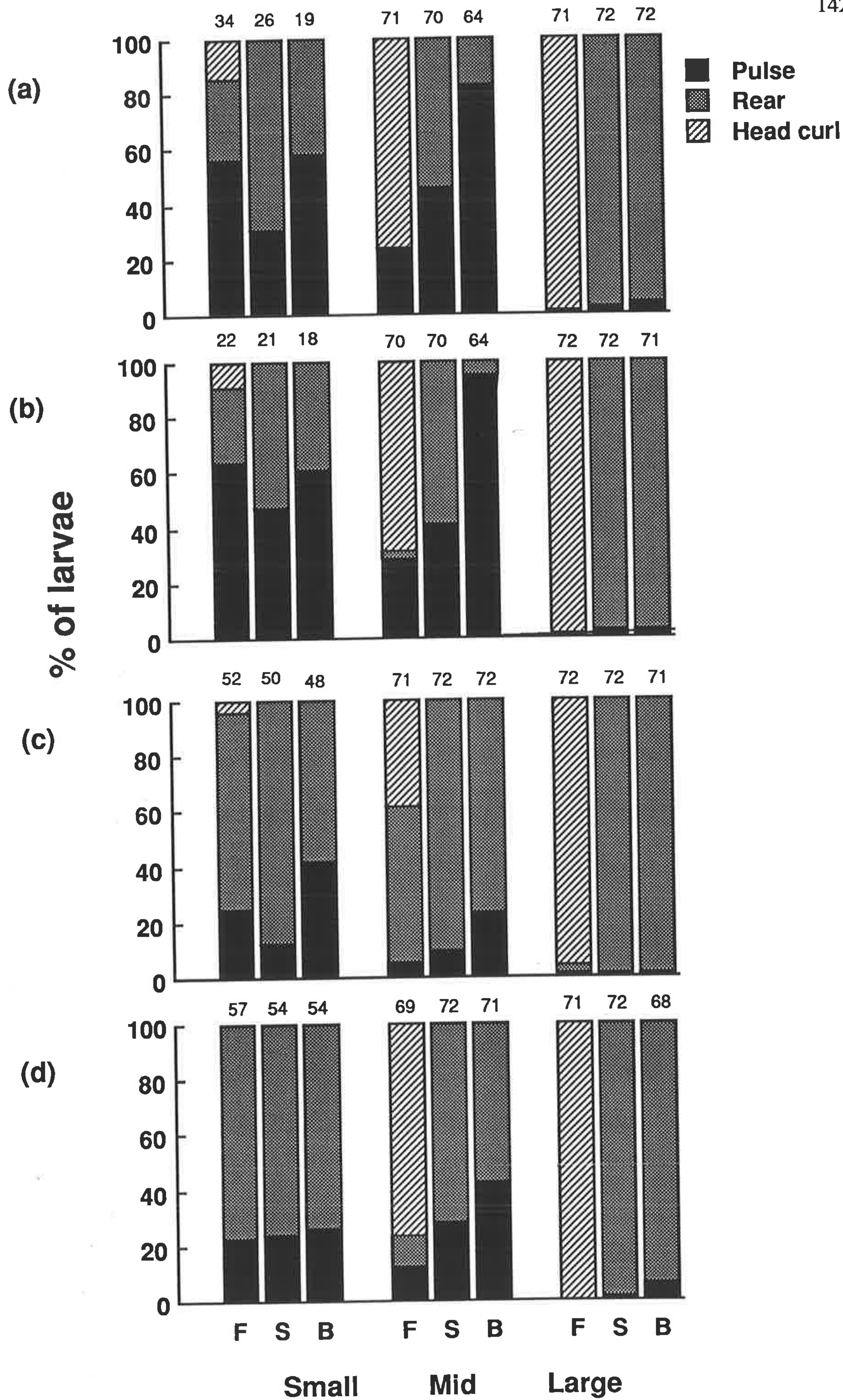

(a)
(b)
(c)
(d)
% of larvae
34 26 19 71 70 64 71 72 72
22 21 18 70 70 64 72 72 71
52 50 48 71 72 72 72 72 71
57 54 54 69 72 71 71 72 68
0
20
40
60
80
100
Pulse
Rear
Head curl
F S B F S B F S B
Small
Mid
Large

Table 2. Outcome of LLCTA and Fisher's exact tests on the frequency of pulsing, rearing, and head curling when attacked: (a) between the three attack directions (front, side, and behind) made to larvae of *U. lugens*, and (b-d) between the four differing occasions that attacks were made on larvae (a-d of Fig. 3) and between small, mid, and large larvae for the upright, 10 min. and two hr tests for attacks: (b) to the front of larvae, (c) to the side of larvae, and (d) from behind larvae. Refer to Fig. 3 for overall frequencies. Symbols as for Table 1.

| (a) | | Small | | | Mid | | | Large | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Front | Side | Behind | Front | Side | Behind | Front | Side | Behind |
| Upr | Front | - | ** | ns | - | ** | ** | - | ** | ** |
| " | Side | | - | ns | | - | * | | - | ns |
| Und | Front | - | ns | ns | - | ** | ** | - | ** | ** |
| " | Side | | - | ns | | - | ** | | - | ns |
| Ten | Front | - | * | ns | - | ** | ** | - | ** | ** |
| " | Side | | - | ** | | - | * | | - | ns |
| 2hr | Front | - | ns | ns | - | ** | ** | - | ** | ** |
| " | Side | | - | ns | | - | ns | | - | ns |

| (b) | | Small | | | | Mid | | | | Large | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2h |
| Small | Upr | - | ns | ** | ** | ** | - | - | - | ** | - | - | - |
| " | Ten | | | - | ns | | | ** | - | | | ** | - |
| " | 2hr | | | | - | | | | ** | | | | ** |
| Mid | Upr | | | | | - | ns | ** | ** | ** | - | - | - |
| " | Ten | | | | | | | - | ** | | | ** | - |
| " | 2hr | | | | | | | | - | | | | ** |
| Large | Upr | | | | | | | | | - | ns | ns | ns |
| " | Ten | | | | | | | | | | | - | ns |
| " | 2hr | | | | | | | | | | | | - |

| (c) | | Small | | | | Mid | | | | Large | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2h |
| Small | Upr | - | ns | ns | ns | ns | - | - | - | ** | - | - | - |
| " | Ten | | | - | ns | | | ns | - | | | * | - |
| " | 2hr | | | | - | | | | ns | | | | ** |
| Mid | Upr | | | | | - | ns | ** | * | ** | - | - | - |
| " | Ten | | | | | | | - | ** | | | ns | - |
| " | 2hr | | | | | | | | - | | | | ** |
| Large | Upr | | | | | | | | | - | ns | ns | ns |
| " | Ten | | | | | | | | | | | - | ns |
| " | 2hr | | | | | | | | | | | | - |

| (d) | | Small | | | | Mid | | | | Large | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2h |
| Small | Upr | - | ns | ns | * | * | - | - | - | ** | - | - | - |
| " | Ten | | | - | ns | | | * | - | | | ** | - |
| " | 2hr | | | | - | | | | ns | | | | ** |
| Mid | Upr | | | | | - | * | ** | ** | ** | - | - | - |
| " | Ten | | | | | | | - | * | | | ** | |
| " | 2hr | | | | | | | | - | | | | ** |
| Large | Upr | | | | | | | | | - | ns | ns | ns |
| " | Ten | | | | | | | | | | | - | ns |
| " | 2hr | | | | | | | | | | | | - |

**Fig. 4.** Frequency of regurgitation, thrashing and both these responses occurring together when in conjunction with pulsing (P), rearing (R) and head curling (H) for small, mid and large larvae of *U. lugens*. Figures **(a)**-**(d)** as for Fig. 2. Numbers above bars are sample sizes.

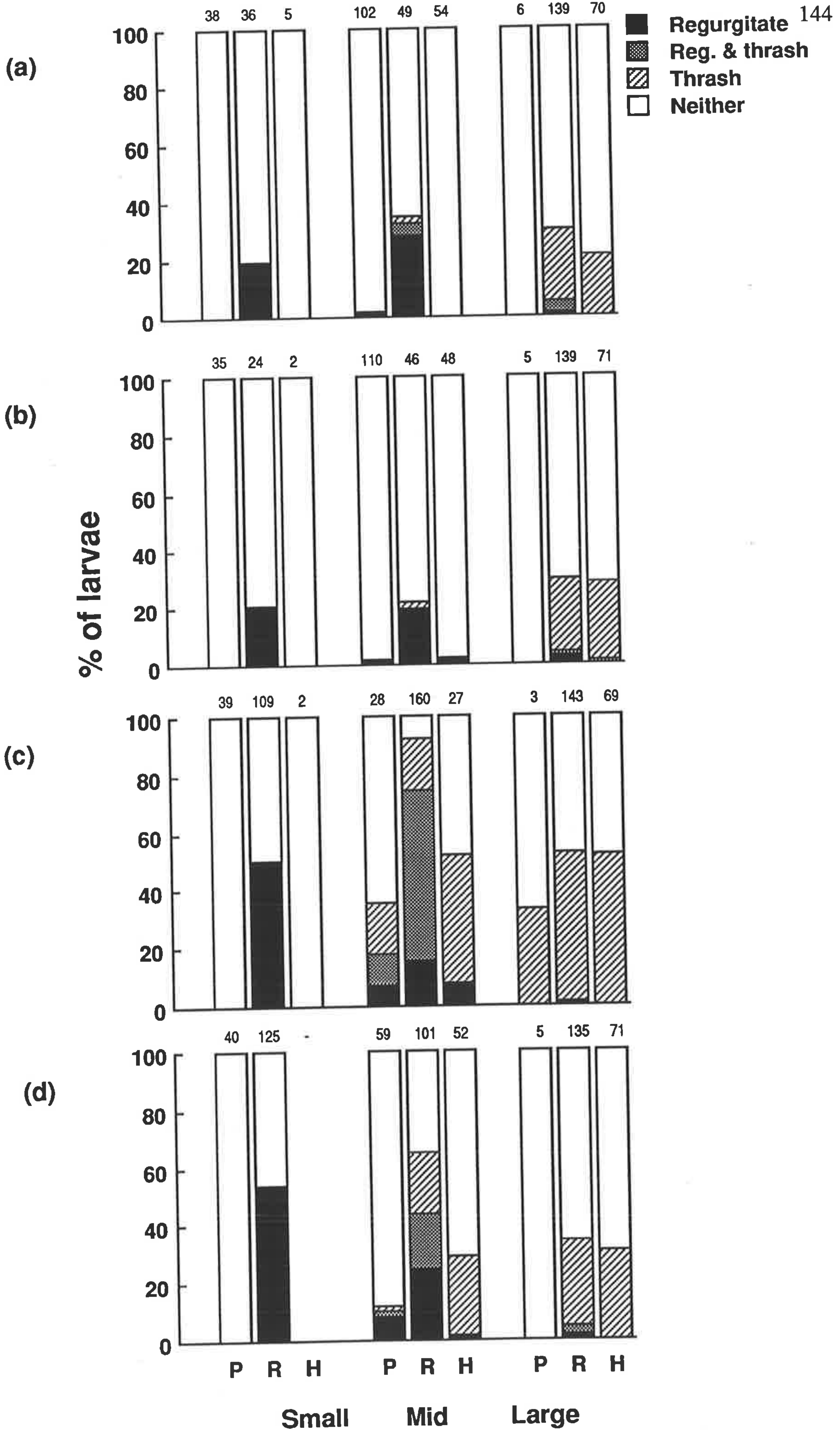

Regurgitate
Reg. & thrash
Thrash
Neither
(a)
(b)
(c)
(d)
% of larvae
0
20
40
60
80
100
38 36 5
102 49 54
6 139 70
35 24 2
110 46 48
5 139 71
39 109 2
28 160 27
3 143 69
40 125 -
59 101 52
5 135 71
P R H
P R H
P R H
Small
Mid
Large

**Table 3. Outcome of LLCTA and Fisher's exact tests on the frequency of regurgitation of *U. lugens* larvae: (a) between pulsing, rearing, and head curling, and (b-d) between the four differing occasions that attacks were made on larvae (a-d of Fig. 4) and between small, mid, and large larvae for the upright, 10 min. and two hr tests for the defensive responses (b) pulsing, (c) rearing, and (d) head curling.**

Refer to Fig. 4 for overall frequencies. Symbols as for Table 1.

| (a) | | Small | | | Mid | | | Large | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Pulse | Rear | Head curl | Pulse | Rear | Head curl | Pulse | Rear | Head curl |
| Upr | Pulse | - | ** | ns | - | ** | ns | - | ns | ns |
| " | Rear | | - | ns | | - | ** | | - | ns |
| Und | Pulse | - | * | ns | - | ** | ns | - | ns | ns |
| " | Rear | | - | ns | | - | ** | | - | ns |
| Ten | Pulse | - | ** | ns | - | ** | ns | - | ns | ns |
| " | Rear | | - | ns | | - | ** | | - | ns |
| 2hr | Pulse | - | ** | ns | - | ** | ns | - | ns | ns |
| " | Rear | | - | ns | | - | ** | | - | ns |

| (b) | | Small | | | | Mid | | | | Large | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2h |
| Small | Upr | - | ns | ns | ns | ns | - | - | - | ns | - | - | - |
| " | Ten | | | - | ns | | | * | - | | | ns | - |
| " | 2hr | | | | - | | | | ns | | | | ns |
| Mid | Upr | | | | | - | ns | ** | * | ns | - | - | - |
| " | Ten | | | | | | | - | ns | | | ns | - |
| " | 2hr | | | | | | | | - | | | | ns |
| Large | Upr | | | | | | | | | - | ns | ns | ns |
| " | Ten | | | | | | | | | | | - | ns |
| " | 2hr | | | | | | | | | | | | - |

| (c) | | Small | | | | Mid | | | | Large | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2h |
| Small | Upr | - | ns | ** | ** | ns | - | - | - | * | - | - | - |
| " | Ten | | | - | ns | | | ** | - | | | ** | - |
| " | 2hr | | | | - | | | | ns | | | | ** |
| Mid | Upr | | | | | - | ns | ** | ns | ** | - | - | - |
| " | Ten | | | | | | | - | ** | | | ** | - |
| " | 2hr | | | | | | | | - | | | | ** |
| Large | Upr | | | | | | | | | - | ns | ns | ns |
| " | Ten | | | | | | | | | | | - | ns |
| " | 2hr | | | | | | | | | | | | - |

| (d) | | Small | | | | Mid | | | | Large | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2h |
| Small | Upr | - | ns | ns | - | ns | - | - | - | ns | - | - | - |
| " | Ten | | | | - | | | ns | - | | | ns | - |
| " | 2hr | | | | - | | | | - | | | | - |
| Mid | Upr | | | | | - | ns | ns | ns | ns | - | - | - |
| " | Ten | | | | | | | - | ns | | | ns | |
| " | 2hr | | | | | | | | - | | | | ns |
| Large | Upr | | | | | | | | | - | ns | ns | ns |
| " | Ten | | | | | | | | | | | - | ns |
| " | 2hr | | | | | | | | | | | | - |

Table 4. Outcome of LLCTA and Fisher's exact tests on the frequency of thrashing of *U. lugens* larvae: (a) between pulsing, rearing, and head curling, and (b-d) between the four differing occasions that attacks were made on larvae (a-d of Fig. 4) and between small, mid, and large larvae for the upright, 10 min. and two hr tests for the defensive responses (b) pulsing, (c) rearing, and (d) head curling.
Refer to Fig. 4 for overall frequencies. Symbols as for Table 1.

| (a) | | Small | | | Mid | | | Large | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Pulse | Rear | Head curl | Pulse | Rear | Head curl | Pulse | Rear | Head curl |
| Upr | Pulse | - | ns | ns | - | ns | ns | - | ns | ns |
| " | Rear | | - | ns | | - | ns | | - | ns |
| Und | Pulse | - | ns | ns | - | ns | ns | - | ns | ns |
| " | Rear | | - | ns | | - | ns | | - | ns |
| Ten | Pulse | - | ns | ns | - | ** | ns | - | ns | ns |
| " | Rear | | - | ns | | - | ** | | - | ns |
| 2hr | Pulse | - | ns | ns | - | ** | ** | - | ns | ns |
| " | Rear | | - | ns | | - | ns | | - | ns |

| (b) | | Small | | | | Mid | | | | Large | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2h |
| Small | Upr | - | ns | ns | ns | ns | - | - | - | ns | - | - | - |
| " | Ten | | | - | ns | | | ** | - | | | ns | - |
| " | 2hr | | | | - | | | | ns | | | | ns |
| Mid | Upr | | | | | - | ns | ** | ns | ns | - | - | - |
| " | Ten | | | | | | | - | ** | | | ns | - |
| " | 2hr | | | | | | | | - | | | | ns |
| Large | Upr | | | | | | | | | - | ns | ns | ns |
| " | Ten | | | | | | | | | | | - | ns |
| " | 2hr | | | | | | | | | | | | - |

| (c) | | Small | | | | Mid | | | | Large | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2h |
| Small | Upr | - | ns | ns | ns | ns | - | - | - | ** | - | - | - |
| " | Ten | | | - | ns | | | ** | - | | | ** | - |
| " | 2hr | | | | - | | | | ** | | | | ** |
| Mid | Upr | | | | | - | ns | ** | ** | ** | - | - | - |
| " | Ten | | | | | | | - | ** | | | ** | - |
| " | 2hr | | | | | | | | - | | | | ns |
| Large | Upr | | | | | | | | | - | ns | ** | ns |
| " | Ten | | | | | | | | | | | - | ** |
| " | 2hr | | | | | | | | | | | | - |

| (d) | | Small | | | | Mid | | | | Large | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2hr | Upr | Und | Ten | 2h |
| Small | Upr | - | ns | ns | - | ns | - | - | - | ns | - | - | - |
| " | Ten | | | - | - | | | ns | - | | | ns | - |
| " | 2hr | | | | - | | | | - | | | | - |
| Mid | Upr | | | | | - | ns | ** | ** | ** | - | - | - |
| " | Ten | | | | | | | - | ns | | | ns | |
| " | 2hr | | | | | | | | - | | | | ns |
| Large | Upr | | | | | | | | | - | ns | ** | ns |
| " | Ten | | | | | | | | | | | - | * |
| " | 2hr | | | | | | | | | | | | - |

to frontal attacks and its frequency was significantly different to other directions for mid and large larvae on all four occasions that attacks were made to groups. Attacks to the side of a larva produced significantly more rearing than attacks from behind a larva with mid larvae (except at the two hr test), and with small larvae at the 10 min. test. The size of a larva also affected its response, with head curling in response to frontal attacks increasing with increasing larval size. Rearing in response to attack from behind and from the side was significantly greater in large larvae than in small or mid larvae (except for mid larvae in the 10 min. test).

Exposure to a parasitoid significantly altered the relative frequencies of pulsing, rearing, and head curling in small and mid but not large larvae (Fig. 3: Table 2). For small larvae exposure to a parasitoid resulted in significantly more rearing to subsequent attacks from the front and from behind, but not from the side, since the latter level was already high prior to exposure to a parasitoid. These high levels of rearing by small larvae were maintained for at least two hrs. Mid larvae also greatly increased their levels of rearing after exposure to a parasitoid in response to attack from all directions but this level began to drop after two hrs.

Biting was always associated with rearing and occurred in 14-17% of all rears in small larvae, infrequently in mid larvae and never in large larvae. Regurgitation was associated with pulsing, rearing, and head curling and often with thrashing (Fig. 4: Table 3). Regurgitation was significantly more frequent with rearing, than either pulsing or head curling, in small and mid larvae but was infrequently observed in large larvae. Exposure to a parasitoid significantly increased regurgitation in small and particularly mid larvae but in mid larvae it began to drop after two hrs.

Small larvae were never observed to thrash, whilst mid and large larvae thrashed most frequently after rearing (Fig. 4; Table 4). Exposure to a parasitoid significantly increased the frequency of thrashing in large and particularly mid larvae, but after two hrs the level of thrashing for large larvae had returned to that prior to exposure to a parasitoid.

In summary head curling was unique to frontal attacks whereas side attacks produced more rearing than attacks from behind. Large larvae consistently showed very high levels of rearing but small and mid larvae increased rearing after exposure to a parasitoid. Regurgitation was infrequently seen with large larvae but was significantly increased in small and mid larvae after exposure to a parasitoid. Thrashing was never observed with small larvae whilst large larvae always showed high levels of thrashing even prior to exposure to a parasitoid. Two hrs after exposure to a parasitoid the levels of rearing, regurgitation and thrashing remained high for small larvae (excluding thrashing), declined for mid larvae and returned or remained at the levels prior to exposure for large larvae.

*c. Prior* U. lugens *behaviour and response to attack*

For small and mid larvae whether the larva attacked was (i) at the edge or within the group, and (ii) feeding or not feeding immediately prior to attack, did not significantly affect its response, nor type of defensive response displayed to attack. There was no consistent significant difference in response, nor type of defensive response between the first half and last half of larvae attacked in each group. Feeding behaviour was affected by simulated and particularly parasitoid attack. Initially 53% of small and 27% of mid larvae were feeding, but 30 min. after the first simulated attack these figures dropped to 45% and 18% respectively. Ten min. after parasitoid attack these figures greatly dropped to 6% and 0% respectively, only slightly recovering after two hrs to 11% and 2% respectively.

*3. Experiment 3: Response of* U. lugens *to repeated attack*

Not all groups responded similarly within each size class in this experiment. The immediate response in small and in mid larvae, and the ultimate response in small and in large larvae each had a group that displayed dissimilar responses to the remaining groups. However only small larvae had a group that was consistently different in immediate and ultimate response, this group having more locomotory activity than other

groups. Since this one difference was consistent it did not unduly affect the overall results and all groups within a size class were pooled for further analyses.

Each successive attack to small, mid, and large larvae increased the level of locomotory response (Fig. 5). LLCTA showed a significant difference was evident, for both immediate and ultimate response, between the six attacks to each of the three size classes. For small larvae the increase in locomotory response was offset by a corresponding decrease in those showing 'no response' to attack. For mid and particularly large larvae it was offset by a decrease in the number of larvae showing a defensive response. Relatively more small larvae began locomotory response earlier than mid and large larvae in response successive attack. The type of defensive response displayed to successive attacks did not significantly change with each attack.

## Discussion

The body size and weight ratios of *U. lugens* larvae relative to *C. urabae* and *D. eucalypti* began to exceed one by around the 4$^{th}$ instar. This is the smallest size of *U. lugens* from which both parasitoid species are known to emerge (Chapters 3 and 4). Evans (1976) found greater capture efficiency by the predator *Anthocoris nemorum* (L.) upon the aphid *Acyrthosiphon pisum* (Harris) when body size ratio of predator to prey began to exceed one. However, since parasitoids, unlike predators, do not have to subdue their 'prey' a direct extension of pivotal ratios to parasitoids is more difficult to make.

Ovipositor length of *D. eucalypti* was around twice that of *C. urabae* but the ovipositors of both species would still be obstructed by the long setae of *U. lugens*. The region where setae of *U. lugens* were shortest was above the 'mid' region of the dorsal surface, but this region was where stiff envenomating spines were predominant and setae dense. Both density and length of setae would be important in obstructing ovipositor penetration. The value of setae to defense has been demonstrated by Ayre and Hitchon (1968) who showed that removal of setae from *Malacosoma americanum* (F.) increased ant predation. The presence of long setae to the anterior, side, and posterior of *U. lugens*

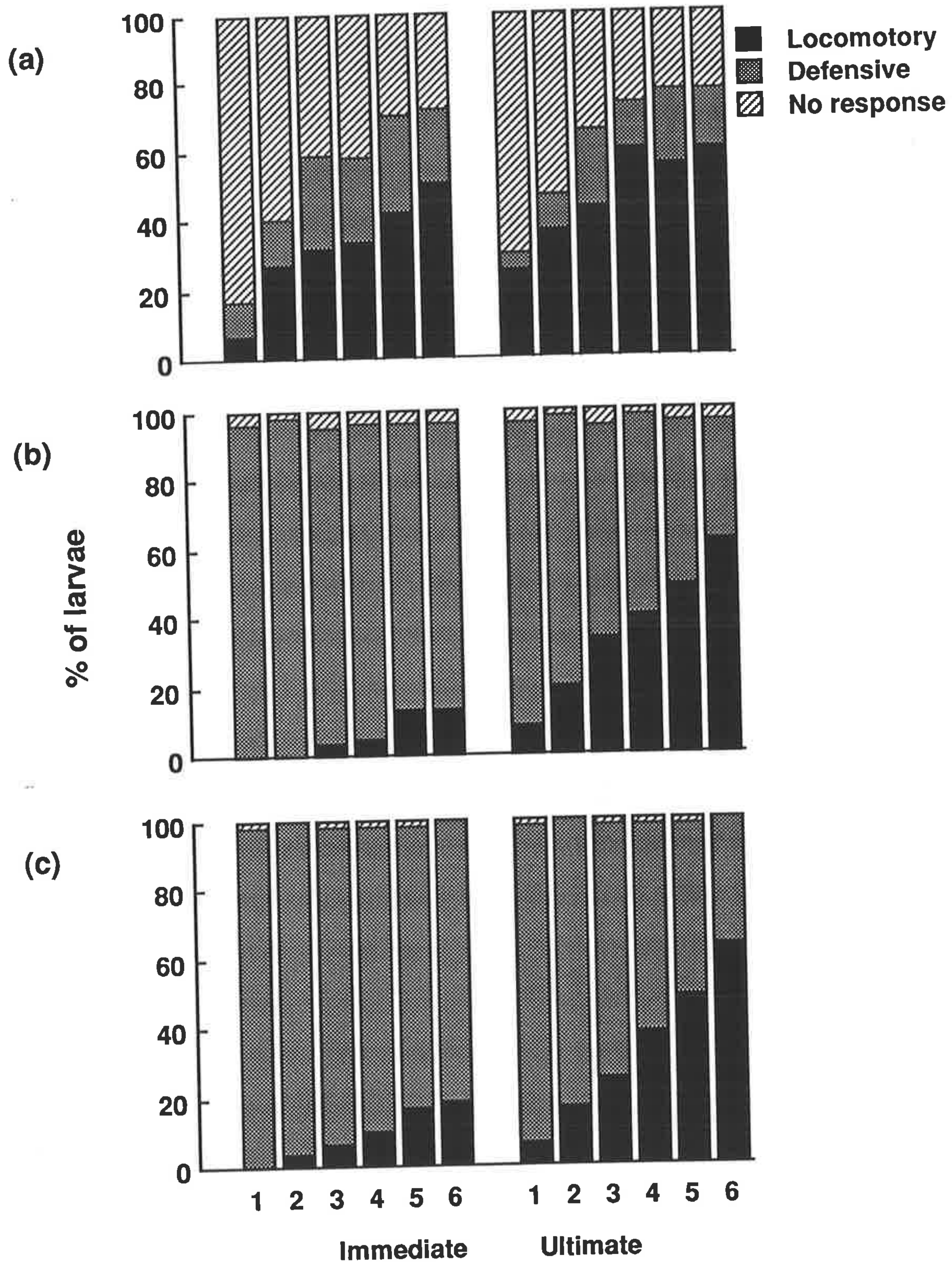



**Fig. 5.** Frequency of defensive, locomotory and no response recorded as immediate and ultimate response, to six successive attacks (labelled 1-6) from behind a *U. lugens* larva with a micropin 15 sec. apart. Figures are for: **(a)** small larvae, **(b)** mid larvae, and **(c)** large larvae. Sample size is 60 for each bar.

would not only be of potential obstructive value towards ovipositor penetration (and predator attack), but would aid the larvae in detecting unseen predators or parasitoids before such enemies make contact with their general body surface.

It was only the type of defensive response not the levels of responses that changed according to the direction of attack direction in *U. lugens*. A principal difference between attack directions was head curling, which was only seen in response to frontal attacks. Head curling was not observed during experiments with *C. urabae* and *D. eucalypti* in the previous chapter, and along with pulsing, shows less movement than the other defensive responses that involved body orientation. Both head curling and pulsing appear the least effective of the defensive responses observed. Despite the 'poor defense' of head curling, attack by a natural enemy to the head of *U. lugens*, would incur other risks to the natural enemy, such as a higher chance of being bitten or contacted by regurgitate. Attacks from behind and to the side of *U. lugens* frequently produced rearing, particularly attacks to the side. The latter, which was generally observed in small and mid larvae, would favour natural enemies that attacked *U. lugens* from behind. Stamp (1982) observed directed attack behaviour by *Cotesia euphydryidis* Muesebeck which generally attacked 2-3rd instar larvae of *Euphydryas phaeton* Drury from behind. For large larvae of *U. lugens*, the typical response to attack from either direction was to rear and its effectiveness was enhanced by the stacked head capsules. Stacked head capsules increased the area behind a larvae defended during rearing. As with *Battus philenor* (L.) (Stamp 1986) the number of prolegs attached to the leaf surface during rearing by *U. lugens* largely determined how far behind itself the caterpillar 'defended'.

Larvae of *U. lugens* were, with the exception of small larvae, unaffected by position above or below a leaf in their response to attack. Only small larvae dropped more frequently from the leaf on silk when positioned underneath the leaf, even though dropping would be most easily achieved from this position by all larvae. Dropping on silk would not incur the disadvantages of desiccation, starvation or increased predation that face other insects such as aphids (McAllister and Roitberg 1987) once they drop from a plant, as they can easily return via the silk thread. A stronger test of the influence

of position would have been to test larvae after parasitoid attack when they showed elevated levels of response or, to use parasitoids instead of an artificial stimulus to attack larvae when situated underneath the leaf.

Small larvae were the least responsive to attack and if they did respond most frequently responded with locomotion. Along with their absence of thrashing and apparent ability to rear only in one plane, small larvae were the least capable of individually defending themselves. Poor defence in small larvae was also a probable cause in Chapter 6 of most ovipositions by *C. urabae* and *D. eucalypti* occurring in small larvae. There was a strong association between rearing and biting, regurgitation, and thrashing in all larvae. Biting and regurgitation may possibly enhance the defense of rearing but thrashing was more of a 'post-attack' response. Thrashing may help prevent subsequent attacks or decrease a larva's chance of being parasitized once re-encountered (Chapter 6). The lower frequency of biting and regurgitation seen with increasing *U. lugens* size was not so apparent when larvae were attacked by parasitoids (Chapter 6). With increasing size the volume of regurgitate produced, and thus its effectiveness, should increase as would that of biting with increasing mandible size.

There was no evidence in this experiment that the response of the first half of larvae attacked within a group affected the response of those larvae yet to be attacked although *U. lugens* not yet attacked do rear and thrash when other larvae are being attacked by a parasitoid (Chapter 6). Cornell *et al.* (1987) hypothesized that the defensive behaviour of *Hemileuca lucina* Hy. Edw. may relate to its degree of aggregation which declines with increasing developmental stage. For this species it was thought that the observed decline in defensive behaviour with increasing stage was related to the decreased effectiveness of displaying defensive behaviour when not in a group. However for *U. lugens* which is also gregarious in its early instars, the opposite pattern to *H. lucina* occurs with defensive responses displayed more frequently as body size increases. This pattern is similar to that of the solitary caterpillar *B. philenor* (Stamp 1986) and most probably reflects the more effective defence of larger larvae.

Gregariousness could help small larvae of *U. lugens* by enabling some of the larvae in a group to detect and attempt to escape natural enemies earlier than if alone (Chapter 6).

Changes in defensive response to attack after exposure to a parasitoid were most pronounced in small and mid larvae and less pronounced in large larvae. Small larvae increased rearing and regurgitation, mid larvae rearing, regurgitation and thrashing and large larvae thrashing. In small larvae this elevated level of response was maintained for at least two hrs, whilst in mid larvae it began to drop after two hrs. An increased responsiveness to attack may help prevent parasitism if larvae avoid an initial attack by a parasitoid. Since the majority of larvae stopped feeding after parasitoid attack the production of kairomones produced by feeding would also decrease. These kairomones may be used by natural enemies to locate larvae and thus ceasing to feed may help prevent further parasitism (Weseloh 1981).

There was no evidence of habituation during repeated attacks to *U. lugens*, but the response of larvae to repeated attacks differed from those larvae that had been exposed to 'continual' (20 min.) parasitoid attack (Chapter 6). The latter resulted in larvae showing more defensive responses whilst the former resulted in larvae showing more locomotion. After parasitoid attack all larvae had been allowed 10 min. 'rest' before being attacked by the micropin which may explain this difference. Although changes occurred in larvae that had not been directly attacked by parasitoids, it is possible that some of the changes observed after parasitoid attack may also have been directly due to parasitism. Parasitoids at oviposition inject other substances along with the egg which may subtly affect a hosts physiology and behaviour (Vinson and Iwantsch 1980; Strand 1986).

Differences in response before and after parasitoid attack may also indicate, not unexpectedly, that a micropin may not be very analogous to parasitoid attack. In preliminary experiments I tried small, two-haired paint brushes similar to those used by Stamp (1986) and Cornell *et al.* (1987) to simulate parasitoid attack, but these infrequently evoked any response from *U. lugens*. Other alternatives that have been used in previous work to simulate natural enemy attack include substrate vibration (Evans

1984; 1986) and forceps (Stamp 1986; Cornell *et al.* 1987). Nevertheless some of the differences in response between larvae exposed to parasitoid attack and those probed with a micropin may be that responses during the latter are relevant to attack by other natural enemies. There are at least four other species of parasitoid, including two ectoparasitoids, which attack the larvae of *U. lugens* in South Australia ( Austin and Allen 1989; Chapter 2). Additionally the stacked head capsules, seen to increase defensive area when rearing and thrashing, may also be beneficial in other ways to encounters with differing natural enemies. Eggs of the ectoparasitoid *Exorista flaviceps* Macquart (Family Tachinidae), for example, were often seen in the field on the stacked head capsules of larvae, which would 'waste' these eggs and so prevent parasitism.

Of the parameters tested direction of attack, position of a larva with respect to gravity, and recent (under two hrs ) exposure to a parasitoid may all influence the outcome of a host-parasitoid (and predator-prey) encounter involving *U. lugens*. As developmental stage increased defensive responses increased and locomotory responses decreased when larvae were attacked. This change probably parallels the increased effectiveness of defensive behaviour against parasitoids with increasing body size that has been observed for *U. lugens* (Chapter 6). Thus for *U. lugens*, response to attack does differ with developmental stage and along with the forementioned parameters are important co-variables in host-parasitoid and predator-prey interactions.

# Chapter 8. General discussion

Although this thesis has concentrated on *C. urabae* and *D. eucalypti* their ecology and behaviour must be set in the wider context of the large parasitoid complex associated with *U. lugens* in South Australia. The success or failure of both species in the field interrelates directly and indirectly with that of the other parasitoids in the complex, especially of their associated hyperparasitoids. For the high proportion of polyphagous parasitoids within the complex, the relative abundance of alternative hosts may, for example, complicate any attempts at modelling the biocontrol potential of the parasitoids of *U. lugens*. However, since *U. lugens* is an important outbreak pest of forests throughout its Australia-wide distribution, the comparative approach of studying the effectiveness of its associated parasitoid complexes, which may include fewer or more parasitoid species, in other climatic and geographic regions, may prove rewarding. It must be stressed that the selection of *C. urabae* and *D. eucalypti* in this thesis did not reflect on their promise as biocontrol agents but rather on the opportunity to study the adaptations of two 'similar' parasitoids to exploiting the same host in an environment within which they had evolved.

One unforeseen barrier to understanding the adaptations of these two species of parasitoid to their host was the lack of scientific data, other than anecdotal observations, on the developmental biology of *U. lugens*. The possibility of subspecies of *U. lugens* does not seem improbable, but subdivision into biological morphs based on number of larval instars, pattern of egg deposition and/or degree of multivoltinism (Campbell 1969; Harris 1974) uses characters that may have too much biological plasticity to be of any use. Furthermore the number and sizes of instars seen in the field and laboratory negates the usefulness of the term instar as a relative measure of size. The range of instars is so wide that I have been careful to specify a further parameter, such as head capsule width, when referring to a particular instar of *U. lugens*. The method of determining instar by the counting of stacked head capsules must be used with caution because the first head capsule stacked may differ between individuals reared under different conditions (as

observed between summer and winter generations in Adelaide) and because head capsules, especially the earliest ones, may be lost by larvae in the field.

The behaviour of head capsule stacking is not unique to *U. lugens*, occurring in at least four other Nolids (*s.l.* ) of the old world (McFarland 1978), but its obvious morphological prominence is of interest. Stacked head capsules may be a secondary defence enabling prey to escape a predators bite by only losing the superfluous exuviae in that first bite (McFarland 1978). In this respect the stacked head capsules are analogous to the 'false head' found in some other insects and fish that can be removed without mortally wounding the animal (Alcock 1984). However *U. lugens* appears to be unlikely to have any prevalent, if indeed any, avian predators (Chapter 1) ; the most conspicuous type of vertebrate predator present on *Eucalyptus* trees. At least two further possible functions of head capsule stacking were observed during this study. The first was in increasing the 'area of defence' during rearing or thrashing in response to predator or parasitoid attack. The second was that stacked head capsules may act as a 'sink' for ectoparasitoids eggs, such as those of *Exorista flaviceps* Macquart, effectively wasting those eggs that may otherwise successfully develop if placed on the host's general body surface. The instar at which head capsule stacking began may be a function of the size of the head capsule and the relative size and flexibility of the prothoracic setae, upon which head capsules are stacked, of that individual rather than of its life stage. Thus the instar may differ when head capsules are first stacked but the size of the differing instars and their head capsule widths may be very similar. This seems a more credible explanation of why head capsule stacking behaviour occurs in larva beyond a certain instar rather than inferring some functional value only relevant to latter stages of *U. lugens*.

The transition from gregarious to solitary larval feeding behaviour is another important behavioural variable in *U. lugens* and of direct importance to parasitoids. *D. eucalypti* only parasitizes gregarious (small and mid) larvae whilst *C. urabae* also parasitizes solitary or large larvae when it commence its second generation in winter. This change from gregarious to solitary feeding behaviour in *U. lugens* also marks a transition from skeletonizing to edge feeding behaviour. Thus adults of the first

generation of *C. urabae* in winter are foraging for a less numerous host (Chapter 2), that is commencing to feed in a different manner, and most importantly is more dispersed (or less clumped) than ever encountered at any other time in the field when this species is present as an adult. Gregariousness, in this thesis, was only examined for its potential influence on host acceptance but at least as important may be its influence on host finding behaviour which is the second of Vinson's five steps towards host selection by parasitoids.

Just analysing host and parasitoid behaviour during and immediately after host contact ignored many of the wider differences between *C. urabae* and *D. eucalypti* that may occur in the chain of steps before (host habitat finding and host finding) and after (host suitability and host regulation) host-parasitoid contact. Even the differences observed during and immediately after host contact may have other functions relating to the forementioned steps. Hosts situated underneath a leaf as well as influencing host defensive behaviour may influence parasitoid visiting behaviour differently to those on top of a leaf. A parasitoid dislodged from a leaf by a host rearing or thrashing may as a consequence be too far away from the host to easily relocate and attack it. The affect of a host rearing or thrashing on a parasitoid revisiting it after taking flight may differ according to whether host movement is a cue used by *C. urabae* or *D. eucalypti* to locate hosts. The cessation of feeding behaviour observed after parasitoid attack may decrease kairomone production and in turn affect host location by parasitoids. Thus the success of *C. urabae* and *D. eucalypti* should not ultimately be judged on the differences observed within the context of this thesis.

Although *C. urabae* and *D. eucalypti* were rarely seen as adults in the field some baseline information about their degree of dispersal in the field would be advantageous. Indeed the evidence for poor dispersal ability of *U. lugens* is at best circumstantial (Chapter 1) and some better 'hold' on this would add to the understanding of the dispersal ability of *C. urabae* and *D. eucalypti*. Determination of how many hosts they attack in a batch of larvae before they leave it, and how frequently they revisit the same batch of larvae after taking flight are two key concepts to understanding their relative

success which are unknown at present. Another unknown factor is the behaviour of the adults during the period that follows each host generation when hosts are unavailable.

Most differences in the phenology of *C. urabae* and *D. eucalypti* resulted from their differing rates of egg-larval development in hosts of different sizes. Egg-larval development of *C. urabae* was rapid in mid hosts but slower in those hosts where size was initially smaller than that of the mature larvae of *C. urabae*. In a host of a size inbetween small and mid larvae, the egg-larval development rate of *C. urabae* may lie inbetween that of these two host sizes (Chapter 5). Although intermediate sizes are not relevant to the phenology of *C. urabae*, they may lead to understanding how *C. urabae* develops in small hosts. Similarly the long delay observed in the development *D. eucalypti* from mid hosts in summer, and from small hosts in winter, needs further elaboration as to how and why such delays occur. Parasitized larvae show no obvious morphological or behavioural differences to unparasitized larvae although what constitutes 'normal' development in the latter is hard to precisely determine.

Comparison of the behaviour of *U. lugens* larvae showed little difference in their response to attack by *C. urabae* and *D. eucalypti*, but indicated significant differences between host ages and between the behaviour of the two species of parasitoid. *D. eucalypti*, unlike *C. urabae*, improved its oviposition success in small larvae by using its front and/or mid pair of legs to hold small larvae during ovipositor insertion. *C. urabae* visited patches of mid larvae more often than *D. eucalypti* and frequently proceeded to move through the patch rather than retreat from it after an encounter. Overall the differences in parasitoid behaviour resulted in greater oviposition success in mid and large larvae by *C. urabae* than by *D. eucalypti*. A precaution to this is that the level of some of the observed differences may alter according to temperature, which was standardized for comparisons in this study at 20°C.

Host acceptance thus involves a complex interplay of parasitoid and host behaviours. On top of this, the changing array of defensive responses of *U. lugens* with increasing host age complicates direct comparison of parasitoid behaviours between host sizes. *C. urabae* and *D. eucalypti*, for example, must alter their responses according to

the increasing levels and array of defensive responses that occur with increasing host age. To infer conclusions about host acceptance or host preference from the level of parasitism after exposure of differing host sizes to a parasitoid for a fixed period of time is unsound as this 'end' result ignores the behavioural interactions occurring during oviposition. These behavioural interactions must be observed in order to truly elucidate the host preference and host acceptance of a parasitoid.

Unfortunately I was unable to measure the 'end' result of the observed acts of oviposition in Chapter 6. However a comparison of the number of visits made to larvae relative to the number of ovipositions highlights potential discrepancies. Based on the number of acts of oviposition, *C. urabae* would appear to accept small hosts more than mid or large hosts and *D. eucalypti* to accept almost exclusively small hosts. Yet *C. urabae* made an equivalent number of visits to each host size with its failure to oviposit in mid and particularly large hosts being a consequence of increasingly effective host defensive behaviour rather than a 'decision' on behalf of the parasitoid not to attempt oviposition.

It is the behavioural interactions that take place during and immediately after a host-parasitoid encounter that are therefore stressed as important quantifiable variables in this thesis. Hosts which display active host defensive behaviour cannot be assumed to be passive receptacles for parasitoid eggs. Instead their behaviour as well as that of the parasitoid should be included within programs aimed at determining the biocontrol potential of a parasitoid or at modelling parasitoid host selection.

# Appendix 1

See pocket inside the back cover for copy of the paper:

Austin, A.D., and Allen G.R. (1989). Parasitoids of *Uraba lugens* Walker (Lepidoptera: Noctuidae) in South Australia, with description of two new species of Braconidae. *Trans. R. Soc. S. Aust.* **113**,

## Appendix 1

Uncorrected page proofs of paper to be published in *Trans. R. Soc. S. Aust.* in November 1989.

# PARASITOIDS OF *URABA LUGENS* WALKER (LEPIDOPTERA: NOCTUIDAE) IN SOUTH AUSTRALIA, WITH DESCRIPTION OF TWO NEW SPECIES OF BRACONIDAE

by A. D. Austin & Geoff. R. Allen*


### Summary

Austin, A. D. & Allen, G. R. (1989) Parasitoids of *Uraba lugens* Walker (Lepidoptera: Noctuidae), with description of two new species of Braconidae. *Trans. R. Soc. S. Aust.* 113( ), 00–00. 30 November, 1989.

Information is presented on the large complex of hymenopteran and dipteran parasitoids associated with *Uraba lugens* Walker (the gumleaf skeletonizer) in South Australia. A key to the 22 species involved is presented, along with notes on identification and relationships with their host. Two species of microgastrine braconids are described, *Cotesia urabae* sp. nov. and *Dolichogenidea eucalypti* sp. nov.; both are parasitoids of the larval stages of *U. lugens*.



Key Words: *Uraba lugens*, Noctuidae, parasitoids, hyperparasitoids, Braconidae, Ichneumonidae, Aphelinidae, Chalcididae, Elasmidae, Eulophidae, Eurytomidae, Eupelmidae, Trichogrammatidae, Tachinidae.


### Introduction

*Uraba lugens* Walker, the gumleaf skeletonizer, is a native noctuid moth and has been collected from all states of Australia (Turner 1944). It has been recorded as damaging stands of eucalypt species in eastern Australia (Brimblecombe 1962; Campbell 1962; Harris 1972[1], 1974; Harris *et al.* 1977[2]), the Adelaide region and south-western Western Australia (pers. comm. F. D. Morgan; Strelein 1988). Occasionally outbreaks of this species can defoliate large areas of native forests. Several such outbreaks have occurred in stands of *Eucalpytus camaldulensis* Dehnh. along the Murray Valley region of N.S.W. and Victoria, where, on at least four occasions, more than 30,000 ha have been affected (e.g. Campbell 1962; Harris 1974; Harris *et al.* 1977[2]). Apart from the widespread damage associated with this species, it is also responsible for the partial defoliation of individual eucalypt trees planted as ornamentals in parks and gardens.

Other than the work of the above authors and those of Morgan & Cobbinah (1977) and Cobbinah (1983), very little has been published on the biology and ecology of *U. lugens*, while even less has been reported on its parasitoids. Brimblecombe (1962) reported five species of primary parasitoids as attacking *U. lugens* and Campbell (1962) ten species. However, in most cases these parasitoids were not identified further than family level and, if so, their identification was not reliable. One of us (G.R.A.) has recently completed a major study on the interaction of this insect and its parasitoids in the Adelaide region. This work shows that the immature stages of *U. lugens* support a diverse complex of hymenopteran and dipteran parasitoids (22 species — Table 1), which includes both primary parasitoids and hyperparasitoids. In this paper we provide a taxonomic framework for the information on the behaviour and ecology of this parasitoid complex and its interaction with *U. lugens*, which will be published elsewhere by G.R.A. Here we present a key to identify all the parasitoid species involved, and provide notes on their taxonomic position and biology, including information on the stage attacked and place of pupation. Two of the more common species reared from *U. lugens* larvae, which are members of the braconid subfamily Microgastrinae, and are the subject of detailed behavioural studies by G.R.A., are described here as new.

### Materials and Methods

All life history stages of *U. lugens* were collected from eucalypts (mostly *E. camaldulensis*, *E. leucoxylon* F. Muell. and *E. microcarpa* (Maiden) Maiden) between 1985 and 1988 at several sites within 7 km of Adelaide G.P.O. They were brought to the laboratory, held at 20°C in rearing cages, and larvae provided with new foliage, so that any parasitoids present could develop and emerge normally. Parasitoid cocoons found with their dead host in the field were also collected and reared in the laboratory at 20°C until adults emerged.

[1]**Harris, J. A. (1972) The effect of flooding on population density of the gum leaf skeletonizer moth,** *Uraba lugens* Walk., in Barmah State Forest. Forest Commission, Victoria, Research Branch Report, No. 25 (unpubl).

[2]**Harris, J. A. Neumann, F. G. & Ward, B. (1977) An outbreak of the gum leaf skeletonizer,** *Uraba lugens* Walker, in river red gum forest near Barmah. Forest Commission, Victoria, Research Branch Report, No. 87 (unpubl).

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TABLE 1. *Summary of relationships between* U. lugens *and its parasitoids and hyperparasitoids*

| *Parasitoid* species | Family | *Primary (P) or Hyperparasitoid (H)* Solitary (S) or Gregarious (G) | *U. lugens* stage attacked | Stage emerges from |
|---|---|---|---|---|
| *Trichogramma* sp. | Trichogrammatidae | P;S | egg | egg |
| *Cotesia urabae* | Braconidae | P;S | larva | larva |
| *Dolichogenidea eucalypti* | Braconidae | P;S | larva | larva |
| *Euplectrus* sp. | Eulophidae | P;S | larva | larva |
| *Casinaria micra* | Ichneumonidae | P;S | larva | larva |
| *Exorista flaviceps* | Tachinidae | P;S | larva | larva |
| *Eriborus* sp. | Ichneumonidae | P;S | larva | pupa |
| *Xanthopimpla rhopaloceros* | Ichneumonidae | P;S | pupa? | pupa |
| *Antrocephalus* sp. | Chalcididae | P;S | pupa | pupa |
| *Brachymeria* sp. 1 | Chalcididae | P;S | pupa | pupa |
| *Winthemia lateralis* | Tachinidae | P;S | ? | pupa? |
| *Eurytoma* sp. | Eulophidae | P;G/H;S | pupa/— | pupa/parasitoid cocoon |
| *Centrodora* sp. | Aphelinidae | H;G | — | parasitoid cocoon |
| *Brachymeria* sp. 2 | Chalcididae | H;S | — | parasitoid cocoon |
| *Elasmus australiensis* | Elasmidae | H;G | — | parasitoid cocoon |
| species indet. | Eulophidae | H;G | — | parasitoid cocoon |
| *Pediobus* sp. | Eulophidae | H;G | — | parasitoid cocoon |
| species indet. | Eupelmidae | H;S | — | parasitoid cocoon |
| *Anastatus* sp. | Eupelmidae | H;G | — | parasitoid cocoon |
| *Mesochorus* sp. | Ichneumonidae | H;S | — | parasitoid cocoon |
| *Paraphylax* sp. | Ichneumonidae | H;S | — | parasitoid cocoon |
| *Pteromalus* sp. | Pteromalidae | H;S | — | parasitoid cocoon |

Parasitoids were stored in a freezer or in 70% ethanol prior to mounting on pins or card points.

Material for S.E.M. study was washed in half strength concentrated liquid soap, rinsed in distilled water, dehydrated in an alcohol series and critical-point dried using an Emscope CPD 750, before being examined under a Cambridge Stereoscan 250 (Mk 3B) electron microscope. Terminology for morphology follows Boucek (1988), Eady (1968), Gauld (1984), Harris (1979), Mason (1986) and van Achterberg (1979). The term 'alitrunk' is used for the thorax plus propodeum, and 'gaster' is used for the post-propodeal segments. The abbreviation 'T' refers to the gastral tergites. Abbreviations for collections are: ANIC, Australian National Insect Collection, CSIRO, Canberra; WARI, Waite Agricultural Research Institute, Adelaide. Voucher specimens of all species are lodged in the Waite Institute collection.

### Key to the parasitoids of *Uraba lugens* in South Australia

1. Two pairs of wings developed; dorsal surface very rarely with stout bristles; wasp-like in appearance (ovipositor always developed in female and usually clearly visible (Figs 5, 7, 29)) (Hymenoptera)..........2
   Only one pair of wings developed (fore wings); dorsal surface with numerous stout bristles; blowfly-like in appearance (Figs 48, 49) (scutum with several black longitudinal bands) (Tachinidae)..............21
2. Fore wing with relatively complete venation (e.g. Figs 1, 2, 6, 19).................................3
   Fore wing with pigmented venation reduced to anterior margin (e.g. Figs 21, 23, 27, 39)...............9
3. Fore wing with venation distal to pterostigma wanting (Figs 1, 2); vein 2mcu absent (Braconidae)......4
   Fore wing with distal veins present and well-pigmented; vein 2mcu present (Figs 4, 20) (Ichneu monidae)..................................5
4. Propodeum with longitudinal medial carina, coarsely sculptured at least anteriorly (Fig. 12); legs red to red-yellow..................*Cotesia urabae* sp. nov.
   Propodeum with large carinate areola and horizontal carinae extending to lateral margins of propodeum (Fig. 13); legs dark brown to black with distal parts reddish........*Dolichogenidea eucalypti* sp. nov.
5. Fore wing with an areolet (Figs 4, 6)...........6
   Fore wing without an areolet (Figs 19, 20)......7
6. Scutum and propodeum coarsely punctate or rugulose; ovipositor very short (Fig. 5); ♂ genitalia without long rods protruding posteriorly (body dark brown to black, legs reddish).....*Casinaria micra* Jerman & Gauld Scutum and propodeum generally unsculptured (except for propodeal carinae); ovipositor extending well past posterior gaster (Fig. 7); ♂ genitalia with pair of long rods (gonosquama) protruding posteriorly (body yellow-brown with darker markings)......................*Mesochorus* sp.
7. Body bright yellow with black markings; T1 short and broad basally (Fig. 16)........................ ...................*Xanthopimpla rhopaloceros* Krieger Body not so coloured; T1 narrow basally (Figs 17, 18).....................................8
8. Fore wing with radial cell short and broad (Fig. 19); T1 flat, broadening distally (Fig. 17) (small species, length 2.3–3.2 mm not including ♀ ovipositor; body black except for T2 which is yellow-brown) ..............................*Paraphylax* sp.
   Fore wing with radial cell long and narrow (Fig. 20); T1 tubular in basal half, bulbous in distal half (Fig. 18) (large species, length 6.5–8.7 mm not including ovipositor for ♀; head and alitrunk black, gaster reddish-brown.......................*Eriborus* sp.

9. Femur of hind leg greatly expanded, toothed or serrated along lower margin (Fig. 24) (Chalcididae) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 10
   Femur of hind leg normal, smooth along lower margin (Figs 25, 35) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 12
10. Fore wing with marginal vein much longer than postmarginal vein (Fig. 22); apex of hind tibia tapering into strong spine (*Brachymeria*) . . . . . . . . . . . . . 11
   Fore wing with marginal vein about same length as postmarginal vein (Fig. 21); apex of hind tibia perpendicularly truncate (Fig. 24) (large species, 4.8 mm in length; body black, hind leg dark red-brown marked with black) . . . . . . . . . . . *Antrocephalus* sp.
11. Body black with red hind femur and tibia; 4.2–4.4 mm in length . . . . . . . . . . . . . . . . . . . *Brachymeria* sp. 1
   Body black with white-yellow marking on tegulae and legs; 1.8–2.3 mm in length . . . . . *Brachymeria* sp. 2
12. Hind coxa developed as large flat disc; hind tibia with distinct criss-cross pattern of setae (Fig. 25) (fore wing with stigmal vein very short (Fig. 23); body dark, tegula and legs except for hind coxa pale; body length of ♀ 1.8–2.7 mm, ♂ 1.3–1.9 mm) (Elasmidae) . . . . . . . . . . . . . . . . . *Elasmus australiensis* Girault
   Hind coxa, hind tibia and stigmal vein not as above . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 13
13. Pronotum (seen dorsally) large and quadrangular; dorsal surface of alitrunk coarsely sculptured (Fig. 26); fore wing venation as in Fig. 27 (body black and non-metallic, legs with some pale markings) (Eurytomidae) . . . . . . . . . . . . . . . . . . . *Eurytoma* sp.
   Pronotum not large and quadrangular and alitrunk without such sculpturing; body often metallic in colour . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 14
14. Body length greater than about 1.5 mm; gaster separated from alitrunk by narrow waist (Figs 28, 29) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 15
   Body length less than 1.0 mm (minute species); gaster broadly attached to alitrunk or appearing so (Fig. 44) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 20
15. Tarsi 5-segmented (cf. Figs 24, 25) . . . . . . . . . . . 16
   Tarsi 4-segmented (Fig. 35) . . . . . . . . . . . . . . . . . . 18
16. Mesopleuron not enlarged and shield-like (Fig. 28); body rather robust with large head and alitrunk (♀ gaster in lateral view sharply angled; ♂ gaster small and flattened; body length of ♀ 2.3–3.3 mm, ♂ 1.7–2.4 mm; metallic green in colour with yellow-brown legs and antennae) (Pteromalidae) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . *Pteromalus* sp.
   Mesopleuron large and shield-like; body somewhat elongate (Fig. 29) (Eupelmidae) . . . . . . . . . . . . . . . 17
17. Dorsal surface of scutum flattened with raised anterior triangular area (Fig. 31); fore wing with broad pigmented bands (Fig. 30) (mostly dark in colour with metallic green tinge; body 2.1–2.5 mm in length) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . *Anastatus* sp.
   Dorsal surface of scutum not particularly flattened, without anterior raised area (Fig. 32); fore wings hyaline (head and alitrunk bright metallic green, gaster darker) . . . . . . . . . . gen. & species indet. (♂ only)
18. Fore wing with stigmal and postmarginal veins very short (Fig. 34); anterior scutellum longitudinally striate (Fig. 33) (body dark with metallic green tinge, 1.1–1.5 mm in length) . . . . . . . . . . . . . *Pediobius* sp.
   Fore wing with stigmal and postmarginal veins long (Fig. 37); scutellum smooth or with fine reticulate sculpturing . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 19
19. Dorsal head and alitrunk with scattered long bristle-like hairs (Fig. 36) (body mostly dark and non-metallic; eyes red; antennae, legs and broad patch on dorsal gaster yellow-brown) . . . . . . . . *Euplectrus* sp.
   Dorsal head and alitrunk with shorter finer hairs (dorsal alitrunk with distinctive metallic green and yellow markings (Fig. 38)) . . . gen. & species indet.
20. Tarsi 3-segmented (Fig. 40); antennae 5-segmented (Figs 41, 42); fore wing very broad (Fig. 39) (Trichogrammatidae) . . . . . . . . . . *Trichogramma* sp.
   Tarsi 5-segmented (Fig. 47); antennae 6-segmented (Figs 45, 46); fore wing narrower (Fig. 43) (Aphelinidae) . . . . . . . . . . . . . . . . . . . . . . . . *Centrodora* sp.
21. Hairs covering occiput (posterior part of head) silver-grey; abdomen in dorsal view with anterior, medial and posterior parts black, lateral areas brown (Fig. 48) . . . . . . . . . . . . . *Winthemia lateralis* (Macquart)
   Hairs covering occiput golden brown; 1st segment of abdomen black, other segments black with patches of silver (Fig. 49) . . . . *Exorista flaviceps* Macquart

## Treatment of species

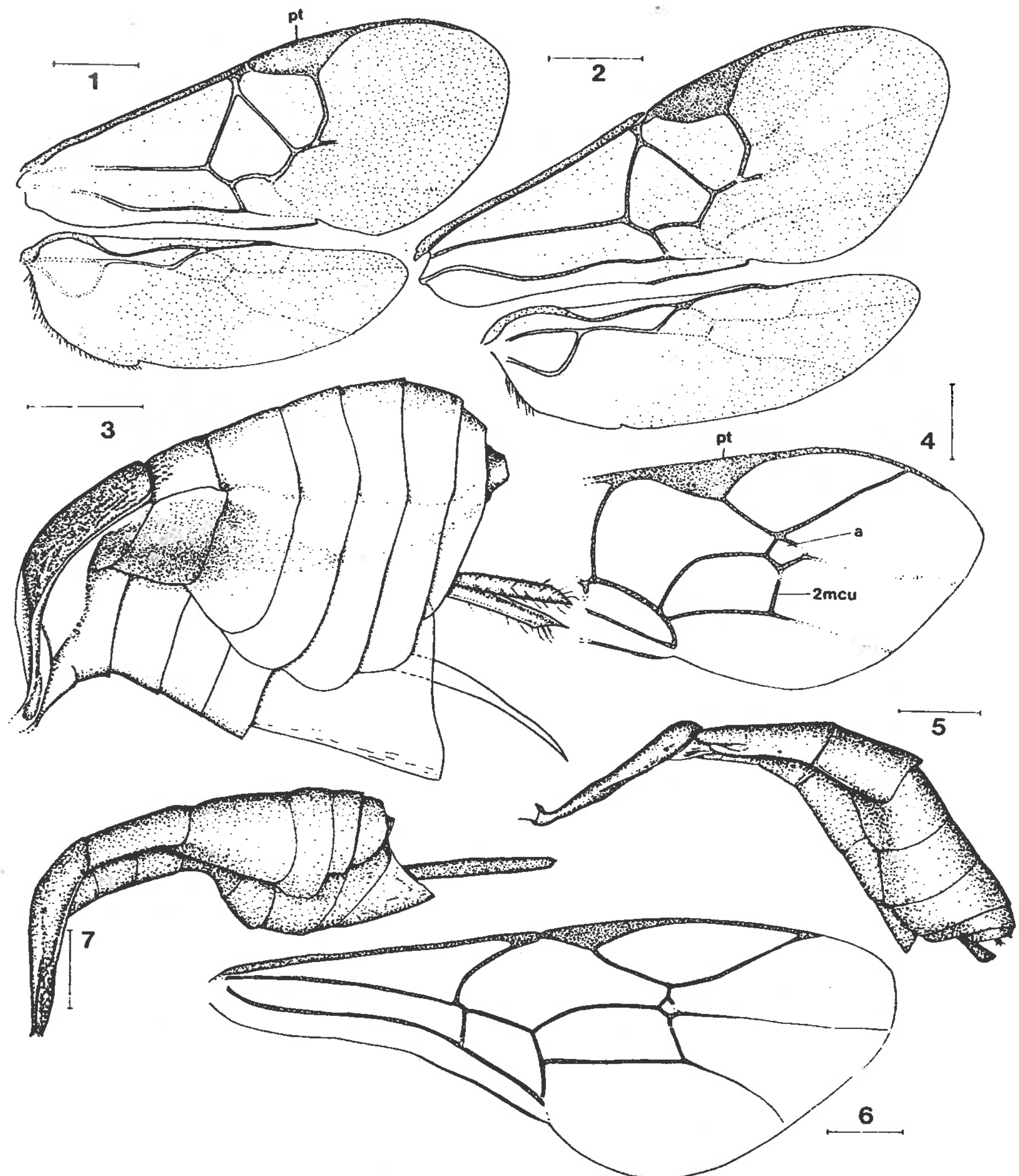
## HYMENOPTERA

### Family Braconidae

### *Cotesia urabae* sp. nov.
FIGS 2, 8, 10–12, 14

*Holotype:* ♀, ANIC, S. Aust., Adelaide (Mitcham), reared ex *Uraba lugens* on *Eucalyptus microcarpa*, coll. 14.x.1985, emerged 3.xi.1985, G. R. Allen. Paratypes: 8♀♀, 20♂♂, same data as holotype except some with different suburbs of Adelaide and different dates; 17♀♀, 13♂♂, S. Aust., Adelaide (Waite Institute campus), various collecting and emergence dates during 1964, L. Hope; 5♀♀, 1♂, S. Aust., National Park, Belair, coll. 20.xi.1964, emerged 25.xi.1964, F.D.M. (5♀♀, 5♂♂, ANIC; rest of material including 1♀ gold coated for SEM (wings slide-mounted) in WARI).

*Female*
*Length.* 2.9 mm (2.5–3.2 mm, n = 10) including ovipositor.
*Colour.* Body black; palps yellow; legs except for coxa yellow-brown, hind leg infuscate distally, distal end of hind tibia with dark patch; anterior pleural-sternal region of gaster dark red; wings hyaline, stigma evenly and darkly pigmented, as is rest of fore wing venation.
*Head.* In anterior view vertex arched so that head is somewhat circular; face and gena with longish white hairs and associated fine colliculate sculpturing; inner margins of eyes virtually parallel; in dorsal view ocelli in wide triangle, posterior tangent of median ocellus coincident with imaginary line across the anterior margins of lateral ocelli; frons




FIGS 1–7. 1–2, Fore and hind wings: 1, *Dolichogenidea eucalypti* sp. nov., ♀; 2, *Cotesia urabae* sp. nov., ♀; 3, Lateral view of gaster of *Dolichogenidea eucalypti* sp.nov., ♀; 4, Distal fore wing of *Mesochorus* sp., ♀; 5–6, *Casinaria micra* Jerman & Gauld, ♀: 5, Lateral view of gaster; 6, Fore wing; 7, Lateral view of gaster of *Mesochorus* sp., ♀. Scales: Figs 1, 2, 4 and 6 = 0.5 mm; Fig. 3 = 200 μm; Figs 5 and 7 = 1.5 mm. Abbreviations: a = areolet; pt = pterostigma.

and medial occiput smooth and hairless; temples with white hairs and associated colliculate sculpturing which is slightly coarser than on face; vertex with few scattered short hairs otherwise smooth; antennae slightly longer than body, distal 4–5 segments only slightly longer than wide.

*Alitrunk*. Scutum punctate with covering of shortish hairs, punctation denser along courses of notauli and along lateral margins (Fig. 8), punctation along posterior margin becoming slightly longitudinally elongate; courses of notauli faintly depressed, these faint depressions broadening posteriorly; scutellum faintly punctate; phragma of scutellum exposed along posterolateral margins (Fig. 8); propodeum

coarsely rugose to rugose-straite in anterior half, generally smooth with faint rugose punctation in posterior half; medial longitudinal carina well developed with associated short horizontal and oblique carinae (Fig. 12); mesopleuron finely punctate in anterior half with associated short hairs, smooth and hairless in posterior half except for compact group of 5–6 foveae medially; metapleuron smooth anteriorly, rugose-punctate posteriorly (Fig. 14); hind coxa faintly punctate on dorsal surface with associated short hairs, this sculpturing becoming coarser on ventral surface.

*Wings.* Fore wing with veins r and 2-SR sharply angled, r slightly longer than 2-SR; cu-a almost straite; distal part of basal cell and anterior part of sub-basal cell devoid of hairs or almost so; discal cell sparsely covered with hairs; hind wing with vein r (spectral) present (Fig. 2).

*Gaster.* T1 as wide as long, broadening posteriorly, surface in posterior two-thirds coarsely punctate to rugose-punctate, becoming coarsely striate in posterior one-quarter (Fig. 10); sclerotized part of T2 rectangular, slightly wider than T1, coarsely rugose-punctate with a few longitudinal striations, sculpturing fading to nearly smooth in postero-lateral corners, longitudinal midline smooth; T3 usually rugose-punctate in anterior one-quarter to two-thirds, with scattered hairs, posterior part smooth, in some specimens T3 virtually smooth throughout but always with at least anterior margin with band of punctation; rest of tergites smooth with scattered hairs; in lateral view hypopygium pointed, extending past posterior gaster, with scattered fine hairs, ventroapical margin not indented (Fig. 11); ovipositor sheaths with few apical hairs.

*Male*

As for female except for length, 2.8 mm (2.6–3.1 mm, n = 10) and sexual differences (genitalia and development of hypopygium).

*Other material examined:* S. Aust., suburbs of Adelaide, various dates and collectors, 10♀♀, 16♂♂ (excluded from type series because material is damaged or inadequately labelled).

*Comments:* The sculpturing on the propodeum and T1–T3, the shape of these sclerites, the form of the hypopygium and ovipositor, clearly place this species in *Cotesia* Cameron. *Cotesia* has previously been referred to as the *glomeratus* species-group of *Apanteles* s.l. (see Mason 1981; Nixon 1965), and is the largest generic level taxon in the subfamily Microgastrinae, the latter comprising some 1500–2000 described species world-wide (Mason 1981). In Australia *Cotesia* is common and diverse, but other than several species introduced from Europe and North America as bio-control agents for certain lepidopteran pests (viz. *C. flavipes* Cameron, *C. glomerata* (L). *C. kazak* (Telenga), *C. marginiventris* (Cresson), *C. plutellae* (Kurdijumov), *C. rubecula* (Marshall) and *C. ruficrus* (Haliday)), the Australian fauna remains unstudied. A few poorly characterized species, which presently remain under the name *Apanteles* s.l., may turn out to belong in *Cotesia.* These species are unlikely to be conspecific with the present species as their type localities are outside the known distribution of *C. urabae* sp. nov. or they are associated with other hosts. There are no workable keys to Indo-Australian species of *Cotesia.* However, the key in Nixon (1974) to the north-western European fauna can be used to separate *C. urabae* sp. nov. from four of the seven introduced species mentioned above. Of the other three species, *C. flavipes,* is very different to *C. urabae* sp. nov. in that its body is strongly flattened dorsoventrally (see Austin 1989), while *C. kazak* and *C. marginiventris* can only be identified reliably in association with their hosts, *Helicoverpa* spp. and *Mythimna convecta* (Walker), respectively.

*Biology: C. urabae* sp. nov. is a solitary, primary endoparasitoid and oviposits into early to intermediate larval instars of its host, emerging from intermediate to late instars before pupating. The pupal cocoon is alongside the host and is sulphur yellow-green with a surrounding silk matrix. This species has only been reared from *U. lugens.*

### *Dolichogenidea eucalypti* sp. nov.

FIGS 1, 3, 9, 13, 15

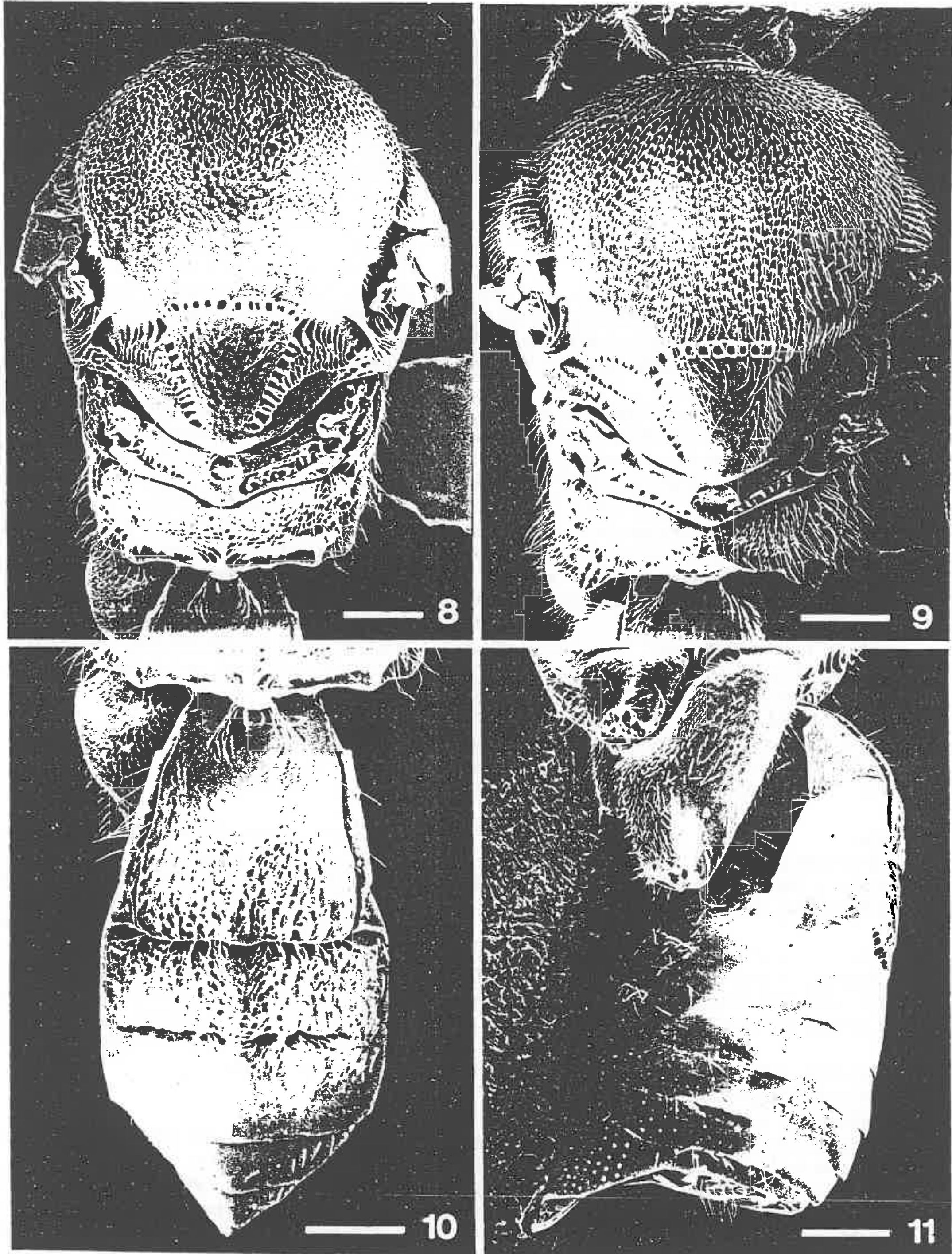
*Holotype:* ♀, ANIC, S. Aust., Adelaide (Highgate), reared ex *Uraba lugens* on *Eucalyptus camaldulensis,* collected 1.xi.1985, emerged 17.xi.1985, G. R. Allen. Paratypes: 15♀♀, 20♂♂ same data as holotype except some with different suburbs of Adelaide and different dates; 13♀♀, 5♂♂, S. Aust., Adelaide (Waite Institute campus), various collecting and emergence dates during 1964, L. Hope (5♀♀, 5♂♂, ANIC; rest of material including 1♀ gold coated for SEM (wings slide-mounted) in WARI).

*Female*

*Length.* 2.9 mm (2.5–3.1 mm, n = 10) including ovipositor.

*Colour.* Body black, wings hyaline with darkly pigmented venation, palps brown, legs black with distal fore legs, tarsi of mid and hind legs and tibio-femoral joint yellow-brown.

*Head.* Mostly smooth except for fine colliculate sculpturing associated with dense covering of short




FIGS 8–11. 8–9, Dorsal view of alitrunk: 8, *Cotesia urabae* sp. nov., ♀; 9, *Dolichogenidea eucalypti* sp. nov., ♀; 10–11, *Cotesia urabae* sp. nov., ♀; 10, Dorsal view of gaster; 11, Lateral view of gaster. Scales: = 200 μm.

hairs; surface with characteristic dull lustre; in anterior view inner margins of eyes virtually parallel; in dorsal view ocelli forming wide triangle, posterior tangent of median ocellus coincident with imaginary line across the anterior margins of lateral ocelli; fine colliculate sculpturing and associated pilosity slightly denser across vertex and occiput, except for smooth narrow band around posterior margins of eyes; antennae reaching to posterior gaster or slightly beyond, distal three segments slightly longer than wide and sometimes slightly compressed.

*Alitrunk.* Scutum coarsely punctate with punctures mostly closer to each other than their own diameter, except along posterior border and along courses of notauli, which are thus faintly indicated (Fig. 9); scutellum smooth; scutum and scutellum densely covered with short hairs; metanotum rather broad, anterolateral margins emarginate so that phragma of scutellum well exposed; carinae forming propodeal areola raised well above surface, carinae extending laterally below horizontal midline, these carinae with dorsal and ventral extensions forming cristulae, but not enclosing spiracles (Fig. 13); anterior part of propodeum mostly smooth and setose, posterior part with faint rugose-punctate sculpturing which becomes more obvious laterally; mesopleuron setose in anterior half, smooth posteriorly; metapleuron mostly smooth, except for ventroposterior one-third which is rugose-punctate (Fig. 15); distal fore tarsus without spine opposed to tarsal claw.

*Wings.* Fore wing venation as in Fig. 1; costal and basal cells bare posteriorly; hind wing broad; vein 1-SC+R deeply bowed; r present but faint; cubitellan cell moderately broad; submediellan cell rounded posteriorly.

*Gaster.* T1 as wide as long, slightly widened in posterior half, lateral margins slightly emarginate, surface mostly punctate, striate-punctate along lateral margins and striate in posterolateral corners and along posterior margin (Fig. 13); sclerotized part of T2 slightly wider than T1, 2.5 x wider than long, mostly smooth with faint scattered punctures; T3 slightly longer than T2 (14:11); T4–T6 shorter than T2 (8:11); T7 very short, about one-quarter length of T2; T3–T6 all smooth; T2–T7 each with single transverse row of hairs; ovipositor and sheaths short, not extending far past posterior gaster; ovipositor with strong distal attenuation (Fig. 3); hypopygium lacking obvious lateral creases though weakened normally in ventral midline.

*Male*

As for female except as follows: Length 2.7 mm (2.5–2.8 mm, n = 10); alitrunk very slightly flattened dorsoventrally; fore wing stigma unpigmented in medial area so that it is transparent; rest of wing venation generally with less pigmentation than female; T1 sometimes with dense rugose-punctate sculpturing merging with posterior striations, otherwise same as female except for male genitalia and lacking hypopygium.

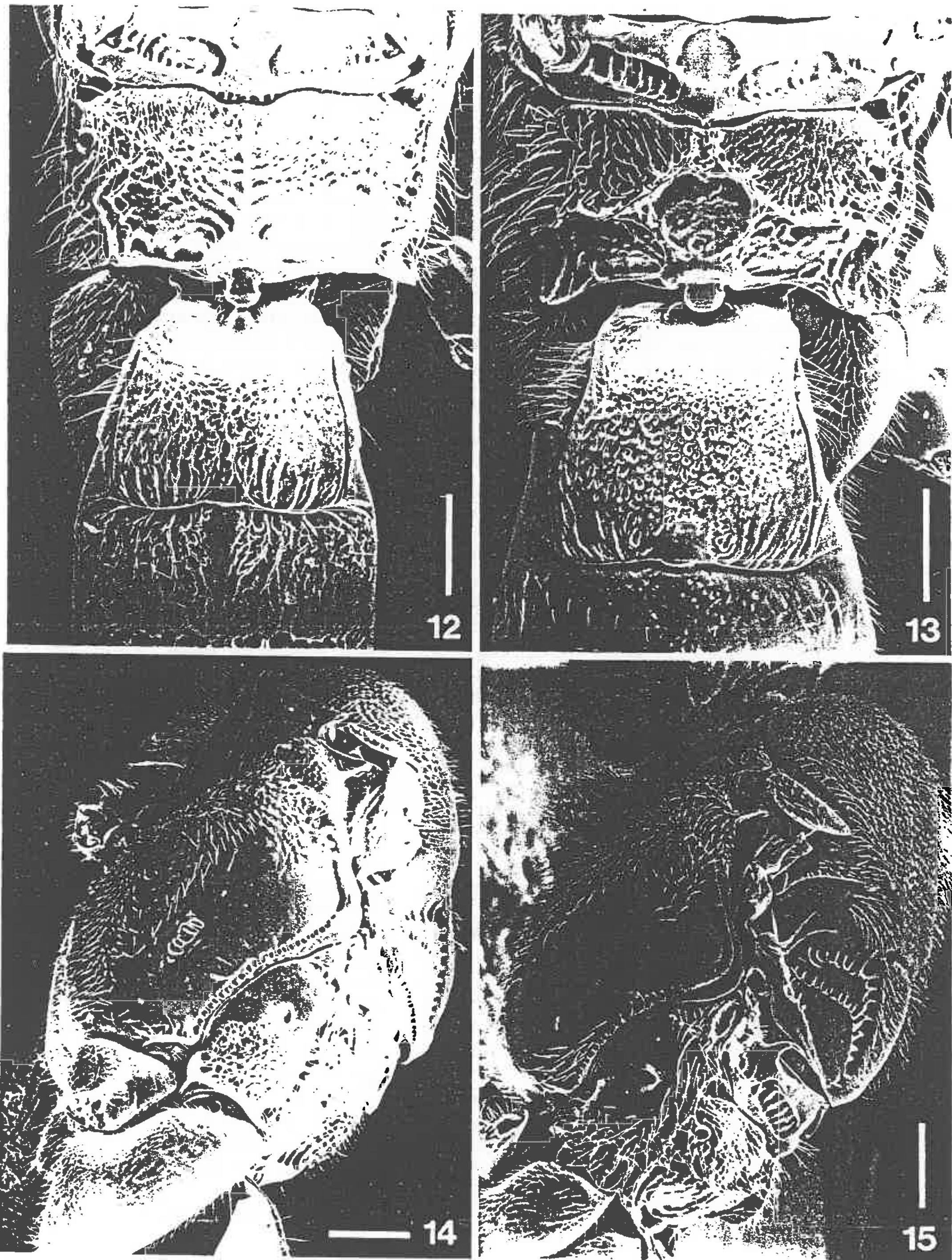
*Comments:* The sculpturing of the scutum and propodeum, shape of T1 and T2, form of the hypopygium, and shape and fringe of the vannal lobe of the hind wing clearly place this species in *Dolichogenidea* Viereck. Previously *Dolichogenidea* was considered as three related species-groups in the genus *Apanteles* s.l., viz. the *ultor, laevigatus* and *longipalpis* species-groups (see Mason 1981; Nixon 1965, 1967). This species falls into the *ultor* group which was revised by Nixon (1967) for the Indo-Australian region. In this work *D. eucalypti* sp. nov. keys out to *D. cleo* (Nixon) (couplet 23), a species known only from India and associated with a nymphalid host *Eriboae arja* Felder, or with some difficulty it keys as *D. caniae* (Wilkinson) (couplet 31), which is known only from Java and associated with a limacodid, *Cania bandura* Moore (Austin 1987). Apart from having different hosts, these species differ from *D. eucalypti* sp. nov. in that *D. caniae* has an unusual striate sculpturing pattern on T1 and T2, and *D. cleo* has the sub-basal cell evenly and darkly setose, the hind femur yellow in colour, the proximal half of the ovipositor very broad, and the mesopleuron coarsely rugose-punctate anteriorly. This is the fourth species of *Dolichogenidea* recorded from Australia, the others being *D. lipsis* (Nixon) comb. nov., *D. miris* (Nixon) comb. nov. and *D. tasmanica* (Cameron) comb. nov. Examination of the holotypes of these species shows that they differ from *D. eucalypti* sp. nov. in a number of important characters. All three have the ovipositor much longer, being at least as long as the hind tibia, and, in addition, *D. lipsis* and *D. tasmanica* have a white spot on the cheek and a much reduced propodeal areola. These species can be readily separated from *D. eucalypti* sp. nov. using the key in Nixon (1967).

*Biology: D. eucalypti* sp. nov. is a solitary, primary endoparasitoid which oviposits into early to intermediate larval instars of its host, emerging from intermediate to late instars before pupating. The pupal cocoon is spun alongside the host and is white in colour and lacks a surrounding silk matrix. This species has only been reared from *U. lugens.*

## Family Ichneumonidae

### *Xanthopimpla rhopaloceros* Krieger

FIG. 16




FIGS 12–15. 12–13, Propodeum and first and second tergites of gaster: 12, *Cotesia urabae* sp. nov., ♀; 13, *Dolichogenidea eucalypti* sp. nov., ♀; 14–15, Lateral view of alitrunk: 14, *Cotesia urabae* sp. nov., ♀; 15, *Dolichogenidea eucalypti* sp. nov., ♀. Scales: = 200 μm.

This species is easily identified by its distinctive colour pattern and fore wing venation. In South Australia it is known from two specimens (WARI), one collected as an adult in the Adelaide region, the other reared from *U. lugens* at Keith. It is also known from Queensland and Tasmania where it has been recorded from *U. lugens*, and the tortricids *Epiphyas postvittana* (Walker) and *Merophyas divulsana* (Walker) (Brimblecombe 1962; Dumbleton 1940; Gauld 1984; also see Townes & Chui 1970). It is a solitary, primary endoparasitoid and emerges from the pupal stage of its host. See Gauld (1984) for additional taxonomic information and list of synonyms.

*Paraphylax* sp.
FIGS 17, 19

*Paraphylax* is a large Old World genus with more than 50 recognized species from Australia, the majority of which are undescribed (Gauld 1984). Where their biology is known *Paraphylax* spp. have been recorded as primary and hyperparasitoids, mostly of lepidopteran hosts. Apart from the characters given in the key this species is notable in comparison to other parasitoids associated with *U. lugens* for its relatively smooth unsculptured body (except for propodeal carinae) and lateral teeth on the propodeum. The species here belongs to the *corvax* species-group (see Gauld 1984) and is only known from the Adelaide region, where it has been reared as an obligate, solitary hyperparasitoid through *C. urabae* and *D. eucalypti.*

*Eriborus* sp.
FIGS 18, 20

This is a distinctive species when compared to the other ichneumonids associated with *U. lugens.* In addition to the characters given in the key this species has distinctive reticulate-punctate sculpturing on the scutum, scutellum and propodeum. *Eriborus* sp. is a solitary primary parasitoid of *U. lugens*, ovipositing into the larval stages and emerging from the pupa. It is only known from the Adelaide region and has been reared from its host on various occasions since 1965 (WARI, unpublished records).

*Casinaria micra* Jerman & Gauld
FIGS 5, 6

This species is a solitary, primary endoparasitoid easily recognized by its fore wing venation, short ovipositor and colour. It has been recorded from all states in Australia and, although it has been most commonly associated with *U. lugens*, *C. micra* has been reared from species belonging to three other distantly related lepidopteran families — Geometridae, Oecophoridae, Notodontidae (see Gauld 1984; Jerman & Gauld 1988). The pupal cocoon is constructed near to (Jerman & Gauld 1988) or underneath (observations in this study) its dead larval host and is attached firmly to the leaf surface. It is grey-brown and marked with characteristic black spots. See Jerman & Gauld (1988) for additional taxonomic information and list of synonyms.

*Mesochorus* sp.
FIGS 4, 7

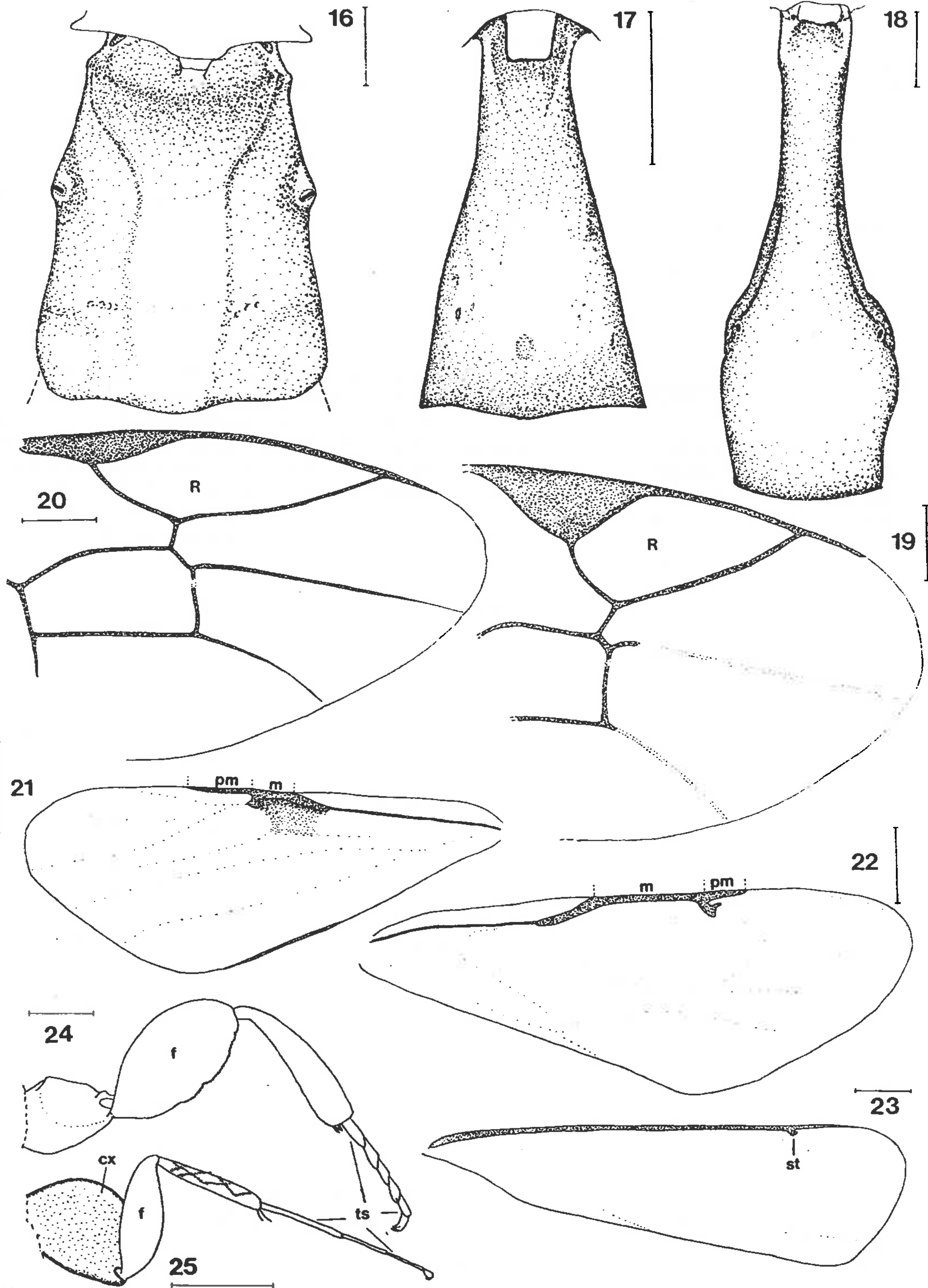
This is a large cosmopolitan genus of primary and hyperparasitoids of lepidopteran and coleopteran hosts; most Indo-Australian species are undescribed (Gauld 1984). The species recorded here is a solitary obligate hyperparasitoid of *U. lugens* through *C. micra*, *C. urabae* and *D. eucalypti*, and known only from the Adelaide region. It is a pale coloured delicate species with fine thread-like antennae. The male is distinctive in having the gonosquama of the genitalia extending from the posterior gaster as a pair of long rods.

Family Chalcididae
*Brachymeria* spp.
FIG. 22

This is a large genus in Australia with nearly 70 described species, the majority of which are primary, pupal parasitoids of Lepidoptera (Boucek 1988). The two species recorded here (both unidentified) can be separated easily by the characters in the key. Species 1 is a solitary, primary parasitoid of *U. lugens* and emerges from the host pupa. Species 2 is solitary and hyperparasitic through *C. urabae* and *D. eucalypti*, though it is not known whether this relationship is facultative or obligatory. In eastern Australia two species of *Brachymeria* have been reared from *U. lugens*, viz. *B. froggatti* (Cameron) (Brimblecombe 1962) and *B. rubripes* Girault (Campbell 1962) (*B. rubripes* is considered a junior synonym of *B. teatua* (Walker): see Boucek 1988). However, the material from these records would have been identified at a time when the Australian species in the genus were confused by most authors and hence might be misidentified. These names should thus be used with some care, especially since Boucek (1988) did not see any material in Australian collections or elsewhere reared from *U. lugens* that he could assign to either *B. froggatti* or *B. teuta*.

*Antrocephalus* sp.
FIGS 21, 24

This species is represented here by a single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17
18
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25

specimen reared as a primary parasitoid from the pupa of *U. lugens* in the Adelaide region. It is easily distinguished by the characters in the key. There are more than 60 described Australian congeners which are discussed by Boucek (1988).

Family Eurytomidae
*Eurytoma* sp.
FIGS 26, 27

This is a large cosmopolitan genus with more than 60 described Australian species (Boucek 1988). Biologically the group is very diverse including phytophagous species, primary parasitoids (mostly of lepidopteran hosts) and hyperparasitoids. The species recorded here develops as either primary gregarious parasitoid of *U. lugens,* or as a solitary hyperparasitoid through *C. urabae.*

Family Pteromalidae
*Pteromalus* sp.
FIG. 28

This species is relatively easily separated from other Chalcidoidea associated with *U. lugens* by its robust body and metallic green colour. The genus is taxonomically very complex and the Australian species are in need of revision (Boucek 1988). The species recorded here is an obligate, solitary hyperparasitoid reared from *U. lugens* through *C. micra, c. urabae* and *D. eucalypti* in the Adelaide region.

Family Elasmidae
*Elasmus australiensis* Girault
FIGS 23, 25

A distinctive species recorded here as an obligate, solitary hyperparasitoid of *U. lugens* through *C. micra, C. urabae* and *D. eucalypti.* Previously it was known to be hyperparasitic and occasionally gregarious through an unknown ichneumonid associated with *U. lugens* in the A.C.T., and also has been collected from N.S.W., and northern and southern Qld (type locality: Gordonvale, Qld). This is the first record of *E. australiensis* from S. Aust., indicating that it is probably distributed throughout south-eastern Australia. See Riek (1967) for additional taxonomic information and list of synonyms.

Family Eulophidae
*Euplectrus sp.*
FIGS 36, 37

*Euplectrus* is a cosmopolitan genus of gregarious ectoparasitoids of lepidopteran larvae, which is represented in Australia by 13 described species (Boucek 1988). The species recorded here is solitary and only known from the Adelaide region. It attacks the early to intermediate larval stages, killing them before they pupate while pupating itself underneath the dead host.

*Pediobius* sp.
FIGS 33–35

This is a large, cosmopolitan genus of primary and hyperparasitoid species that attack a wide range of insect groups (Boucek 1988). There are more than 30 described Australian species, most of which do not have associated host information (Boucek 1988). The unidentified species recorded here is easily distinguished from other Hymenoptera associated with *U. lugens* by its distinctive venation and sculpturing on the scutellum. It is represented by four specimens (WARI) reared in the Adelaide region from *U. lugens* and developed as solitary hyperparasitoids through *Euplectrus* sp. and as a gregarious hyperparasitoid through *D. eucalypti.*

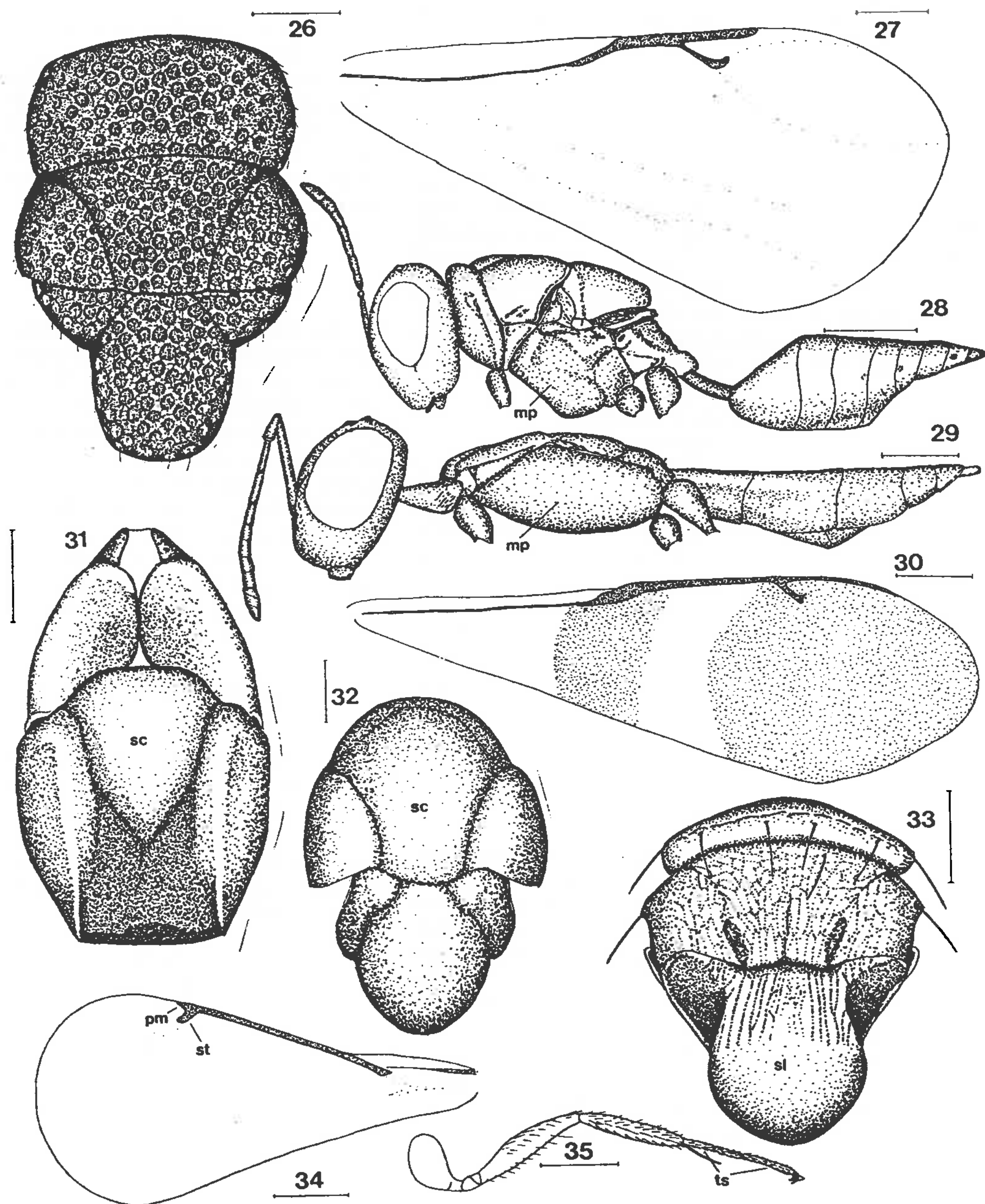
Eulophidae (genus & species indet.)
FIG. 38

The two specimens reared as hyperparasitoids of *U. lugens* through *D. eucalypti* in the Adelaide region could not be identified to genus due to the poor condition of the material. They are different from the other eulophids recorded here and can be distinguished by the dorsoventrally flattened body and distinctive colour pattern.

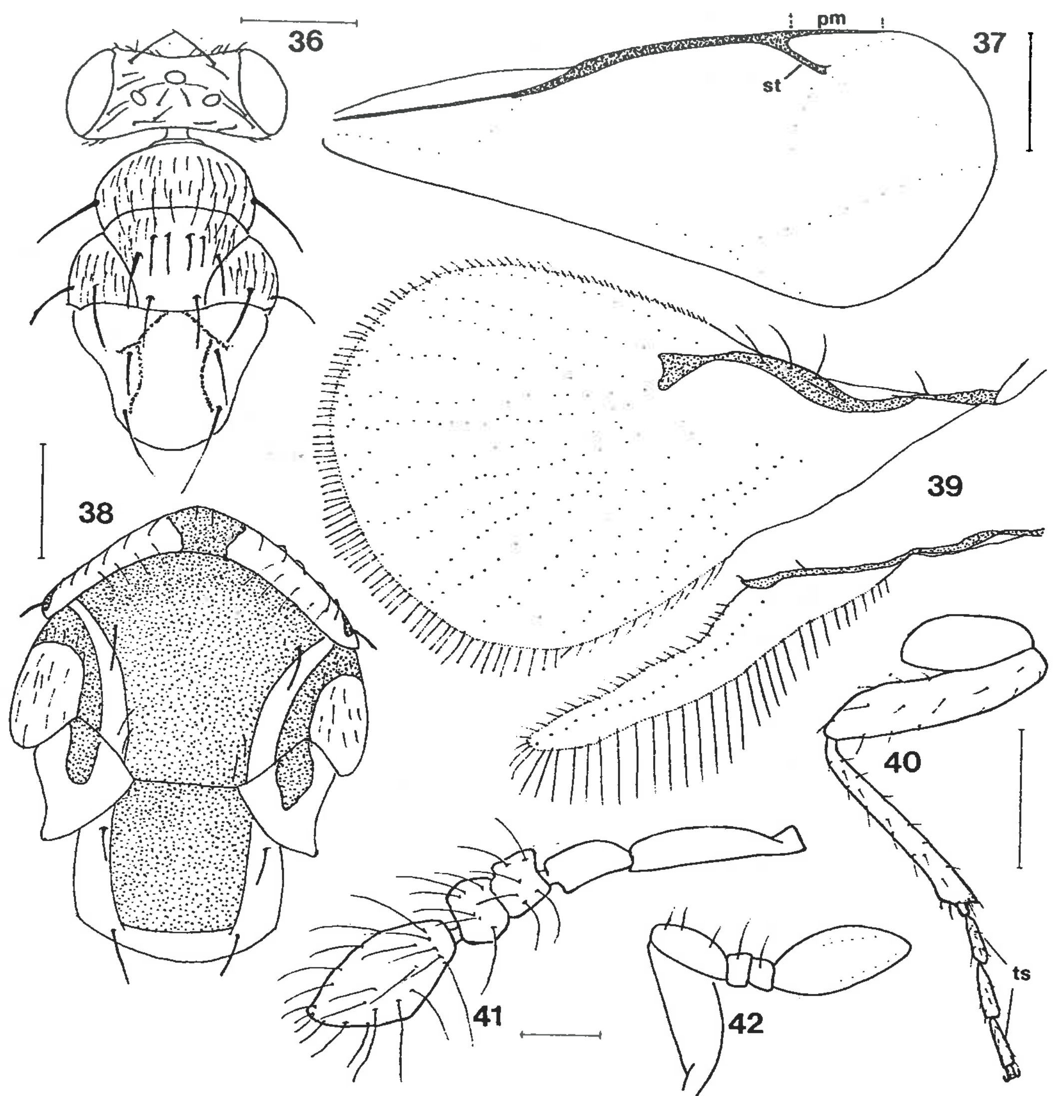
Family Eupelmidae
*Anastatus* sp.
FIGS 29–31

This species is easily separated from the other chalcidoids associated with *U. lugens* by its distinctive body shape and banded wings. The genus is cosmopolitan and known mainly as parasitoids of the eggs of Lepidoptera and Heteroptera,

FIGS 16–25. 16–18, first tergite of gaster: 16, *Xanthopimpla rhopaloceros* Krieger, ♀; 17, *Paraphylax* sp., ♀; 18, *Eriborus* sp., ♀; 19–20, Distal fore wings: 19, *Paraphylax* sp., ♀; 20, *Eriborus* sp., ♀; 21-23 Fore wings: 21, *Antrocephalus* sp., ♀; 22, *Brachymeria* sp. 2, ♀; 23, *Elasmus australiensis* Girault, ♀; 24–25, Hind legs: 24, *Antrocephalus* sp., ♀; 25, *Elasmus australiensis* Girault, ♀. Scales: Figs 16–19 = 250 μm; Figs 20 and 24 = 0.5 mm; Fig. 21 = 0.8 mm; Fig. 22 = 250 μm; Fig. 23 = 200 μm; Fig. 25 = 1.0 mm. Abbreviations: cx = coxa; f = femur; m = marginal vein; pm = postmarginal vein; R = radial cell; st = stigmal vein; ts = tarsal segments.




FIGS 26–35. 26–27, *Eurytoma* sp., ♀: 26, Dorsal view of alitrunk; 27, fore wing; 28–29, Lateral view of body: 28, *Pteromalus* sp., ♀; 29, *Anastatus* sp., ♀; 30, Fore wing of *Anastatus* sp., ♀; 31–33, Dorsal view of alitrunk: 31, *Anastatus* sp., ♀; 32, Eupelmidae, genus & species indet., ♂; 33, *Pediobius* sp., ♀; 34, fore wing of *Pediobius* sp., ♀; 35, Hind leg of *Pediobius* sp., ♀. Scales: Figs 26, 27, 29, 34 and 35 = 250 µm; Fig. 28 = 1.0 mm; Fig. 30 = 200 µm; Figs 31–33 = 150 µm. Abbreviations: mp = mesopleuron; pm = postmarginal vein; sc = scutum; sl = scutellum; st = stigmal vein; ts = tarsal segments.




FIGS 36–42. 36–37, *Euplectrus* sp., ♀: 36, Dorsal view of head and alitrunk; 37, Fore wing; 38; Dorsal view of alitrunk of Eulophidae, genus & species indet., ♀ (stippling indicating colour pattern); 39–42, *Trichogramma* sp.: 39, fore and hind wings, ♂; 40, hind leg, ♀; 41, ♂ antenna; 42, ♀ antenna. Scales: Figs 36 and 37 = 250 µm; Figs 38 and 39 = 150 µm; Fig. 40 = 100 µm; Figs 41 and 42 = 50 µm (same scale line). Abbreviation: pm = postmarginal vein; st = stigmal vein; ts = tarsal segments.

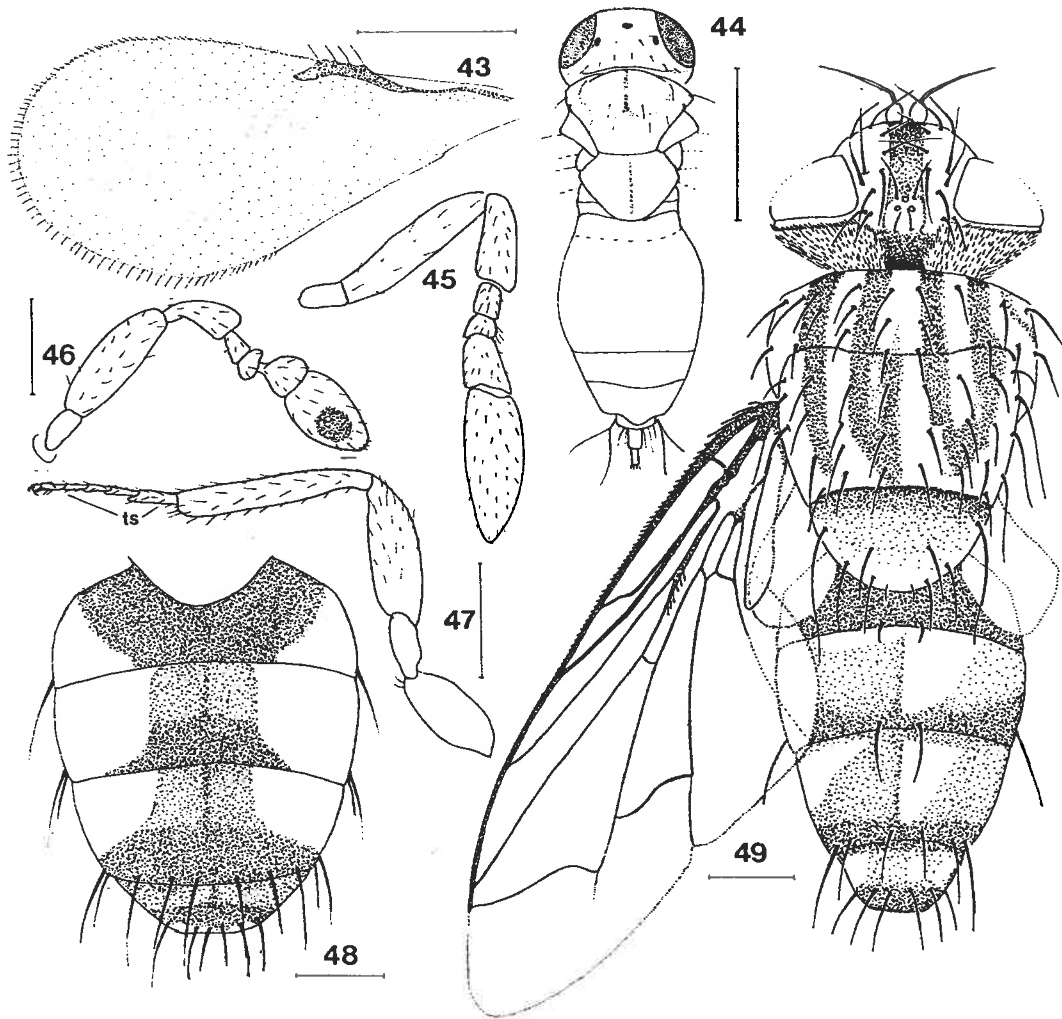
although a few are recorded as hyperparasitoids through braconids. *Anastatus* is represented in Australia by 40 described species (Boucek 1988). The species recorded here is represented by three specimens reared as a gregarious hyperparasitoid through *C. urabae* and *D. eucalypti* in the Adelaide region.

Eupelmidae (genus & species indet.)
FIG. 32

A single male specimen reared as a hyperparasitoid of *U. lugens*through *C. urabae* in the Adelaide region could not be identified to genus, but it is not *Anastatus* sp., from which it can be separated by the characters in the key.

Family Trichogrammatidae
*Trichogramma* sp.
FIGS 39–42

The members of this cosmopolitan genus are




FIGS 43–49. 43–47, *Centrodora* sp.: 43, Fore wing, ♀; 44, Dorsal view of whole body, ♀; 45, ♀ antenna; 46, ♂ antenna; 47, hind leg, ♀; 48, abdomen of *Winthemia lateralis*, ♀ (Macquart); 49, Dorsal view of *Exorista flaviceps* Macquart, ♀ (stippling showing colour pattern in Figures 46 and 47). Scales: Fig. 43 = 200 μm; Fig. 44 = 300 μm; Figs 45–47 = 50 μm (same scale line for Figs 45 and 46); Figs 48 and 49 = 1.0 mm. Abbreviation: ts = tarsal segments.

obligate, primary parasitoids of insect eggs, most frequently those of Lepidoptera. It is the only egg parasitoid of *U. lugens* so far recorded, and it can be identified by the characters in the key, as well as its minute size, distinctive fore wing venation, fore wing setal pattern, and very narrow hind wing. This species is a solitary parasitoid known from the Adelaide region. We have not seen material of the *Trichogramma* sp. reared from *U. lugens* in the Murray Valley in N.S.W. (Campbell 1962), which may be the same species to that recorded here.

Family Aphelinidae
*Centrodora* sp.
FIGS 43–47

This is a cosmopolitan genus of about 40 described species (Hayat 1983), most of which are primary parasitoids of the eggs of Orthoptera and Homoptera, although at least one species is reported to be hyperparasitic (Gordh 1979; also see Viggiani 1984). The species recorded here is an obligate, gregarious hyperparasitoid of *U. lugens*

through *C. urabae* or *D. eucalypti*. Apart from the characters in the key and the life history stage attacked, *Centrodora* sp. can be separated from the other parasitoids associated with *U. lugens* by its minute size, wing venation, and ovipositor which is more than half the length of the gaster (the ovipositor being significantly less than half the gastral length in *Trichogramma* sp.).

DIPTERA
Family Tachinidae
*Winthemia lateralis* (Macquart)
FIG. 48

Previously this species has been collected at various localities in all states of Australia. It has been reared from host species belonging to seven lepidopteran families, viz. Arctiidae, Noctuidae, Pieridae, Notodontidae, Nymphalidae, Saturnidae and Geometridae (Crosskey 1973; Cantrell 1986, 1989). The only record from *U. lugens* is from specimens in this study (3 specimens, Waite Institute campus, J. Cobbinah, 1975, WARI). *W. lateralis* oviposits onto the external surface of a host larva. After hatching the fly larva penetrates the host larva and usually emerges from the host pupa, although we were not able to confirm its biology in this study. See Crosskey (1973) and Cantrell (1986, 1989) for additional taxonomic information and list of synonyms.

*Exorista flaviceps* Macquart
FIG. 49

This species has been recorded from all states of Australia and the N.T. (Cantrell 1985), and has been reared from members of nine lepidopteran families, viz. Lymantriidae, Anthelidae, Pieridae, Agaristidae, Sphingidae, Geometridae, Notodontidae, Lasiocampidae and Noctuidae (Crosskey 1973; Cantrell 1986). It oviposits onto the surface of *U. lugens* larvae. After hatching the fly larva burrows into the host to feed and develop internally, finally emerging from late larval instars to pupate outside the dead host. The colour pattern on the abdomen and occiput of the head is the easiest way to distinguish this species from *W. lateralis*. See Cantrell (1985) for additional taxonomic information and list of synonyms.

**Other Parasitoids**

From *U. lugens* in Queensland Brimblecombe (1962) reared two ichneumonoid species which have not been recorded in S. Aust., viz. *Irabatha* sp. (Ichneumonidae) and *Campyloneura* sp. (Braconidae). Also Gauld (1984) reports the following ichneumonids as having been reared from *U. lugens*: *Stiromesostenus* spp., *Campoplex* sp. and *Pristomerus* sp., but again, they were not reared during this study and may not be found in S. Aust.

**Acknowledgments**

We thank Dr Ian Naumann (Australian National Insect Collection), Mr Geoff Holloway (Australian Museum) and Dr Bryan Cantrell (Queensland Department of Primary Industries) for assistance with identifications; Mr Paul Dangerfield for the line drawings and scanning micrographs, and Dr Mike Keller for reading a draft of the manuscript. This work was supported by a Commonwealth Postgraduate Research Award to GRA and a grant from the Australian Biological Resources Study participatory program to ADA.

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# Appendix 2 Laboratory rearing of *U. lugens, C. urabae and D. eucalypti.*

## Procedures for *U. lugens*

Laboratory cultures of *U. lugens* began in September 1985 by collecting larvae in the field and returning them to the laboratory. However periodically throughout the four years that laboratory cultures were maintained further larvae were brought back from the field to supplement the laboratory culture and thereby maintain genetic diversity.

1. *Adult* U. lugens *(mating and oviposition)*

Adult *U. lugens* were placed in 20x20 cm plastic cylinder cages that had three 13x13 cm holes, covered with gauze cloth, cut in the cage walls. Paper towel was hung from the sides and also placed on the bottom of each cage and the inside of the lid covered with plastic food wrap. Covering the internal cage walls prevented females from ovipositing on the cage wall, from which eggs were difficult to remove. All cages were held at a constant 20ºC and 12L:12D and up to 40 adults kept in each cage.

Copulation occurred soon after lights out and usually within 24 hrs of emergence. Oviposition took place within 24-48 hrs of emergence although eggs laid 24 hrs after emergence were not checked for viability. This preoviposition period of 24-48 hrs is much less than that of ten days reported by Campbell (1962), Harris *et al.* (1977) and Southcott (1978) under unspecified conditions. All eggs were laid around the start of scotophase or if the cage was held under natural day-night conditions around twilight. Females deposited eggs sequentially across rows rather than within rows. Insufficient observations were completed to confirm whether this egg laying pattern was usual but it had been previously assumed that eggs were always laid within rows (Cobbinah 1978; 1983). Eggs were deposited on all sides of the cage.

Longevity at 20ºC and 12L:12D of females that were allowed to mate and oviposit was 3-11 days (6.4±0.40 (SE); n=28), and for males that were allowed to mate was 3-12 days (9.2±0.66 (SE); n=16).

2. *Larval and pupal rearing*

Eggs were allowed to develop in the cage until just prior to eclosion at which time egg batches were cut, as close to the perimeter of the egg batch as possible, from the paper towel. Cutting close to the egg batch meant that hatched larvae had only a small distance to wander before reaching the leaf surface on which they fed. A commercial glue " Selleys Kwik Grip ®" was used to attach the paper towel with the eggs upon it to *Eucalyptus* leaves. This glue was not affected by moisture, unlike water-based glues, and kept the perimeter of the paper towel flush to the leaf surface. Using paper clips (Morgan and Cobbinah 1977) damaged eggs and did not hold the paper towel sufficiently close to the leaf surface to enable hatched larvae easy access off the paper towel to the leaf surface.

If potted trees (*Eucalyptus camaldulensis* Dehnh.) were available, egg batches were attached to the leaves of these trees held in an insectary. Potted trees instead of cut foliage were preferred for young larvae as the latter necessitated frequent transfers of larvae to new cut foliage thereby increasing mortality. Larvae were generally allowed to feed on potted trees until the breakdown of gregarious feeding, at which time, they were transferred to cut foliage.

*Eucalyptus leucoxylon* F. Muell. was used for cut foliage as accessible foliage was more abundant than that of *E. camaldulensis* around the Waite Institute. Furthermore *E. camaldulensis* foliage was often damaged by the psyllid *Glycaspis brimblecombei* Moore and the gum tree scale *Eriococcus coriaceus* Maskell (pers. comm. G.S. Taylor). The cut ends of foliage were placed through slits in the lids of 7x5 cm plastic vials which were sealed with "Parafilm ®" (American National Can, Greenwich CT) to slow evaporation. To reduce fungal and bacterial levels in the water used for cut foliage all water was boiled twice prior to use. Observations showed that foliage kept in unboiled water deteriorated quicker and had higher fungal and bacterial levels around the cut stem.

The cut foliage and its associated water vial were placed in 20x20 cm plastic cylinder cages which had 7x7 cm holes, covered with fine stainless steel mesh, cut in the cage wall and lid for ventilation. To kill any residual pathogens left in cages from previous cultures all cages, before use, were pre-treated for 24 hrs in 1% sodium hypochlorite and then washed in 1% sodium thiosulphate. Frass was removed and cut foliage replaced when leaf quality deteriorated or more than 75% of foliage in the cage had been eaten. Groups of larvae were transferred to new leaves by stapling the leaf on which they resided to a fresh leaf whereas individual larvae were transferred by hand or by placing the old foliage in with the new. When larvae were approaching pupation paper towel was placed on the cage floor which larvae incorporated into their cocoon.

After pupation commenced the paper towel bearing cocoons was removed from the cage at each leaf change, replaced, and the pupae held at 20°C and 12L:12D until adult emergence. Adult emergence generally occurred around the start of scotophase or if the cage was held under natural day-night conditions around twilight. If necessary the pupal or egg stage of *U. lugens* could be held at lower temperatures (10°C) for short periods to stagger the eventual distribution of larval sizes in the colony.

### Procedures for *C. urabae* and *D. eucalypti*

The rearing procedures for *C. urabae* and *D. eucalypti* were identical and cultures were begun in January 1986. Again as with *U. lugens*, cultures were regularly supplemented with individuals collected from the field to maintain genetic diversity.

1. *Parasitization of* U. lugens

Most larvae were parasitized in 12.5x15 cm plastic cylinder cages with 5x5 cm holes, covered with fine stainless steel mesh, cut in the cage walls and lid. Larvae and their associated foliage were placed in these cages and honey streaked on the cage walls for parasitoid food. One or two mated female parasitoids were introduced into the cage for 24-48 hrs and then removed. First instar larvae that were still feeding on the one leaf

were parasitized in a smaller cage of 5x7 cm which enclosed the leaf rather than in the larger cages. Hosts of varying sizes were used to maintain the cultures.

Parasitized larvae were reared on cut foliage, as for unparasitized larvae, until parasitoids emerged to pupate. Parasitoid pupae were collected daily from cages and individually placed in no 00 clear gelatin capsules. All pupae were held at 20°C, 12L:12D and checked daily for emergence.

2. *Handling and mating of adult parasitoids*

Most adults emerged from cocoons around the start of photophase and were sexed in the gelatin capsule. Females were left in the capsule at 12°C for 24-48 hrs whilst males were placed individually in 4x1.6 cm plastic vials with a perforated lid and provided with honey.

Matings were carried out in the laboratory under natural daylight conditions. Many sizes of mating cages were tried but very small cages were preferred as males located females quicker in small cages. Thus 4x1.6 cm plastic vials were used for mating but were modified so that one side was cut away and covered with fine mesh cloth. Pre-starvation of females and the use of cloth cage walls were adapted from the methods used by Weseloh (1977) for the mating of *Cotesia melanoscelus* (Ratzeburg). A starved female was first introduced into the mating cage and then a male. If more than one male was used interference between courting males resulted, so only a single pair was placed in each cage. Pairs were observed until the male began wing vibration which is characteristic of most braconid courtship (Matthews 1974). Once males began wing vibration, honey was streaked on the cloth of the cage wall. Females upon locating the honey spent time feeding upon it during which time the male was able to mount and successfully copulate with the female. Fed females typically moved away from males when the latter attempted to mount them. Copulation times in the laboratory over a temperature range of 17-25°C ranged between 34 and 93 sec. for *D. eucalypti* (59±2.8 (SE); n = 29) and between 27 and 121 sec. for *C. urabae* (52±2.8 (SE); n = 50) . Females were unreceptive to further mating but males were polygamous and could be

used for several matings if required. Most mating success using the above method was achieved with *C. urabae* because female *D. eucalypti* often continued to successfully avoid the courting male.

Female *C. urabae* and *D. eucalypti* were arrhenotokous and capable of mating and oviposition within several hours of emergence. Thus their preoviposition period (if any) is less than a few hours, but this was not further tested. Once mated, females and males were provided with honey and held in 4x1.6 cm vials until further use.

# Appendix 3

**The relationship between hind tibial length (mm) and the cube root of the weight (mg) of an adult female parasitoid for *C. urabae* and *D. eucalypti*.** The model used was that for simple linear regression of y=a(x) + b where x= tibial length, and y= the cube root of adult parasitoid weight. Range of tibial lengths for *C. urabae* = 0.89-1.06mm and for *D. eucalypti* = 0.68-0.89mm.

| Species of parasitoid | n | Gradient±SE | Intercept±SE | $r^2$ | F | Prob. |
|---|---|---|---|---|---|---|
| *C. urabae* | 54 | 1.5040±0.1536 | -0.0367±0.1520 | 0.64 | 95.84 | <0.001 |
| *D. eucalypti* | 47 | 1.5051±0.1608 | 0.0824±0.1334 | 0.66 | 87.63 | <0.001 |

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