The larval parasitoid guild of *Chrysophtharta agricola* (Coleoptera: Chrysomelidae): Host - parasitoid ecological and developmental interactions.

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School of Agricultural Science

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for Leila

DECLARATIONS

This thesis contains no material that has been accepted for a degree or diploma by the University of Tasmania or any other institution. To the best of my knowledge and belief this thesis contains no material previously published or written by another person except where due acknowledgement is given in the text of the thesis.

Anthony D. Rice

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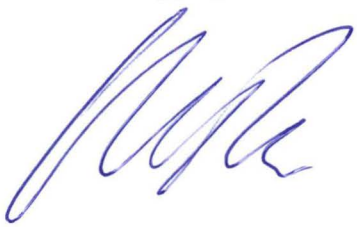

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ABSTRACT

Chrysophtharta agricola Chapuis (Coleoptera: Chrysomelidae), an economically important pest of plantation eucalypts in Tasmania, is parasitised in its larval stage by the solitary primary parasitoids *Eadya paropsidis* Huddleston and Short (Hymenoptera: Braconidae), *Paropsivora australis* (Macquart) and *Balde striatum* gen., sp., nov., (Diptera: Tachinidae: Blondeliini). Hyperparasitoids included in the guild are *Perilampus tasmanicus* (Cameron) (Perilampidae), *Mesochorus* sp. (Ichneumonidae) and possibly *Meteorus* sp. (Braconidae). A taxonomic key was developed for the adults and pupae of all parasitoid species, and brief notes on their biology provided. Formal taxonomic descriptions are provided for two species in the guild. One species is a new species of tachinid fly with a new genus (*Balde*) erected to accommodate it whilst the other species *P. australis*, is re-described. The taxonomic status of the third primary parasitoid *E. paropsidis* was unambiguous and not dealt with further.

The lower developmental thresholds for *E. paropsidis, P. australis* and *B. striatum* were experimentally determined and estimated at 5.9 °C, 294 DD (egg to pupa), 6.2 °C, 384.6 DD (egg to adult), and 6.3 °C, 344.8 DD (larvae to adult) respectively. The developmental thresholds of all three parasitoids were lower than that of their host, and all three required fewer day-degrees for development than their host. The developmental threshold for *Mesochorus* sp., a hyperparasitoid of *B. striatum*, was 9.7 °C, which was higher than *B. striatum*, the primary host and *C. agricola*, the ultimate host. The thermal constant of 333.3 DD for *Mesochorus* sp. was also lower than its primary and much lower than its ultimate host.

These thermal thresholds were used to successfully predict the phenology and thermal constraints of all three parasitoids in the field. *E. paropsidis* and *P. australis* overwinter as pupae within the plantation in the soil near the tree where the host larva fed, but *B. striatum* did not. Despite there being no thermal constraint to a second generation within the host's field-active larval period *E. paropsidis* had an obligate pupal diapause and hence only one generation per year. The two tachinid flies both had second generations but second generation *B. striatum* adults always emerged in the same season even when pupation was very late in the season. *P. australis* were found to be bet-hedging, with approximately half of the population emerging same-season, and half remaining as pupae in the soil until the following season.

E. paropsidis displayed a preference for early instar hosts with attempts at ovipositor insertion being twice as long and only half as successful when attacking large hosts compared to small hosts. Apart from a taking slightly longer to develop from first instars, small hosts offered the best potential for *E. paropsidis* to maximise fitness via increased body size and reduced host handling time. Both tachinid flies, which have different oviposition strategies, also showed strong host stage preferences. *P. australis,* which is the larger but less fecund of the two tachinids, shows a preference for fourth instar hosts, and *B. striatum* prefers second instars, in laboratory and field tests. The stage of host preferentially attacked was found to be constrained by the reproductive biology of the flies, and by interactions between life-histories and host defensive behaviour.

The developmental strategy of *P. australis,* which attaches unembryonated macrotype eggs onto the integument of its host, favoured body size over developmental rate, with delays in development from all host stages except the ultimate instar. Male and female *P. australis* developed at the same rate, and were roughly equal in size. In contrast, *B. striatum,* which is ovolarviparous and deposits its larvae onto the underside of the host, exhibited a trade-off between body size and developmental rate. Male *B. striatum* were smaller and developed more rapidly than females.

Finally, the effectiveness of all three parasitoids as biocontrol agents in plantations is discussed and practical recommendations for leaf beetle management strategies that may enhance the effectiveness of all three parasitoids in the field are explored.

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My room mates Helen Nahrung and Marie Yee provided an instant well of knowledge, advice, friendship and support during this project. Thanks to Helen Nahrung (now Queensland University of Technology) also for comments on early drafts of chapters and collections of *B. striatum* in Victoria.

Don Colless (CSIRO Entomology) spent many hours teaching me the taxonomic eccentricities of the Tachinidae and dissection techniques. He also reviewed the description herein, helped me identify aberrant forms, and confirmed the identity of post-abdominal structures. Monty Wood (Agriculture and Agri-Food Canada, Ottawa) taught me collecting and preservation techniques, reviewed the description herein and gave advice on identifications and type status. Thanks also to Jim O'Hara (Agriculture and Agri-Food Canada, Ottawa) for helpful discussions on species determination; Norm Woodley (Smithsonian Institute, Washington) for loan

of *Lixophaga* specimens; Hiroshi Shima (Kyushu University, Japan) for examining my material; Nigel Wyatt (BMNH) for loan of *Paropsivora* specimens; Chris Burwell (Queensland Museum) for loan of *Lixophaga sphenophori* specimens. Mark Short, John La Salle (CSIRO Entomology) and A.D. Austin (University of Adelaide) kindly identified some of the wasps, Peter McQuillan (University of Tasmania) for advice on taxonomic procedural protocols.

Judy Sprent obtained many references including some obscure ones, Jean Richmond provided vital administrative assistance, and Steve Patterson assisted with field trials. I am also indebted to Industry Partners David de Little (formerly of Gunns Ltd), Dick Bashford, Jane Elek and Simon Grove (Forestry Tasmania), and Sandra Hetherington and Chris Berry (Norske Skog) for various discussions and access to field sites and collections.

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PREFACE

In this thesis data is presented on the biology and ecology of three larval parasitoids of *Chrysophtharta agricola* Chapuis (Coleoptera: Chrysomelidae), in plantations of immature *Eucalyptus nitens* in Tasmania. The chapters in this thesis fall into three main areas: taxonomy; temperature, development and phenology; and developmental interactions. Chapter 1 introduces the host, its parasitoids and places this study into context. Chapter 2 is purely related to the taxonomy of the parasitoids. Chapters 3 and 4 deal with temperature, development and phenology. Chapters 5, 6 and 7 deal with parasitoid-host developmental interactions. Chapter 8 is a general discussion and is comprised of the conclusions from this work.

Each of the data chapters have been prepared as a publishable manuscript, except that here, figures and tables have been numbered to fit with the thesis format, references collated at the end, and acknowledgements at the beginning. Chapter 2 has already been accepted and is in press for publication in the November 2005 issue of the Australian Journal of Entomology. A list of conference presentations arising from this work appears in the appendices, along with tips for collecting, identifying and rearing the three species of parasitoids.

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CHAPTER 1.

Introduction to the Ecological and Developmental Interactions Between *Chrysophtharta agricola* and its Larval Parasitoid Guild.

1.1 Background

Tasmanian plantation eucalypts comprise over one fifth of the total Australian hardwood plantations, with around 15 000 ha planted annually in Tasmania (Wood *et al.* 2001). *Eucalyptus nitens* (Deane and Maiden) Maiden or shining gum, is endemic to Victoria but has replaced *Eucalyptus globulus* Labill. as the favoured plantation species in cooler high altitude sites in Tasmania due to its frost tolerance and high growth rates (de Little 1989, Baker *et al.* 2002). In 2004 there were approximately 151 000 ha of plantation hardwoods in Tasmania (Parsons and Gavran 2005).

E. nitens is attacked by a number of pests and pathogens including fungi (Keane *et al.* 2000), vertebrates (Bulinski & McArthur 1999) and insects (de Little 1989, Elliott *et al.* 1998). Historically, the major insect pest of eucalypt plantations in Tasmania has been the leaf beetle, *Chrysophtharta bimaculata* (Olivier) (Coleoptera: Chrysomelidae: Paropsini) (de Little 1989), and due to its pest status has been the subject of considerable scientific study. However, the expansion of plantings of *E. nitens* has facilitated an elevation in pest status of another paropsine, *Chrysophtharta agricola* (Chapuis) (Fig. 1.1) whose abundance and geographical range has increased coincident with that of its eucalypt host (Nahrung 2004). *E. nitens* is a heterophyllous species and *C. agricola* differs from *C. bimaculata* in that it preferentially oviposits onto, and its larvae feed

almost exclusively on, juvenile foliage (Nahrung and Allen 2003). Presently, broad spectrum synthetic pyrethroid pesticides are the preferred option for control of this pest. However, a growing recognition that pesticide usage may be able to be reduced by natural enemies that give effective biological control, and a move towards Forest Certification Systems has led to focused research into the taxonomy, biology and ecology of native natural enemies in forestry environments (Short and Steinbauer 2004, Elek 2005).

Figure 1.1 *Larvae of the paropsine leaf beetle,* Chrysophtharta agricola *on juvenile* Eucalyptus nitens *foliage. (Photo courtesy of H.F. Nahrung)*

1.2 Study Aims

The overall aim of this study was to gather basic biological data on the primary parasitoid guild that attacks the larval stages of *C. agricola.* In turn, it is hoped that this data may be used by forest managers to establish and maintain eucalypt plantations in such a way as to enhance the contributions of these natural enemies to pest regulation. In

particular, it aimed a) to determine the taxonomic status of the larval parasitoid guild; b) to assess relative parasitoid abundance, levels of parasitism, and seasonal phenology; c) to develop temperature dependant developmental models for predicting seasonality and local abundance peaks and d) to investigate developmental interactions with their host in terms of preferred host stages and the possible selection pressures and fitness consequences of such preferences.

It uses a combination of laboratory experimentation and a variety of population monitoring techniques as well as field experiments. Field work was carried out at two sites in Tasmania (Fig. 1.2).

1.2 Field Sites.

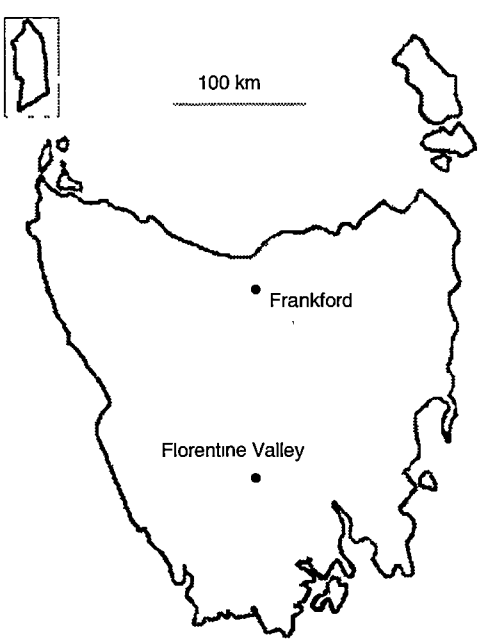

Figure 1.2 *Map of Tasmania showing the approximate location of the two field sites used in this study.*

Florentine Valley. (42°38S 146°29E) Elevation 400m. Coup # FO31VI. *Eucalyptus nitens* planted in 1996; mean annual maximum temperature 16.2 °C and mean annual minimum temperature 5.2 °C (40 years) (Nahrung and Allen 2004). Mean monthly rainfall between December 1999, when field trials began, and March 2002, when they finished, was 96.7 ± s.e.10.8 mm (range 24 – 241.3 mm) (Nahrung and Allen 2004). Bordered by native forest, older *E. nitens* plantation, logged native forest and an *Acacia dealbata* plantation.

Figure 1.3 *Two year old* Eucalyptus nitens *at the Frankford study site.*

Frankford. (41°20S 146°45E) Elevation 240 m. *Eucalyptus nitens* planted in 1996; mean annual maximum temperature 16.9 °C and mean annual minimum temperature 4.6 °C (130 years). Mean monthly rainfall between December 1999 and March 2002 was 79.6 ±

s.e.9.5 mm (range 18.6 – 213.2 mm) (Nahrung and Allen 2004). Bordered by older *E. nitens* trees and a new planting of *E. nitens* in 1999. In the summer season of 2001/02 the study site was expanded to include the trees planted in 1999, which were further bordered by older plantation trees (Fig.1.3).

1.4 The Host, *Chrysophtharta agricola.*

Chrysophtharta agricola has gained its status as a pest by feeding on the foliage of plantation *Eucalyptus nitens,* and to a lesser extent *E. globulus* (Rapley, *et al.* 2004). *C. agricola* oviposits on flushing shoots of juvenile foliage from late October to March where they usually place their egg mass on the tip of the leaf (Ramsden and Elek 1998). There are four larval instars after which the pre-pupal stage drops to the ground (Clarke 1998) and pupate in a chamber excavated in the soil and leaf litter (Ramsden and Elek 1998). Fourth instars can reach up to 14 mm in length (de Little 1979) and 81.7 mg $\pm$ 4.0 SE (n = 15, range = 60.4 – 114.8) in weight (Rice unpublished data). Adults emerge in the same season and feed on adult foliage to build up their fat body before going into overwintering diapause as adults in the leaf litter (Nahrung 2003). Under certain climatic conditions the new generation adults may oviposit before going into diapause (Nahrung *et al.* 2004). Eggs and larvae are present on foliage from November to late March where they are attacked by a range of natural enemies.

1.5 Natural Enemies.

Natural enemies can have a significant impact on *C. agricola* populations in the field (Allen and Patel 2000, Nahrung and Allen 2004). Generalist predators such as coccinellids and cantharids are known to prey upon paropsine eggs and larvae (Greaves

1966, Tanton and Kahn 1978, Elliott and de Little 1980) as are spiders (Nahrung 2003, pers. obs.). Bugs from the families Reduviidae and Pentatomidae are occasionally observed feeding on larvae, but an abundant undescribed species of mirid bug (Orthotylinae) was observed to cause high mortality in eggs and to a lesser extent larvae (Nahrung 2003, pers. obs).

The egg parasitoids *Enoggera nassaui* Girault (Hymenoptera: Pteromalidae) have been collected from *C. agricola* eggs in Tasmania and *Neopolycystus* sp. (Hymenoptera: Pteromalidae) from Victoria, though only at low levels in Tasmania (Nahrung and Murphy 2002).

The larval parasitoid guild of *C. agricola* was largely unknown prior to this study and data have been gathered by extensive larval collections and by examination of various collections within Australia. Collections visited include the Australian National Insect Collection (ANIC), Canberra, collections held at University of Tasmania, Australian Museum, Sydney, Melbourne Museum, Forestry Tasmania, Gunns Limited, Tasmanian Museum and Art Gallery, Hobart, and the CRC for Sustainable Production Forestry. The primary parasitoids include *Eadya paropsidis* Huddleston and Short (Hymenoptera: Braconidae), *Paropsivora australis* (Macquart), *Anagonia* sp., and *Balde striatum* Rice (Diptera: Tachinidae) (Rice 2005). Once preliminary identifications were complete, final formal identification of the two tachinid flies require borrowing material from institutions within Australia and overseas. More formal taxonomic descriptions and details on biology are provided in Chapter 2; in this chapter I will overview their biology

and provide photographs were possible. Voucher specimens of all species covered in this study are held at CRC SPF, Sandy Bay, Hobart Tasmania.

1.5.1 *Eadya paropsidis*

Eadya paropsidis is a medium sized (10 mm) black braconid wasp with a bright orange head (Fig. 1.4). Once a host has been selected *E. paropsidis* oviposits small (0.3 mm), hydropic eggs directly into the haemocoel of its hosts. Such eggs expand by absorbing water and nutrients from the host (Quicke 1997) and hatch in around five days at 22 °C (Fig.1.5). First instar larvae have a well-developed set of mandibles, which are presumably used for fighting with competing parasitoids within the host (Fig. 1.6). *E. paropsidis* undergoes an obligate pupal diapause which greatly complicates its

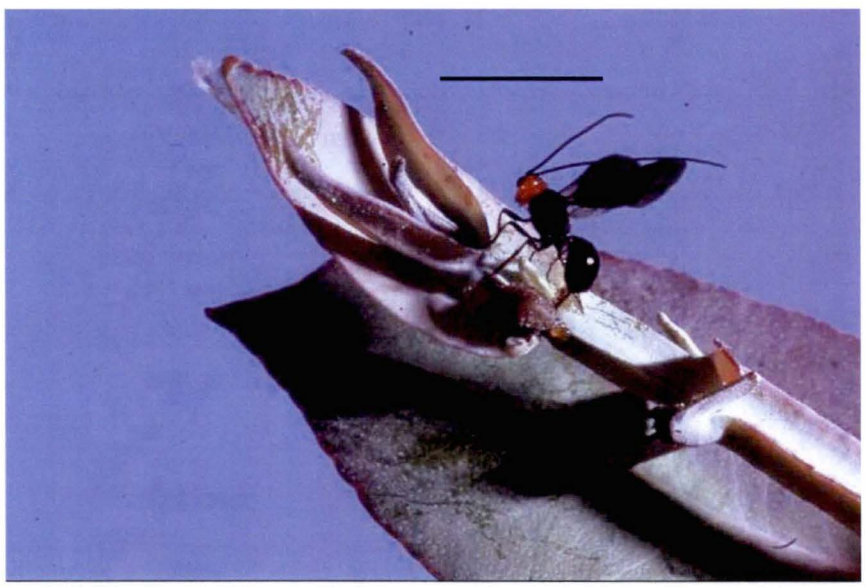

Figure 1.4 *The braconid wasp* Eadya paropsidis *parasitising* Chrysophtharta agricola *larva on* Eucalyptus nitens *foliage. Scale bar represents approximately 10 mm.*

experimental manipulation in the laboratory. For this reason, our laboratory experiments were conducted using gravid field collected females, which were often difficult to find.

Besides *C. agricola, E. paropsidis* is also known to parasitise the larvae of *Paropsis atomaria* (Tanton and Epila 1984, ANIC labels), and *Chrysophtharta bimaculata* (de Little 1982, de Little *et al.* 1990, Bashford 1997, Simmul and de Little 1999). Only low levels of hyperparasitism by *Perilampus* sp. (Hymenoptera: Pteromalidae) were detected in this study.

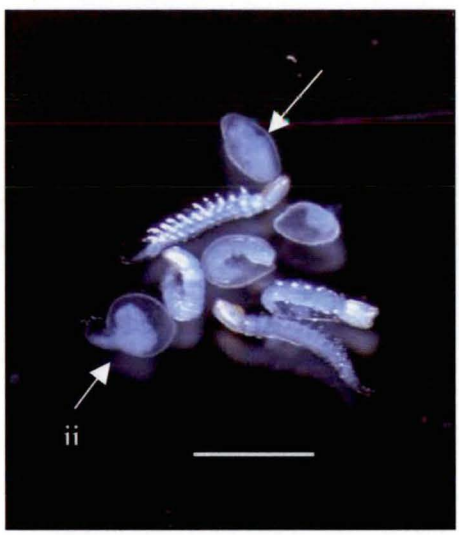

Figure *1.5* *Newly hatched* Eadya paropsidis *larvae i) indicates unhatched eggs ii) hatching egg. Scale bar = 1 mm.*

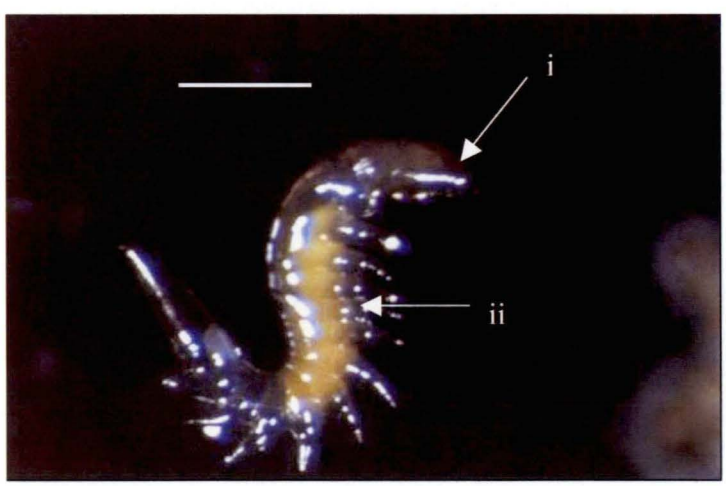

Figure 1.6 Eadya paropsidis *first instar larva at about two days old. Note i) sclerotised mandibulate head capsule and ii) ingested fat body material from host. Scale bar = 0.25 mm*

1.5.2 *Paropsivora australis.*

P. australis (Fig.1.7a) deposits unembryonated macrotype eggs onto the integument of its host (Fig. 1.7b). The eggs hatch within a few days, and provided the host hasn't moulted, the neonate larvae burrow into the haemocoel of the host. The larvae remain just under the integument of the host with their posterior spiracles in

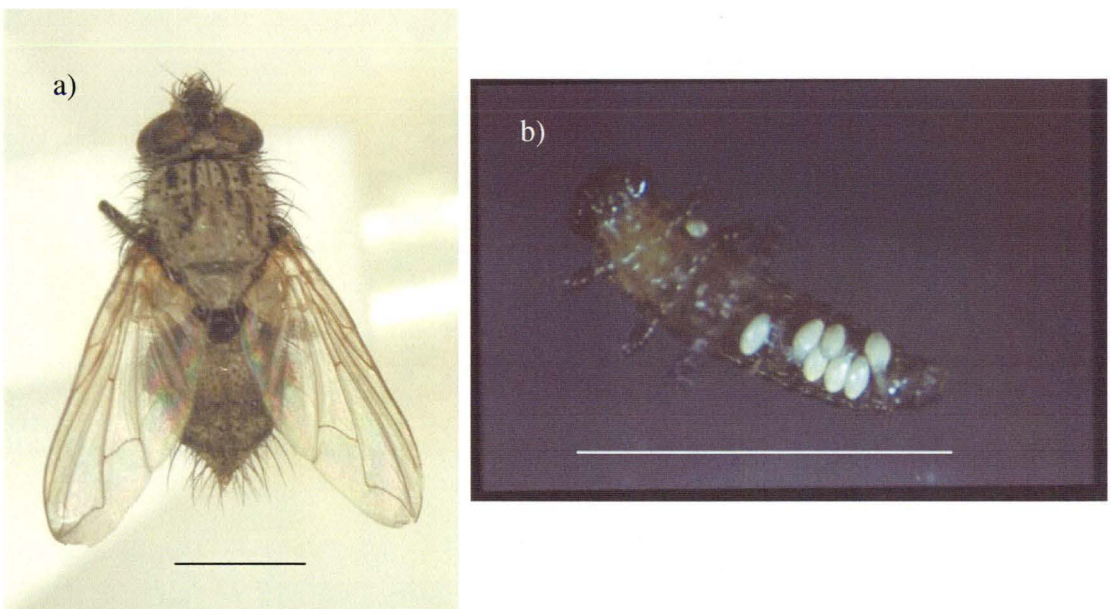

Figure 1.7 *a) Female* Paropsivora australis, *and b)* P. australis *eggs on a first instar host. Scale bars = 2.5 mm.*

contact with the atmosphere. A darkened melanised ring forms around the posterior of the tachinid larvae that extend inwards as the larvae grow to form a respiratory funnel similar to that described by Salt (1968). *P. australis* is recorded from *C. bimaculata* (Crosskey 1973) but not from *C. agricola* until this study. Undescribed flies from this genus (*Paropsivora* sp.) have been reared from *Paropsis atomaria* in the Australian Capital Territory (Tanton and Khan 1978, Tanton and Epila 1984), and *Chrysophtharta*

bimaculata in Tasmania (de Little 1982, de Little *et al.* 1989). No hyperparasitism was detected in this study.

1.5.3 *Balde striatum*.

Balde striatum (Fig. 1.8) is formally described in this thesis, and placed into a new genus. Prior to this study, this species had not been identified as a parasitoid of *C. agricola*. It has also been collected by H.F. Nahrung on the Australian mainland from *C. agricola* at Jindabyne (36° 24S 147° 37E), Mt Bulla (37° 8S 146° 25E), and Piccadilly Circus (35° 22S 148° 48E).

Figure *1.8* *Female* Balde striatum. *Scale bar = 2.5 mm.*

This tachinid fly, at around 5 mm in length, is smaller than both *P. australis* and *Anagonia* sp., with males being on average, smaller than females. It deposits live larvae directly onto the host which then burrow into the host where they remain free-living in the host's haemocoel. This species was particularly difficult to manipulate in the laboratory. It would not parasitise hosts in the same containers that were successful for the other two parasitoids, it would only rarely mate, and parasitism rates were typically low in the laboratory. However, by collecting gravid females from the field and keeping cohorts of around 15 females in a larger cage (0.125 m^3) we could achieve sufficient levels of parasitism for manipulative experiments, although they would only continue parasitising hosts for 4 to 5 days. In the field, *B. striatum* was heavily hyperparasitised by a species of *Mesochorus* (Hymenoptera: Ichneumonidae).

1.5.4 *Anagonia* sp.

Collection of *C. agricola* from mainland Australia by H.F. Nahrung yielded tachinid parasitism by *Anagonia scutelata.* I mistakenly identified these as *Paropsivora* sp. for Nahrung (2002). Another species form this genus, *A. rufifaces* is a common parasitoid of *C. bimaculata* in Tasmania (de Little 1982, de Little *et al.* 1989, *pers. obs*), where it is heavily parasitised by the hyperparasitoid, *Mesochorus* sp. Despite extensive collections of *C. agricola* larvae, there was no parasitism of *C. agricola* by *Anagonia* in Tasmania, and this genus is not dealt with further in this work.

1.5.5 *Mesochorus* spp.

Two species of *Mesochorus* (Mark Short, CSIRO Entomology Canberra) were encountered during this study, one parasitising *Anagonia rufifaces* in *Chrysophtharta*

bimaculata and one parasitising *Balde striatum* in the primary host *C. agricola*. Both species became abundant towards the end of the active summer season. Only the species parasitising *Balde striatum* is featured in this thesis but its reluctance to oviposit under any controlled conditions provided has limited the attention it received in this study.

1.5.6 *Perilampus tasmanicus*

Perilampus tasmanicus has been collected from *Paropsis atomaria* where it parasitises the braconid wasp *Eadya paropsidis (Aridelus)* and at least one of three species of tachinid flies (Tanton and Epila 1984). In this study, only a small number of *P. tasmanicus* (Identified to genus by John la Salle, CSIRO Entomology) were collected (Chapter 2).

1.5.7 *Meteorus* sp.

One specimen of the braconid wasp *Meteorus* sp. (Identified by A. D. Austin, University of Adelaide) was found in a large sample of what I took to be pupae of *E. paropsidis.* Several more specimens were collected from the field but none oviposited in host larvae that they were given.

1.6 Areas of Study.

Chapter 2 - Taxonomy. The taxonomy of the Australian Tachinidae is notoriously difficult (Colless and McAlpine 1970). One species that was encountered was undescribed, and the other, *Paropsivora australis,* was known only from one type specimen, the holotype, located in the British Museum of Natural History. These two

species are formally described in this thesis. However, the taxonomic status of *Eadya paropsidis* is not in doubt and consequently, is not discussed to any depth. The identity of the two species of *Mesochorus* encountered here is not yet determined to species level but formal description of these two species is beyond the scope of this thesis. This chapter has been accepted for publication by the "Australian Journal of Entomology".

Chapter 3 - Temperature and development. The relationship between temperature and development is used extensively to make predictions regarding insect population dynamics, especially for insect pests (Dent 1991). Because thermal requirements usually differ between species, ambient temperature can impact on ecological interactions between species (Campbell *et al.* 1974, Frazer and Gilbert 1976, Nealis *et al.* 1984). For parasitoids, developmental thresholds and day-degree requirements are subject to selection (Gilbert and Raworth 1996) and may be used to maximize synchrony with their host (Campbell *et al.* 1974). In this study, I collected developmental data over several constant temperatures for the parasitoid guild and used this data to calculate lower thresholds and DD requirements for *E. paropsidis, P. australis* and *B. striatum* to aid in understanding parasitoid phenology in the field.

Chapter 4 - Phenology and Host Synchrony. Host synchrony is essential for successful parasitoid relationships (Mackauer and Sequeira 1993). In this chapter, I used four different monitoring techniques to determine the timing of early season parasitoid emergence, estimate second generation timing, to check for pupal bet-hedging, and to monitor field parasitism rates and adult abundance. Finally, data-loggers were deployed to record ambient temperatures to provide data to test whether our predictive

temperature models from chapter 3 accurately predicted parasitoid phenology in the field.

Chapters 5, 6 and 7 - Host parasitoid Developmental Interactions. The last three chapters are concerned with developmental interactions between the three larval parasitoids and their host. Since Salt's pioneering work laid the broader foundations for modern insect parasitology, insect parasitoids have been favoured in the modeling of ecological theories (Charnov and Skinner 1985, Harvey *et al.* 2000, Hawkins 2000). Parasitoids are largely suited to such studies because all the resources required for immature development and growth, and hence adult size, are derived from one host individual. Here I determine the preferred host-stage of *C. agricola* for each of the three parasitoids. Then, using various manipulation experiments, I examine how oviposition into each instar of the host affects various developmental measures and reproductive fitness of the parasitoid. These parameters included the likelihood of successful parasitism, development times, and the size of resulting adult parasitoid.

Chapter 8. Conclusion and Recommendations to Industry.

In the final chapter I integrate the findings of the previous chapters. Furthermore, I also assess the potential of each of the parasitoids as biocontrol agents for *C. agricola*, and discuss my findings in terms of pest management options that may improve the efficacy of these natural enemies in plantations.

CHAPTER 2. (In press for publication in the `Australian Journal of Entomology,' November 2005).

The parasitoid guild of larvae of *Chrysophtharta agricola* Chapuis (Coleoptera: Chrysomelidae) in Tasmania, with notes on biology and a description of a new genus and species of tachinid fly.

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Abstract

The taxonomic status of the parasitoid guild associated with the larvae of Southern Eucalypt Beetle (*Chrysophtharta agricola* Chapuis) in Tasmania is discussed. The primary larval parasitoid complex comprised the tachinid flies *Balde striatum* gen. n., sp. n. and *Paropsivora australis* (Macquart) (Diptera: Tachinidae: Goniinae: Blondeliini), and *Eadya paropsidis* Huddleston & Short (Hymenoptera: Braconidae), while the hymenopteran hyperparasitoids included *Perilampus tasmanicus* (Cameron) (Perilampidae), *Mesochorus* sp. (Ichneumonidae) and possibly *Meteorus* sp. (Braconidae). Keys are provided to the three adult primary parasitoids and two adult hyperparasitoids, and to the pupae of the primary parasitoids. *Balde striatum* gen. n. sp. n. is described, and *P. australis* is re-described. Brief notes on biology are included.

Key Words: Tachinidae, Braconidae, Taxonomy, *Eucalyptus,* Hyperparasitoid

2.1 *INTRODUCTION.*

The paropsine chrysomelid *Chrysophtharta agricola* Chapuis is a native defoliating pest of two-to-five year old *Eucalyptus nitens* plantations in Tasmania (Bashford 1993, Elliott *et al.* 1998). *Chrysophtharta agricola* has recently been noted as a pest, and its increased activity in Tasmania has been facilitated by rapid expansion in plantings of *E. globulus* and *E. nitens*, the latter being exotic to Tasmania. In 1997, *C. agricola* costs the timber industry around A$12 000 annually in pesticide applications (see Chapter 1 for more recent figures), and up to $870 per hectare in lost production (Nahrung 2003). These costs could be expected to rise because *E. nitens* is becoming the preferred plantation species for colder, high altitude sites in Tasmania (Baker *et al.* 2002). Within the genus *Chrysophtharta* Weise, another pest species, *C. bimaculata* (Olivier), has been the main focus of research in Tasmania, including some work on its larval parasitoids by de Little (1982) and de Little *et al.* (1990). However, until recently, little has been published on *C. agricola,* except by Nahrung (2003), or on its natural enemies, and even less on its parasitoids. *Chrysophtharta agricola* has three larval parasitoids- two tachinid flies and one braconid wasp – and two egg parasitoids- *Enoggera nassaui* Girault and a species of *Neopolycystus* Girault. (Hymenoptera: Pteromalidae) (Nahrung and Murphy 2002, Nahrung 2003).

The Tachinidae is one of the largest families of Diptera (Wood 1987, Cantrell & Crosskey 1989) and an immensely diverse and taxonomically difficult group (Colless & McAlpine 1970) which is relatively poorly studied (Crosskey 1973, Barraclough & O'Hara 1998). The two tachinid flies dealt with here belong to the tribe Blondeliini (subfamily Goniinae) which is a somewhat heterogeneous grouping with great diversity

of body form and chaetotaxy (Crosskey 1973), and an almost world-wide distribution (Wood 1985). However, blondeliines can be distinguished reliably from other goniines by having a small pre-alar seta, strong subapical scutellar setae and an obtuse evenly rounded bend in vein *M* (Crosskey 1973). Blondeliines commonly parasitise larval and adult Coleoptera and larval Lepidoptera (Wood 1985).

This paper forms part of a larger study of the biology and host interactions between *C. agricola* and its larval parasitoids, the results of which will be published elsewhere, and aims to provide a taxonomic framework to relate to other biological data. A new species of tachinid fly is described and another re-described. I also provide a key to the adults of the primary parasitoids and hyperparasitoids and to the pupae of the primary parasitoids, as well as brief notes on their biology.

2.2 *MATERIALS AND METHODS.*

Cohorts of the four larval instars of *C. agricola* were collected fortnightly from 2-5 year old *E. nitens* plantations at Frankford (41°20'S 146° 45'E) and Florentine Valley (42°38'S 146° 29'E), Tasmania, over the active summer seasons of 2000/01 and 2001/02. Larvae were reared in the laboratory at 22°C and 16 light: 8 dark photoperiod on flushing shoots of juvenile foliage of *E. nitens* to allow parasitoids to develop normally and emerge from their hosts for identification. The fecundity of each parasitoid species was determined from field collected females, and their longevity estimated from females held at ambient temperature (max 22 °C) and 16 light: 8 dark photoperiod. Terminalia were removed from freshly pinned specimens with a hooked needle and from dried specimens by severing the abdomen at around T4. Terminalia were soaked

overnight in 10% KOH to remove softer tissues. Dissections were carried out and stored in 70% ethanol or 100% glycerol. Drawings of wing venation were made with the wing pressed flat between two microscope slides and descriptions of the terminalia were completed according to the format of Cantrell (1988). The tachinid flies were identified to tribal level using the keys in Crosskey (1973). Measurements were made with an eyepiece micrometer at 10x magnification. Body lengths were measured from foremost point of antennal segment 2 to tip of abdomen. Specimens that had the abdomen curled forward were measured in two steps and summed. Wing lengths were taken from wing tip to proximal edge of tegula. Frons widths were taken at apex of head. The terms setae, setulae and hairs are used in the same manner as Crosskey (1973) as are other morphological terms. Setae are described in the singular even though there is obviously a matching one as a result of symmetry, unless referred to as `paired medial', in which case all possible setae are covered.

Specimen depositories are as follows:

ANIC, the Australian National Insect Collection, CSIRO Entomology, Canberra;

BMNH, Natural History Museum, London;

MVM, Museum Victoria, Melbourne;

FT, collection of Forestry Tasmania, Hobart;

CRC SPF, Cooperative Research Centre for Sustainable Production Forestry, Hobart, Tasmania.

2.3 *RESULTS.*

2.3.1 Key to Adult Parasitoids of *Chrysophtharta agricola* in Tasmania

1 One pair of wings 2

Two pairs of wings.....3

2 Bend in vein M evenly rounded forming a gentle obtuse angle (Fig. 2.1d); when viewed without microscope, thoracic and abdominal markings forming transverse bands; relatively small fly rarely exceeding 5-6 mm in length; eyes sparsely and inconspicuously haired .....*Balde striatum* gen. n., sp. n.

Bend in vein M abruptly angulate (Fig. 2.1e), body without obvious transverse bands; larger fly, rarely smaller than 8 mm in length; eyes conspicuously haired .....*Paropsivora australis.*

3 Gaster sessile; wing venation much reduced; body stout and head large relative to body; wasp entirely black in colour with metallic sheen.....

.....*Perilampus tasmanicus*

Gaster petiolate; wing venation not reduced; body slender and head not large relative to body; not all black.....4

4 Fore wing with only one recurrent vein (Fig. 2.1g).....5

Fore wing with two recurrent veins (Fig. 1f);.....*Mesochorus* sp.

5 Gaster globular and nearly as large as thorax when viewed in profile (slightly flattened dorsoventrally in males); colour black except head which is bright orange; head large and subcubic, frons carinate, clypeus tuberculate; tergite 1 polished and punctate.....*Eadya paropsidis*

Gaster elongate, nearly twice as long as wide; colour orange/brown; head not large or subcubic, frons without carinae, clypeus without tubercles; tergite 1 not as above .....*Meteorus* sp.

2.3.2 Key to Pupae of the Primary Parasitoids of *Chrysophtharta agricola* in Tasmania

1. - Outer pupal case fibrous, usually straw coloured or yellow but occasionally lighter, sometimes white; host remains always associated with pupa, and reduced to head capsule and skin only.....*Eadya paropsidis*

   - Pupa not fibrous but smooth, brown and barrel shaped; host remains not always associated with pupa.....2

2 - Pupae with two visible respiratory structures (Fig. 1 h) located about one third of the length of the pupa from anterior end (i.e. non-terminal), in the form of gentle mounds with a respiratory horn emerging from apex .....*Balde striatum*

- Pupae without such structures.....*Paropsivora* sp.

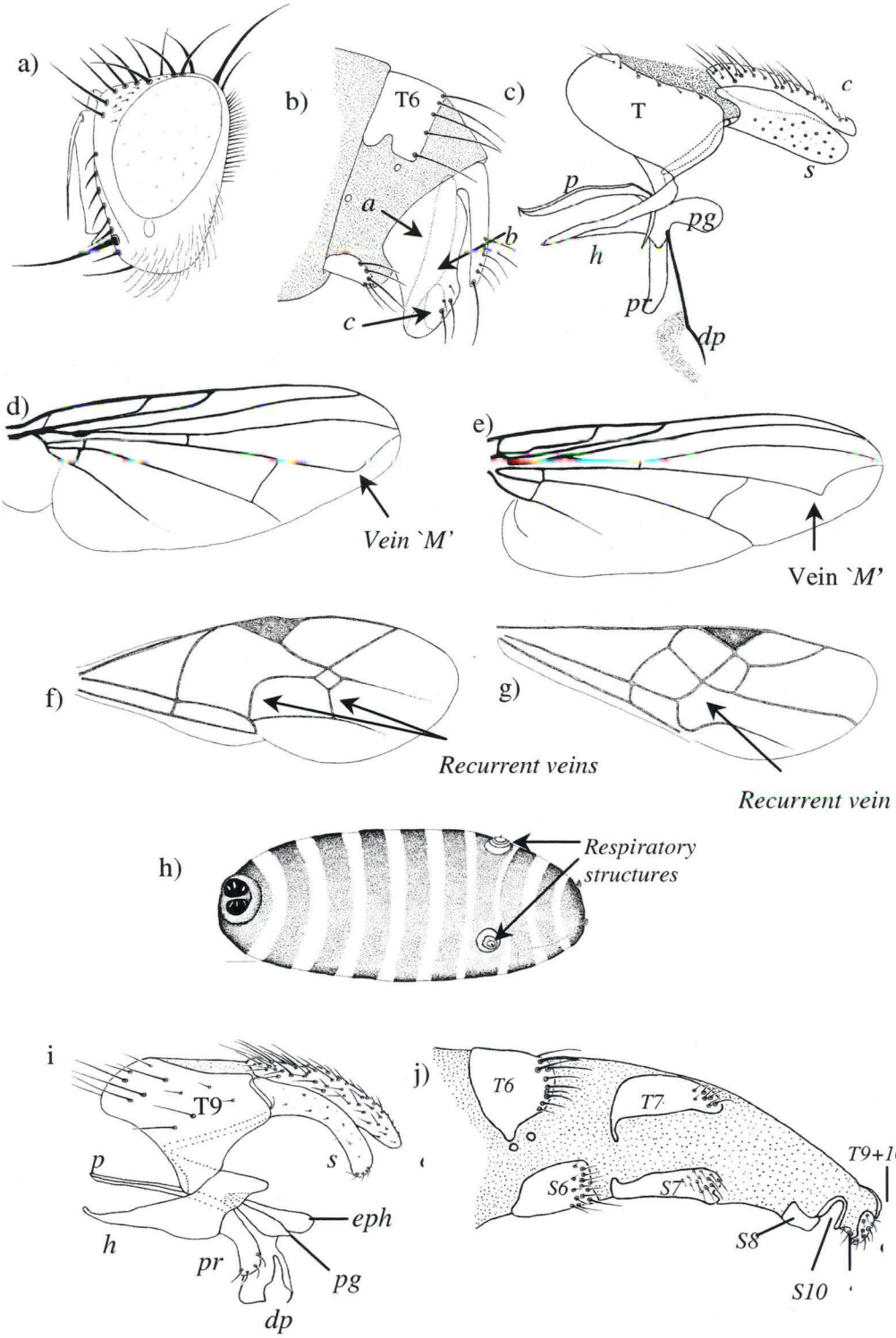

Figure 2.1 a) Left profile of *Balde striatum.* b) *B. striatum* female terminalia (*a, b* and *c* post abdominal sclerites). c) *B. striatum* male terminalia; T9 tergite 9 (epandrium); *c*, cercus; *s*, surstylus; *pg*, postgonite; *eph,* epiphallus: *dp*, distiphallus; *pr*, pregonite; *h*, hypandrium; *p*, phallopodeme (after Cantrell 1988). d) Right wing of *B. striatum* e) Right wing of *Paropsivora australis.* f) Right wing of *Mesochorus* sp. g) Right wing of *Eadya paropsidis*. h) Pupal case of *Balde striatum.* i) Male terminalia of *Paropsivora australis*. J) Female terminalia of *P. australis.*

2.4 Treatment of Species

Balde gen. n. (Diptera: Tachinidae: Blondeliini)

Type species. *Balde striatum* sp. n., designated here.

Diagnosis.

Head: (Fig. 2.1a) dichoptic and without heavy facial carina; eyes sparsely and inconspicuously haired; male and female with two proclinate and two reclinate orbital setae. Vibrissae inserted level with epistomal margin, not projecting past vibrissal angle, and not visible in profile. Antennal axis inserted well above level of middle of eye. Gena narrower than length of third antennal segment. Parafacials bare.

Thorax: two supra-alar setae; pre-alar seta small; 3 post-intra-alar setae, apical scutellar setae small, often reduced to one and sometimes absent; sub-apicals long and strong, weakly convergent and sometimes crossing at tips; 2 + 3 or 3 + 3 dorsocentral setae. Propleuron bare and prosternum setulose. Infrasquamal hairs absent; humeral callus with three setae arranged in a line; 3 sternopleural setae.

Wings: Bend in vein *M* evenly rounded; cell r_5 narrowly open or closed, never petiolate; distance between bend in vein *M* and cross-vein *m-cu* conspicuously less than distance between *m-cu* and *r-m*; 2 or 3 setulae at basal node of r_{4+5}; second costal sector haired ventrally.

Abdomen: intermediate abdominal tergites without discal setae; T_{1+2} non-excavate to hind margin and without paired medial setae; post-abdomen neither forcipate nor recurved under abdomen, lobes of S_5 without setae.

Legs: fore-tibia with two posteroventral setae. Hind tibia with posterodorsal pre-apical setae; mid tibia with strong sub-median ventral seta.

Etymology. The genus, *Balde,* is named in honor of a life-long friend Peter U. Balde of Coodanup, Western Australia. The name is Latvian, is pronounced B**a**lduh, with the emphasis on the `a' as in band, and gender is masculine.

Discussion. The setose prosternum, small pre-alar setae, strong sub-apical scutellar setae, and an obtuse and evenly rounded bend in vein M which ends near the wing tip along with cell r_5 unambiguously place this fly in the tribe Blondeliini of the sub-family Goniini. However, most blondeliines have divergent sub-apical scutellar setae, but Wood (1985) notes some exceptions, and states that divergent subapical scutellar setae are "not an absolutely reliable indicator of tribal affinity", and I propose that this type species will represent another exception.

The following combination of characters possessed by *Balde striatum* exclude it from all of the Australian blondeliine genera except the introduced *Lixophaga* Townsend (Crosskey 1973). Mid tibia with submedial ventral seta; three post-sutural dorsocentral setae, two supra-alar setae, humeral callus with three setae, distance between bend in vein *M* and cross-vein *m-cu* conspicuously less than distance between *m-cu* and *r-m*; basal node of r_{4+5} with two or three setae, and T_{1+2} non excavate to hind margin; propleuron bare, vibrissae inserted almost level with epistomal margin and parafacials bare.

There is no doubt that this species shares many character states with the predominantly New World genus *Lixophaga*. However, the lack of paired medial setae on T_{1+2}, the absence of a single long seta on each lobe of sternite 5 in males, different placement of setae on the humeral callus, and markedly different genital structure of the males [comparisons made with *L. sphenophori* (Villeneuve), *L. parva* Townsend, *L. mediocris* Aldrich, and *L. variabilis* (Coquillett)] separates this species from *Lixophaga* (confirmed by D.M. Wood, Agriculture and Agri-Food Canada, N. Woodley, Smithsonian Institution, and Hiroshi Shima, Kyushu University). Members of the genus *Lixophaga* are among the most widely distributed of the blondeliine genera, found from the eastern Palaearctic and Alaska to Argentina, with two isolated species, *L. sphenophori* (examined) and *L. beardsleyi* Hardy, from Papua New Guinea (Wood 1985). However, *Lixophaga* is not an Australian genus, and Crosskey's (1973) definitive work on the Australian Tachinidae, included *Lixophaga* in his keys in order to accommodate the introduced *L. sphenophori*.

There are three reported instances of *Lixophaga* parasitising paropsine leaf beetles in Australia: Elliott (1978), Simmul and Clarke (1999), and Tribe (2000). Simmul and Clarke (1999) reported a parasitoid of *Acacicola orphana* (Erichson) as *Lixophaga* sp. in Tasmania. These specimens were dissected and identified as *Balde striatum,* using the structure of the male genitalia. The fly collected by Elliott (1978) is likely to be *B. striatum* but has not been examined. He reports two species, *Deltomyza australiensis* (Malloch) and *Lixophaga* sp., that parasitise *A. orphana* in Tasmania and deposit small, white, oval eggs onto their host, but as *B. striatum* deposits live young, it

is likely that *D. australiensis* is responsible for the eggs he observed. The third report of *Lixophaga* in Australia is by Tribe (2000) who reports *Lixophaga* sp. parasitising *Trachymela tinticollis* (Blackburn), *Paropsis geographica* Baly and *Paropsisterna picta* (Chapuis) in Western Australia but these specimens have not been examined.

Balde striatum sp.n.

Holotype: ♀ ANIC, Florentine Valley Tasmania, coll. Dec. 11th 2002. Netted on *E. nitens* foliage. A. D. Rice

Paratypes: 21♀♀ ANIC, CRC SPF, FT, same collection details as holotype; 15♂♂ reared from laboratory cultures established from gravid field-collected females from Florentine Valley via *C. agricola,* emerged Jan/Feb 2000.

Female

Dimensions: Body length 5.1 mm (SE = 0.5, n = 10, range = 4.7 – 5.4); wing length 4.5 mm (SE = 0.6, n = 10, range = 4.2 – 5.0); frons width = 0.58 mm (SE = 0.0006, n = 10, range = 0.51 – 0.63); right rear tibial length = 1.48 mm (SE = 0.57, n = 10, range = 1.35 – 1.6).

Colour: Head mainly bright grey, changes to brown on rotation, parafrontal grey to yellow/grey, parafacial mainly grey to brown depending on direction viewed and with a faint to faint brown triangular patch of pigmentation, extending from just below eye and coming to a point between first and second peristomal setae. Small dark brown patch below eye. Gena, postorbit mainly grey to yellow/grey. Face bright grey pollinose.

Third antennal segment black. Interfrontal area brown ocellar triangle grey to yellow/grey. Occiput grey/yellow, sometimes dark, almost black with brown vertical medial band delineated by vertical setae. Eyes brown, brightness variable, usually light brown after drying, with up to one half of facets towards centre of eye dark. Hairs and setae black except on lower occiput and gena, where they are white. Thorax blue/grey to yellow/grey (sometimes grey/brown in darker individuals) ground colour; setation black; humeral callus, mesopleuron, pteropleuron, propleuron, sternopleuron, barette, hypopleuron, pleurotergite, mediotergite and postscutellum grey. Prescutum blue/grey to yellow/grey with pair of narrow longitudinal vittae situated between presutural acrosticals and dorsocentrals, strong anteriorly but fading before reaching suture and resuming postsuturally as dark patches; two dark patches located subcentrally around anterior posthumeral setae. Scutum mostly dark with grey to yellow/grey rim around edge except anterior edge which is dark up to suture. Scutellum dark yellow/brown fading to grey at posterior tip. Legs dark, usually with ventral, anterior and posterior surfaces of femur grey. Abdomen dark grey/black, T_3-T_5 with light grey band on anterior third of tergite dorsally; laterally narrow yellow band on hind margin of T_2 and T_3, continuing ventrally along length of tergite and along medial longitudinal margin, less conspicuous on T_4 and T_5.

Head. Eyes inconspicuously and sparsely haired. Ocellar triangle just visible in profile above upper limit of eye, nearest ocelli also visible in profile. Antennal axis inserted a quarter down from top edge of eye. Gena narrower than length of third antennal segment. Epistome not strongly developed, projecting level with vibrissal angle and only sometimes visible in profile. Parafrontal hairs sparse, not extending posteriorly

past half way along ocellar triangle, usually confined to a single line anterior of foremost orbital seta, extending down level with beginning of third antennal segment, rarely slightly lower. Parafacial bare. Two pairs of reclinate and two pairs of proclinate orbital setae, (anterior reclinate orbital seta inserted in line with frontal setae and rotated to curve towards a point at 45° between frontal setae and other reclinate orbital setae), two strong divergent proclinate setae originating between anterior and the two posterior ocelli. Small ocellar setae variable, some larger ones confined to region posterior of ocellar triangle, sometimes extending into triangle and rarely past anterior margins, and then minute. Three pairs of vertical setae arranged in a row at apex of occiput; innermost smallest, situated posterior of ocellar setula, angled posteriorly but curved as if proclinate; next pair laterally strongest, reclinate; next pair situated posterior and laterally to inner margin of eye, also reclinate. Post-ocular row of setae erect, beginning just laterally to outermost vertical setae and extending down two thirds of eye. 2nd row of irregular small black setae on occiput, just below and extending at least half length of post-ocular row, usually 2 – 3 setae wide at medial end which is situated at edge of vertical medial brown band; rest of occiput and gena setulose, setae small and white. Five to seven frontal setae, quite strong, cruciate, often crossing at tips; anterior penultimate pair located at point of profrons (sometimes slightly forward), markedly stronger than rest. Facial ridge with row of stout downward curving (until one above vibrissae) setae, beginning half way along parafacial region and continuing past (but including) vibrissae and becoming peristomal setae.

Thorax. Acrostichals 3 + 3; presutural dorsocentrals variable, usually 3 with posterior seta longest and strongest but sometimes middle setae reduced or lost,

postsutural dorsocentrals 3. Two supra-alar setae, 1 + 3 intra alars, rarely 1 + 4; presutural one smallest; pre-alar small, situated near suture; anterior supra-alar long and strong, 2nd smaller; presutural setae long and strong; 2 post humeral setae, medial one strongest, lateral one often reduced to hair. Humeral callus with three setae arranged in lateral row towards anterior margin when viewed from above, with smallest medially increasing in size laterally, anteriorly rest of callus setulose. Three post-alars arranged in line parallel to anterior edge of post-alar callus, middle setae longer and stronger than other 2, at least as long and strong as anterior supra-alar (among longest and strongest on thorax). Scutellum with small apicals often reduced to one and rarely absent: long and strong sub-apicals convergent and crossing at or before tips. Laterals inserted lower than sub-apicals on lower margin of scutellum, and are approximately half length of sub-apicals, weakly convergent. Basals stronger than laterals but weaker than sub-apicals; convergent initially but curving posteriorly forming a lyre-shape, often with a more vertical aspect than apicals. Discal setae sometimes present and then often irregular and non-symmetrical. Notopleuron with two setae; anterior slightly stronger than posterior. Propleuron bare except for two setae on lower extremity, anterior setae sometimes reduced to hair, and rarely not present. One strong prostigmatic seta and one weaker setula inserted directly below, both directed outward and upward. Mesopleuron with 5 or 6 posterior marginal setae arranged in vertical row, posterodorsal region sparsely haired becoming more dense anteriorly where 2 or 3 setulae have been differentiated. Usually one but sometimes two strong pteropleural setae surrounded by patch of long slender hairs which continue in loose band towards posterior margin. 2 + 1 sternopleural setae; lower one smaller than upper two and positioned anterior to midway point between upper two, occasionally almost below anterior setae. Hypopleuron bare except row of

setae on posterior margin; barette bare; pleurotergite micro-pubescent; mediotergite bare and prosternum with 2-4 pairs of setae.

Legs. Fore-tibia with one submedial posterior seta, one dorsal pre apical seta, and one posteroventral apical seta. Mid-tibia with two submedial setae, one anterodorsal and one anteroventral, two apical setae, one anteroventral and one posteroventral, and one posterior pre-apical; hind tibia also with two submedial setae, one anterodorsal and one posterodorsal; two pre-apical setae, one anterodorsal and one posterodorsal as well as one anteroventral apical setae.

Abdomen. Abdomen without discal setae. T_{1+2} non-excavate to hind margin, with marginal setae towards outer edge of abdomen but without paired median marginal setae. T_3 with setae confined to margin, always with paired medial setae and at least one seta towards outside of tergite, sometimes an unbroken row of marginal setae. T_4 and T_5 with row of marginal setae.

Wings (Fig. 2.1d). Second costal sector haired ventrally; cell r_5 usually narrowly open, but rarely closed at margin, never petiolate; bend in vein *M* rounded, and distance to wing margin 2 times length of cross-vein *r-m*. Two or 3 setulae at basal node of r_{4+5}. Distance between bend in vein *M* and cross-vein *m-cu* conspicuously less than distance between *m-cu* and *r-m*.

Terminalia. Female postabdomen much reduced; tergite 6 complete dorsally and continuing laterally to dorso-ventral midpoint of abdomen; lateral margin notched with

two spiracles situated in membrane just below. Structure `a' in Fig. 2.1b is quite unlike any terminalia described by Cantrell (1988). It forms a sclerotised tube that is deeply and broadly incised laterally almost to the anterior margin, circumvents the terminal portion, is not fused dorsally, and houses two other sclerites (b and c in Fig. 2.1b).

Remarks. The derivations of structure `a' in Fig. 1b is uncertain. It is similar in shape and position to T_8 of *Myotrixa prosopina* and T_7 of *Hyleorus* sp. (Cantrell 1988). However, it may also be derived from St_8 as the hypogynium, as it is the last pregenital piece (of Dupuis 1963, cited in Cantrell 1988). Herting (1957, summarised by Cantrell 1988) notes that in the Goniini T_6 and T_7 are usually complete, T_8 is present, and T_{9+10} is only present in primitive forms. It is likely then, that structure 'a' is derived from T_7 (similar to *Hyleorus* sp.), and the two sclerites within are T_8 and the cerci, with T_{9+10} and the remaining sternites lost.

MALE

Same as female with the following exceptions

Dimensions: body length 4.5 mm (SE = 0.5, n = 10, range = 4.1 – 4.7 mm); wing length = 3.8 mm (SE = 0.2, n = 10, range = 3.5 – 4.2 mm); frons width = 0.54 mm (SE = 0.01, n = 10, range = 0.48 – 0.57 mm); right rear tibial length = 1.3 mm (SE = 0.3, n = 10, range = 1.2 – 1.4 mm).

Colour: Head generally darker than female, parafrontals brown to light brown, triangular patch of pigmentation below eye sometimes dark. Gena, post orbit bright grey, sometimes dark. Occiput dark, especially ventrally. Thorax generally darker than

females; scutum sometimes entirely dark, scutellum dark, sometimes with light grey posterior tip. T_{1+2} and T_3 yellow on lateral and ventral surfaces, with yellow patch extending into T_4 but only on lateral surface.

Terminalia. S_5 with 3 setulae on each lobe and partly fused with S_{6+7}, which is asymmetrical and reduced on right. T_6 fused with T_{7+8} but defined by suture line, spiracle 7 near anterior margin of T_{7+8} with a row of setae located dorsally on posterior margin. Cerci fused, pointed and same length as surstyli that are bluntly rounded. Pregonites elongate pointing downwards and curved anteriorly, bearing no setulae; postgonites rounded lobes (Fig. 2.1c).

Etymology. To the naked eye, the transverse bands on thorax and abdomen give the fly a distinctly striped appearance and the species name `*striatum*' comes from the common name `stripey' that was used to refer to the fly in the laboratory.

Notes on Biology. *Balde striatum* is a solitary endoparasitoid that has been reared from *Chrysophtharta agricola* and *Acacicola orphana* in Tasmania, and also was collected by H.F. Nahrung from *C. agricola* in Mt Bulla in Victoria (examined CRC SPF). It is ovolarviparous, depositing first instar larvae onto the underside of the host, which then burrow into the host's hemocoel where they are free living, developing without the need to maintain direct contact with the atmosphere and hence do not produce a respiratory horn. It has a fecundity of around 100 larvae $\pm$ 38 SE (estimated as instantaneous egg load, n = 5) and field collected females have an average longevity in the laboratory of 23 days $\pm$ 3.9 SE (n = 13, range = 10 - 37). Once the host and larval

parasitoid are ready to pupate, the host drops to the soil where pupation occurs. Adult flies are active over the summer period, when females are abundant within eucalypt plantations. However, males are difficult to find and may be either hilltopping or forming leks nearby.

2.4.2 *Paropsivora australis* (Macquart) (Diptera: Tachinidae: Blondeliini). This description is intended to augment the original by Macquart (1847) that is typically brief. Although the holotype was examined, many of its diagnostic features are missing, and hence I have used some recently collected specimens to improve this description. The original holotype was matched with these specimens in every character state that it still possessed.

Holotype. Female. Condition – poor. (Thorax slightly crushed; antennal segments missing; some orbital and frontal setae missing; scutellum and subscutellum severely damaged by pin; some thoracic setae missing, distal half of right wing, front and mid legs on left side missing; some abdominal setae missing). M. Bigot. Tasmania. (examined BMNH).

Female. *Dimensions.* Mean body length = 6.9 mm (SE = 0.3, n = 10, range = 6.2 to 8.2 mm); mean wing length 5.7 mm (SE = 0.1, n = 10, range = 5.1 to 6.2 mm); mean frons width = 0.56 mm (SE = 0.01, n = 10, range = 0.50 to 0.65 mm); mean right rear tibial length = 1.89 mm (SE = 0.03, n = 10, range = 1.7 to 2.0 mm).

Colour. The holotype was not used in description of colour as it is not apparent what effect nearly 160 yrs of storage has had on the original colour, and it now appears more orange/brown than the freshly collected specimens. Head pollinose; mainly bright greyish/white that changes to darker grey depending on direction of viewing; interfrontal area brown; eyes brown. Thorax grey to silver grey with five dark longitudinal vitae; medial vitae thin and presutural, other four symmetrical and continuous across suture, lateral vitae broken presuturally; vitae terminate pre scutellum; scutellum grey, darker posteriorly. Remaining thoracic sclerites bright grey. Setae black, legs grey, femur darker proximally and ventrally; tibia grey with brown or black ground colour. Abdomen patchy, tergites silver anteriorly but tending darker towards posterior margins often with patches of golden and silver iridescence.

Head. Eyes haired; ocellar triangle visible in profile; antennal axis inserted slightly above middle of eye; gena half as wide as length of third antennal segment. Epistome not strongly developed, not visible in profile, and situated below level of vibrissae. Parafrontals sparsely haired; parafacials haired in top half. Two proclinate and two reclinate orbital setae, with posterior reclinate seta smaller and more strongly curved; two strong proclinate divergent ocellar setae originating between anterior and the two posterior ocelli, remainder of ocellar triangle setulose. Paired erect and weakly proclinate medial setae situated immediately posterior to ocellar triangle; two vertical setae, innermost reclinate and longest and strongest, outermost situated lateral to inner margin of eye and curved laterally. An additional proclinate setula situated posterior to vertical setae that is initially angled posteriorly. Post-ocular row of stout setulae, weakly proclinate and stoutest medio-dorsally and becoming finer and straighter half way down

eye with alternating sized setulae in top half of row. A second less regular row of smaller setulae lies posterior to first post-ocular row, and both rows becoming more slender and straighter and extending into gena to peristomal setae. Rest of occiput setulose with straight slender white hairs. Five to seven frontal setae, cruciate posterior to antennal axis and anterior of this point reclinate and not descending below insertion of arista. Facial ridge with a row of four to seven downward curving setulae, becoming longer and stronger descending to vibrissae and continuing past vibrissae as peristomal setae.

Thorax. Acrostichals 3+3 with pre-sutural setae situated close to suture; dorsocentrals 3+3, with pre-sutural setae placed further forward of suture than pre-sutural acrostichal; post-sutural dorsocentrals increasing in size posteriorly. Intra-alars 1+3 increasing in size posteriorly; 2 supra-alar setae, with anterior seta long and strong; pre-alar small placed anterior of formost supra-alar so that pre- and supra alars form a straight row with anterior supra alar placed almost equidistant between other two setae. Pre-sutural setae long and strong; 3 post humeral setae; 4 humeral setae (occasionally five), with three placed forward of and parallel to posterior humeral suture with other seta forming apex of a triangle: 3 post-alar setae with middle one long and strong; lateral setae intermediate and medial one weakest, often with a setula proximal and medial to middle strong seta. Scutellum with small divergent apicals often with a distinct vertical aspect; long and strong divergent sub-apicals; basal setae next strongest on scutellum, convergent initially but never meeting or crossing; laterals inserted towards ventral margin of scutellum and weaker than sub-apical and basal setae; a pair of discal setulae inserted immediately anterior to sub-apicals rest of scutellum setulose with posterior

setulae strongest. Notopleuron with 2 sub-equal setae ventrally, rest setulose. 1 strong prostigmatic seta with various weaker setulae around it; mesopleuron with 6 to 8 posterior marginal setae arranged in a vertical row; one strong seta on anterio-dorsal corner of sclerite, often with at least one more proximal differentiated setula; rest of mesopleuron setulose except anterio-ventral corner which is bare. One pteropleural seta surrounded by a patch of long slender hairs. 2+1 sternopleural setae with the lower seta smallest and placed anterior to midway point between the two upper setae. Hypopleuron with a row of 5-6 setae in vertical row on posterior margin and a scattering of fine slender hairs on posterio-dorsal region; barette setulose anteriorly; pleurotergite micropubescent; mediotergite bare, infrasquamal hairs absent; prosternum setose on lateral margins.

Legs. Fore-tibia with a submedial posterio-ventral (*pv*) setae, a pair of equal prepical dorsal setae and an apical posterio-ventral seta; mid-tibia with a submedian ventral seta, 2 anterio-dorsal (*ad*) setae with the more distal of the two the longest on mid-tibia; 2 equal posterio-dorsal (*pd*) setae; apical setae as follows; paired subequal dorsal (anterodorsal [*ad*] strongest), paired subequal ventral (anteroventral [*av*] strongest), one anterior and one posterior setae. Rear tibia with a double row of well separated dorsal setae increasing in strength distally, except in the ultimate pair (pre-apicals). 1 pre-apical in the true dorsal position, 1 *ad* and 1 *av* seta.

Abdomen. Abdomen with paired discal and marginal medial setae; T_{1+2} non-excavate to hind margin, and with paired marginal setae. T_{1+2} and T_3 without a row of

marginal setae on dorsal surface but with marginal setae laterally; T_4 with a row of marginal setae.

Terminalia. T_6 pointed ventrally and complete dorsally, with two pairs of spiracles below. T_7 not complete dorsally but present as two lateral plates; T_8 absent; T_{9+10} narrow elongate plate. S_6, S_7, S_8, and S_{10} present; S_{10} reduced to narrow curved plate; cerci setulose.

Comments. Female terminalia are similar to *Paropsivora* sp. of Cantrell (1988) but also with an additional small, lightly sclerotised ventral plate between S_{10} and cerci. (labeled '?' in Fig. 2.1j). The origin of this plate is uncertain but Cantrell (1988) proposes that a similar plate on the female terminalia of another blondeliine, *Protaporia galerucae* Townsend, located in a similar position, may be detached lingulae.

Wings (Fig 2.1e) Second costal sector bare below; cell r_5 narrowly open at wing margin; Bend in vein *M* sharply angulate; 2 to 4 setulae at basal node of r_{4+5}; distance between bend in vein *M* and cross-vein *m-cu* discernibly less than distance between *m-cu* and *r-m*.

Male. Same as female with the following exceptions:

Dimensions. Mean body length = 7.7 mm (SE = 0.1, n = 10, range = 7.2 to 8.3 mm); mean wing length = 6.1 mm (SE = 0.07, n = 10, range = 5.8 to 6.4 mm); mean frons width = 0.5 mm (SE = 0.01, n = 10, range = 0.45 to 0.6 mm) mean right rear tibial length = 2.15 mm (SE = 0.04, n = 10, range = 2.0 to 2.3 mm).

Colour. Overall darker than female. Head grey to dark grey depending on direction viewed with a more yellowish aspect than female. Scutellum, abdomen darker than female T_3 with a lateral dark yellow patch that extends ventrally but does not reach margin of tergite; colour extends into T_{1+2} and T_4 laterally. Legs mostly dark grey to black.

Head. Eyes are more densely haired, have a more frontal aspect, and are larger and occupy more of the face than in the female. Parafrontals more densely haired and parafacials often with hairing descending lower but never completely haired. No proclinate orbital setae; 3 to 5 orbital setae weakly reclinate in a row that continues anteriorly as frontal setae, sometimes with some additional differentiated setulae on parafrontal area level with antennal axis. Facial ridge with 7 to 9 setae.

Thorax. Thorax often with more than 3 differentiated setulae in post humeral region.

*Terminalia.*T6-8 fused, S6+7 reduced and obsolete on right. Cerci setulose, not fused, and bluntly rounded at apex. Surstyli sparsely haired and strongly down-curved; epiphallus bluntly rounded; postgonite pointed posteriorly; pregonite gently curved

anteriorly with rounded apex, setulose distally; microstructures not visible on distiphallus (Fig. 2.1 i).

Discussion. Macquart's holotype was examined but not dissected due to the limited information in female terminalia, and because the specimen is in poor condition and would probably have been irreparably damaged by dissection. Final determination relied on matching morphological character states of the holotype with a female specimen from this study and dissecting the terminalia of male siblings and matching to the specimens that I have dissected. Comparisons of male genitalic structure were made with specimens borrowed from the BMNH (labelled as *Paropsivora australis* but without determination labels) and the ANIC (collected by Greaves in the Florentine Valley in 1964 and reared from *Chrysophtharta bimaculata*), specimens collected by de Little in 1984, and specimens from this study. All were the same species which is different to the *Paropsivora* sp. dissected by Cantrell (1988) because the terminalia of *P. australis* differ slightly from those illustrated by Cantrell and have cerci that are not fused.

This endemic Australian genus may be and may increasingly become, economically important to the expanding plantation forestry industry as it parasitises many paropsine (ten reported species so far from six genera) defoliators of eucalypts, and is in need of revision. There are four species currently described, *Paropsivora australis, P. graciliseta* (Macquart), *P. grisea* (Macquart) and *P. tessellata* (Macquart) (Crosskey 1973), and some awaiting descriptions (D. Colless, ANIC, *pers. comm.*). The genus *Paropsivora* was erected by Malloch (1934) to accommodate *P. grisea. P.*

australis was described by Macquart and placed in *Degeeria* Meigen (Macquart 1847). However the description is brief and does not distinguish this species from the multitude of species that are recognised today. Macquart also created the genus *Phorocera* in 1846, and included four species therein, *Pherocera lateralis* Macquart, *Ph. Scutelata* Macquart, *Ph. tessellata* Macquart and *Ph. tenuiseta* Macquart, adding *Ph. cilipes* Macquart, *Ph. graciliseta* Macquart, *Ph. biserialis* Macquart, and *Ph. acutangulata* Macquart in 1847 (Macquart 1846, 1847). In his major revision of the Australian Tachinidae, Crosskey (1973) synonomised *Ph. acutangulata* with *Ph. graciliseta* and placed *Ph. australis, Ph. graciliseta* and *Ph. tessellata* into Malloch's genus *Paropsivora.*

Notes on Biology. Paropsivora australis attach unembryonated macrotype eggs onto the integument of its host, have a mean fecundity (estimated as instantaneous egg load) of around 95 eggs $\pm$ 19.2 SE (n = 5; range, 37 – 142), and field collected females have an average longevity in the laboratory of 11.5 days $\pm$ 1.8 SE (n = 40, range = 2 - 47). The eggs hatch within a few days and, provided that the host has not moulted, the neonate larvae burrow into the haemocoel of the host using their sharp mouth hooks. The larvae remain just under the integument of the host with their posterior spiracles in contact with the atmosphere. A melanised ring forms around the posterior end of each tachinid larva and, as the larva grows, the ring extends inwards to form a respiratory funnel. The funnel is produced from the products of the host's immune system (Salt 1968), and allows the larva to maintain contact with fresh air (Feener & Brown 1997).

Paropsivora spp. have been reared from: *Paropsis atomaria* Olivier in the Australian Capital Territory (Tanton & Khan 1978, Tanton & Epila 1984); *Chrysophtharta bimaculata* in Tasmania (de Little 1982, de Little *et al.* 1989); and *Trachymela tinticollis* (Blackburn), *Chrysophtharta amoena* (Clark), *Paropsis geographica* Baly, *P. obsoleta* Olivier, *Paropsis* sp., *Paropsisterna picta* (Chapuis), *Trochalodes mimula* (Blackburn) and *Pyrgoides* sp. in Western Australia (Tribe 2000). *Paropsivora australis* is recorded from *C. bimaculata,* and *P. grisea* is recorded from *Paropsis atomaria* (Malloch 1934, Crosskey 1973), although this material was not examined. No hyperparasitism of *P. australis* was detected in this study.

2.4.3 *Eadya paropsidis* (Hymenoptera: Braconidae: Euphorinae) was placed into a new genus by Huddleston and Short (1978) and had previously been placed in *Aridelus* Marshall by Riek (1970) and McInnes *et al.*(1976), *Meteorus* Haliday by Wilson (1963), and *Westwoodiella* Szepligeti by Reik (1970) (Huddleston & Short 1978). However, *Eadya* Huddleston and Short possess a petiolate gaster which differentiates it from *Westwoodiella* and *Aridelus* as well as a 'large subcubic head with carinate frons and tuberculate clypeus' and 'polished punctate tergite 1' which distinguish it from *Meteorus* (Huddleston & Short 1978). *E. paropsidis* can be distinguished from its only described congener, *E. falcata* Huddleston and Short, by possessing a transverse carina on the propodeum, a breadth of face about 1.5 times the height of eye, and a straight ovipositor of the same length as T1 (Huddleston & Short 1978). There are three undescribed species of *Eadya* held in MVM: 1 x '*Eadya* sp. nr *paropsidis* det S. Shaw 1982',

collected on King Island Tasmania, 2 x '*Eadya* n. sp. det S. Shaw 1982', collected in Victoria. There were no host records for these species.

Type specimens of both described species, *E. paropsidis* and *E. falcata*, were examined at the Australian National Insect Collection (ANIC) in Canberra and are sufficiently distinctive in morphology and colouration as to not be confused with other related species. *E. paropsidis* has been collected previously from the hosts *Paropsis atomaria* (*P. reticulata*) in ACT and NSW (Huddleston & Short 1978, Tanton & Epila 1984, ANIC labels), and *Chrysophtharta bimaculata* in Tasmania (de Little 1982). *E. falcata* has been collected only from WA.

Notes on Biology. Eadya paropsidis is a solitary endoparasitoid of larval paropsine leaf beetles which injects small (length = 0.16 mm) hydropic eggs directly into the haemocoel of its host, and has a mean fecundity (estimated as instantaneous egg load) of around 975 eggs $\pm$ 68 SE, (n = 5, range = 804 – 1193). Field collected females have an average longevity in the laboratory of 13.6 days $\pm$ 1.1 SE (n = 28, range = 2 - 24). Once the eggs are deposited, they expand, by absorbing nutrients and water from the host (Jervis & Copeland 1996, Quicke 1997), and hatch in about five days at 22 °C. The first instar larva possesses well-developed mandibles that are retained for about half of the larval stage but are lost in the moult to second instar. The mandibles are probably used in competitive combat with larval tachinids and con-specifics within the host, evidenced by encapsulation around wounds of supernumerary parasitoid larvae.

E. paropsidis overwinters as pupae in the soil and adults can become abundant in the field during the summer season, and although they are relatively weak flyers males can often be seen in flight forming congregations downwind of females sitting on foliage. Mature parasitised host larvae drop to the ground to excavate a chamber and pupate, whereupon the parasitoid emerges from the host and itself pupates.

Hyperparasitoids:

2.4.4 *Mesochorus* spp. (Hymenoptera: Ichneumonidae). Two species of *Mesochorus* Gravenhorst (identified to genus by Mark Short, ANIC) were encountered during this study; one parasitised *Anagonia rufifaces* (Macquart) (Diptera: Tachinidae: Blondeliini) in *Chrysophtharta bimaculata* and the other parasitised *Balde striatum* in the primary host *C. agricola*. Both *Mesochorus* species can become abundant towards the end of the active summer season.

2.4.5 *Perilampus tasmanicus*. (Hymenoptera: Perilampidae). *P. tasmanicus* Cameron has been collected from *Paropsis atomaria* where it parasitises *Eadya paropsidis* and at least one of three species of tachinid flies in the Australian Capital Territory (Tanton & Epila 1984). In this study, only a small number of *P. tasmanicus*. were collected; some came from *E. paropsidis* and some from a mixed culture of pupae of *B. striatum* and *P. australis*. The perilampids were identified to genus by John La Salle (CSIRO Entomology). The species I encountered possessed a truncate second tooth on the bidentate mandible, and ornamentation on the third abdominal segment consistent with *Perilampus tasmanicus* - features which distinguish this from other species of *Perilampus* (Reik 1966).

2.4.6 *Meteorus* sp. (Hymenoptera: Braconidae). I found one specimen of *Meteorus* sp. (identified by A.D. Austin, University of Adelaide) in a large sample of what I thought were pupae of *E. paropsidis*, and therefore their pupae may be similar in appearance or maybe they are hyperparasitising *E. paropsidis*. Several more specimens were collected from the field but none oviposited into host larvae in the laboratory.

CHAPTER 3

Temperature and Development: Host-Parasitoid Comparisons.

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Abstract

We compare lower developmental thresholds and thermal constants (day-degree requirements) of three larval parasitoids and one hyperparasitoid with those of their host, the eucalypt defoliating leaf beetle, *Chrysophtharta agricola* Chapuis (Coleoptera: Chrysomelidae). The lower developmental thresholds and thermal constants for *Eadya paropsidis* Huddleston and Short (Hymenoptera: Braconidae), *Paropsivora australis* Macquart and *Balde striatum* Rice (Diptera: Tachinidae) were estimated at 5.9 °C, 294 DD (egg to pupa), 6.2 °C, 384.6 DD (egg to adult), and 6.3 °C, 344.8 DD (larvae to adult) respectively. Developmental thresholds of all three parasitoids were lower than their host, and all three required fewer day-degrees for generational development than their host. The developmental threshold for *Mesochorus* sp., a hyperparasitoid of the tachinid fly, *B. striatum*, was 9.7 °C which is higher than *B. striatum*, the primary host and *C. agricola*, the ultimate host. The thermal constant of 333.3 DD for *Mesochorus* sp. is also lower than its primary and much lower than its ultimate host.

A survey of the literature yielded developmental thresholds on 76 host-parasitoid partnerships, and thermal constants on 40, with nearly half of these being aphid-based systems. Overall, parasitoids had significantly higher developmental thresholds and significantly lower thermal constants for development than their hosts. In 75% of aphid-parasitoid partnerships, parasitoids had higher developmental thresholds, and in 90% parasitoids had higher thermal constants. The difference between host and parasitoid developmental thresholds were significantly different between parasitoid families, with the Aphelinidae having significantly greater thresholds than their hosts compared to the other families studied, and the Tachinidae significantly lower threshold than their hosts compared to than the other families.

3.1 *INTRODUCTION.*

Ambient temperature not only impacts on the rate of insect development (Campbell *et al.* 1974, Taylor 1981) but also on ecological interactions between species with different thermal requirements (Campbell *et al.* 1974, Frazer and Gilbert 1976, Nealis *et al.*1984). Therefore to fully understand the interactions between hosts and their parasitoids requires knowledge of their thermal developmental requirements.

Developmental thresholds of insects are both plastic and subject to selection (Gilbert and Raworth 1996, 1998), and their plasticity may enable adaptation to climatic conditions (Nealis *et al.* 1984, Kamphampati and Mackauer 1989) and to improve parasitoid synchrony with their hosts (Campbell *et al.* 1974). In variable climates, parasitoid synchrony with their hosts, especially early in the season, is essential in a successful host-parasitoid relationship (Campbell *et al.* 1974, Mackauer and Sequeira 1993) and developmental thresholds are thought to be the mechanism by which host synchrony is achieved. For example, in aphid-parasitoid systems, Campbell *et al.* (1974) reports that the threshold temperatures of aphid parasitoids were nearly always higher than that of their hosts, and speculated that this may allow the host aphid population to develop sufficiently to provide a more stable host resource for the parasitoids, especially early in the season. More rapid parasitoid development, facilitated by lower thresholds would cause the parasitoid population to peak too early to maximise the number of over-wintering individuals, a characteristic which is subject to selection (Gilbert 1984a,b). While there exists a considerable body of literature on temperature and development for parasitoids in aphid systems there is little available on

others. Aphid-based systems are characterised by cyclic population explosions and crashes and are inherently unstable (Lamb *et al.* 1987, Dixon 2000) and the relationship between the thresholds of host and their parasitoids is thought to offer potential stability to otherwise unstable systems (Campbell *et al.* 1974, Nealis *et al.*1984). Consequently, it does not necessarily follow that what holds for aphid systems will also apply to other systems, especially more stable ones, and in particular for holometabolous hosts where generational synchrony is more difficult due to pupal and adult stages not being suitable hosts. Additionally, as developmental thresholds are not fixed but subject to selection, it is likely that there are selection pressures, other than synchrony, acting on them. Traits such as parasitoid and host voltinism, specialist vs generalist parasitoids, idiobiont vs koinobiont, parasitoid host ranges, and developmental interactions between parasitoid and host, may impact on this relationship. For example, idiobiont parasitoids may delay development in smaller hosts (Chapters 5 & 7) which may ultimately affect the threshold estimates observed (e.g., Allen and Keller 1991).

Nahrung *et al.* (2004) have published development rate data on the leaf beetle *C. agricola,* and provided predictive models for forecasting population phenology. Here we provide data on the lower developmental thresholds and thermal constants of the three larval primary parasitoids and one hyperparasitoid of *C. agricola* to enable the development of phenological models of this system (Chapter 4). We also aimed to examine whether the developmental thresholds of three parasitoids utilising the same host species show similar solutions to the problem of host synchrony. To see how our data fits with host-parasitoid systems on a broader scale, and to test whether the development thresholds of parasitoids are universally higher than their host we

conducted a survey of the recent literature for host-parasitoid temperature-development data and compared the temperature and development relationship between parasitoids and their hosts.

3.2 *MATERIALS AND METHODS*

The study system. The paropsine chrysomelid *Chrysophtharta agricola* is a pest of eucalypt plantations in Tasmania, especially of two to five year old *Eucalyptus nitens* (Dean and Maiden) Maiden and *E. globulus* Labill. (Nahrung 2004). *C. agricola* larvae are present on foliage from mid Spring to early Autumn where they are parasitised by two tachinids from the tribe Blondeliini, *Paropsivora australis* and *Balde striatum* and a braconid wasp *Eadya paropsidis* (Chapter 2). The tachinid *B. striatum* is itself hyperparasitised by an ichneumonid wasp, *Mesochorus* sp. The host oviposits continuously throughout spring and summer and has four larval instars that are present simultaneously over the active season. After the fourth instar it drops to the ground where it spends several days as a pre-pupa, then pupates. The host usually has only one generation per year, although new season adults may mate and oviposit if seasonal conditions are favourable (Nahrung *et al.* 2004). However, it is not clear whether there are sufficient heat units available for this second generation to fully develop to a stage that they can successfully emerge post-winter.

The three parasitoids vary in body size and life histories. *E. paropsidis* oviposits small hydropic eggs directly into the haemocoel of the host via a piercing ovipositor, has an obligatory pupal diapause, a preference for early instars (Chapter 5) and one generation per year (Chapter 4). *P. australis* is a medium sized tachinid fly that adheres

large white anhydropic eggs on the integument of its host that hatch and burrow into the haemocoel of the host where it remains just under the integument and retains contact with the atmosphere via a respiratory funnel (Chapter 2). *P. australis* has a preference for fourth instar hosts (Chapter 6), and has at least two generations per year (Chapter 4). *Balde striatum* is a smaller tachinid that deposits first instar larvae onto the underside of its host (Chapter 2), prefers first and second instar hosts (Chapter 6), and also has more than one generation per year (Chapter 4). *P. australis* is also recorded from the host *Chrysophtharta bimaculata* (Olivier), and there are numerous rearing records for unidentified species of *Paropsivora* from paropsine leaf beetle larvae but it is not clear if they are *P. australis* (Chapter 2). Alternative hosts for *B. striatum* consist of a single rearing from *Acacicola orphana* (Erichson) (Chapter 2). *Eadya paropsidis* has also been reared from *Paropsis atomaria* in ACT (Huddleston and Short 1978, Tanton and Epila 1984), and *Chrysophtharta bimaculata* in Tasmania (de Little 1982). Little is known of the biology or host range of *Mesochorus* sp. except that it can achieve great abundance late in the season (Chapter 4).

3.2.1 Thresholds and thermal constants in this system. Host larvae were reared from eggs collected from cultures of *C. agricola* that had been established using adults collected from *E. nitens* plantations in Frankford (41°.20'S 146°.45'E) and the Florentine Valley (42°.38'S 146°.29'E), Tasmania. Up to three adult pairs were held at 22 °C and 16:8 L:D photoperiod in 500-ml cylindrical plastic tubs containing a fresh shoot of *E. nitens* foliage which protruded through the bottom of the tub into a reservoir of water. Ventilation was provided via a large hole in the lid that was covered with fine gauze material. Eggs were collected and held under the same conditions until the beetle

larvae were ready to be parasitised. The supply of eggs was augmented by egg batches collected from the field sites described above. Gravid adult females of all three parasitoid species were collected by net from both field sites on *Eucalyptus nitens* foliage. *E. paropsidis* and *P. australis* were held individually in inverted 500-ml cylindrical plastic tubs whose open base was covered in gauze. A 20-ml plastic vial containing paper wadding, water and a sprig of eucalyptus blossoms (various species) was attached to the lid by a velcro pad. The cages were held at 22 °C and 16:8 L:D until required and honey was provided to supplement the blossoms. *Balde striatum* females were held in a cage 50 cm x 50 cm x 50 cm constructed from aluminium fly-screen frames with black light-weight netting to reduce wing tattering. Water was provided via a paper towel wick inserted into a bottle of water and by daily sprays with a spray bottle. Honey was smeared into the netting panels and replaced when needed. The cage was kept on the laboratory bench at ambient temperature and photoperiod, but over peak summer temperatures an air-conditioning unit kept the maximum temperature to around 18-20 °C. *Mesochorus* sp. were either collected as gravid females from the field or reared from parasitised hosts collected from the field. Males and females were held together in cages of the same design as those used to hold *E. paropsidis.* As the parasitoids can delay their development through some host stages (Chapter 5 and 7), we used the preferred host stage of each parasitoid to estimate developmental rate, as for each species of parasitoid, the preferred host stage also caused no developmental delays.

Six female *E. paropsidis* were each exposed to second instar *C. agricola* larvae in a 100 x 25-mm glass tube, where they oviposited readily. Each host larva was introduced separately into the tube using a fine brush, and once parasitised, was removed

and placed into individual petri-dishes for rearing. Each petri-dish had a paper towel disc in the bottom and a fresh shoot of *E. nitens* foliage which was replaced every 2-3 days or earlier where necessary. The parasitised larvae from each female parasitoid were evenly distributed to controlled temperature cabinets set to 26, 23, 19, 15, and 12 $\pm$1°C, and 16:8 L:D. No parasitoids pupated at 26 °C, and our method yielded 33 *E. paropsidis* pupae evenly distributed over the four temperatures.

Twelve gravid female *P. australis* were each given fourth instar hosts on foliage in their holding cage as described above and females were allowed to oviposit freely. Parasitised hosts were removed as they received an egg and placed in individual petri-dishes with foliage for rearing. Parasitised larvae from each female were distributed evenly across controlled temperature cabinets set at 24, 21, 18, 15, and 12 $\pm$ 2 °C, and yielded 44 *P. australis* pupae distributed roughly evenly across the five temperatures.

Balde striatum would only deposit its larvae within a few days of collection and only in a large cage. Therefore, females were left in their holding cage and the second instar host larvae were introduced and exposed to multiple gravid females at once. The larvae were placed on shoots of *E. nitens* and allowed to settle before being placed in the cage for oviposition, following which they were placed in individual petri-dishes for rearing at 14, 17, 20 and 23 $\pm$ 2 °C, yielding 28 *B. striatum* pupae. *Mesochorus* sp. were also given second instar hosts in their holding cage that had previously been exposed to *B. striatum* for four hours and were allowed to oviposit for 3 hours and reared at 14, 17, 20 and 23 $\pm$ 2 °C, which yielded 12 hyperparasitoids across three temperature (none emerged from 14 °C).

Measurement of the duration of all life stages for all parasitoids was not possible: *E. paropsidis* has an obligate pupal diapause so pupal duration was not measured, and adults could not be used to determine sex; laboratory reared *B. striatum* would not oviposit so egg maturation time within the uterus was not measured, and *Mesochorus* sp develops entirely within *C. agricola* and the pupal case of *B. striatum* so only egg to adult times were measured.

Mean developmental rates (1/days) were regressed against rearing temperatures to give a more rigorous test than using the individual data points, and lower developmental thresholds and thermal constants estimated. Significance levels of differences between regression lines were determined using multiple regression and difference parameterisation.

3.2.2 Thresholds and thermal constants from the literature. We surveyed the recent literature to obtain developmental thresholds for other parasitoids and their hosts. Parasitoid data were only included if it was reared on a host for which developmental data were also available. As few studies report the thresholds of both parasitoid and host, much of the host data have been collected at different times and geographic locations to that of the parasitoid, and therefore some differences in both parasitoid and host are probable due to local adaptations to climate. It was not unusual to find more than one study on developmental rates of particular parasitoids or hosts, often from different locations, and often with different estimates of developmental thresholds and thermal constants. Single studies that covered both parasitoid and its host and were given priority

over partnerships derived from different studies, and the first report of either parasitoid or host that we encountered was included. Although our search was not exhaustive, we obtained developmental threshold data for 76 partnerships which, when grouped into families, formed sample sizes sufficient for basic analysis, and thermal constants for 10, which we considered too few for any but rudimentary overall comparisons.

There are many characters and traits that can potentially impact on the thermal relationships between parasitoids and their hosts, such as parasitoid and host voltinism, generalist vs specialist, idiobiont vs koinobiont, and stage of host attacked. Furthermore, phylogenetic relatedness can mean that a lack of statistical independence exists between species within family groups (Harvey and Pagel 1991, Freckleton *et al.* 2002) raising issues of pseudoreplication. As there are no suitable phylogenies published for the hymenopteran families used here that may be used to control for the lack of independence between our species, rigorous analysis between parasitoid and host would require phylogenetic correlation and meta-analysis which is beyond the scope of this work. Therefore, our analysis was kept to a minimum, and we used paired t-tests to test whether parasitoid thresholds and thermal constants were significantly different to their host. However, we held to the view that it was valid to gauge the level that phylogenetic thermal constraints may be acting at a family level in parasitoids, by comparing the variation in thermal differences within families compared to the variation across all comparisons. To do this we calculated the absolute value of the difference in the lower developmental thresholds for each partnership and compared the coefficient of variation (CV) for each family for which we obtained more than five partnerships. We used

ANOVA to look for any effect of parasitoid family on the difference between host and parasitoid

developmental thresholds, and used LSD pothook tests to discriminate differences between families. Lastly, because aphids and their parasitoids have featured so prominently in the literature (29 of the 74 partnerships involved aphids), we used the host family Aphididae to briefly examine the thermal relationship between aphids and their parasitoids.

3.3 *RESULTS.*

3.3.1 Thresholds and thermal constants in this system. The lower developmental thresholds for all of the immature stages that we measured combined together for each of three primary parasitoids were within 0.4 °C of each other (Table 3.1). The developmental threshold for *E. paropsidis* (egg to pupation) is estimated at 5.9 °C, *B. striatum* (larviposition to adult eclosion) at 6.3 °C, and *P. australis* (oviposition to adult eclosion) at 6.2 °C. These thresholds were almost 2 °C below that of their host *C. agricola* at 7.8 °C (egg to adult eclosion). The hyperparasitoid *Mesochorus* sp. however, had a threshold of 9.7 °C that is higher than both its primary host (400 DD) *B. striatum* and the ultimate host, *C. agricola*. The thermal constants were also all lower than that of their host, with *E. paropsidis* at 294.1 DD (not including pupae), *Mesochorus* sp. 333.3 DD, *B. striatum* 344 DD (not including egg development) to *P. australis* at 384.6 DD (Table 3.1).

Table 3.1 *Developmental thresholds and day degree requirements for* Chrysophtharta agricola *and three of its larval parasitoids,* Balde striatum, Paropsivora australis, Eadya paropsidis, *and the hyperparasitoid* Mesochorus sp.

	Regression equation	R^2	Developmental threshold (°C)± SE	Thermal Constant ± SE
*Chrysophtharta agricola**				
Eggs	$y = -0.154 + 0.0180x$	0.996	8.6 ± 0.5	55.6 ± 3.7
Larvae	$y = -0.034 + 0.0042x$	0.995	8.2 ± 0.2	238.1 ± 9.6
Pupae	$y = -0.065 + 0.0089x$	0.992	7.2 ± 0.2	112.3 ± 5.6
Egg – pupa	$y = -0.028 + 0.0034x$	0.993	8.2 ± 0.3	294.1 ± 13.0
Larva – adult	$y = -0.023 + 0.0029x$	0.995	7.9 ± 0.2	344.8 ± 13.1
Egg – adult	$y = -0.020 + 0.0025x$	0.994	7.8 ± 0.2	400.0 ± 17.6
Balde striatum				
Larvae	$y = -0.027 + 0.0059x$	0.972	4.6 ± 0.2	169.5 ± 19.8
Pupae	$y = -0.050 + 0.0063x$	0.975	8.0 ± 0.3	158.7 ± 17.9
Larva – adult	$y = -0.018 + 0.0029x$	0.997	6.3 ± 0.2	344.8 ± 23.3
Paropsivora australis				
Egg – pupa	$y = -0.035 + 0.0064x$	0.991	6.1 ± 0.1	149.0 ± 6.9
Pupae	$y = -0.029 + 0.0043x$	0.996	6.7 ± 0.3	232.6 ± 10.3
Egg – adult	$y = -0.016 + 0.0026x$	0.999	6.2 ± 0.2	384.6 ± 3.5
Eadya paropsidis				
Egg – pupa	$y = -0.020 + 0.0034x$	0.987	5.9 ± 0.2	294.1 ± 22.5
Mesochorus sp.				
Egg – adult	$y = -0.029 + 0.0030x$	0.869	9.7 ± 0.4	333.3 ± 4.2

* Data from Nahrung *et al.* (2004)

The tachinid *Balde striatum* developed fastest overall of all the parasitoids in the guild (Fig. 3.1a). Both tachinids developed significantly faster from oviposition to adult emergence than their host *C. agricola* and the hyperparasite *Mesochorus* sp. across the temperature range tested. Furthermore, the regression line from oviposition to adult emergence for *Mesochorus* sp. and its ultimate host, *B. striatum* also differed significantly (F-ratio = 3338, df = 6, $P << 0.001$). For the interval from oviposition to pupation, *E. paropsidis,* and both of the tachinids developed significantly faster than their host, *C. agricola* (F-ratio = 1707.7, df = 5, $P << 0.001$) (Fig. 3.1b), indicating that at least one parasitoid larval generation is likely to be possible for all three parasitoids in the guild within the hosts larval development time. Once pupated though, both tachinid species' pupae developed significantly slower than that of their host (F-ratio = 785.2, df = 4, $P < 0.000001$) (Fig. 3.1c).

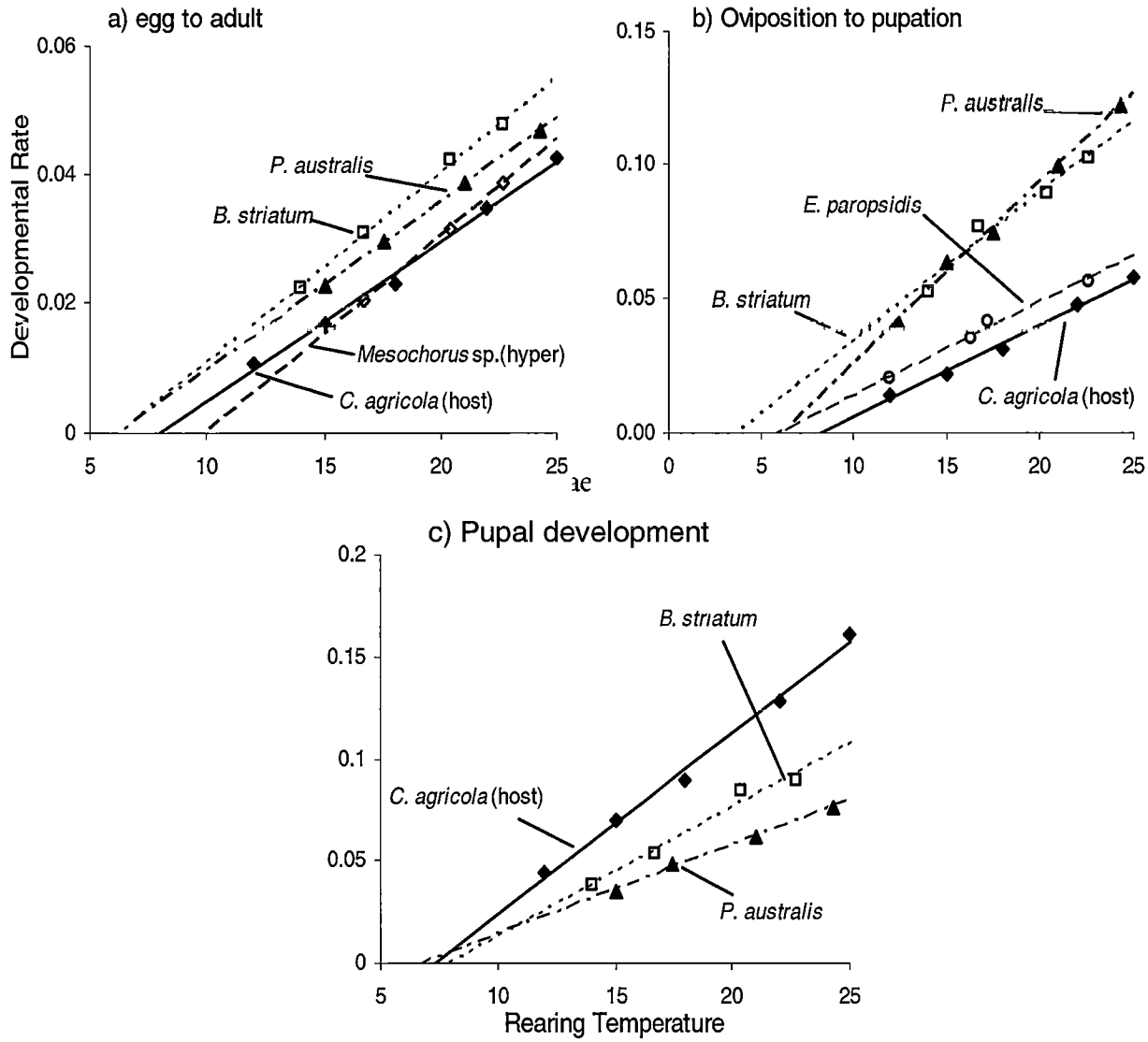

Figure 3.1 *Developmental rates vs. temperature for a) egg to adult, b) oviposition to pupation and c) pupal development, for the primary larval parasitoids* E. paropsidis, P. australis *and* B. striatum, *the hyperparasitoid of* B. striatum, Mesochorus sp. *and their host* C. agricola.

Thresholds and thermal constants from the literature.

There was a significant correlation between the lower developmental thresholds of parasitoid and their hosts (Pearsons $r^2 = 0.41$, $\chi^2 = 38.5$, df = 1, $p < 0.001$) (Fig. 3.2). The difference between host (mean = 7.8 °C) and parasitoid (mean = 8.8 °C) developmental thresholds was significant (t = 2.98, df = 73, p = 0.004). In 39 of the 74 partnerships examined, the parasitoid had higher developmental thresholds than their hosts, in 18, the host had higher thresholds, and in 17 the thresholds were equal (within ± 1 °C of each other).

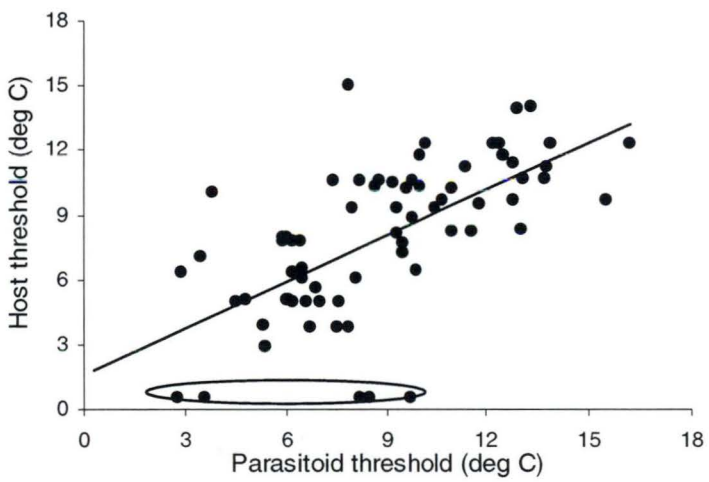

Figure 3.2 *Lower developmental thresholds (ldt) of parasitoids vs ldt of their host. Data sourced from literature. The five outliers (circled) are from the same host species (see appendix 1).*

Parasitoids of aphids had mean lower threshold temperature of 6.6 °C which was significantly (t = 4.05, df = 28, p = 0.004) higher than their hosts', at 4.5 °C. In non-aphid systems, parasitoid (10.2 °C) and host (10.0 °C) were not significantly different (t

= 0.61, df = 44, p = 0.54) (Fig. 3.4). Of the 29 parasitoids attacking aphids that we examined, 22 had higher thresholds than their aphid hosts, seven had thresholds lower than their hosts and in one partnership thresholds were equal.

The difference thermal constants between host (mean = 340 DD) and parasitoid (mean = 262 DD) was also significant (t = -2.29, df = 43, p = 0.026). In 21partnerships hosts had higher thermal constants, in 17, the parasitoids required more heat for development and in two, thermal constants were equal. Of the 17 aphid-based partnerships that we obtained thermal constants for, 15 had parasitoids with higher thermal constants than their hosts, and two had lower.

Parasitoid family had a significant effect on the difference between developmental thresholds of parasitoids and their host (F-ratio = 4.95, df = 5, p = 0.0008). The Aphelinidae had mean thresholds that were 4 °C above that of their hosts which was significantly higher than the other families tested , but the Tachinidae were on average 2 °C lower than their hosts which was significantly different from other families tested except the Eulophidae (Fig. 3.3). The CV for the parasitoid families were around the same order of magnitude as the overall CV (Table 3.2), indicating that pseudoreplication on the basis of phylogenetic relatedness did not compromise the validity of these findings.

Table 3.2 *Coefficients of variation (CV) for five families of parasitoids calculated from the absolute value of difference between developmental thresholds between parasitoid and their hosts as reported in Appendix1. (n's are the same as in Fig. 3.3)*

Parasitoid Family	Family CV	Overall CV
Aphlelinidae	0.709	0.890
Braconidae	0.656	
Eulophidae	0.313	
Pteromalidae	0.888	
Tachinidae	0.767	

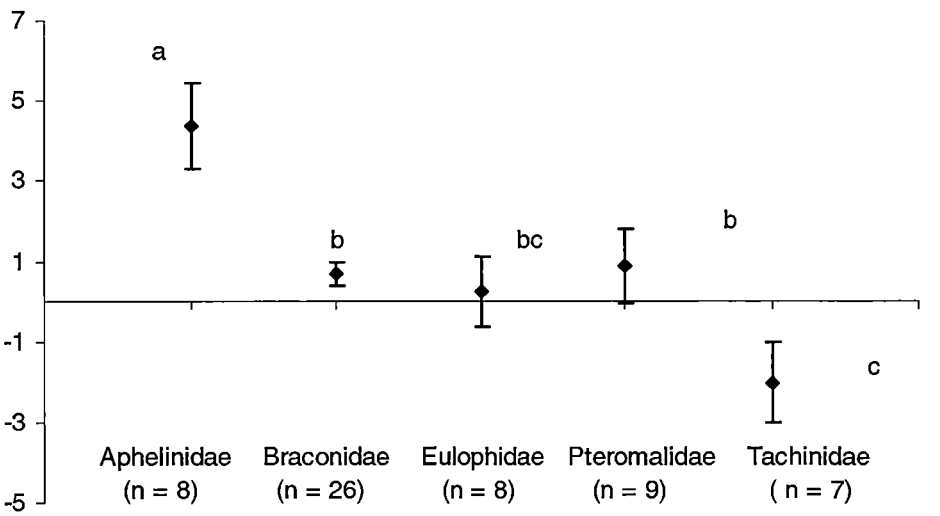

Figure 3.3 *Mean difference in lower developmental thresholds between parasitoid families and their hosts ± SE, calculated by subtracting host thresholds from parasitoid thresholds as reported in Appendix1. Those data points adjacent to the same letter are not significantly different (LSD).*

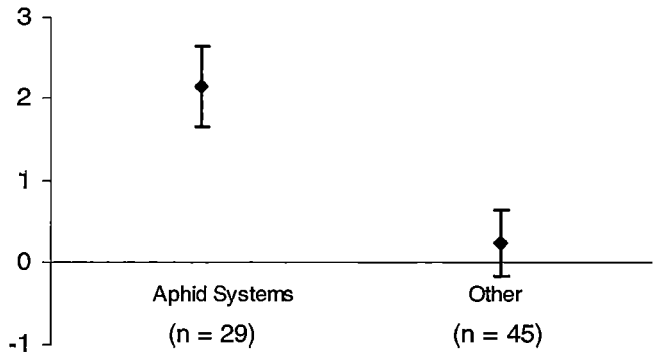

Figure 3.4 *Mean difference in lower developmental thresholds between aphids and their parasitoids ± SE, calculated by subtracting host thresholds from parasitoid thresholds as reported in Appendix1.*

3.4 *DISCUSSION.*

The thresholds and thermal constants of *E. paropsidis*, *P. australis*, and *B. striatum* were all lower than those of their host. However, although the threshold of the hyperparasitoid *Mesochorus* sp. was higher than both its primary host, *Balde striatum* and its ultimate host, *C. agricola*, its thermal constant was different in being higher than its primary host but lower than its ultimate host. Furthermore, the parasitoids' having lower thresholds than their host would allow them to continue to develop when the ambient temperature is too low for the host to develop, and when coupled with a lower thermal constant, may enable the primary parasitoids to be multivoltine on the essentially univoltine host.

Nahrung *et al.* (2004) report a drop in developmental thresholds for every increase in instar for the host, *C. agricola* with instars 1, 2, 3 and 4, having thresholds of 10.3, 8.9, 7.8, and 2.8 °C respectively. It is in the fourth instar that the host feeds most

voraciously and is when the greatest (80%, Rice unpublished) increase in body size occurs and when most resources are therefore accumulated that parasitoids can exploit. Campbell *et al.* (1974) suggest that the declining thresholds of *Pieris rapae* (L.) (Lepidoptera: Pieridae) ensures that early instars don't 'outgrow' their host plant early in the cool start of the season and cold weather cannot prevent development completion. *C. agricola* emerges from diapause when the average field temperatures are roughly equal to their lower thresholds, as predicted by Gilbert and Raworth (1996), and begins ovipositing well before there is sufficient new flush of foliage to support large larval populations (*Pers. obs.* see also Paterson *et al.* 2003). Therefore, slow development early in the season facilitated by thresholds close to ambient temperature, may as with *P. rapae*, ensure a reliable food supply is present to complete larval development.

The developmental relationship between host and parasitoid can also be affected by host developmental stage. Allen and Keller (1991) reported differences in both thresholds and thermal constants for the braconid wasp *Cotesia urabae* reared through small and mid-sized larvae of its noctuid host *Uraba lugens*. Also, Greenburg *et al.* (2000) reports an increase of over 3 °C in threshold temperature and a decrease of over 100 DD for the aphelinid *Eretmocerus eremicus*, Rose and Zolnerowich parasitising different host species. The developmental thresholds of all three primary parasitoids fall below those of the first three instars but above that of the fourth. *P. australis* is a relatively large tachinid fly and has a strong preference for fourth instar hosts which it parasitises up to five times more frequently than any of the other three instars (Chapter 6). With a preference for fourth instars and larval threshold of 6.2 °C compared to 2.8 for fourth instar hosts, *P. australis* will no not be able to grow too fast and kill the host

before it maximises the resources available for parasitoid growth. For *B. striatum* and *E. paropsidis,* by having lower thresholds than their preferred (early) host instar they will be able to develop more rapidly than their host while the parasitoids are small, but when the parasitoids themselves reach maturity they are constrained from out growing their hosts by developmental thresholds that are higher than their hosts'.

Our survey of host parasitoid thermal relationships generally supports the claim by Campbell *et al.* (1974) that parasitoid thresholds are higher than that of their hosts. Moreover, in aphid-parasitoid systems, as was used by Campbell *et al.* (1974), nearly 75% of parasitoids had higher developmental thresholds, and almost 90% had greater thermal constants than their hosts, which would cause the parasitoids to develop more slowly than their hosts throughout the season. The higher developmental thresholds for parasitoids compared to their hosts should allow the host population to gain a 'head start' early in the season and impart stability to the host-parasitoid relationship (Nealis *et al.* 1984). Such stability is particularly important on a local scale for parasitoids with narrow host ranges, because host synchrony requires parasitoids to cease development or utilise alternative hosts as host abundance declines (Mackauer and Sequeira 1993). Aphid phenology is characterised by rapid growth rates (Lamb *et al.* 1987) and, often localised, population build-up and crashes (Dixon 2000) - an inherently unstable system to utilise as a resource. If slower development by parasitoids does impart stability to such systems it may explain our findings. However, outside aphid systems the data reveal a different pattern altogether, with parasitoids generally having lower thresholds and lower thermal constants than their hosts, and therefore generally developing at the same rate or faster than their hosts, particularly the Tachinidae. Without more data,

explaining these patterns is premature, but the Tachinidae have a diversity of life-histories unmatched in other parasitoid groups, particularly the ubiquitous Hymenoptera (Chapter 6). Perhaps we should expect distantly related parasitoids to behave differently, and more data are required to reveal how host-parasitoids relationships are affected by temperature, to fully understand the parasitoid life style.

CHAPTER 4

Phenology and Host Synchrony of the Larval Parasitoid Guild of the Southern Eucalypt Beetle

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Abstract

Host-parasitoid synchrony is essential in parasitic relationships, and knowledge of parasitoid phenology is fundamental to understanding the population dynamics of host-parasitoid systems. Here, we examined the phenology and host synchrony of three larval parasitoids of *Chrysophtharta agricola* Chapuis (Coleoptera: Chrysomelidae) - *Eadya paropsidis* Huddleston and Short (Hymenoptera: Braconidae), *Paropsivora australis* (Macquart) and *Balde striatum* Rice (Diptera: Tachinidae), and one hyperparasitoid, *Mesochorus* sp. Cameron (Hymenoptera: Ichneumonidae). We used a variety of techniques to monitor the emergence of parasitoids, as well as diapause behaviour, voltinism, and abundance at two sites in Tasmania. Ambient field temperatures were used to measure heat unit accumulation by the parasitoids, and to test for thermal constraints on voltinism. We also tested different events in the phenology of the system as starting points for heat unit accumulation to find the best correlation between predictive models from Chapter 3, and our phenological data collected here.

E. paropsidis and *P. australis* both overwinter as pupae in the soil below the natal tree, but *B. striatum* does not. *E. paropsidis* and *B. striatum* were the most abundant species achieving up to 30 and 47% parasitism respectively. These two species displayed temporal partitioning with *E. paropsidis* becoming abundant early in the season and targeting the host population in its first and main peak in abundance, and *B. striatum* becoming abundant later in the season and targeting the second and smaller peak in host population. There were no thermal constraints on more than one generation within the host's field active larval period for any of the parasitoid species, but *E. paropsidis* has an obligate diapause and only one generation per year. The two tachinid flies both had second generations but *B. striatum* adults always emerged in the same season even when pupation was late in the season. *P. australis* was bet hedging, with approximately half of the population emerging same-season, and half remaining as pupae in the soil until the following season.

4.1 *INTRODUCTION*

Parasitoids that either emerge from diapause too early or do not cease reproduction as their host population is in decline at the end of the season may have insufficient host to maximise lifetime reproductive success. However, perfect synchrony can have a destabilizing effect on parasitoid interactions, and both phenological and spatial asynchrony offer refuges for hosts that ensure their persistence as a resource (Munster-Swendsen and Nachman 1978, Godfray *et al.* 1994, van Nouhuys and Tay 2001). Nevertheless, some degree of phenological synchrony is essential for successful parasitic relationships (Campbell *et al.* 1974, Mackauer and Sequeira 1993), and the degree of synchrony can directly affect parasitoid population size (van Nouhuys and Lei 2004), and their impact on host populations (Weseloh 1976). Parasitoids with wide host ranges may not necessarily show good synchrony with any particular host but phenological constraints on specialists that target particular stages of their host may be tight.

For parasitoids that diapause over winter the timing of emergence to coincide with the presence of susceptible host stages synchronises them with their hosts (Godfray 1994). Many insects that diapause over winter emerge when the average field temperature is roughly equal to their lower developmental thresholds (Campbell *et al.* 1974, Gilbert 1988). Consequently, insect herbivores tend to develop slowly immediately after diapause which gives host plants a 'head start' early in the season and ensures adequate supply of foliage (Gilbert and Raworth 1996). Similarly, parasitoids often have higher thresholds than their hosts (Chapter 3), and develop more slowly than

their hosts, which may provide stability to host-parasitoid interactions, especially in inherently unstable aphid systems (Campbell *et al.* 1974, Nealis *et al.*1984).

By having more than one generation per year, parasitoids can increase the number of over-wintering individuals in a population, which makes voltinism an important aspect of host-parasitoid population dynamics. Potential for second generations of parasitoids may be constrained by persistence of hosts, which may not be as critical for generalists, and availability of field heat units. Although insect developmental rate is governed largely by ambient temperature, insects can regulate their body temperature and hence developmental rate via behavioral, physiological and morphological traits (May 1979, Heinrich 1993). Dark coloured lepidopteran larvae can significantly increase their body temperatures above ambient by basking in the sun (Stamp and Bowers 1990, van Nouhuys and Lei 2004) and Maddox (1995) showed that similar behaviour in another paropsine, *Paropsisterna tigrina* Chapuis, can increase its body temperature by up to 8 °C above ambient by basking (cited in Clark 1998). Because parasitoids experience the same temperature as their hosts, any increase in host body temperature can translate into increased developmental rate for the parasitoid (Gould and Elkington 1990).

The work reported here follows on from Chapter 3 where we determined the relationship between temperature and developmental rate of the larval parasitoid guild of the paropsine chrysomelid *Chrysophtharta agricola.* The guild is comprised of two tachinid flies, *Paropsivora australis* and *Balde striatum,* a braconid wasp *Eadya paropsidis*, and hyperparasitoid *Mesochorus* sp that parasitises *B. striatum* . Our first

aim was to determine the phenology of the parasitoids in terms of timing and spread of emergence from diapause, voltinism and the degree of synchrony that they displayed with their host as the host population fluctuated over the season. Secondly we aimed to test the predictive phenological models developed in Chapter 3 by using data loggers to measure heat unit accumulation in the field and test the coincidence of predicted versus realized, phenological events.

4.2 MATERIALS AND METHODS

4.2.1 The Study System. The paropsine host, *Chrysophtharta agricola* feeds on juvenile eucalyptus foliage where it oviposits continuously throughout its active summer season. *C. agricola* undergoes four larval instars that are usually present simultaneously on foliage from mid November to mid March (Nahrung and Allen 2004). There are generally two population peaks of immature stages, with the first one early in the season, around November to December being the largest, with another minor peak late in the season, around late January to February (Nahrung and Allen 2004). Depending on environmental conditions newly emerged adults may mate and lay eggs resulting in the late season peaks in larval abundance being more substantial (Nahrung *et al.* 2004). Mature larvae drop to the ground where they construct a chamber in the soil or leaf litter and pupate.

Eadya paropsidis is a solitary endoparasitoid that oviposits small hydropic egg directly into the haemocoel of its host. First instar larvae possess well developed mandibles which are lost at the first larval moult. Beside *C. agricola*, *E. paropsidis* has

also been reared from *C. bimaculata* in Tasmania (de Little 1982) and *Paropsis atomaria* Ol. (Tanton and Epila 1984) on mainland Australia. *Paropsivora australis* is a medium sized tachinid fly (ca. 7- 8 mm in length) that adheres large white anhydropic eggs on the integument of its host which hatch within two or three days and burrow into the haemocoel of the host. *P. australis* has a strong preference for fourth instar hosts (Chapter 6) and has also been reared from several other paropsines (chapter 2). *Balde striatum* is a smaller tachinid fly than *P. australis* (ca. 5mm) and deposits first instar larvae onto the underside of its host. It has a preference for early host instars (Chapter 6) and there is a single record of rearing from *Acacicola orphana* (Erichson) (Chapter 2).

4.2.2 Field Monitoring. The majority of field work was carried out at two Tasmanian field sites - one in the Florentine Valley and the other at Frankford over two summer seasons of 2000/01 and 2001/02. In the first season, in 2000/01, small numbers of emergence traps were also set up in two other *E. nitens* plantations located near Ellendale (42° 37' S 146° 42' E) and Tyenna (42° 43' S 146° 40' E) in southern Tasmania.

Site 1: Florentine Valley (42° 38'S 146° 29'E) Elevation 400m. Coup # FO31VI. *Eucalyptus nitens* planted in 1996; mean annual maximum temperature 16.2 °C and mean minimum temperature 5.2 °C (40 year average) (Nahrung and Allen 2004). The mean monthly rainfall between December 1999 and March 2002 was 96.7 ± s.e.10.8 mm (range 24 – 241.3 mm) (Nahrung and Allen 2004). The site was bordered on one side by native forest, on the second by an older *E. nitens* plantation, on the third by a road and logged native forest and on the last by an *Acacia dealbata* plantation.

Site 2: *Frankford.* (41° 20'S 146° 45'E) Elevation 240 m. *Eucalyptus nitens* planted in 1996; mean maximum temperature 16.9 °C and mean minimum temperature 4.6 °C (130 year average) (Nahrung and Allen 2004). The mean monthly rainfall between December 1999 and March 2002 was 79.6 ± s.e.9.5 mm (range 18.6 – 213.2 mm) (Nahrung and Allen 2004). Bordered by older *E. nitens* trees and a 1999 planting of *E. nitens*. In the summer season of 2001/02 the study site was expanded to include trees planted in 1999, which were further bordered by older plantation trees.

Early season emergence. The emergence of parasitoids from the soil at the beginning of the season was monitored by the use of 'tent' traps (Fig. 4.1a). The tent trap was constructed of four triangular panels of green or white insect netting sewn together to form a pyramid. The traps measured 1 m by 1 m at a point 10 cm from the bottom edge. The bottom 10 cm was buried directly downwards into the soil so that the trap covered 1 m^2 of soil surface. At the apex of the trap was an opening into which a flexible plastic tube was inserted and held in place with plastic electrical ties. A two litre plastic bottle was fixed to the other end of the tube on its side containing about 50-ml of 70% ethanol. Emerging insects moved up the sides of the tent and into the ethanol in the bottle where they were preserved for collection. Traps were fenced, sited under the canopy of *E. nitens* trees that were known to have been attacked the previous year by *C. agricola*, and cleared, with the ethanol replaced, every two weeks. We set up 5 traps per site, in September of each year, except in 2001/02 in Frankford where we added another five traps to include a 1999 planting of *E. nitens* that was heavily attacked by *C. agricola* the previous year to give us a total of 25 traps over two years. Traps were

erected in different locations to those of the previous year, and were monitored until the end of the season in April of the following year.

Same season emergence. The emergence of a second generation of parasitoids within the season was examined using 'bucket' traps (Fig. 4.1b). The bucket trap was also used to confirm diapause and bet hedging of emergence times as well as temporal changes in bet-hedging as the season progressed. As soon as late fourth instar larvae were present in the field, approximately 200 were collected and half placed into the bucket trap, and half taken back to the laboratory for rearing to determine parasitism levels. Larvae were reared in the same manner as for field parasitism monitoring, described below.

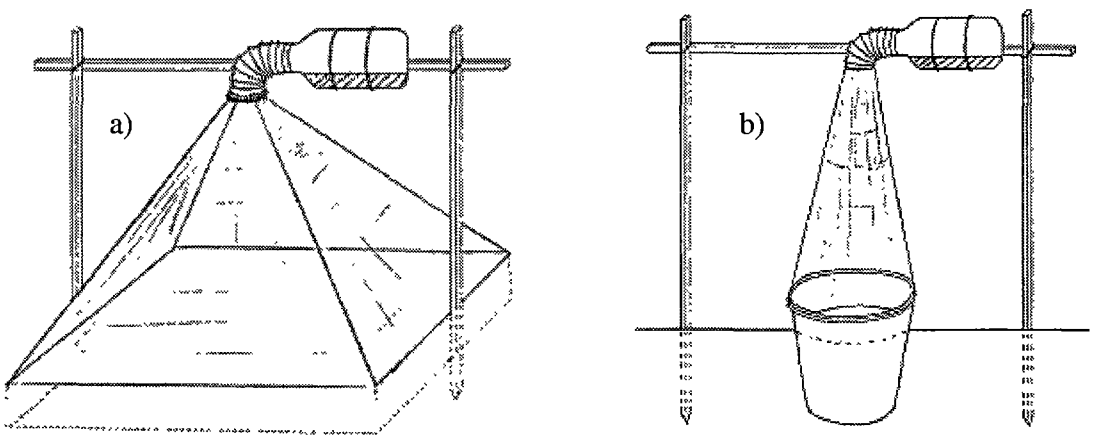


Figure 4.1 *Two types of emergence traps used to monitor emergence of adult parasitoids. a) tent trap, used to monitor early season emergence from diapause. b) bucket trap used to monitor same-season emergence and diapause/emergence bet hedging. Scale bar = 1 m.*

The bucket trap consisted of a plastic 10 L bucket with ten 8 mm holes drilled into the bottom for drainage and lined with fine nylon mesh to exclude ants. The bucket was half filled with soil collected from outside the plantation to ensure that it contained no parasitoid pupae. This arrangement was then buried halfway into the soil and 100 late 4^{th} instar/prepupae placed on the soil surface. Larvae were collected for this experiment by placing a container under a cohort of fourth instars and touching them lightly. Those larvae ready to pupate dropped from the foliage into the container below. By using this method, the larvae placed on the soil in the bucket trap immediately burrowed beneath the surface. A conical net was placed over the top of the bucket and held in place with a draw-string. Emerging insects were collected in alcohol in the same manner as the tent trap and any parasitoids collected in the same season indicated a new generation. In the first field season, one trap was set up at Florentine Valley, and another two in plantations at Ellendale and Tyenna. In the second season we set up traps only at Florentine Valley and Frankford, but set them up every two weeks provided sufficient late fourth instar larvae were available. The first traps were erected as soon as late fourth instars were available in the field. By setting up these traps every two weeks, we aimed to detect temporal changes in bet-hedging, i.e., the proportion of parasitoids that were emerging in the current season versus the proportion that were entering diapause to emerge next season. Twelve bucket traps in total were erected and monitored every 2 weeks over the two seasons.

Field parasitism. Parasitoid oviposition activity was monitored by collecting host larvae, rearing them in the laboratory and scoring them for species emerging. Five cohorts of each of the four instars (if they were available) were collected every two

weeks at each field site for the entire season (November – March) resulting in 2515 larvae from 97 cohorts (mean size = 26, range 4 – 85) from Florentine Valley, and 2730 larvae from 119 cohorts (mean size = 22, range 5 – 100) from Frankford. Cohorts were collected without conscious bias throughout the plantation but selection was not randomised to a strict protocol. Only one cohort was sampled from the same tree on the same day, all larvae on a shoot were considered to be from one cohort and only cohorts consisting of a single instar were collected.

Collected larvae were held in plastic bags and placed in a cooled icebox on warm days for transport back to the laboratory. In the laboratory larvae were counted and placed in 50 or 100-ml plastic containers depending on cohort size and instar. A paper towel was moistened with a few drops of water and placed on the bottom of the container along with sufficient *E. nitens* to last three days, after which time it was replenished. Ventilation was provided via a 5 cm hole in the lid of the container covered with a perforated plastic film. Larvae were reared at 22 ^{O}C and 16L:8D photoperiod, and parasitism rates noted. We calculated mean percent parasitism across all cohorts and all instars (for parasitism rates of each instar see chapters 5 and 6).

Adult population monitoring. Parasitoid population levels were monitored using Malaise traps as they are recognised for their efficiency for sampling tachinid flies (Belshaw 1993). We set one up at each site in the 2001/2002 season, which were cleared fortnightly. The Malaise traps were erected between the rows of trees within the plantation.

4.2.3 Field Heat Units. A Tiny Tag [tm] data logger was placed at both field sites over the two seasons of 2000/01 and 2001/02. They were suspended 1.5 m above the ground within the canopy a *E. nitens* tree covered with an inverted plastic 10 litre bucket and recorded ambient temperature. Logistical difficulties resulted in the data logger being deployed two weeks after field activity began in 2000/01 at Frankford, and loggers were removed at the end of field activity. In 2001/02, when the data indicated that *B. striatum* may still be accumulating heat units well after most activity in the field had ceased, loggers were left for longer, until April. We calculated average temperatures at both sites for both field seasons for December, January and February for making general site and season comparisons.

Field day degrees were determined by the formula

$$DD \text{ per day} = \Sigma[(T_1 - t)/24]$$

where T_1 = hourly temperature reading and t = lower developmental threshold (determined in chapter 3).

To determine whether there were sufficient day degrees available for more than one generation per year, and to predict the time of emergence at each field site of each parasitoid species, temperature readings that were above the lower threshold for each species were summed until day degree requirements for each parasitoid was reached. We used the lower developmental thresholds of 5.9 °C, 6.2 °C, an 6.3 °C, and thermal constants of 294 DD, 385 DD, and 345 DD for *E. paropsidis, P. australis* and *B. striatum* respectively. For parasitoids that diapause as pupae we did not include pupation

times for the second generation in this calculation. We used several starting points for day degree accumulation, in order to test which gave the best prediction of second generation activity. Because *P. australis* emerges from diapause before hosts are abundant and *B. striatum* does not emerge in the plantations and we don't know when it emerges, we used starting points based on host phenology. The first starting point was the first appearance of larval *C. agricola* in the field (from Nahrung *et al.* 2004), the second was the first appearance of the parasitoids' preferred host stage, which was first instars for *B. striatum* and fourth instars for *P. australis* (Chapters 6), and the last was the first detection of parasitism of cohorts during larval collections described earlier. However, *E. paropsidis* emerges when hosts are abundant and the population is already composed of all four instars, and therefore we used the time of peak parasitoid emergence and peak parasitism as starting points for accumulation. We used an end point of mid March in the first season and the end of April in the second season which roughly corresponded to the end of *C. agricola* larvae in the field..

To assess whether the two seasons in which we monitored the parasitoid populations, were typical of past summer seasons, temperature data were obtained from the Bureau of Meteorology for mean summer temperatures for the past 120 years, and compared to our seasons. Although the data obtained were for Hobart, it does give an indication of long-term summer temperatures and how our two monitoring seasons fit into the long-term patterns.

We used data on host larvae abundance and population structure provided by H.F. Nahrung (Fig. 4.2) who sampled the same sites in the same seasons, to compare

parasitoid activity patterns with host abundance, in order to assess synchrony between parasitoids and their host.

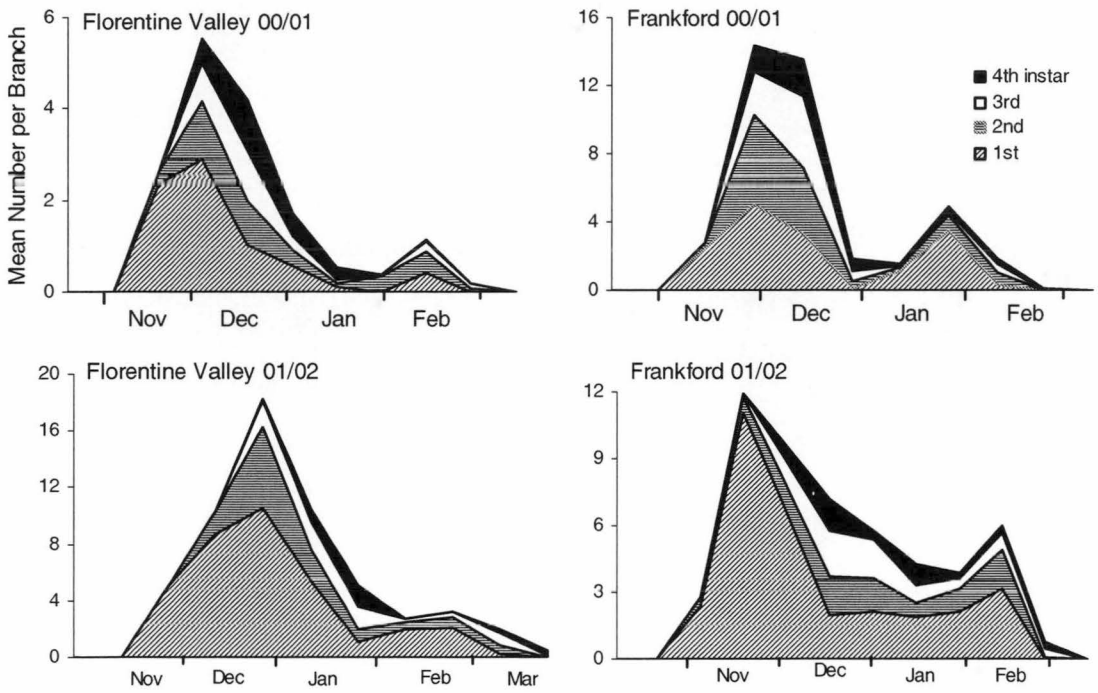


Figure 4.2 *Abundance and population structure of larval* Chrysophtharta agricola *over the two seasons at Florentine Valley and Frankford. Courtesy of H.F. Nahrung*

4.2 *RESULTS*

4.3.1 Field Monitoring. *Early Season Emergence. Eadya paropsidis* and *P. australis* were the only parasitoids collected in the tent traps, and we only detected them in Frankford. Both species emerged from diapause early in the field season during a relatively narrow window (Fig. 4.3a,b). *E. paropsidis* emerged in November and early

December in both seasons, which coincides with the peak host population, and also when it is largely comprised of first and second instars (Fig. 4.2), the preferred host stage of *E. paropsidis*. *P. australis* was only detected in the second season, and emerged earlier than *E. paropsidis* with first appearance seen by October 8th which is nearly one month before any hosts were available for parasitism, and nearly two months before its favoured and more suitable fourth instar hosts were available in the field. However, parasitism by *P. australis* was not detected until four weeks after adult emergence had finished at this site, though this time also corresponds with the season's peak in its preferred fourth instar hosts. At Florentine Valley, no emergence was detected, but peak adult population of *P. australis* (detected via Malaise traps) roughly corresponded to the time when its preferred fourth instar hosts were most abundant. However, parasitism levels detected by larval collections remained low.

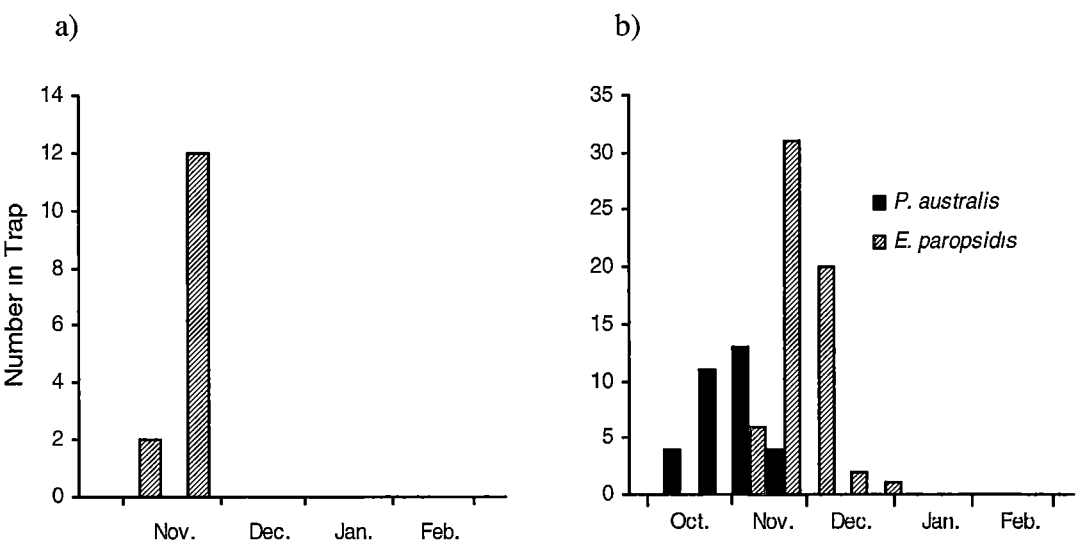


Figure 4.3. *Total early season tent trap catches of* Eadya paropsidis *and* Paropsivora australis *at Frankford, Tasmania in a) 2000/01(n = 5) and b) 2001/02 (n = 10) summer seasons. No parasitoids were caught at Florentine Valley (n = 10). Traps were deployed in September each year.*

Same season emergence. In the first season, only *B. striatum* and its host *C. agricola* were recovered from the traps, and all emergence was from within the same season (Fig. 4.4a). However, in the second season where more traps were set up, all three species of parasitoids and their host were collected (Fig. 4b). *E. paropsidis* only emerged post-winter, which corroborated the obligate diapause observed in the laboratory (Chapter 3).

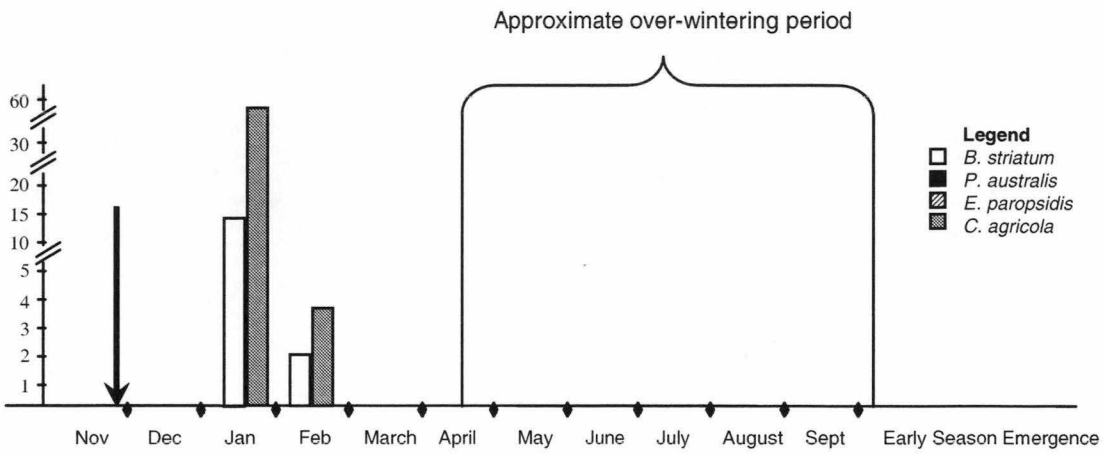


Figure 4.4(a) *Same season emergence of the larval parasitoids and their host* Chrysophtharta agricola *over the summer season of 2000/01 from Florentine Valley, Tyenna and Ellendale in southern Tasmanian (pooled). The arrow indicates time of trap deployment.*

Only *P. australis* and possibly *Mesochorus* sp. were bet hedging with some individuals emerging same season, and some diapausing to emerge post-winter (Fig. 4b). The *Mesochorus* sp. specimens recovered post-winter were in an advanced state of decay indicating that they may have emerged much earlier but the traps were not monitored after mid April until late October. *Mesochorus* sp. was only recovered at Frankford, and only in low numbers.

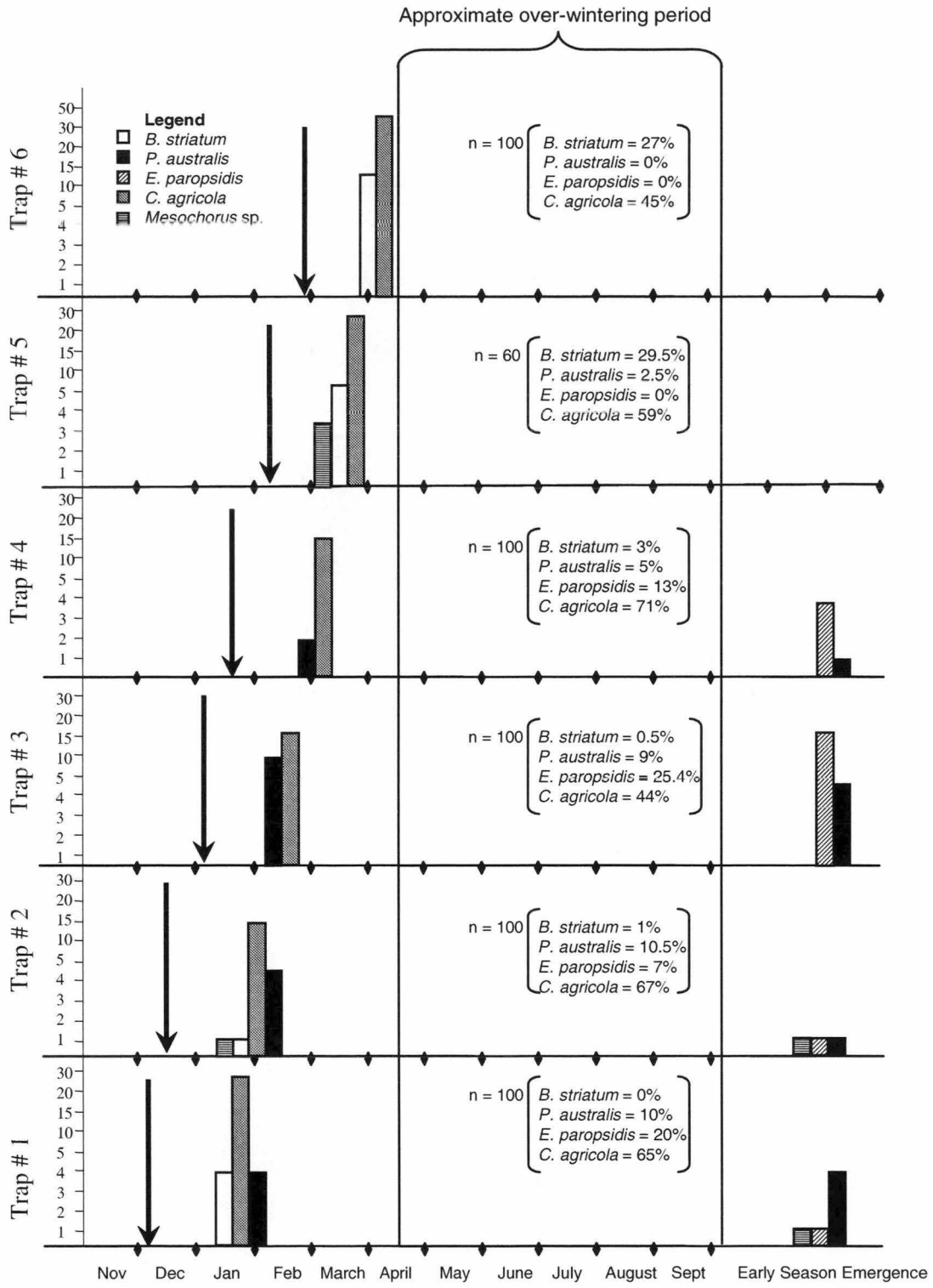


Figure 4.4b. *Same season and post-winter emergence, and bet hedging of larval parasitoids and their host* Chrysophtharta agricola *over 2001/02 from Frankford, Tasmania. Arrow indicates time of trap deployment. Figures in large brackets indicate percent parasitism from half of sample reared in the laboratory plus mortality. Multiple collections in each month were pooled.*

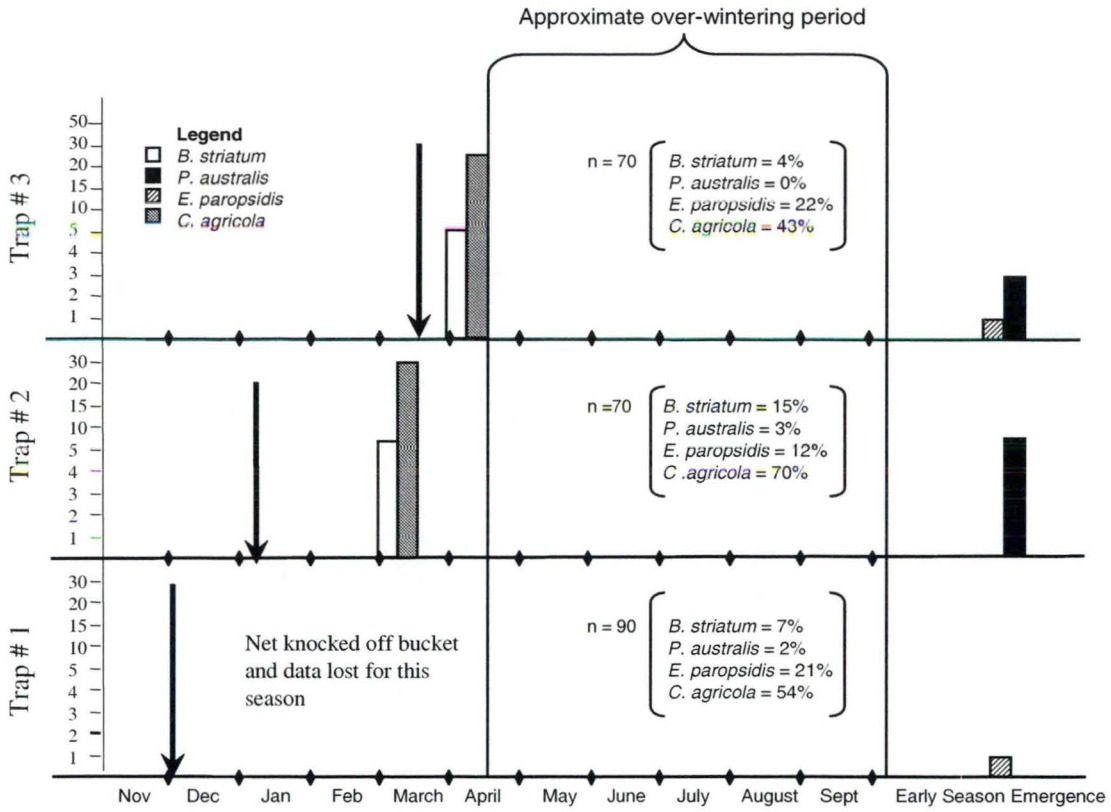


Figure 4.4c *Same season and post-winter emergence, and bet-hedging of larval parasitoids and their host* Chrysophtharta agricola *over 2001/02 from Florentine Valley, Tasmania. Arrows indicate time of trap deployment. Figures in large brackets indicate percent parasitism from half of sample reared in the laboratory plus mortality. Multiple collections in the same month were pooled.*

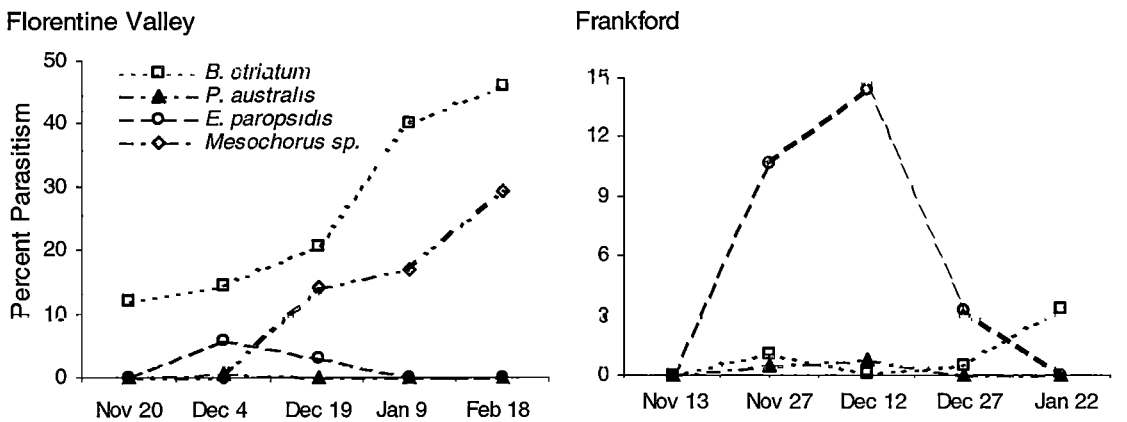
Approximately equal numbers of *P. australis* individuals emerged same season as entered diapause, and this did not vary greatly as the season progressed. At the Florentine Valley, even though *E. paropsidis* reached high levels of parasitism, few survived to emerge after diapause and conversely, *P. australis* which was only detected at low levels still emerged post-winter in relatively high numbers (Fig.4.4c). *C. agricola* always emerged same season. *P. australis* emerged in both the same and following seasons for as long as it was parasitising *C. agricola*, and *B. striatum* was only recovered in the same season with none entering diapause to emerge in the following season. All

C. agricola emergence occurred in the same season, at about the same time as the same season emergence of the two tachinid flies.

Field parasitism. Over the two seasons, at both sites, we collected a total 6,962 *C. agricola* larvae from 309 cohorts (mean size 22.5 ± 1.8 SE; range 4 to 100).

In the first season, overall, parasitism rates were higher at Florentine Valley (Fig. 4.5) even though the host population was smaller (Fig. 4.2). *Balde striatum* was the dominant parasitoid at Florentine Valley and parasitism was detected as soon as hosts were available for collection. Thereafter a steady increase in percent parasitism by *B. striatum* occurred, eventually reaching 47% toward the end of the season when the host population was well past its highest level. Parasitism of *B. striatum* by the hyperparasitoid also increased steadily to peak at the end of the season. In comparison, *E. paropsidis* and *P. australis* at Florentine Valley only achieved low levels of parasitism and *E. paropsidis* only parasitised the main early host population peak. In the second season at the Florentine Valley *B. striatum* was again the dominant parasitoid, and again was detected as soon as hosts were available for collection, and then showed a steady increase in parasitism as the season progressed with the second peak in host population subject to the highest levels of parasitism (Fig.4.5). The hyperparasitoid, *Mesochorus* sp. was less abundant than the first season. Parasitism rates by *E. paropsidis* declined earlier than the tachinid, although it persisted longer than the previous year, and further declined just prior to the second peak in host abundance.

2000/01



2001/02

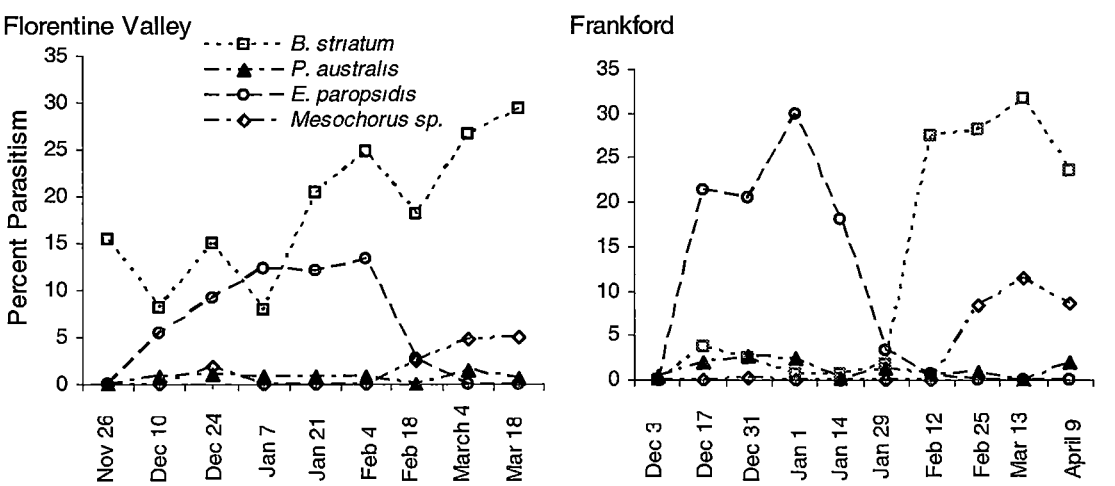


Figure 4.5 *Mean percent parasitism for three larval parasitoids of* Chrysophtharta agricola, *and* Mesochorus sp. *over the 2000/01 and 2001/02 summer seasons. Data for* B. striatum *also includes individuals parasitised by* Mesochorus sp. *and data for* Mesochorus sp. *is percentage parasitism of* B. striatum.

At Frankford, in the first season, *E. paropsidis* was the predominant parasitoid during both seasons, and the rise in parasitism rates coincided with their emergence from diapause (Fig. 4.5 and 4.3). Again, *E. paropsidis* targeted the early, large peaks in host population.

Levels of parasitism by *B. striatum* followed a similar pattern as at the Florentine Valley with the highest level late in the season during the hosts' second peak in abundance (Fig. 4.5 and 4.2). However, in the second season at Frankford, the tachinid *B. striatum* did not reach high rates of parasitism until early February, by which time the braconid *E. paropsidis* had almost finished its season. These two parasitoids, that both prefer early instar hosts displayed clear temporal partitioning in percentage successful parasitism, with *E. paropsidis* successfully parasitising the first main peak in host population abundance and again *B. striatum* mainly successfully parasitising the second peak. Both parasitoids reached rates of around 30%. *Mesochorus* sp. peaked at both sites late in the season, and *P. australis* was not abundant at either site.

Adult population monitoring. Adults of all three species of parasitoid were recorded at both sites but generally only in low numbers. At Florentine Valley, there was evidence of a second generation emergence of both species of tachinid fly but not in *E. paropsidis* which was present only in low numbers (Fig.6a). The population of *P. australis* showed a steady increase towards a peak in late January, a crash in early February, and a steady rise towards a second, albeit smaller, peak in early to mid March. *B. striatum* was not recorded until later in the season than *P. australis* but peaked at

around the same time and level, and also showed a similar trough in abundance in early February and second peak shortly after.

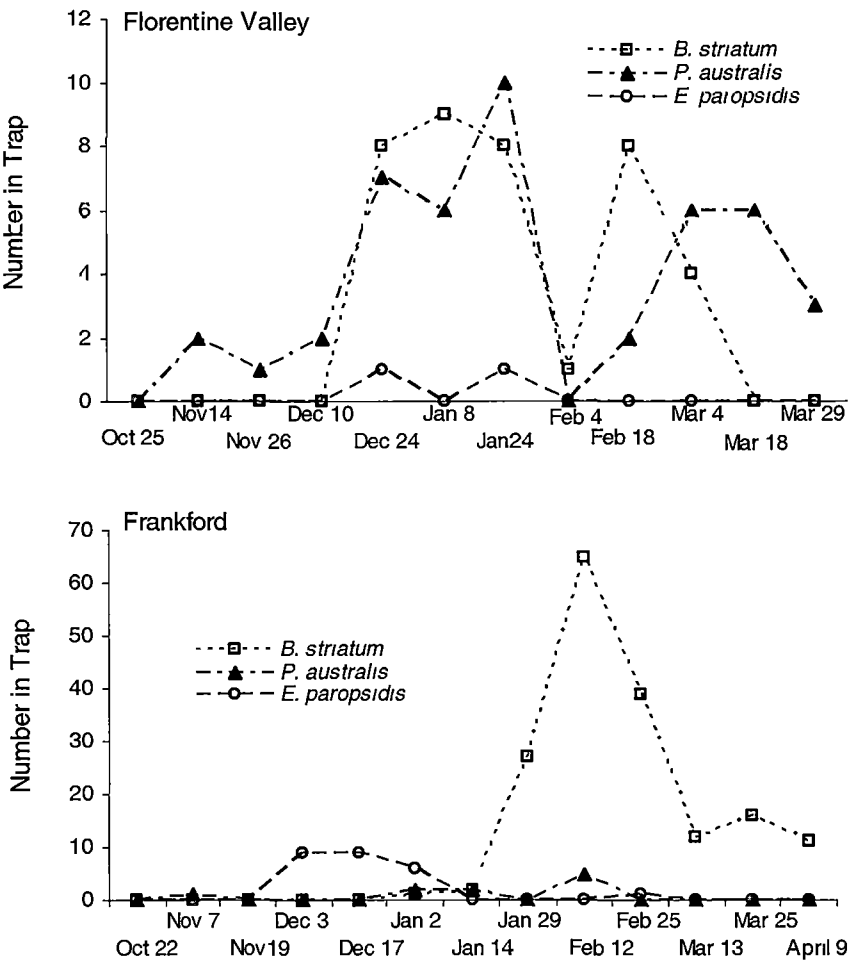


Figure 4.6 *Malaise trap catches of males and females of the three larval parasitoids of* Chrysophtharta agricola, *over the 2001/02 summer season at Florentine Valley and Frankford.*

At Frankford, a massive population peak of *B. striatum* collected on February 12 dominated the Malaise trap catches (Fig. 4.6), which is mainly attributable to males, which accounted for over 90 % of the population caught at this time. At the same time a smaller peak of *P. australis,* which was only recorded in low numbers, suggests that the two tachinids were having a second generation emergence at this site also. *E. paropsidis* became abundant throughout December but declined afterwards and showed no evidence of same season emergence. Only two specimens of *Mesochorus* sp. were recovered from the Malaise traps, both in Frankford, one on January 2nd and the other on March 25th.

4.3.2 Field Heat Units.

Using the appearance of first instar hosts in the field as a starting point for day degree accumulation, there were sufficient day degrees available for two generations of all three species of parasitoid at both sites in both seasons (Table 1). Mean summer temperatures were higher at Frankford in both seasons (mean 2000/01; 16.9 °C and 2001/02; 13.4 °C) than at Florentine Valley (mean 2000/01; 13.9 °C and 2001/02; 11.2 °C), and at both sites, the first season was hotter than the second. These differences are reflected in the greater heat units available in Frankford for all three species, and even though the period that temperatures were measured in the first season was much shorter, heat units were comparable.

Table 1. Day degree (DD) requirements for one and two generations of three larval parasitoids of *C. agricola* compared to field day degrees at two sites in Tasmania.

			DD available in field from three accumulation starting points					
			Florentine Valley			Frankford		
	DD for one generation	DD for two generations	Appearance of first instar hosts	Appearance of preferred host stage	From first parasitism	Appearance of first instar hosts	Appearance of preferred host stage	From first parasitism
2000/01			(November 20 to March 14)			(November 27 to March 19)		
Balde striatum [1]	345	690	904	904	904	1192	1192	1192
Paropsivora australis	385	534[3]	914	786	786	1204	1204	1204
Eadya paropsidis [2]	294	588	943	943	812	1235	1235	1235
2001/02			(November 25 to April 30)			(November 5 to April 28)		
Balde striatum [1]	345	690	730	730	730	1199	1199	980
Paropsivora australis	385	534[3]	742	617	683	1214	1062	992
Eadya paropsidis [2]	294	588	780	780	780	1260	1260	1218

[1] Does not include time from adult eclosion to larviposition.[2] Does not include pupal duration. Dates in brackets indicate period over which data loggers measured temperature, and heat units accumulated. [3] This DD for two generations is not 2X DD for one generation because this species overwinter as pupae in the soil and therefore the second generation pupae do not necessarily need to develop in the same season to complete that generation

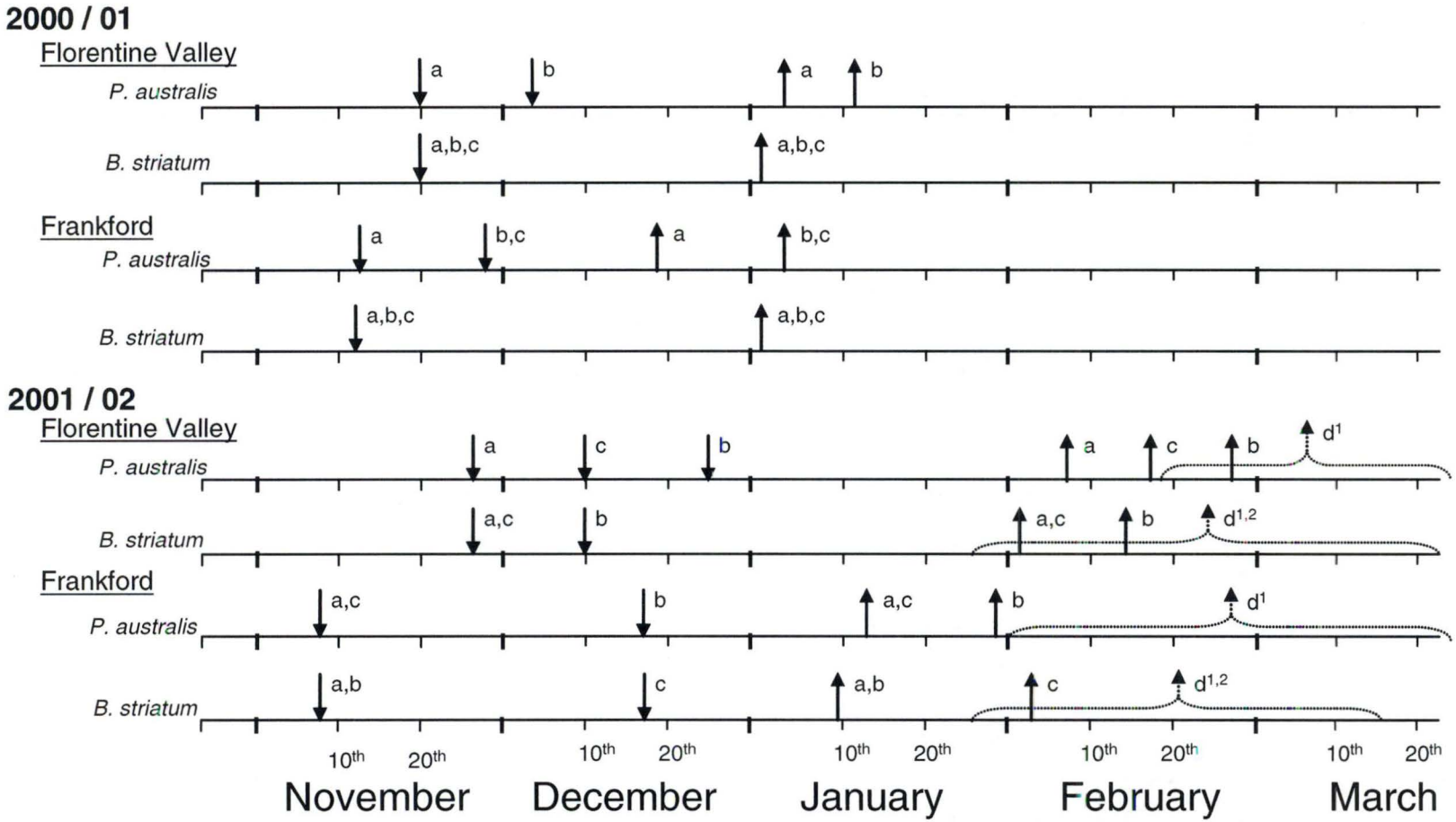


Figure 7a. *Heat unit accumulation and predicted emergence times of second generations for the two tachinid flies* Paropsivora australis *and* Balde striatum *parasitising* Chrysophtharta agricola. *a = appearance of first instar hosts in the field, b = appearance of preferred host stage, (Nahrung and Allen 2004) c = detection of first parasitism, d = estimated activity of second generation, estimated from; [1] adult abundance and [2] parasitism rates. ▼ = starting point of accumulation, ▲= predicted emergence point.*

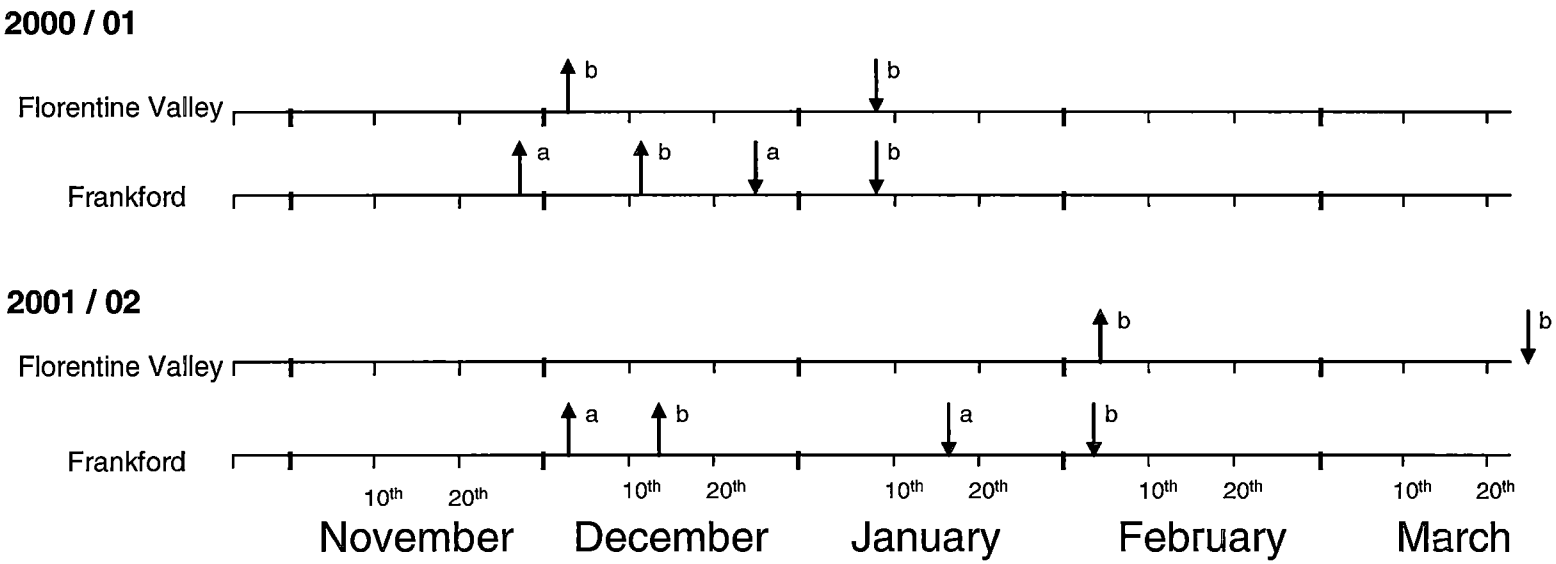


Figure 7b. *Heat unit accumulation and predicted pupation times from two accumulation starting points for the braconid wasp* Eadya paropsidis *parasitising* Chrysophtharta agricola. *a = emergence trap peaks, b = parasitism peaks.* ▲ *= starting point of accumulation,* ▼ *= predicted time that most of the population has pupated.*

Predictions of emergence of second generations of the tachinids were generally close with the starting points of first appearance of preferred host stage and first detection of parasitism giving the best predictions (Fig. 4.7a). Accuracy was hampered by fortnightly sampling and defining second generation activity. The predictions for pupation of *Eadya paropsidis* varied greatly between sites and seasons (Fig. 4.7b). In the first, warmer season, our model predicted most of the population would be pupating by 7th January at both sites. However, due to a long period of activity at Florentine Valley in the second season and cooler conditions, our model predicted that entering of the pupal stage hadn't finished until near the end of March.

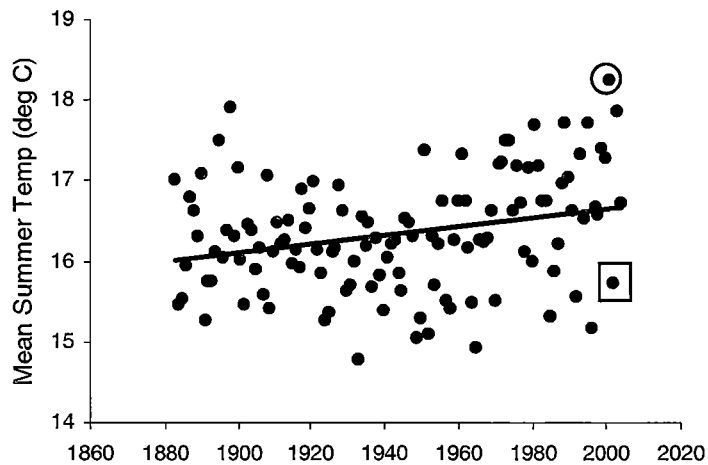


Figure 4.8 *Mean summer temperatures (December, January and February) for Hobart Tasmania. Circle denotes 2000/01, and the square denotes 2001/02.Courtesy Bureau of Meteorology.*

Neither of the two seasons during which our field trials were carried out, were close to average in terms of temperature. Temperatures at Hobart indicated that the first season (2000/01) was much warmer than average, and the second (2001/02) was cooler (Fig. 8).

DISCUSSION

The emergence of *Eadya paropsidis* and *Paropsivora australis* from the tent traps, and from the bucket traps the season after collection, confirms that these two species overwinter as pupae within the soil. *E. paropsidis* consistently entered into pupal diapause, confirming that it is univoltine in Tasmania, which agrees with Tanton and Epila (1984) who report that *E. paropsidis* is also univoltine in the Australian Capital Territory. However, *P. australis* is bet-hedging with about half of the parasitoids emerging same-season to parasitise new hosts and half staying in the soil to emerge in the following season. During its period of activity, the proportion of *P. australis* that emerged same-season compared to those that entered diapause did not vary as the season progressed.

In contrast, *Balde striatum* never emerged the following season and even if parasitism occurred late, all adults emerged in the same season, some as late as April. Together with the complete absence of this species in the tent traps, this indicates that this tachinid fly does not overwinter as pupae in the soil within the plantation. With a life-span of 2-3 weeks in the laboratory (A.D. Rice *pers obs*, Chapter 2), it is unlikely that *B. striatum* is overwintering as an adult but may be using other host species during the autumn and entering diapause at a later date. It is known to parasitise the winter-active paropsine *Acacicola orphana* (Erichson) (Chapter 2), although reportedly only at low levels (Elliott 1978, Simmul and Clarke 1999). A large collection (ca 200-300) of *A. orphana* in September 2000 yielded a correspondingly large emergence of *Mesochorus* sp. from tachinid pupae (Rice unpublished). The primary parasitoid host of these *Mesochorus* sp. is not known but this shows that parasitism in *A. orphana* can reach high

levels, and more work is needed to establish whether *A. orphana* is an alternative host that is utilized by *B. striatum* to significant levels. While we are not certain that this is the same species of *Mesochorus* that we have reared from *C. agricola,* the specimens recovered from the bucket traps in October may have emerged during winter, and may be following *B. striatum* across to *A. orphana. A. orphana* is a significant defoliating pest of *Acacia dealbata*, and is abundant in the Florentine Valley in a plantation bordering the study site used here. Therefore, if *A. orphana* is an alternative host it may explain why parasitism rates by *B. striatum* were higher at this site compared to Frankford where there were few *A. dealbata* trees available to sustain a large population of *A. orphana* and *B. striatum.*

Eadya paropsidis appeared to be well synchronized with the first and greatest population peak of its host, and although diapause-enforced univoltinism is not rare in insects (Danks 1994), it is not clear why *E. paropsidis* only has one generation per year. There appeared to be adequate heat units for more than one *E. paropsidis* generation in a season but, due to obligate pupal diapause, the development time of pupae was not included in the calculation of heat unit accumulation. If we use thermal requirements for pupal development of other braconid species obtained from the literature (Allen and Keller 1991, Nealis *et al.* 1984) we obtain a mean estimate of 85 day degrees (n = 5, range = 76-97, mean = 85.4). Assuming that this may also apply to *E. paropsidis,* there still is sufficient time for at least two generations per year at both sites over both seasons, so there are no apparent thermal constraints on the voltinism of *E. paropsidis.*

Likewise for the two tachinid flies, there were no thermal constraints on two generations.

Paropsivora australis showed poor synchrony with its host, as when emergence from diapause was detected, it occurred long before any hosts were available for parasitism. However, its peak in adult activity in the plantation did coincide with the period of time that fourth instars, its preferred host stage, were most abundant. This peak in adult flies occurred before sufficient heat units were available in the field for *P. australis* to have completed a generation, which suggests that there was a significant immigration of adults into the system, which may be a consequence of *P. australis* being polyphagous and utilizing hosts that are outside this system. At Florentine Valley, *B. striatum* showed good apparent host synchrony, parasitising hosts as soon as they were available, however as this fly was not collected from emergence traps we cannot pinpoint its emergence from diapause or indeed if it undergoes diapause at all. The pattern found here supports Belshaw (1994) who suggests that the formation of a respiratory funnel, such as is produced by larvae of *P. australis*, may allow tachinids to be polyphagous. We could expect then that although they may not show good synchrony with any particular host, they may be well synchronised, overall, to the guild of species that they parasitise. *B. striatum* on the other hand does not form a respiratory funnel and therefore may be constrained to a narrower rage of hosts and hence be more likely to show good synchrony with them.

Testing our predictive models was complicated in the first season by an inability to discern second generation parasitoid activity. However, in the second season the

addition of Malaise traps to the monitoring techniques meant that we could detect the second generation emergence of *B. striatum*, and to a lesser degree, *P. australis*. All three of the accumulation starting points gave reasonable estimates of the second generation, and generally fell within the two week sampling period. Similar to the findings of Nahrung *et al.* (2004), we did not find any compelling evidence of additional heat units being absorbed by the host basking in the sun.

The sharp rise in abundance of *B. striatum* in Frankford and the low rates of parasitism within the plantation prior to this peak, suggest that there may have been an influx of this fly into the plantation. The population peak also coincides with an abrupt rise in parasitism rates, indicating that it was not just males that became abundant as the Malaise trap catches suggest. The increased abundance and rise in parasitism rates also coincide with the predicted emergence of a second generation based on heat unit accumulation beginning from the first recording of parasitism. Therefore, the increased abundance of *B. striatum* is likely to be due to a combination of immigration into the system and emergence of a second generation. Additionally, the rise in parasitism rates at both sites by *B. striatum* coincides with the end of parasitism and adult abundance of *E. paropsidis* which achieved high rates of parasitism. This may be partially due to the competitive advantage that mandibles possessed by first instar larvae of *E. paropsidis* affords them over the non-mandibulate tachinid larvae (See Chapter 8), but low adult abundance when parasitism rates were also low suggests that *B. striatum* was simply not present in large numbers.

At Florentine Valley, parasitism rates by *P. australis* are consistently low even when adult populations appear to be relatively high, which may indicate the use of an alternative host. *P. australis* has been reared from several paropsine hosts including *C. agricola's* congener *C. bimaculata*, which is also a known host of *P. australis* (Chapter 2) and abundant at this field site (ADR *pers obs*, J. Madden *pers comm* etc). *Eadya paropsidis* also uses *C. bimaculata* (and other paropsines) as a host (de Little 1982) and therefore what we see occurring within the plantation may only be a part of the whole picture.

CHAPTER 5

Intraspecific Host Choice and Suitability in the Solitary Koinobiont Parasitoid *Eadya paropsidis*: Bigger Hosts are not Necessarily Better.

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Abstract

Although host stage selection in koinobiont endoparasitoids is frequently measured, the mechanisms that underlie parasitoid oviposition preferences are often overlooked. Here, we determine the preferred host stage of the braconid wasp *Eadya paropsidis* Huddleston and Short (Hymenoptera: Braconidae: Europhorinae) when attacking the leaf beetle *Chrysophtharta agricola* Chapuis (Coleoptera: Chrysomelidae: Paropsini) in the field and laboratory, and examine how behavioural and developmental interactions may be mediating host stage preferences and parasitoid reproductive fitness.

Observed rates of parasitism by *E. paropsidis* in the field indicated a preference for early instar hosts. During field choice tests, *E. paropsidis* parasitised significantly more small hosts than large, and in laboratory tests, deposited 30% more eggs into first instars than any other instar. Parasitism doubled the probability of premature host death across all instars, though the host's internal ability to kill a developing parasitoid larva did not vary with host instar. Multiple ovipositor insertions by the wasp reduced the chances of the host beetle surviving parasitism by 2.5 fold for every additional insertion, but did not significantly increase host mortality. Attempts at ovipositor insertion were twice as long and only half as successful when attacking large hosts compared to small hosts. Larger host were over three times more likely to tail flick and 27 times more likely to walk away from the attacking parasitoid than small hosts. Parasitoid larval weights were initially lower for those individuals originating from first instars than those from fourth instars but towards the end of parasitoid larval development, those from first instars were larger. Parasitoid pupal weights showed the same pattern, that is, a trend towards larger parasitoids from early instars with parasitoid development taking significantly longer from first instars.

There were no fitness advantage for *E. paropsidis* in attacking larger hosts, and apart from a taking slightly longer to develop from first instars, small hosts offered the best potential for the parasitoid to maximise fitness via increased body size and reduced host handling time.

5.1 *INTRODUCTION*

Female parasitoids that maximise their genes in subsequent generations by choosing the most suitable host for oviposition and juvenile development can accrue considerable fitness benefits. Since hosts vary, both inter– and intraspecifically in their suitability for parasitoid development, variations in size and age between hosts can be critical in deciding the outcome of host-parasitoid interactions (Vinson and Iwantsch 1980, Gross 1993). Hence, for parasitoids that attack several developmental stages of their host, variation in host size and age may result in host stage preferences (Hopper and King 1984, Hopper 1986), as has been demonstrated in many parasitoid species (e.g., Mackauer 1973, Liu *et al.*1984, Gerling *et al.* 1990, Wang and Liu 2002).

Host behavioural defences prior to ovipositor insertion and physiological defences following oviposition, may also influence parasitism success (Berberet *et al.* 1987, Vinson 1990, Gross 1993, Godfray 2000). A host's ability to survive oviposition by parasitoids often improves with age, with older individuals better able to injure, repel or escape from ovipositing females (Allen 1990, Harvey 1996, Brodeur *et al.* 1996, Al-Saffar & Aldrich 1998, Kairo and Murphy 1999) or encapsulate a parasitoid's eggs or larvae (van Alphen and Vet 1986, de Bolt 1991, Bratti *et al.* 1992, Strand and Pech 1995). Parasitoids are also able to counter internal host defences through inherent external properties that prevent encapsulation, by suppression of the hosts' immune system, or by location of immatures in tissues that afford some protection (ganglion, fat bodies, glands etc.) (Vinson and Iwantsch 1980, Strand 1986, Belshaw 1994, Quicke 1997, Theopold *et al.*2000). There is also a suggestion that parasitoids' counter measures may be modified to suit variability in expected resistance, such as ovipositing more than

once in older hosts or different host species with more effective immune systems to improve parasitoid survival (Blumberg and Luck 1990, Godfray 1994).

Adult parasitoid body size is often positively correlated with fitness (Mackauer and Sequeira 1993), with larger individuals having higher fecundity, living longer, searching more efficiently for hosts, and having improved mating success (Gunasena *et al.* 1989, Visser 1994, Kazmer and Luck 1995, West *et al.* 1996). Therefore, maximising body size has often been gauged to be an important selective force (Charnov and Skinner 1985, King 1989). However, Harvey *et al.* (2004) challenged the assumption that size alone is prioritised above other correlates of fitness, and argued that host mortality can be a stronger selective force than size.

Whether to grow large or rapidly is arguably the most important trade-off an organism faces (Volrath and Parker 1992), and for parasitoids, the limited resources available for parasitoid development in small hosts can constrain growth. However, developmental plasticity may enable parasitoids to maximize selected developmental characteristics from smaller hosts (Mackauer and Sequeira 1993, Harvey *et al.* 2000). Mackauer and Sequeira (1993) hypothesised that there were three developmental strategies that parasitoids could use, dependent upon maximizing either body size, developmental rate or a trade-off between the two. They predicted that if body size is the selective priority, development is delayed in smaller hosts to allow time for the host to grow to a sufficient size for parasitoid development, which results in decreased developmental times as the developmental stage of the host at parasitism increases, and same-sized offspring across host stages. If however, developmental rate is prioritised,

developmental rates are similar irrespective of the size of the host at oviposition, resulting in smaller offspring from less developed hosts. The third strategy represents a trade-off between developmental rate and adult body size (Mackauer and Sequeira 1993). Furthermore, a host's risk of mortality from other external causes frequently shifts with host age (Price 1975) and any immature parasitoid inside its host will thus also be subject to the same changes in risk and selection pressures that mortality risk exerts on the host.

Hence, host sizes that apparently optimise parasitoid reproductive fitness may not reflect host sizes successfully utilised in the field. Indeed, assumptions of reproductive fitness based on observed rates of field parasitism fail to consider any of the behavioural or developmental interactions that underlie, and ultimately determine, a parasitoid's choice of host. Therefore to gain insight into fitness returns from different host sizes it is important to compare a wider range of determinants of fitness than just size (Price 1972, Harvey *et al.* 2004). In this study we used the solitary koinobiont endoparasitoid, *Eadya paropsidis* Huddleston and Short (Hymenoptera: Braconidae) parasitising larvae of the paropsine chrysomelid beetle *Chrysophtharta agricola* to examine parasitoid potential fitness returns in relation to intraspecific differences in host age and size. We firstly test whether the host stage utilised in the field reflects host preferences in the laboratory. We then test whether there is any impact of host defences on parasitism rates of host stages, by quantifying the success of oviposition bouts and test whether the number of ovipositor insertions influences the developmental success of the parasitoid across host instars. Finally we test whether the observed host stage

preferences result in potential fitness benefits via final adult body size or developmental time.

5.2 MATERIALS AND METHODS

5.2.1 The Study System. The host, *Chrysophtharta agricola* feeds on juvenile eucalyptus foliage where it undergoes four larval instars, with the final larval weight at the end of the fourth instar averaging 81.7 mg $\pm$ 4.0 SE (n = 15, range = 60.4 – 114.8) (Rice unpublished data), before it drops to the ground to pupate (Nahrung 2004). Larvae are gregarious and possess a pair of defensive eversible glands on their eighth abdominal tergite that are thought to contain hydrogen cyanide, benzaldehyde and glucose (Moore 1967). Larvae are present on foliage from mid November to mid March.

Eadya paropsidis is a solitary endoparasitoid that deposits small hydropic eggs directly into the haemocoel of its host. First instar larvae possess well developed mandibles which are lost at the first larval moult. *E. paropsidis* larvae begin destructive feeding and kill the host at the prepupal stage, and consume all of their host's tissues prior to pupation (Rice unpublished data). *E. paropsidis* overwinters as a pupa in the soil, undergoes an obligate pupal diapause, and has only one generation per year (Chapter 4). It is active in the field from early December to early February and therefore is exposed to all four instars of its host, which makes it an ideal model to test intraspecific host choice. Beside *C. agricola*, *E. paropsidis* has also been reared from *C. bimaculata* in Tasmania (de Little 1982) and *Paropsis atomaria* Ol. (Tanton and Epila 1984) on mainland Australia.

5.2.2 Host Stage Parasitism in the Field.

Parasitism rates of all four instars were determined using two methods, and yielded parasitism rate data on three larval parasitoids: *Eadya paropsidis* and two tachinid flies. Here we only report on parasitism by *E. paropsidis*, and do not attempt to account for any competition or interference that may have occurred between the three larval parasitoid species.

Sentinel larvae. In this experiment, cohorts of unparasitised (sentinel) larvae of each instar, were simultaneously exposed to *E. paropsidis* in the field and then brought back to the laboratory for rearing and assessment of parasitism rates. For laboratory rearing we used either 50 or 100-ml plastic containers depending on the cohort size and instar. In each container moistened paper towel and cut *E. nitens* was added and replenished every three days until parasitoid or beetle pupation was complete. Ventilation was provided via a 5cm hole in the lid of the container covered with a perforated plastic film. Larvae were reared at 22 °C and 16L:8D photoperiod. Experiments were conducted at two field sites that were both *Eucalyptus nitens* plantations from three to five years of age.

Site 1 (Florentine Valley: 42°38S 146° 29E). *C. agricola* egg batches were bagged on trees along both sides of a break of 300 m that ran through a 5 yo plantation. Fine mesh bags (30 cm x 50 cm), were placed over the shoot containing the egg batches and held in place with flagging tape. Twenty egg batches were bagged every two weeks over 4 site visits in November and December 2001. Only cohorts consisting of one instar were exposed (larvae of different instars within cohorts were removed) and Tanglefoot™

applied to the branch prevented larvae from leaving or invading the shoot and also prevented spiders from preying on the host larvae. The bags were removed, exposing the larvae for 3 days on the 8th of January 2002. Losses of cohorts from broken branches, damaged branches, hatch failure, and the presence of predatory mirids within the bags meant that only a total of 588 larvae from 38 cohorts were successfully retrieved (mean size 15 $\pm$ 2.1 SE; range 3 to 68).

Site 2 (Frankford 41°20S 146°45E) Six egg batches (5 + 1 control) were bagged within each of four 100m rows in a three y.o. plantation. This was done three times over consecutive two week visits during November and December 2001. On the 14th December 2001 bags were removed and the range of larval instars from firsts to fourths, exposed to parasitoids for three days. A total of 1388 larvae from 54 cohorts (mean size 26 $\pm$ 2.0 SE; range 4 to 79) were successfully exposed to *E. paropsidis* attack for three days and retrieved, eight were lost and a further 12 remained bagged to serve as controls to ensure that the parasitoids had not accessed the hosts within the bags prior to the commencement of the exposure of larvae.

We pooled data from first and second instars together to form the category 'small hosts' and third and fourths together into 'large hosts', which allowed us better comparisons with behavioural interaction data collected later and also enabled us to use mixed cohorts that arose from moulting during exposure. One additional cohort (n = 37) was not included in analysis because it was exposed as small hosts and retrieved as large. We used ANOVA to look for effects between host size and site on arcsine transformed parasitism rates.

Larval Collections: The second method used to determine host stage parasitism was to collect cohorts of each instar from each field site and rear them in the laboratory as for sentinel larvae. Any changes in parasitism as instar increased indicated parasitism of that instar. Five cohorts of each of the four instars were collected every two weeks at each field site for the entire season resulting in 2658 larvae from 99 cohorts (mean size 27 $\pm$ 1.7 SE; range 4 to 85) being collected from Florentine Valley, and 2998 larvae from 122 cohorts (mean size 25 $\pm$ 1.7 SE; range 5 to 100) collected from Frankford. Cohorts were collected randomly throughout the plantation and as far as possible towards the end of each instar. Only one cohort was sampled from a given tree on the same day, all larvae on a shoot were considered to be from one cohort and only cohorts consisting of a single instar were collected. Collected larvae were held in plastic bags and placed in a cooled icebox for transport back to the laboratory.

5.2.3 Host Stage Preference

Chrysophtharta agricola larvae were reared from eggs collected weekly from the Frankford and the Florentine Valley field sites. Eggs batches were hatched and reared as per returned sentinel larvae.

Eadya paropsidis were collected as gravid females from both field sites and held individually in gauze-topped 500-ml cylindrical plastic holding cages. A 20-ml plastic vial containing paper wadding, water and a sprig of fresh *E. nitens* foliage was attached to the base of the cage by a velcro pad. A drop of honey was smeared onto the gauze and replaced as necessary.

Ten gravid *E. paropsidis* females were each presented in their cages, with twenty larvae consisting of five larvae of each instar on a shoot of *E. nitens* foliage. Larvae were exposed to parasitism for five hours, reared for three days at 23 °C and then frozen at – 20 °C. Larval dissections were carried out in distilled water, examined under 30x magnification and parasitoid eggs counted.

We used chi-square analysis to test for equality of parasitism levels between host instars to overcome the assumption of independence inherent in parametric analyses that was violated by exposing all four instars to the same females.

5.2.4 Host defence and parasitism success.

Host behavioural defences: To test whether host size can impact on parasitoid oviposition success, we confined each of seven gravid *E. paropsidis* females to an arena that contained either four small (first instar) or four large (fourth instar) hosts. We recorded host defensive behaviour and parasitoid oviposition behaviour on video tape until the parasitoid had successfully inserted her ovipositor into hosts a total of ten times and analysed tapes using the "Observer" event recording software. We used a give-up time of one hour, and if the parasitoid had not inserted her ovipositor ten times by then we recorded the time as one hour. The arena consisted of a clean plastic petri-dish (90 mm dia.) with a filter paper floor. We recorded the frequency and duration of both oviposition and grooming bouts, and the success rate of oviposition attempts. An oviposition bout or attempt was considered to have been initiated when the parasitoid approached the host and drew her antennae back towards her wings. Such behaviour was generally followed by pointing her abdomen forward, beneath her body, and attempting

to insert her ovipositor into the host. We used non parametric tests to compare parasitoid behaviours between the two host sizes due to the non-normality of data.

Overcoming internal defences: We tested how, following one or more ovipositor insertions, host age and increasing the number of ovipositor insertions changed a parasitoid's success rate. To do so, we used 11 gravid field collected females, held them in glass collecting tubes (100 x 25 mm) and presented them with one of the four instars every day for four days in a no-choice design. Females were not exposed to hosts for a day before initially being used. Only one instar was presented per day and the order was randomised for each female. Four larvae of each instar were presented to each female individually on each occasion with the female allowed to insert her ovipositor no times (control), once, twice or three times, so that all four larvae were eventually utilised. This resulted in 16 host larvae per female – four instars x four parasitism treatments.

We carried out two separate analyses both using logistic regression (logits) with host instar and number of ovipositor insertions as treatments, and used alpha = 0.01. The first, a binary logit, examined whether parasitism increased the probability of host death prior to either beetle or parasitoid pupation, and included controls, i.e., no ovipositor insertion, and two only possible outcomes: host dead or not dead. The second, a multinomial logit, examined whether the probability of premature death or the likelihood of parasitoid pupation was altered by host instar or the number of ovipositor insertions greater than one. The second logit used three possible outcomes: premature death, beetle pupation or parasitoid pupation, and did not include the controls. As there was no

significant variability between females in regards to either host mortality (G = 10.29, df = 10, p = 0.42) or the likelihood of a parasitoid pupating (G = 20.46, df = 10, p = 0.43), we did not include female as a treatment in analysis.

5.2.5 Host instar, development time and body size.

To test whether our observed host stage preferences resulted in increased fitness via either final adult body size or developmental time, we exposed host larvae to parasitism in a glass tube as before. Five females were presented with five larvae of each instar in random order and removed after one ovipositor insertion, giving 100 'parasitised' larvae, which realised 47 parasitoid pupae across the four host instars. Host larvae were reared individually on *E. nitens* foliage at 21 °C and 16L:8D photoperiod, development times recorded to the nearest 24 hrs and parasitoid pupae weighed immediately following pupation to the nearest 0.0001g. As there was no significant maternal effect on larval duration (F-ratio = 1.06, df = 3, p = 0.31) or pupal weights (F-ratio = 1.04, df = 3, p = 0.31), maternal origin was not included as a treatment in subsequent analysis, and as pupae could not be sexed due to obligate pupal diapause in this species, nor was sex.

To determine immature growth patterns of the wasp, four gravid field collected females were provided with first and fourth instar hosts in a glass tube as before. Host larvae were reared on *E. nitens* foliage at 22 $\pm$ 2 °C and 16L:8D photoperiod, and after seven days we began removing parasitised larvae daily and storing them at –20 °C until dissection. We removed 12 host larvae from each sample per day which resulted in 66

immature parasitoids from the two host instars that ranged in age from 7 to 15 days old, with the latter being the point that they had reached pupation. Host larvae were dissected in saline at 20x magnification, and any parasitoid larva found was collected, oven dried at 38 °C for seven days, and weighed using an electronic balance (Mettler M3) to the nearest 0.00001g. We transformed the data to approximate linearity, and compared regression lines representing growth patterns from the two host instars using multiple regression and difference parameterization.

5.3 *RESULTS*

5.3.1 Host stage parasitism in the field

At both field sites, nearly twice as many sentinel cohorts of small larvae were parasitised by *E. paropsidis* than large larvae cohorts (F. ratio = 5.3, df = 1, p = 0.024) (Table 5.1). Parasitised small hosts accounted for 86% of all parasitism at the Florentine Valley, where no fourth instars were parasitised within the large host group, and for 78% of all parasitism at Frankford. Parasitism levels were significantly higher at Frankford than Florentine Valley (F ratio = 4.6, df = 1, p = 0.034). Parasitism levels across all cohorts of small hosts were on average at least double that of large hosts (Fig 5.1).

Table 5.1. *Patterns of parasitism by* Eadya paropsidis *of its host* Chrysophtharta agricola *in sentinel cohorts.*

	Florentine Valley		Frankford	
	Small	Large	Small	Large
Cohorts exposed (parasitised)	19 (5)	19 (3)	30 (18)	23 (8)
Larvae exposed (parasitised)	293 (19)	265 (7)	801 (90)	550 (24)
% parasitism per cohort [overall] (range)	4 (0 - 23)	1 (0 - 30)	10 (0 - 44)	4 (0 - 26)
% parasitism per cohort [parasitised only] ± SE (range)	13.9 ± 3.6 (7 – 23)	16.3 ± 8.1 (3 – 30)	16.9 ± 2.9 (3 – 44)	10.9 ± 3.0 (2 – 26)

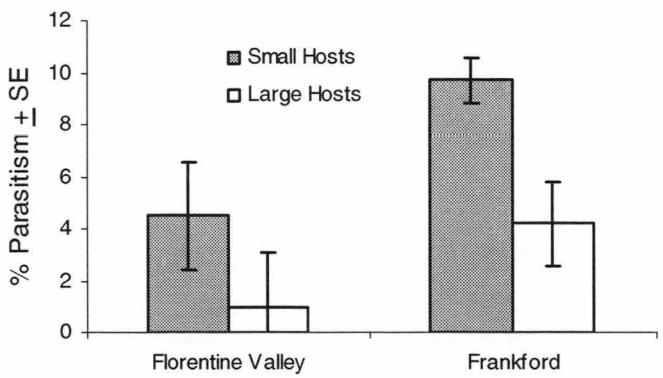

Figure 5.1. *Mean percent parasitism by* Eadya paropsidis *per cohort of small (first and second instars) and large (third and fourth instar) sentinel* Chrysophtharta agricola *larvae in field trials at Frankford and Florentine Valley.*

The pattern of parasitism by *E. paropsidis* revealed by larval collections from the field was similar between sites, and generally showed use of early instars and no additional parasitism by the time the host finished its third instar (Fig. 5.2). There was a significant difference in parasitism rates between instars at Frankford (F-ratio = 8.05, df = 3, p = 0.0001) where parasitism levels were higher than at Florentine Valley, though in the Florentine Valley, parasitism rates between instars approached significance (F-ratio = 2.46, df = 3, p = 0.07). Parasitism levels of early instars were similar to those seen for sentinel larvae in the previous experiment at each field site.

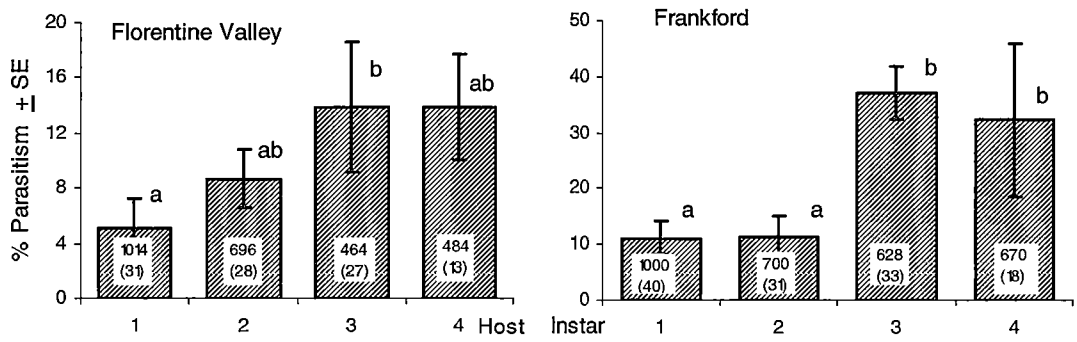

Figure 5.2. *Mean percent parasitism per cohort by* Eadya paropsidis *of field collected* Chrysophtharta agricola *larvae at Frankford and the Florentine Valley. Numbers indicate number of larvae collected and numbers in parentheses, the number of cohorts.*

5.3.2 Host Stage Preference

Parasitism levels were significantly different between instars ($X^2 = 10.8$, df = 3, $0.025 > p > 0.01$). Many more eggs were placed into first instar hosts, and more first instars were parasitised than any other host stage (Table 5.2). Despite there being unparasitised hosts available, superparasitism was common. Over half of parasitised first

instars had more than one egg, but superparasitism was independent of instar (X^2 = 1.075, df = 3, N.S). There was no evidence of encapsulation of eggs by the host larva.

Table 5.2. *Oviposition choice of* Eadya paropsidis *(n = 10) after exposure to twenty* Chrysophtharta agricola *larvae (5 of each instar) for five hours during host stage preference tests. .*

Instar	Number of larvae exposed	Total eggs deposited	Number larvae parasitised	Received more than 1 egg	Mean eggs per parasitised larvae ± SE	Mean eggs per cohort of 5 larvae ± SE
1	50	34	15	9	2.3 ± 0.6	3.4 ± 1.3
2	50	11	8	2	1.4 ± 0.2	1.1 ± 0.5
3	50	15	9	4	1.7 ± 0.3	1.5 ± 0.7
4	50	22	10	4	2.2 ± 0.5	2.2 ± 1.1

5.3.3 Host defence and parasitism success

Host behavioural defence. The hosts' reaction to an oviposition attempt by the parasitoid was to either flick their abdomen (tail flicking) or walk. The frequency of tail flicking and of walking was significantly greater for large hosts than for small hosts (Table 5.3). When with large hosts parasitoids on average increased the time required to achieve ten ovipositions by over seven fold and doubled their time to undertake each oviposition. Consequently the probability of an oviposition attempt being successful against a large host was only half that seen against a small host. Parasitoids frequently reacted to contact with the hosts' defensive glands (often facilitated by host tail flicking) by immediate cessation of oviposition and intensive bouts of grooming. Whilst

parasitising large hosts, the parasitoids had significantly more grooming bouts that on average each took over 4 times as long to complete.

Table 5.3 *Mean ± SE of host/parasitoid behaviour during parasitism, for the parasitoid* Eadya paropsidis *(n = 7) and small (L1) and large (L4) instars of its host* Chrysophtharta agricola.

Behaviour	Small Hosts (range)	Large Hosts (range)
Host Defense		
Tail flicks during an oviposition attempt	6.4 ± 1 (3-12)	20.0 ± 6.5 (1-46)*
Times host walked away during oviposition attempts	0.3 ± 0.2 (0-1)	8.1 ± 2.3 (2-17)*
Oviposition		
Time for ten ovipositor insertions (sec)	178 ± 51 (92-479)	1309 ± 511 (147-3600)*
Mean duration of oviposition bouts(sec)	6.1 ± 0.9 (3 – 10)	12.1 ± 1.7 (8-22)***
% of oviposition bouts that led to ovipositor insertion	89 ± 3.9% (75-100%)	49 ± 10% (10-89)*
Grooming		
% time spent grooming prior to achieving all ten ovipositor insertions	3.2 ± 2.8% (0-19)	21.4 ± 4.6% (1-40)***
Frequency of grooming bouts	0.9 ± 0.7 (0-5)	14.8 ± 5 (0-36)*
Grooming bout duration	3.3 ± 2.7 (0-19)	13.8 ± 5.4 (0-41)*

*$p < 0.05$ *; $p < 0.01$**; $p < 0.005$ ****

Overcoming internal defence. Overall, the two models, using host instar and number of ovipositor insertions as factors, were significant in either predicting premature host death ($G = 11.76$, $df = 2$, $p = 0.003$), or whether a host or parasitoid would pupate ($G = 14.39$, $df = 4$, $p = 0.006$). In the first model, being parasitised significantly increased the likelihood of premature host death ($t = -3.07$, $p = 0.002$, odds ratio 0.53, (0.36 – 0.79; 95% confidence limits), though this was not affected by host instar ($t = -1.08$, $p = 0.28$, odds ratio = 0.81 (1.18 – 0.56; 95% confidence limits) (Fig. 5.3).

In the second model the number of additional ovipositor insertions (over and above the first) was a significant predictor of whether a parasitoid or beetle pupates ($t = -2.75$, $p = 0.006$) with the likelihood of a beetle pupating decreasing by a factor of 2.5 for every insertion of the ovipositor (odds ratio 0.4 (0.21 –0.77; 95% confidence limit). However, additional ovipositor insertions did not increase the likelihood of premature host death ($t = 0.59$, $p = 0.55$, odds ratio = 1.18; 2.02 – 0.69, 95% confidence limit). The likelihood of premature host death averaged around 20% and was not significantly different between host instars ($t = 0.38$, $p = 0.7$, odds ratio = 1.08; 1.6-0.73; 95% confidence limit). Similarly, the probability of parasitoid pupation ($t = -1.88$, $p = 0.06$, odds ratio = 0.65; 1.02 – 0.41 95% confidence limit), which averaged around 60% was also not significantly different between instars. Finally, the probability of the host surviving parasitism, assuming that all hosts had eggs laid in them, averaged around 20%.

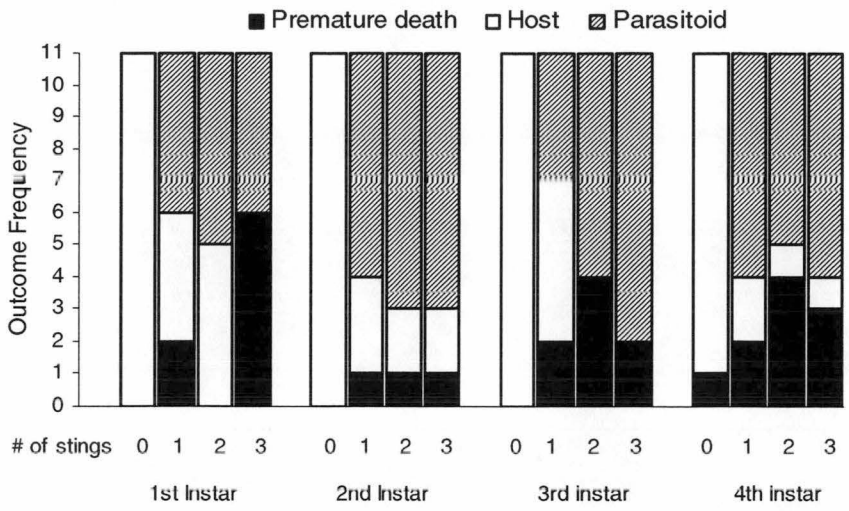

Figure 5.3. *Outcome frequency versus host instar and number of ovipositor insertions by the parasitoid* Eadya paropsidis *on its host* Chrysophtharta agricola *larvae in laboratory tests.*

5.3.4 Host instar, development time and body size.

Host instar had a significant effect on larval duration ($F = 5.6$, $df = 3$, $p = 0.0025$), with parasitoids developing from first instar hosts completing overall larval development on average over one day later (7% longer duration) than those from the other instars (Fig. 5.4a). There was a large variability in pupal weights with a non – significant trend towards lower pupal weights as the instar of the host parasitised increased ($F = 1.04$, $df = 3$, N.S.) (Fig. 5.4b). Parasitoid pupal weights reached an upper average of 36.1mg $\pm$ 1.9 SE which is around 40% of the host's average final instar live weight prior to pupation.

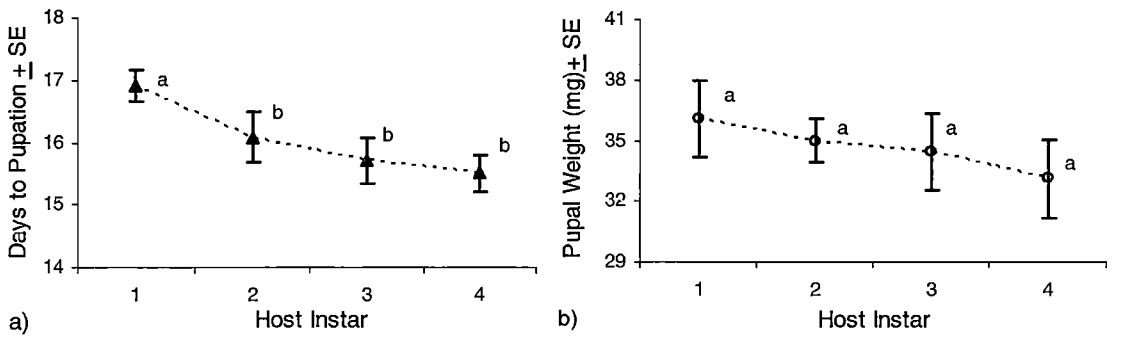

Figure 5.4. *Host instar versus (a) larval duration of the parasitoid* Eadya paropsidis *at 21 °C and (b) pupal weight. Those data points with the same letter are not significantly different (LSD).*

The growth pattern of larval *E. paropsidis* was significantly different for parasitoids that originated from first instar compared to fourth instar hosts (F-ratio = 236.9, df = 4, p<<0.001) (Fig. 5.5a). The development time of parasitoid larvae developing in first instar hosts was longer and resulted in heavier final larval weights, a similar result as was seen in the previous experiment. Host instar had a significant effect on larval dry weights (F = 5.16, df = 1, p = 0.026). We recorded only one parasitoid larval moult, around days 11-12 of development, where the parasitoid larva changed from a mandibulate first instar to a non-mandibulate second instar. Prior to this moult, larvae developing from first instar hosts were smaller than those developing from fourth instars. However, after this moult, those parasitoid larvae developing from first instar hosts were larger than those developing from fourth instars (Fig. 5.5b). In general, the larval weight of parasitoid larvae prior to the end of their first instar when developing from first instar hosts was approximately equal to that of those developing from fourth

instars one day earlier and suggests that the developmental lag (see Fig. 5.4a) occurs early in parasitoid development.

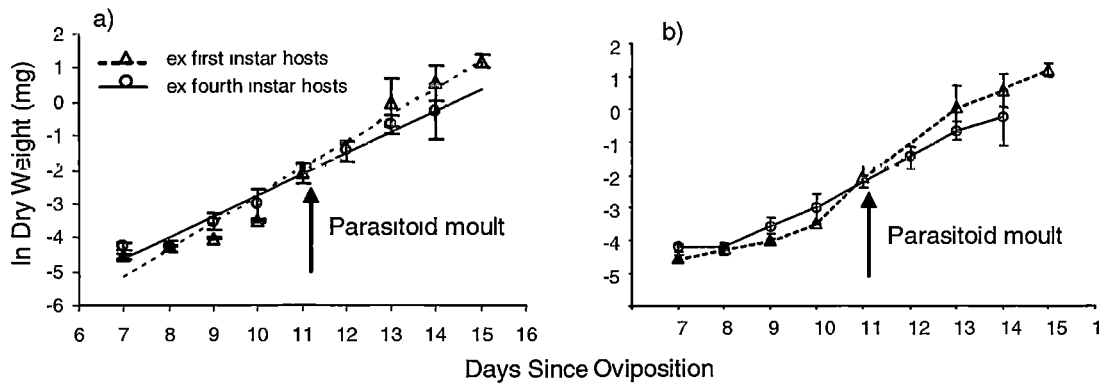

Figure 5.5 *Natural log of larval weight vs age for larval* Eadya paropsidis *developing in first and fourth instars of its host* Chrysophtharta agricola. *a) regression lines used in analysis; b) actual growth patterns. L4s sampled on day 11 and L1s sampled on day 12 contained no parasitoids. By day 14, parasitoids ex L4s had pupated*

DISCUSSION

Eadya paropsidis showed a consistent oviposition preference for early instars of its host *C. agricola*, both in the field and laboratory. *E. paropsidis* was gaining fitness benefits in terms of body size and host handling time by using smaller instars. Furthermore, apart from a developmental delay that extends development time from first instar hosts by ca. 7% over that from other instars, there was no evidence to suggest that using large hosts benefits the parasitoid using the fitness parameters measured here.

The efficacy of host defensive behaviour was greater in large hosts compared to small and effectively halved wasp oviposition success following initiation of an

oviposition attempt. By walking, large hosts were often able to make the parasitoid abandon oviposition attempts. Tail flicking by large hosts also broke contact between ovipositor and host, and facilitated parasitoid contact with larval defensive glands. Such contact led to more frequent and extended bouts of grooming with large hosts. Several studies conclude that there are costs for parasitoids associated with the behavioural defence of larger hosts, which may injure the parasitoid or increase handling time (Allen 1990, Harvey 1996, Brodeur *et al.* 1996, Al-Saffar & Aldrich 1998, Kairo and Murphy 1999). Field collected *E. paropsidis* females have a lifespan in the laboratory of up to 28 days (mean = 13.6$\pm$ 1.1 SE, n = 28, range = 2 to 28) and a fecundity of up to 1193 eggs (n = 5, range = 804 to 1193) (Rice 2005). With a maxima of 16 hours of daylight available each day of the adult season, an *E. paropsidis* would need to average at least 5.5 eggs/daylight hour over her entire lifespan to entirely deplete her egg compliment. In the field, some of this time may be used in other activities such as feeding, mating, host location and egg maturation. Therefore, handling costs associated with large hosts may ultimately impact upon parasitoid fitness in this species, by reducing its realised fecundity.

The developmental delay from first instar hosts yielded parasitoids that are at least as large as those from older hosts as predicted by Mackauer and Sequeira (1993) for parasitoids that are selecting primarily for body size. However, the body weights of parasitoids from fourth instars hosts are lower than those from first instars, suggesting that factors other than straightforward resource limitation are affecting parasitoid growth. Other studies have implicated nutritional incompatibility or other physiological problems that are associated with larger hosts in inhibiting parasitoid growth and

development from large hosts (e.g., Beckage and Templeton 1985, Harvey 1996, Strand 2000). Slansky (1986) reports variable, but generally low growth efficiencies for parasitoids, with a mean growth efficiency, or conversion of assimilated food into parasitoid biomass, of 37% across 15 species, and pupal weights of *E. paropsidis* are around 40% of the mass of unparasitised final instar hosts. For *C. agricola*, from midway through their fourth instar, at 21°C, there are only 2 feeding days left before reaching a pre-pupa where no further feeding occurs. *E. paropsidis* eggs require around five days to hatch at 21°C, so eggs deposited into hosts older than late third instars will not hatch and begin feeding until the host has finished growing. Hence, assuming *E. paropsidis* has average growth efficiency, to achieve a mean pupal weight of 36 mg would require a host of 103 mg which is in the upper range of final instar host weights. Consequently, not only is there no further food input in large instar hosts, but it is also likely that respiration of both the host, and the actively growing parasitoid, will reduce the amount of host resources that are available for parasitoid growth. Furthermore, once the host dies there may be some deterioration in nutritional quality of the host tissue towards the end of parasitoid development.

However, we have assumed that there is no contribution to *E. paropsidis* growth resulting from the parasitoid developing while allowing the host to continue its own feeding and growth and potentially replenish resources consumed by the parasitoid, which would ultimately yield a larger parasitoid. This would be evident if parasitism by *E. paropsidis* caused the host to either grow faster or develop for longer than unparasitised hosts, and would be more pronounced in hosts that are parasitised early in their development. However, Slansky (1986) reports a general tendency for solitary

parasitoids, like *E. paropsidis,* to reduce host growth rates rather than increase it, as is generally found with gregarious parasitoids. Nevertheless, there are exceptions to the rule, and increased food consumption by parasitised hosts may result in larger parasitoid larvae from small hosts compared to those from large hosts, irrespective of the final potential size of the host, and may also explain our findings.

Our study did not find, as many authors report, an improved ability to kill immature parasitoids within the host as host age at parasitism increases (van Alphen and Vet 1986, De Bolt 1991, Bratti *et al.* 1992, Strand and Pech 1995), with *C. agricola* survival averaging around 20%. No host age bias in parasitoid survival was evident in the field collected data as parasitism levels were concordant with those of the choice trials that were determined by dissection. However, self superparasitism did improve the probability of a parasitoid fully developing in the host irrespective of host instar. This may be advantageous when competition for hosts among foraging females is high, as multiple offspring may have a better chance of out-competing other parasitoids (Visser 1993). Certainly discrimination against parasitised hosts was not evident, with *E. paropsidis* continuing to oviposit in parasitised hosts even when unparasitised hosts were still available. If self-superparasitism is being used by *E. paropsidis* to overwhelm host defences, then the increased defensive behaviour of large hosts would be of even more significance as superparasitism would take considerably longer and involve more risk into larger hosts, than into smaller hosts.

Harvey *et al.* (2004) suggest that the fitness-host size relationship for koinobiont parasitoids attacking hosts that may potentially grow much larger than the parasitoid is

distinctly dome shaped, i.e., there are better fitness returns from mid-sized hosts. This is due to increased host-induced parasitoid mortality from larger hosts and, presumably, smaller size and/or increased developmental time (hence risk of premature host death) from smaller hosts. However, with *E. paropsidis* there was a probable decrease in overall fitness with increasing host instar, though this is dependent on how important any selection pressure on having an extended development from first instars is, with any strong selection pressure reducing fitness resulting from early instars. As yet there is no general consensus in the literature on the importance of rapid development to parasitoid fitness. However, for *C. agricola,* Nahrung *et al.* (2001) report that mortality is higher in early instars than it is later in development.

We conclude that the preference for early instar hosts of *E. paropsidis*, both in the field and laboratory, is mediated by the host's improved ability to prevent oviposition with increasing age and selected for at least in part, due to gains in body size. Furthermore, our findings suggest that estimates of functional constraints on parasitoid fitness in terms of host size and quality also need to account for a host's ability to prevent or delay oviposition. We also suggest that age-related host defence prior to oviposition may be an important element in determining the relationship between host age/size and parasitoid fitness especially in those hosts able to injure or repel the attacking parasitoid.

CHAPTER 6

Life History and Host Use in Two Tachinid Flies: I. Host Stage Selection.

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Abstract.

Despite great diversity in insect parasitoids, the parasitic Hymenoptera have enjoyed almost exclusive attention in studies on parasitoid fitness and host use, largely at the expense of other parasitoid groups. The diversity of life histories and impressive host ranges of tachinid flies makes them ideal candidates for testing predictions of optimality theory and for broadening the theoretical framework upon which parasitoid fitness is based. Here, we used two tachinid flies, *Balde striatum* Rice and *Paropsivora australis* Macquart, parasitising the same host, *Chrysophtharta agricola* Chapuis (Coleoptera: Chrysomelidae), to investigate influences of life history traits on host use. First, we tested whether flies, from a widely polyphagous family, show intraspecific host preferences and examined some behavioural interactions that may explain our findings. We then used *P. australis*, which lays unembryonated eggs onto unparalysed hosts, as an example of a life history strategy not found in the Hymenoptera, to see whether life histories not yet considered could contribute to the understanding of parasitoid fitness and host use.

Both species of tachinid fly showed consistent host stage preferences. *P. australis* preferentially oviposited onto large hosts, especially fourth instars, where parasitism was up to five times that of early instars. In contrast, *B. striatum* favoured early instars where parasitism was up to three fold that of large hosts. However, the differing oviposition strategies resulted in vastly different interaction times with the host during oviposition, with *B. striatum* taking on average 15 seconds to larviposit on a host whereas *P. australis* took < 1 second to lay an egg on the host. The oviposition strategy of *B. striatum* may restrict its parasitism success to earlier instar hosts that are less able to defend themselves from parasitoid attack. By placing unembryonated eggs onto the host, *P. australis* faced a 50% chance of eggs being lost during host larval moults, and did not counter this by further maturing eggs within the uterus or by selecting newly moulted hosts. Internal host defence did not vary with instar, with 34% of all hosts parasitised dying prematurely and 37% yielding a parasitoid.

Despite differing reproductive strategies in tachinids, our results clearly indicate intraspecific host preferences for both species of fly and that differences in oviposition strategies impact on tachinid chances of successful parasitism.

6.1 *INTRODUCTION*

Optimality theory (*sensu* Maynard Smith and Price 1973, Maynard Smith 1982) predicts that an animal should behave in a way that maximises its fitness. Recently, parasitoids have predominated as the functional group of choice in the development of much of optimality theory because all the resources required for development and growth are derived from just one host individual (Charnov and Skinner 1985, Feener and Brown 1997, Harvey *et al.* 2000, Hawkins 2000). Thus, parasitoid decisions on host choice, and strategies for their optimal use, have received considerable attention. Optimality theory assumes that insects vary inter- and intraspecifically in their suitability as hosts for parasitoids and consequently, parasitoid reproductive fitness can be greatly influenced by host choice (Harvey *et al.* 2004, Chapter 5).

For solitary idiobiont parasitoids (those that prevent further host growth at parasitism), host quality is assumed to be directly proportional to host size at parasitism because larger hosts tend to yield larger parasitoids, which in turn correlates to other measures of fitness such as fecundity and mating success (Gunasena *et al.* 1989, Visser 1994, Kazmer and Luck 1995, West *et al.* 1996). However, a host's ability to survive oviposition attempts by parasitoids often improves with age, and older individuals are often better able to injure, repel or escape ovipositing females (Allen 1990, Harvey 1996, Brodeur *et al.* 1996, Al-Saffar and Aldrich 1998, Kairo and Murphy 1999, Chapter 5) or encapsulate a parasitoid's eggs or larvae (van Alphen and Vet 1986, De Bolt 1991, Bratti *et al.* 1992, Strand and Pech 1995, Harvey *et al.* 2004). Recent studies focussing on koinobiont parasitoids (those that allow the host to continue to develop after parasitism) have taken this into account, and revealed host quality does not

necessarily increase with host size. For example, Harvey *et al.* (2004) showed that instar-specific mortality can reduce fitness returns from some instars and Chapter 5 demonstrated that host defence behaviour prior to oviposition can also contribute to the quality of a host. Therefore, if fitness returns to parasitoids vary with the instar of the host that they use, variation in host size and age should result in host stage preferences in optimally behaving parasitoids (Hopper 1986, Hopper and King 1984, Kouame and Mackauer 1991). Such preferences have been demonstrated in several hymenopteran species (Mackauer 1973, Liu *et al.*1984, Gerling *et al.* 1990, Wang and Liu 2002).

The use of parasitoids in studies of optimality has been largely confined to insects from just one insect taxon, the Parasitica (parasitic Hymenoptera), resulting in a large portion of parasitoid fitness theory being based on a single taxon, with other parasitoid groups being largely ignored (Waage and Greathead 1986, Godfray 1994, Hawkins and Sheehan 1994, Stireman unpublished). Over-representation of the Parasitica in theoretical work may be detrimental to our overall understanding of parasitoid biology in light of their inherent features that make them unique among parasitoids as a whole (Feener and Brown 1997). The most important feature that sets this group apart is the single evolutionary lineage that gave rise to the Parasitica compared to the multiple (possibly hundreds) of lineages in the other parasitoid groups (Eggleton and Belshaw 1993). Therefore, it is unlikely, for example, that the Parasitica alone can shed light on the circumstances that led to the rise of the parasitoid lifestyle (Feener and Brown 1997), especially considering the much greater diversity of life histories to be found in other parasitoid groups (Eggleton and Gaston 1992).

After the Hymenoptera, with 56 families that have parasitic species, the Diptera contains the next greatest number of parasitoid families with 22, but despite having fewer parasitoid families, the Diptera attack a greater range of taxa, with hosts from 22 orders and five phyla compared to 19 and one for the Hymenoptera (Eggleton and Belshaw 1993). Within the Diptera, the family Tachinidae (who are all parasitoids, Colless and McAlpine 1970) may be the most speciose in the world, with species estimates ranging from 8000 (Wood 1987) to 8200 (Crosskey 1980). The Tachinidae differ from the Parasitica in a number of life history traits with the parasitic life style having arisen numerous times. Oviposition methods of the Tachinidae are more varied (summarised in O'Hara 1985), adult flying abilities are greater, and both location of larvae within the host and larval respiration strategies differ. Such a diversity in life histories enables the Tachinidae to exploit an impressive range of hosts that rivals that of the entire Parasitica (Eggleton and Belshaw 1993).

The Tachinidae are widely polyphagous in comparison to the Hymenoptera (Stireman and Singer 2003), a trait that is possibly made feasible by the use of respiratory funnels which are not used by Parasitica and/or a less intimate relationship with the host than that of the Parasitica (Belshaw 1994). This raises the question of whether members of such a widely polyphagous group will show intraspecific host preferences as predicted by optimality theory, and here we tested for evidence of host stage preferences in two tachinid flies. We chose two tachinid flies, *Balde striatum* Rice and *Paropsivora australis* (Macquart) because they differ markedly in life-history characteristics (Chapter 2), yet parasitise the same host species, *Chrysophtharta agricola* (Chapuis) (Coleoptera: Chrysomelidae: Paropsini). These natural history differences

enabled us to look at ways that life-history traits may shape host use, and to examined interactions between parasitoid oviposition behaviour and changes in host defensive behaviour as the host matures. Lastly, we used *P. australis* that attaches unembryonated eggs to the integument of its host and produces a respiratory funnel as an example of a parasitoid that has a life history strategy not found within the koinobiont Parasitica, to test whether the life history strategy of *P. australis* impacts host use, and also whether there was an effect of host age on success of physiological host defence.

This chapter reports on the first part of this work, and in Chapter 7 we continue our examination of life history and host use in these two flies, but we focus more on the impacts that host size has on the developmental characteristics of these two flies, and make direct comparisons between the two species.

6.2 *MATERIALS AND METHODS*

6.2.1 The Study System. The host, *Chrysophtharta agricola* feeds on juvenile eucalyptus foliage where it undergoes four larval instars before it drops to the ground to pupate (Nahrung 2003). Larvae range in size from 2.7 $\pm$ 0.09 (se) mm long and 1.06 $\pm$ 0.05 mg in weight (first instars) to over 10.20 $\pm$ .25 mm and 81.67 $\pm$ 4.02 mg (fourth instars) (Ramsden and Elek 1998, Chapter 7). They are gregarious and possess a pair of eversible defensive glands on their eighth abdominal tergite that release a liquid probably containing hydrogen cyanide, which is thought to deter natural enemies (Moore 1966). The larvae use several defensive behaviours when parasitoids attack, especially tail flicking and walking away (Chapter 5). Both of these behaviours are more likely to occur in later-stage larvae, with an increase in size and mobility in larger hosts

(Chapter 5). Larvae are present in the field, feeding on foliage, from mid November to mid March.

Paropsivora australis is a medium sized tachinid fly (ca. 7-8 mm in length) that adheres large white unembryonated eggs on the integument of its host which hatch after two to three days and burrow into the haemocoel of the host. Larvae maintain contact with the atmosphere with their posterior spiracles, eventually forming a respiratory funnel. *P. australis* has also been reared from *Chrysophtharta bimaculata* (Crosskey 1973), and unidentified species of *Paropsivora* have been reared from several species of paropsine leaf beetles on mainland Australia (Tanton and Khan, 1978, Tanton and Epila 1984, Tribe 2000) , so the host range of *P. australis* is potentially larger than is presently recognised.

Balde striatum is a smaller tachinid fly than *P. australis* (ca. 5 mm) that deposits first instar larvae onto the underside of its host which burrow into the host where they do not use a respiratory funnel, but rather are free living in the haemocoel. Only one other host of *B. striatum* is known and this only from a single rearing record from *Acacicola orphana* (Erichson) in Tasmania (Chapter 2). Both species of tachinid fly oviposit whilst standing on their host (AD Rice *pers obs*).

6.2.2 Host Stage Parasitism in the Field

Parasitism rates of all four instars were determined using two methods, yielding data on parasitism levels for three larval parasitoids: the two tachinid flies, *P .australis* and *B. striatum*, and a braconid wasp, *Eadya paropsidis*. Here we only report on

parasitism by the two tachinids, and do not attempt to account for any competition or interference that may have occurred between parasitoid species in the field.

Sentinel larvae. Cohorts of unparasitised (sentinel) larvae of each instar were simultaneously exposed to tachinid flies and then taken to the laboratory for rearing and assessment of parasitism rates. Levels of parasitism were determined by the successful pupation of a parasitoid. For laboratory rearing we used either 50 or 100-ml plastic containers depending on the cohort size and instar. In each container moistened paper towel and cut *E. nitens* foliage was added and replenished every three days until parasitoid and beetle pupation was complete. Ventilation was provided via a 5 cm hole in the lid of the container covered with a perforated plastic film. Larvae were reared at 22 °C and 16L:8D photoperiod. Sentinel larvae were exposed to parasitism at two *Eucalyptus nitens* plantations of three and five years of age.

Site 1 (Florentine Valley: 42°38S 146° 29E). *C. agricola* egg batches were bagged on trees along both sides of a break of 300 m that ran through the plantation. Fine mesh bags (30 cm x 50 cm), were placed over the shoot containing the egg batches and held in place with flagging tape. Twenty egg batches were bagged every two weeks over four site visits in November and December 2001. The bags were removed on 8 January 2002, and larvae were exposed to parasitism for three days. Only cohorts comprising one instar were exposed (i.e., larvae of different instars within cohorts were removed) and Tanglefoot™ prevented larvae from leaving or invading the shoot and prevented spiders from preying on the host larvae. Losses of cohorts from broken branches, damaged bags, hatch failure, and the presence of predatory mirids within the bags meant that a total of

only 38 cohorts consisting of 558 larvae (mean size 15 ±2.1 SE; range 3 to 68) were successfully exposed and retrieved.

Site 2 (Frankford 41°20S 146°45E) Six egg batches (5 + 1 control) were bagged within each of four 100 m rows in a three y.o. plantation. This was done three times over consecutive two week visits during November and December 2001. On December14th 2001, 60 bags were removed and the range of larval instars from firsts to fourths was exposed to parasitoids for three days. In total, 1388 larvae from 54 cohorts (mean size 26 ± 2.0 SE; range 4 to 79) were successfully exposed to parasitoid attack for three days and retrieved. Twenty one cohorts were not included in the analysis (or on Table 6.1) because they were comprised of mixed instars, eight were lost and 12 remained bagged to serve as controls to ensure that the parasitoids had not accessed the hosts within the bags prior to the commencement of the exposure of larvae. Thus we had 844 larvae from 31 cohorts from this site included in our analysis.

There were a number of unparasitised cohorts of all four instars which resulted in highly skewed data, so we used nonparametric chi-square analysis to look for instar-dependence of parasitism rates. No parasitoids were reared from the control cohorts and they were not used further, and no retrieved cohorts of five or less individuals were parasitised by either tachinid fly so small cohorts did not contribute unduly to average parasitism rates.

Larval Collections: The second method used to determine host stage parasitism was to collect cohorts of each instar from each field site and rear them in the laboratory

as described above. Changes in parasitism as instar increased indicated parasitism of that instar. Five cohorts of each of the four instars were collected every fortnight at each field site for the entire season (November – March) resulting in 2658 larvae from 99 cohorts (mean size = 27 ± 1.7 SE, range 4 85) from Florentine Valley, and 2988 larvae from 122 cohorts (mean size = 25 ± 1.7 SE, range 5 – 100) from Frankford. Cohorts were collected without conscious bias throughout the plantation, but as far as possible towards the end of each instar. Only one cohort was sampled from a given tree on the same day, all larvae on a shoot were considered to be from one cohort and only cohorts consisting of a single instar were collected. Collected cohorts were held in separate plastic bags and transported in a cooled icebox to the laboratory. For instars two, three and four we subtracted the previous instars' parasitism level to give stage-specific parasitism, and used χ^2 to test for host instar dependence on the percentage of larvae parasitised.

6.2.3 Host Stage Preferences

Chrysophtharta agricola larvae were reared from eggs collected weekly from the Frankford and the Florentine Valley field sites. Eggs batches were hatched and larvae were reared as per sentinel larvae.

Gravid *P. australis* females were collected from both field sites and held individually in gauze-topped 500-ml cylindrical plastic holding cages. A 20-ml plastic vial containing paper wadding, water and a sprig of fresh *E. nitens* foliage was attached to the base of the cage. A drop of honey was smeared onto the gauze and replaced as necessary. Five larvae of each instar were presented simultaneously in a choice design on a single shoot of *E. nitens* foliage to each of 17 gravid *P. australis* females in their

cages. Larvae were exposed to flies for five hours at ambient temperature of ca. 22 °C, larvae were scored for parasitism by counting the fly eggs on each larva under a dissecting microscope at 10x magnification.

Gravid, field-collected *B. striatum* would not oviposit in the cages used for *P. australis* and were instead held in groups of fifteen females in a larger cage (50 cm x 50 cm x 50 cm), made from an aluminum frame and floor with soft black netting forming the top and side panels. Water was provided via a paper towel wick inserted into a bottle of water, and honey was smeared into the netting panels and replaced when consumed. All cages were kept in the laboratory at ambient temperature (15 - 22 ^{O}C) and photoperiod. Despite good oviposition activity, successful parasitism by *B. striatum* was difficult to quantify. Therefore, we recorded oviposition behaviour on videotape and analysed oviposition behaviour using Observer ^{TM} event-recording software. Each instar was presented as a discreet cohort of ten larvae on a separate shoot of *E. nitens* foliage simultaneously in the cage, to a group of 15 gravid female tachinid flies. This was repeated for four different groups of 15 flies, and the tachinids were recorded ovipositing for one hour from the time of the introduction of the larvae. We recorded oviposition frequency into each instar as well as the duration of an oviposition bout. We used ANOVA and post-hoc LSD to test for the effect of host instar on the number of oviposition bouts. Violations of independence were addressed with the use of a rank transformation in conjunction with ANOVA as recommended by Iman *et al.* (1984) and Zar (1996).

6.2.4 Influences on parasitism success.

In the following experiments we aimed to identify the key elements that determine the success or failure of parasitism, and how they changed between host instars. We partitioned the process of parasitism into three stages: before oviposition; between oviposition and egg hatch (egg stage) and after egg hatch when the larvae enter the host (host immune efficacy).

Before oviposition. The likelihood of a parasitoid being dislodged during oviposition before an egg or larva is deposited is directly related to host age and to duration of time needed for oviposition, because the propensity and intensity of host tail flicking increases as the host grows (Chapter 5). Therefore, we measured the duration of oviposition for each species of tachinid fly. We used video data recorded from the previous experiment and Observer^{TM} software to calculate the mean duration of oviposition bouts by *B. striatum* on its preferred host stage, the second instar. A bout was defined as the time between a fly alighting on the dorsal surface of a host, recurving its abdomen around to the ventral surface (presumably to deposit a larva) and the fly leaving the host. For *P. australis,* we presented three gravid field collected females with four larvae individually in a petri-dish and again, recorded oviposition behaviour on videotape. Because oviposition was so quick, we measured the duration of oviposition using slow motion video replays to the nearest second.

The egg stage. To test whether a parasitoid's developmental success following oviposition changed with host instar we used *P. australis* because its externally deposited egg allowed quantification of oviposition. In general, parasitoid larvae are at

risk of mortality from host immune responses, but parasitoids that deposit eggs externally also risk that eggs may be lost at host moult.

The risk posed to an egg by host moulting is determined by the period of time between host moults and the time required for the parasitoid's egg to hatch. There are two strategies that a parasitoid may use to reduce the risk. One is to oviposit only onto hosts that are unlikely to moult before the egg hatches, and the other is to mature eggs within the uterus to reduce the time spent on the integument of the host. Here, we determined the age within each instar that would result in failed parasitism due to host moult, and then tested whether *P. australis* could ameliorate the risks by choosing recently moulted hosts, and/or by further maturing unlaid eggs internally to reduce the time required for eggs to hatch.

We allowed each of six *P. australis* females to oviposit onto a fourth instar host larva for two and half hours which resulted in 60 eggs being deposited on the six hosts. We then killed the host and removed the cuticle with the eggs still attached. The eggs were then kept at 24 °C, checked hourly, and the time taken for the first egg to hatch was recorded to give us the minimum time required for egg hatch. The probability of egg loss, expressed as a proportion of the development time for each instar, was then calculated using development data for *C. agricola* at 24 °C obtained from Nahrung *et al.* (2004).

To test whether gravid females showed a preference for newly moulted hosts, we exposed a cohort of ten third instar hosts (5 just moulted and 5 approaching moult) to

each of nine gravid females as a mixed cohort. We allowed them to oviposit for three hours, counted the eggs that were deposited and compared the two treatments using a Wilcoxon signed ranks t-test. Then, to test whether eggs continued to mature within the uterus if not deposited, we deprived five females of hosts for five days and then provided each of them with five hosts and allowed them to oviposit, and at the same time five females that had been ovipositing regularly were also given five hosts each. Parasitoids were reared to pupation and development times compared using a t-test.

Host age and immune efficacy. To test how *P. australis*' developmental success following oviposition changed with host age, seven gravid, field collected females were presented with five recently-moulted host larvae of the same instar. Larvae were presented in the cages described earlier, were removed as soon as an egg was visible on them, and once all five larvae of that instar were parasitised, we introduced five larvae of another instar, and so on until each female had oviposited on a maximum five larvae of each instar. We used a give-up time of one hour so if all five had not been parasitised in that time, the fly was rested for the remainder of the day and presented with the next instar the following day. The order that each instar was presented to each female was randomised. Host larvae were then reared at 22 ^{o}C and 16L:8D photoperiod and parasitoid emergence was monitored. We used multinomial logistic regression to test for instar dependence of the three possible outcomes of premature host death, and either a parasitoid or host pupating. We did not test for variation between females because female was the basic unit of replication.

6.3 *RESULTS*

6.3.1 Host stage parasitism in the field.

Sentinel larvae: Balde striatum parasitised more second instar sentinel larvae than any other instar at the Florentine Valley, and *P. australis* parasitised more fourth instars at both sites (Fig. 6.1). Levels of parasitism by *B. striatum* varied significantly between instars ($\chi^2 = 14.84$, df = 3, $0.005 > p > 0.001$). The first three instars accounted for 92% of total parasitism by *B. striatum* at Florentine Valley, with 14 of 30 cohorts and 37 of 505 larvae parasitised, but *B. striatum* was not detected at Frankford (Table 6.1). For *P. australis* at Frankford, parasitism levels also varied significantly between instars ($\chi^2 = 147.2$, df = 2, $p << 0.001$) with no first or second instars parasitised at either site, but with ten of 16 fourth instar cohorts and 20 of 215 fourth instar larvae parasitised between the two sites.

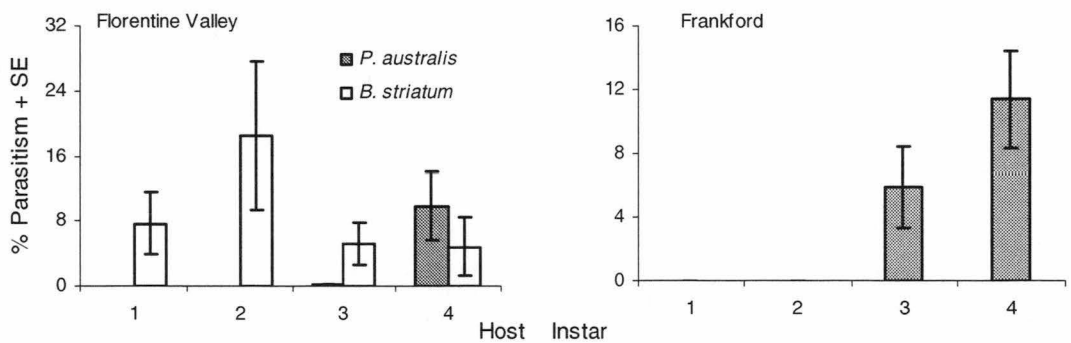

Figure 6.1 *Percent parasitism by host instar for the two tachinid flies* Paropsivora australis (grey) *and* Balde striatum (white) *in their host* Chrysophtharta agricola *during sentinel field trials at the Florentine Valley and Frankford in 2001/02.*

At the Florentine Valley we collected only seven hosts parasitised by *P. australis* from the 558 exposed, with all of these being third or fourth instars. Parasitism of fourth instars accounted for 85% and 80% of total parasitism by *P. australis* at the Florentine Valley and Frankford, respectively.

Table 6.1 *Parasitism levels in different instars of sentinel host cohorts by* Balde striatum *and* Paropsivora australis *in their host* Chrysophtharta agricola

	Cohorts exposed (parasitised)	Larvae exposed (parasitised)	Overall % parasitism per cohort ± SE (range)	% parasitism in parasitised cohorts
Florentine Valley				
B. striatum				
1st instar	9 (4)	115 (7)	8 (0 - 27)	17 (4 – 27)
2nd	10 (6)	178 (24)	19 (0 - 75)	31 (7 – 75)
3rd	11 (4)	212 (6)	5 (0 – 25)	15 (8 – 25)
4th	8 (2)	53 (3)	5 (0 – 28)	19 (10 – 28)
P. australis				
1st instar	9 (0)	115 (0)	0	0
2nd	10 (0)	178 (0)	0	0
3rd	11 (1)	212 (1)	0.1 (0 – 1.5)	1.5
4th	8 (4)	53 (6)	10 (0 – 29)	20 (8 – 28)
Frankford*				
P. australis				
1st instar	14 (0)	425 (0)	0	0
2nd	4 (0)	72 (0)	0	0
3rd	5 (3)	185 (5)	3.7 (0 – 11)	5.9 (2 – 11)
4th	8 (6)	162 (14)	8.6 (0 – 21)	11.4 (5 – 21)

*B. striatum *was not detected at Frankford in this experiment*

Larval collections: Stage specific rates of parasitism by *P. australis* varied significantly between instars at both sites (Florentine Valley: $\chi^2 = 16.8$, df = 3, $p < 0.001$; Frankford $\chi^2 = 71.2$, df = 3, $p < 0.001$). Parasitism for *P. australis* was low in early instars and higher in later instars, especially fourths, where parasitism was around five times higher than in first or second instars (Fig. 6.2).

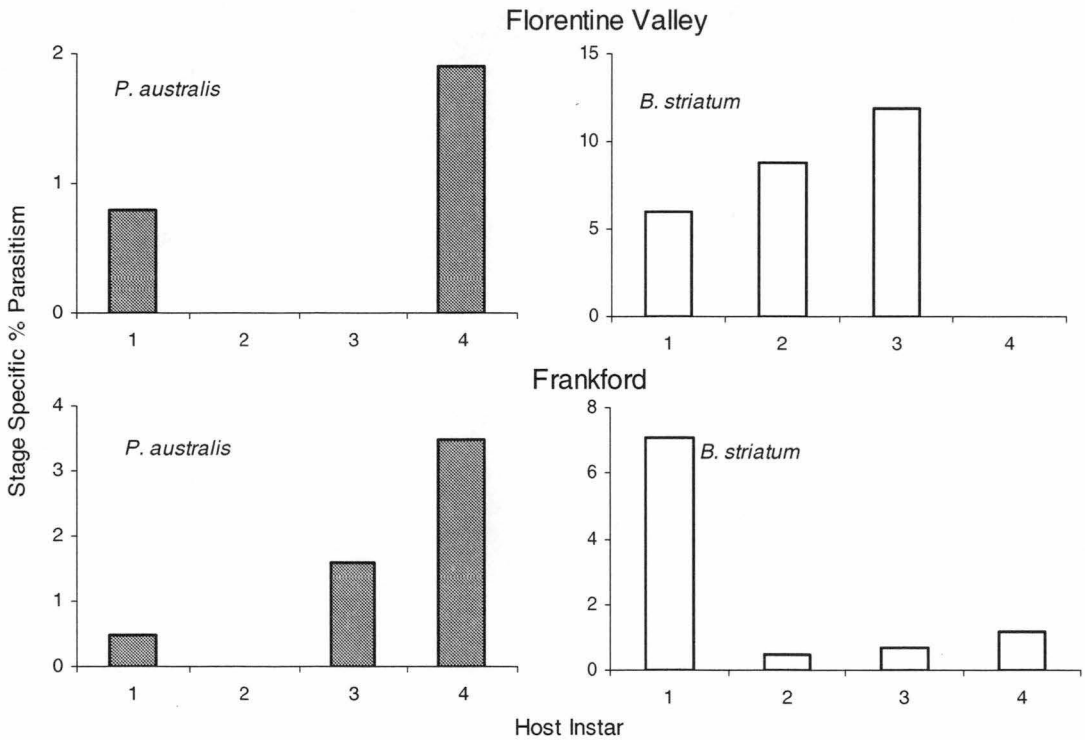

Figure 6.2 *Instar-specific parasitism rates for* Chrysophtharta agricola *by the tachinid flies* Paropsivora australis (grey) *and* Balde striatum *(white) at the Florentine Valley Tasmania*

In contrast to *P. australis*, parasitism of early instars was more common for *B. striatum*, although larger hosts were also used (Figs. 6.2 and 6.3). At the Florentine Valley, parasitism levels varied significantly between host instars ($\chi^2 = 125.8$, df = 3, $p < 0.001$). There was a steady increase in stage specific parasitism rates of 3 to 4% for each instar up to the third and no parasitism of fourth instars (Fig. 6.2). At Frankford, parasitism rates were not significantly different between instars ($\chi^2 = 1.6$, df = 3, ns).

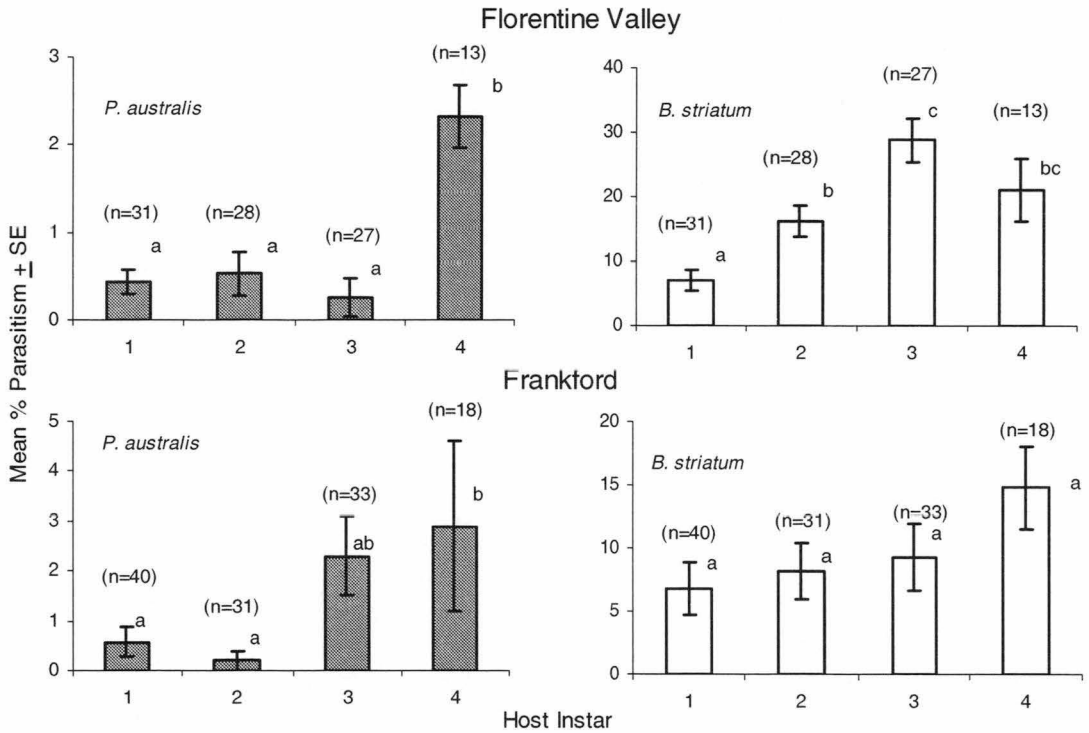

Figure 6.3 *Cumulative parasitism rates for* Chrysophtharta agricola *by the tachinid flies* Paropsivora australis (grey) *and* Balde striatum (white) *at The Florentine Valley and Frankford, Tasmania.*

Host stage specific parasitism levels at Frankford show that first instars were the host stage predominantly parasitised by *B. striatum*. While the specific instar with the majority of parasitism differs between the two sites, these results clearly indicate a field preference for early instars by *B. striatum*.

6.3.2 Host stage preference. *Paropsivora australis* showed a preference for parasitising large hosts, especially fourth instars, and deposited more eggs onto fourth instars, and parasitised proportionally more fourth instar larvae than any other host stage (Table 6.2). The mean number of larvae oviposited onto was significantly different between instars using both the parametric ANOVA ($F = 15.48$, $df = 3$, $p = 0.0001$) and rank transformation ($F_{3,48} = 21.23$, $p << 0.0005$), and although there was no significant difference between first and second instars, parasitism rates were significantly higher in third and fourth instars (Fig. 6.4). Despite there being unparasitised hosts available, the incidence of superparasitism was common and increased with instar, with parasitised fourth instars averaging almost ten eggs per host.

Balde striatum oviposited significantly more often onto small hosts, especially second instars, than any other (ANOVA, $F = 4.93$, $df = 3$, $p = 0.02$; rank transformation $F_{3,9} = 6.54$, $0.025 > p < 0.01$). Although there were significant differences between mean parasitism levels for each instar, no instar was clearly delineated from all other instars (Fig. 6.4).

Table 6.2 Parasitism patterns by *Paropsivora australis* (n = 17) in laboratory host choice tests.

	Instar 1	Instar 2	Instar 3	Instar 4
% larvae parasitised	4.7	16.5	35.3	50.6
Mean eggs per parasitised larva ± SE	3.3 ± 1.2	2.5 ± 0.6	5.1 ± 1.6	9.7 ± 2.2
Total eggs deposited	10	20	72	145

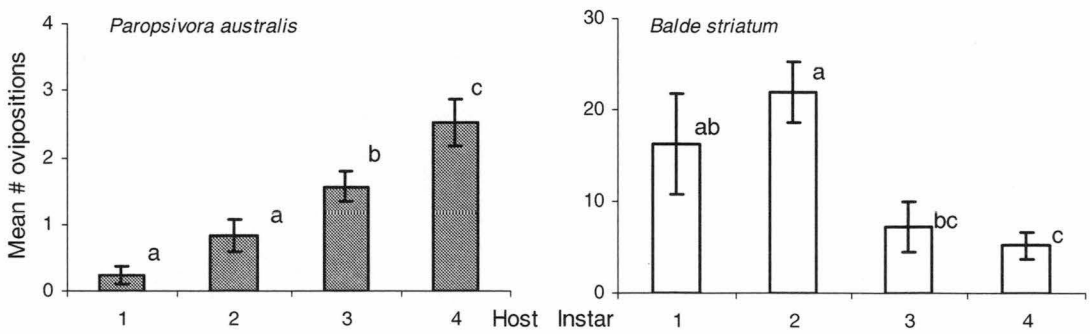

Figure 6.4 *Mean number of ovipositions (± se) on different instars of their host by female* Paropsivora australis *(n = 17) and* Balde striatum *(n =4 replicates of 15 gravid females) during laboratory tests. Data points with the same letter are not significantly different.*

6.3.3 Influences on parasitism success.

Pre-oviposition. For *Paropsivora australis,* of the 12 successful oviposition events we recorded, none took longer than one second, and were often shorter, but with one second being the smallest resolution that we could measure this was not quantified further. *Balde striatum* took considerably longer to oviposit at an average of 15.6 $\pm$ 2.9 SE seconds (range 1 - 66 sec, n = 10). Some bouts may not have been successful but as we could not readily quantify success or failure all were included in estimates.

The egg stage. The minimum time for egg hatch for *P. australis* was 31.2 hours (1.3 days) at 24 °C which is approximately half the duration of host instars one, two and three at 57.6, 57.4 and 64.8 hours respectively, at the same temperature (Nahrung *et al.* 2004). The probability of eggs being lost at each instar is therefore approximately 50% for instars one to three but zero for fourth instars. This is because there is no host moult from fourth instar to pre-pupa, and the pre-pupal stage is longer in duration than the required time for the eggs of *P. australis* to hatch. Maternal oviposition history did not affect the development time to pupation ($t = -0.09$, $df = 12$, $p = 0.9$), with offspring of females with recent oviposition history pupating on average 9.2 days $\pm$ 0.2 SE (n = 8, range = 9 to10) following oviposition, and those deprived of hosts for five days in 9.1 days $\pm$ 0.13 (n = 11, range = 9 to 10). There was no preference for recently moulted hosts, with the number of eggs placed onto recently moulted hosts not significantly different from the number placed on hosts that were due to moult ($z = -1.0890$, $p = 0.2761$), even though there were over twice as many eggs placed on early third instar hosts than late ones (Table 6.3).

Table 6.3 Parasitism rates by *Paropsivora australis* (n = 7) in within-instar host preference tests.

	Early third instar	Late third instar
Total eggs deposited	35	14
Total larvae parasitised	11	9
Mean eggs per parasitised larva ± SE	3.2 ± 0.9	1.5 ± 0.4

Host age and immune efficacy. The frequency of the three possible outcomes following *P. australis* oviposition (premature host death, failed parasitism or successful parasitism) was independent of instar ($\chi^2 = 5.15$, df = 6, ns). Across all four host instars, once an egg had been deposited, the proportion of hosts that died prematurely was 33%, the proportion that escaped parasitism was 37%, and the proportion that were successfully parasitised was 30% (Fig. 6.5).

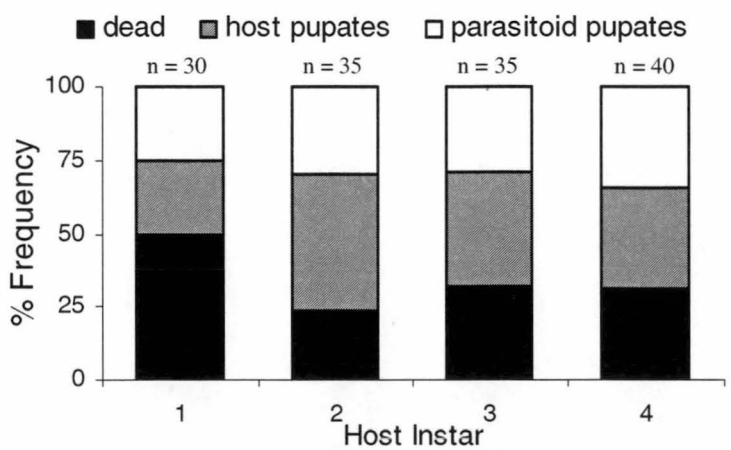

Figure 6.5 *Outcome frequency following oviposition by the tachinid fly* Paropsivora australis *for the three possible outcomes vis. premature host death, host successfully pupates or parasitoid pupates vs instar of its host* Chrysophtharta agricola.

6.4 *DISCUSSION*

This study revealed consistent and measurable intraspecific host preferences in two tachinid fly species, and evidence that their life histories influence intraspecific host use. *Paropsivora australis* showed a strong preference for fourth instars in the laboratory and field, but also readily oviposits onto other instars, particularly thirds, to a greater degree than we recorded in the field. However, our field data were based on rates of successful parasitism, whereas host preference results in the laboratory were based on egg deposition. Although the chance of successful parasitoid development was equal in all instars, the 50% loss due to host moulting in instars one to three may greatly affect estimates of host preferences based on observed parasitism rates. Nevertheless, even with a 50% underestimation of early instars in the field data, the preference tests showed a consistent preference for large hosts, especially fourths. Our results support the prediction from optimality theory that koinobiont endoparasitoids, faced with a range of hosts with highly variable potential fitness returns, should show host stage preferences (Hopper and King 1984, Hopper 1986, Kouame and Mackauer 1991). Such preferences have also been identified in other studies on tachinid flies (e.g., Adam and Watson 1971, Hondo 1992, Morewood and Wood 2002).

Parasitoid egg loss due to host moult was first reported by Salt (1938), and our loss estimates are similar to those in *Winthemia rufopicta* (Diptera: Tachinidae) parasitising *Helicoverpa zea* (Lepidoptera: Noctuidae) where 25-50% of eggs laid on the penultimate host instar are lost by moulting Danks (1975). Nakamura (1994) also identified the relationship between egg hatching time and time to the next host moult as one of the principal determinants of success of parasitism in the tachinid fly *Exorista*

japonica Townsend parasitising *Pseudaletia separate* Walker (Lepidoptera: Noctuidae). Askew (1971) reported tachinid preferences for recently moulted hosts, as a strategy that can reduce losses from host moult.

Maturation of eggs within the uterus and deposition of a fully formed larva is a common oviposition strategy within the Tachinidae (O'Hara 1985). However, Terkanian (1993) reported that in the oviparous tachinid fly *Chetogena edwardsii* juveniles hatching from previously host-deprived females reach pupation faster than those from females with recent oviposition history. Here, there was no difference in development times between offspring from our two oviposition history treatments, suggesting that *P. australis* does not mature eggs within the uterus, and may fertilise eggs just prior to oviposition, or 'dump' them. The larvipositing *Homotrixa allenii* (Diptera: Tachinidae) deposits large numbers of young in cages if hosts are not present (G.R. Allen unpublished data). Host deprived *P. australis* females deposited eggs on the sides of holding cages without hosts present, but not all females did this and the number of deposited eggs was low (A.D. Rice pers obs), which is a similar strategy in *Exorista larvarum* (Diptera: Tachinidae) (Mellini 1990, cited in Dindo and Marchetti 2005). However, acute host deprivation is unlikely to be the normal state in the field due to the abundance of hosts, and therefore high egg loads are not likely to impact on the probability of egg loss at host moult. The deposition of unembryonated eggs which results in premature death of 50% offspring deposited on early instars, may therefore be exerting sufficient selection pressure to select for a preference for fourth instars, and hence shape optimal host use by *P. australis*.

Parasitism of fourth instars involves interaction with the host instar that is best able to physically defend itself from parasitoid attack (Chapter 5). *P. australis* may circumvent this defence to a degree by depositing its egg quickly. Field observations (A.D. Rice unpublished) suggest that *P. australis* may wait for defensive behaviours to subside before ovipositing. Similar behaviour was noted by Prop (1960) in the tachinid fly *Sturmia inconspicua* attacking the sawfly *Diprion pini*. In this case *S. inconspicua* avoided hosts' defensive exudate by waiting beside a host cohort for the hosts' activity to subside before attack.

Balde striatum showed significant use in the field of, and preference in the laboratory for, small hosts, especially second instars which is unusual in the Tachinidae as the majority of reports of intraspecific preferences indicate preferences for larger, late instar hosts (e.g., Danks 1975, Hansen *et al.*1982, Fritz *et al.* 1986, Oshiki and Nakazawa 1987, Martin *et al.* 1989, Hondo 1992, Kumar *et al.* 1993, Terkanian 1993, Morewood and Wood 2002). Oviposition time for *B. striatum* is over 15 times that of *P. australis*, and we suggest that the decline in oviposition success in larger hosts is due to improved efficacy in host defensive behaviour as they grow. In *Uraba lugens* (Nolidae) improved defensive rearing and thrashing of larger larvae decreases the likelihood of parasitism by two braconid wasps by 50% (Allen 1990), and older and larger *Heliothis zea* (Noctuidae) are also better able to repel attack by the braconid *Campoletis sonorensis* (Schmidt 1974). As the tachinid deposits live young, there is no complication arising from the host's moult, but free living larvae may find it more difficult to penetrate the host than those that hatch from eggs firmly attached to the host's integument such as those deposited by *P. australis*. This may be a result of lack of

leverage to push into the host, as reported by Danks (1975) who found a 75% reduction in successful host penetration in the tachinid fly *W. rufopicta* in eggs that were not firmly attached to the host. *Balde striatum* uses the defensive rearing of the host to deposit larvae on the underside of its host where the integument appears to be much thinner, and which may also be necessary to allow the larvae to use the substrate to gain leverage for penetration. *B. striatum* may therefore require more time to oviposit because placement of the larvae may be important. Such placement is important in other tachinid species, and Nakamura (1997) reports that the tachinid fly *E. japonica* selectively deposit their eggs on certain locations of the hosts' body, in this case, to circumvent the hosts' ability to remove eggs from some areas of its body. Whatever the reason, the fly's inability to consistently remain standing on a fourth instar host that is tail flicking is strong evidence that life history is also shaping host use in this fly.

C. agricola larvae did not show an improved ability to kill *P. australis* larvae within the host as instar increases, as is common in the Parasitica (eg, deBolt 1991). This may be due to the tachinid larvae maintaining contact between their posterior spiracles and the atmosphere via a respiratory funnel produced from material deposited by the host's immune system. This strategy may allow tachinids to exploit a wide range of hosts because they do not need the intimate coevolutionary systems needed by Hymenoptera to counter physiological immune responses by the host (Belshaw 1994). However, the host, *C. agricola*, showed a similar lack of improved age related immune efficacy against *Eadya paropsidis* (Hymenoptera: Braconidae) (Chapter 5), so this result may be mediated by the host and not the parasitoid.

We conclude that members of a group that generally have wide host ranges, (although the host ranges of these two species are not yet known) still show strong intraspecific host preferences, as predicted by optimality theory, and also that the life history traits of *P. australis* affect intraspecific host use. Improved efficacy of defensive behaviour in larger hosts may interact with long oviposition times of *B. striatum,* resulting in a preference for, and greater success of parasitism in, smaller hosts. *Paropsivora australis* circumvents this defense by ovipositing rapidly and the deposition of unembryonated eggs makes fourth instars the most suitable as hosts due to the probability of eggs being lost when the host moults in earlier instars.

We have shown that the life history of both flies may be shaping intraspecific host use. Both are life history strategies that are common in the Tachinidae, although they are generally not taken into account in the development of parasitoid fitness models. Furthermore, they are but two of many oviposition strategies that are found in this ubiquitous and amazingly diverse family. To further advance our understanding of parasitoid foraging and oviposition decisions, it is clear from our study that researchers will need to expand the scope to include more groups outside the parasitic Hymenoptera.

CHAPTER 7

Life History and Host Use in Two Tachinid Flies: II. Developmental Strategies, Body Size and Sex.

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Abstract.

Growth takes time, and the slow growth-high mortality hypothesis suggests that in risky environments, selection may favour rapid development over body size and by extension, the mortality risks faced by insect herbivores can impact on the developmental strategies of their parasitoids. Furthermore, different selection pressures faced by male and female parasitoids can results in sexual dimorphisms in size and developmental rate trade-offs. Here, we compared the relationship of host size and development for two tachinid flies, *Balde striatum* Rice and *Paropsivora australis* Macquart (Diptera: Tachinidae), that differ markedly in their life-history traits, but parasitise the same host, *Chrysophtharta agricola* Chapuis (Coleoptera: Chrysomelidae). We investigated whether changes in mortality risks faced by the host, was related to the developmental strategies of its two tachinid parasitoids, and also whether the relationship was the same for both sexes.

P. australis, which mainly parasitises low risk fourth instar hosts, is strongly selected to maximise body size over developmental rate, and *B. striatum*, which preferentially parasitise high-risk early instars, is largely selecting for rapid development over body size, but with some evidence of a trade-off. Although the developmental strategies of the two tachinid flies fits with the host risk – parasitoid development hypothesis, the time saved by *B. striatum* developing quickly does not necessarily reduce their exposure to mortality risks.

The sexual dimorphism between the two species of flies is reversed, with male *P. australis* being larger than females, and female *B. striatum* being larger than males. We discuss our findings in terms of body size /developmental rate trade offs and host use, and in addition, their impact on male mating success, protandry, mating systems and female fecundity.

We conclude that parasitoid life histories are constraining intraspecific host use in these two flies and that host stage preferences have arisen from differential fitness gains between host stages.

7.1 *INTRODUCTION*

The optimizing of an organism's life-history traits almost invariably involves trade-offs between competing fitness parameters (Maynard Smith 1982, Parker and Maynard Smith 1990) and arguably the most important trade off is whether to develop rapidly at a cost in size or develop slowly and become large (Stearns 1989, Volrath and Parker 1992, Begon *et al.* 1996, Harvey and Strand 2002). There are constraints on the maximum size an animal may attain but studies across many animal groups indicate that maximizing size is an important selective force (Charnov and Skinner 1985, Waage and Godfrey 1985, King 1989). However, under conditions of high stress such as resource limitation, intense competition or high risk of predation, fast development may be favoured over larger size (Schaffer 1974, Hirshfield and Tinkle 1975). Maximising adult parasitoid body size is an important target for selection (Charnov and Skinner 1985, King 1989) because it is often positively correlated with other measures of parasitoid fitness (Mackauer and Sequeira 1993). For example, larger individuals often have higher fecundity, live longer, search more efficiently for hosts, or have more mating success than their smaller counterparts (Gunasena *et al.* 1989, Visser 1994, Kazmer and Luck 1995, West *et al.* 1996).

Feeny (1976) suggested that sub-lethal plant defences extended herbivore developmental times and exposed them to greater attack by natural enemies. Called the slow-growth, high-mortality hypothesis (Feeny 1976, Clancy and Price 1987) it has been supported in studies on herbivores that feed in exposed positions (Grossmuller and Lederhouse 1985, Benry and Denno 1997, see also Price 1984), and is widely accepted

in the literature (but see Williams 1999). Harvey and Strand (2002) expand on Price's (1984) exploration of concealment and risk in insect herbivores and suggest that parasitoid development strategies may be under selection pressures from the slow-growth, high-mortality hypothesis, via the feeding ecology of their hosts. That is, variations in mortality risks associated with different herbivore feeding ecologies are impacting on the parasitoid guilds associated with them, with parasitoids attacking high-risk hosts selecting to maximise development rate over size and those attacking low-risk hosts selecting for size over developmental rate.

Mortality risks faced by hosts, above and beyond that posed by any attacking parasitoids, may have further consequences for parasitoid reproductive biology. For example Price (1975) showed that parasitoids that attack hosts that are faced with higher risk of mortality generally have higher fecundities than those that attack low risk hosts, and categorised host mortality risk into two types. In the first type (type 'A'), host mortality is high in the early stages of development compared to later stages, with 70% of mortality occurring by mid stage larvae. In contrast, type 'B' survivorship is characterized by high mortality later in development with only around 40% of overall mortality occurring by mid stage larvae (Price 1975). Ichneumonid parasitoids attacking early stages of type 'A' hosts have over twice the ovarioles and hence an equal magnitude of increased fecundity, over those attacking type 'B' hosts. Therefore, host mortality risks can have wide ranging biological and ecological consequences for parasitoids.

Once a koinobiont endoparasitoid has parasitised a host, the immature parasitoid can take one of two developmental pathways: it may consume the host rapidly, or allow the host to continue to grow to ultimately yield a larger parasitoid (Askew and Shaw 1986, Strand 2000, Harvey and Strand 2002). Hence, the quality, or size of the host, may influence parasitoid developmental strategies. Mackauer and Sequeira (1993) and Harvey *et al.* 2000 (summarised by Harvey and Strand 2002) proposed three patterns of host use by koinobiont parasitoids. They all assume that parasitoids are maximising the use of the resources available in the host, and that either body size or development rate are being selected for, or alternatively there is a trade-off between the two. The first strategy, in which developmental rate is maximised, predicts that parasitoid developmental rate is the same from small and large hosts, and consequently small hosts yield small parasitoids. The second strategy, in which body size is maximised, predicts that parasitoids attacking smaller, immature hosts will exhibit a lag phase in which the host is allowed to continue growing, whereas those attacking larger hosts should develop at a constant rate. The resulting development times vary with host size but yield parasitoids of optimum body size irrespective of the size of the host attacked. The third strategy represents a trade-off between maximising size and developmental rate and may involve developmental lags or small parasitoids from small hosts but not to the same degree as the strategies that maximize body size or developmental rate (Mackauer and Sequeira 1993, Harvey *et al.* 2000, Harvey and Strand 2002).

Insects frequently display sexual size dimorphism and female parasitoid wasps are often larger than males (Hurlbutt 1987) frequently taking longer to develop to attain their greater size (Allen 1990, Sequeira and Mackauer 1992, Mackauer 1996, Mackauer

et al. 1997, Harvey 2000). It follows, therefore, that males and females are subject to different developmental pressures (Mackauer *et al.*1997), and that generally, females may benefit more from increased size than males. Fitness benefits are realised in both sexes by maximising their genes in subsequent generations, with males by maximising mating success and females maximising successful parasitism. In mating systems that involve lekking, males can accrue considerable advantages from arriving early, which can select for rapid male development and lead to protandry (reviewed by Morbey and Ydenberg 2001). Therefore males that sacrifice size for rapid development rate in these types of systems can be selected for, provided their small size is not a disadvantage to them in other ways such as inhibiting their ability to maintain their lek against other males or deleteriously affecting mate choice by females. Furthermore, males that emerge early may be able to mate with more females than those that emerge later (Fagerstrom and Wiklund 1982). It is well documented among the Tachinidae that in captivity, females may only be receptive to mating immediately following eclosion (Quednau 1993, Kuhlmann 1995, Zhang *et al.* 2003), a system in which early eclosing males would be advantaged, and probably reflects protandrous development. Alternatively, in mating systems with strong competition between males for access to females, or where females may be dispersed, large males may have the selective advantage (Bernal *et al.* 2001).

In contrast to males, female parasitoids appear to benefit more from being large compared to males. Larger females may be more fecund, have improved host location abilities and higher levels of successful parasitism (Salt 1941, Boldt 1974, King 1987, Godfray 1994). Moreover, by having longer development times than males or by

delaying eclosion so that females emerge after males can advantage females. For example, by arriving at lek sites after males have become established can allow improved mate assessment (e.g., Wedell 1992) or reduce costs associated with waiting for males to be ready for mating (Tagerstrom and Wiklund 1982). Harvey and Strand (2003) further propose that by delaying eclosion, female parasitoids may spend their pre-oviposition period in a relatively safe environment and hence avoid mortality risks and unnecessary depletion of metabolic energy reserves associated with activity.

In Chapter 6 we discussed the disproportionate representation of the parasitic hymenoptera (Parasitica) in the development of parasitoid host selection theory, and in light of the great variety of life history strategies in other parasitoid groups, the need to expand the range of parasitoid groups used in its development. Here we continue our investigation into developmental interactions between two tachinid flies that parasitise the same host, and expand our scope to include host risk and parasitoid developmental strategies, sexual size and developmental rate dimorphisms and the implications of host stage preferences for parasitoid fitness.

7.2 *MATERIALS AND METHODS*

7.2.1 The Study System.. The host, *Chrysophtharta agricola* feeds on juvenile eucalyptus foliage where it undergoes four larval instars before it drops to the ground to pupate (Nahrung 2003). *C. agricola* oviposit continuously throughout spring and summer and all four instars are generally present on the foliage simultaneously over the host's active season from mid November to mid March. Larvae are preyed upon by a

range of predators, including two species of coccinellids, as well as cantharids, spiders and predacious bugs from the families Miridae, Reduviidae, and Pentatomidae (Nahrung and Allen 2004). Average mortality for *C. agricola* from oviposition to the time larvae reach the fourth instar is around 95% (Nahrung and Allen 2004). About 1% or less of eggs survive to adulthood with mortality for fourth instars, pre-pupae and pupae being about 4% of total generational mortality (Allen 2002). Seventy one percent of larval mortality occurs before the moult to third instar, clearly placing this species into Price's (1974) Type 'A' survivorship curve (data from Nahrung and Allen 2004).

The tachinid flies can both develop through all four instars of their host, but they have strong oviposition preferences for particular instars, with *P. australis* preferring fourth instars, and *B. striatum* preferring early instars (Chapter 6). The flies also have distinctly different life-histories (Table 1). *P. australis* is a medium sized fly and at about 7mm in female body length (males 8mm) is about one and a half times larger than *B. striatum*. *P. australis* adheres large white unembryonated eggs on the integument of its host which hatch within two to three days and burrow into the haemocoel of the host (Chapters 2 and 6). Male *P. australis* are around 11% larger than females and *P. australis* entirely consumes its host before it pupates. *P. australis* has also been reared from *Chrysophtharta bimaculata* (Ol.) (Crosskey 1973) and may also parasitise other paropsine leaf beetles (Chapter 2).

Balde striatum is a smaller tachinid than *P. australis* (ca. 5mm) that deposits first instar larvae onto the underside of its host. It has also been reared from *Acacicola orphana* Erichson (Coleoptera: Chrysomelidae) (Chapter 2). *B. striatum* females are

around 13 % larger than males (Chapter 2). We detected no hyperparasitism for *P. australis* but *B. striatum* suffers high rates of mortality from the hyperparasitoid *Mesochorus* sp., especially late in the season. Both species emerge from late fourth instar hosts to pupate.

Table 1. *Comparison of life-history traits of the two tachinid flies used in this study:* Balde striatum *and* Paropsivora australis. *Data from; [1]Chapter 6; [2]Chapter 2; [3]this study; [4]Chapter 4. A3- third antennal segment*

	Balde striatum	*Paropsivora australis*
Host stage preference [1]	Early	Fourth
Oviposition time[1]	Long	Short
Reproductive unit [2]	Larva	Egg
Larval respiration [2]	No funnel	Respiratory funnel
Adult Size [2]	Small	Medium
Heaviest pupae [3]	Female	Same
Largest sex [2]	Female	Male
Protandrous emergence [3]	Yes	Yes
Sex dimorphism in frons [2]	No	Yes
Sex dimorphism in length of A3 [2]	Yes	No
Fecundity [3]	ca 135	ca 90
Multivoltine [4]	Yes	Yes
Hyperparasitised [2]	Yes	No

To determine whether the parasitoids are prioritising developmental rate or body size we reared each species of tachinid fly through each instar of the host and recorded the time taken to pupate and then eclose as an adult as well as the weight of each pupa. We further recorded the sex of each individual so we could compare developmental strategies and dimorphisms in developmental rate and size between males and females. Lastly, in order to investigate parasitoid fitness on the basis of size we assessed the body size of field caught females and by dissection, determined the egg load of each female.

7.2.2 Developmental Interactions

To obtain hosts for parasitism *C. agricola* larvae were reared from eggs collected weekly from sites within 2 and 5 yo *Eucalyptus nitens* plantations at Frankford (41°20S 146°45E) and the Florentine Valley (42°38S 146° 29E), Tasmania. Eggs were placed on shoots of juvenile *E. nitens* foliage in 50-ml or 100-ml plastic tubs (depending on the size of the cohort), with a moistened paper towel on the bottom. A 50-mm hole cut in the lid and covered with a perforated plastic film provided ventilation. The containers were held at 22 °C until eggs had hatched, and then at 4°C until required. Larvae were fed on *E. nitens* foliage, which was changed every three days or as needed.

P. australis and *B. striatum* were collected as gravid females from the same two field sites at Frankford and Florentine Valley. *P. australis* females were held individually in gauze-topped 500-ml cylindrical plastic holding cages. A 20-ml plastic vial containing paper wadding, water and a sprig of fresh *E. nitens* foliage was attached to the base of the cage by a velcro pad. A drop of honey was smeared onto the gauze and replaced as necessary. *B. striatum* would not oviposit in these cages and so were held in

cohorts of fifteen females in a large cage (50cm x 50cm x 50cm), with an aluminum frame and floor and soft black netting forming the top and side panels. Water was provided via a paper towel wick inserted into a bottle of water and honey was smeared into the netting panels and replace when consumed. All cages were kept on the laboratory bench at ambient temperature (15 - 20 °C) and 16L:8D photoperiod.

To obtain hosts parasitised by *P. australis*, each of ten females were given five host larvae of a given instar which were removed as soon as an egg was visible on them. Once five were parasitised, the next instar was introduced, and five parasitised individuals were again obtained until each female had parasitised five individuals of each instar, all within the same day. Order of parasitism of each instar was randomised, and only host larvae in the early stage of each instar were used to ensure that fly eggs were not shed when the host moulted.

Field collected *B. striatum* females were allowed to parasitise *C. agricola* larvae in their holding larger cage (as above) in a cohort of fifteen females. Hosts were presented in a no-choice situation in loose cohorts of 20 individuals, with all four instars being presented in random order on any given day, and repeated over three days.

Parasitised larvae were reared individually in petri dishes on *E. nitens* foliage at 21 °C $\pm$ 2, and both larval and pupal development times recorded to the nearest 24 hrs. Tachinid pupae were separated from the remains of the host and weighed immediately following pupation to the nearest 0.0001g.

We first used ANOVA to look for significant maternal effects on the developmental rates of immature *P. australis,* and as there were none for larval duration ($F = 1.26$, $df = 7$, $p = 0.29$), pupal duration ($F = 1.43$, $df = 7$, $p = 0.23$) or body size ($F = 1.55$, $df = 7$, $p = 0.19$) each datum was treated as independent. ANOVA was then used for both species to look for any effect that host instar, sex of the developing parasitoid and interactions between these two had on: overall development time, time from oviposition to pupation, time from pupation to adult emergence, and pupal weight. Post hoc LSD tests were used to test for significance of any differences between means. We also used t-test to compare lengths of right rear tibia as an indicator of body size.

7.2.3 Parasitoid Body Size and Fecundity.

To obtain a random sample of foraging gravid females of both species, we erected Malaise traps during the active season, in the field at the same sites that we collected host eggs. Both traps were located between rows of three to five year old *E. nitens* trees within plantations. Females of each species were stored in 70% ethanol until required and then pinned for measurement of the right rear tibia which was used as a measure of body size. Each fly was soaked for five to seven days in soapy water to re-constitute larvae or eggs and to soften abdominal tissues made hard by desiccation in the ethanol. Once softened, each was dissected and larvae or eggs counted.

7.3 *RESULTS*

7.3.1 Developmental Interactions. *P. australis* displayed an average increase of over five days in larval development time when developing from first instar hosts compared to fourth instars, with every increase in host instar at parasitism resulting in a

significant reduction in larval development time (Fig. 7.1a). Consequently larval duration was over 50% longer from first instars than that from 4th instars. The effect of host instar on development time was significant ($F = 95.4$, $df = 3$, $p < 0.001$), but sex was not ($F = 0.05$, $df = 1$, $p = 0.83$) despite males being on average larger than females. There was no significant interaction between host instar and parasitoid sex on larval duration ($F = 0.21$, $df = 3$, $p = 0.89$). All *P. australis* larvae, irrespective of host instar parasitised, emerged to pupate from late 4th instar *C. agricola* larvae. Male pupae developed significantly faster than female pupae irrespective of host instar ($F = 60.95$, $df = 1$, $p < 0.001$) (Fig. 7.1b). As with larval duration host instar had a significant effect on pupal duration ($F = 11.56$, $df = 3$, $p < 0.001$) though there was also a significant interaction between host instar and parasitoid sex ($F = 3.90$, $df = 3$, $p = 0.019$). Female pupae developed significantly slower when reared from either first or fourth instars.

Finally, when larval and pupal durations were combined to overall development time, host instar had a significant effect on development time ($F = 48.4$, $df = 1$, $p< 0.001$) as did parasitoid sex ($F = 32.9$, $df = 1$, $p < 0.001$) resulting in earlier adult male emergence by 1-3 days but there was no significant interaction ($F = 1.3$, $df = 3$, $p = 0.28$) (Fig. 7.1c). There was no significant correlation between individual larval and pupal development times ($\chi^2 = 2.6$, $df = 1$, $p = 0.10$). The overall development times for parasitoids from third instars were not significantly different for those from fourth instars.

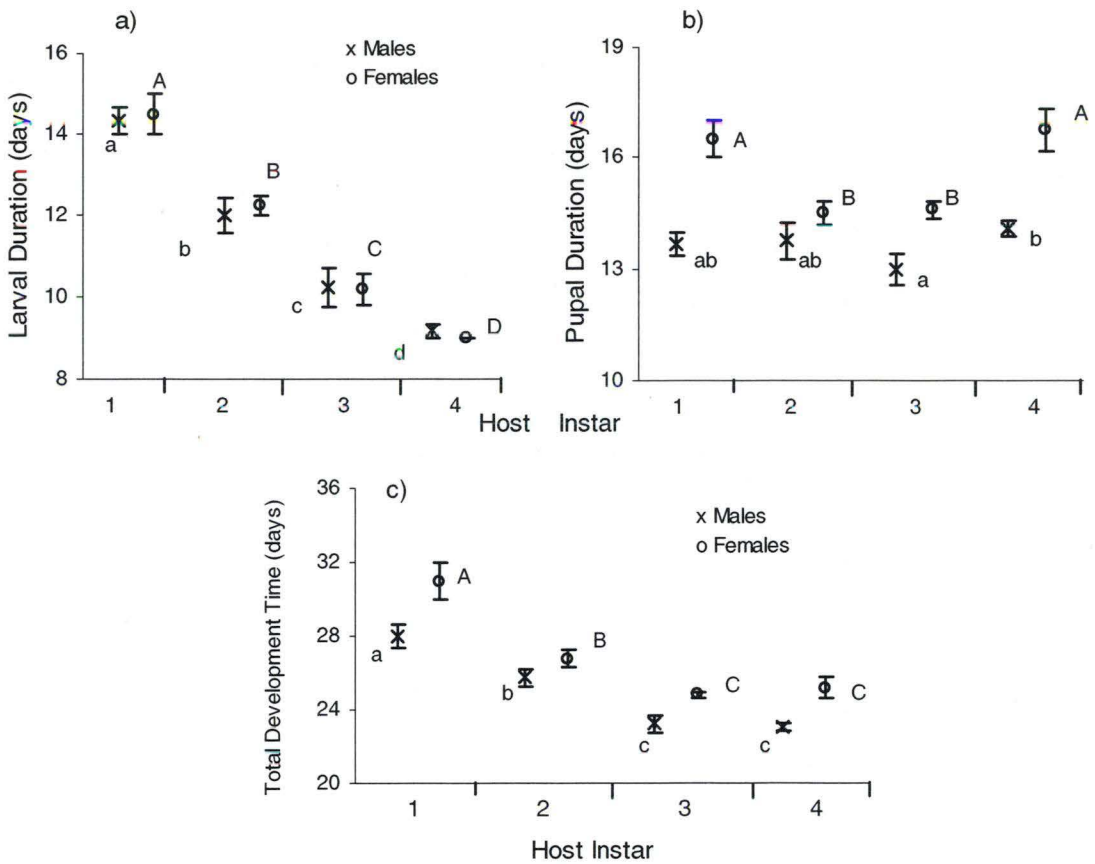

Figure 7.1 *Development times ± SE vs host instar for a) larvae, b) pupae and c) Total development time of* Paropsivora australis *developing in its host* Chrysophtharta agricola.

Despite the differences in development time, host instar had no significant effect on pupal weights ($F = 1.11$, $df = 3$, $p = 0.36$), though males were overall significantly heavier than females ($F = 13.23$, $df = 1$, $p = 0.001$) (Fig. 7.2). There was a strong interaction between host instar and sex ($F = 5.43$, $df = 3$, $p = 0.004$) with females being 10-20% lighter if they originated from the first two instars than if they developed

through third and fourth instars, which contributed to the significant difference between male and female pupal weights. Males did not significantly differ in size according to host instar at parasitism. Because most oviposition by *P. australis* in the field occurs on instars three and four (Chapter 6), we compared male and female pupal weights from instars three and four in order to gain a biologically significant comparison between the sizes of the two sexes, and found no difference ($t = -0.59$, $df = 23$, $p = 0.56$). However, using the same individuals, we did find a significant difference between tibial length of males and females ($t = -4.24$, $df = 21$, $p < 0.001$), indicating that the larger males were eclosing from pupae that were the same size as females.

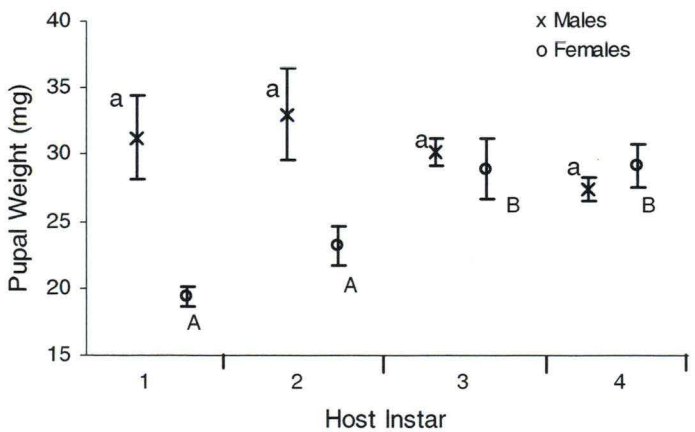

Figure 7.2 *Pupal-weights ± SE for* Paropsivora australis *developing in different instars of its host* Chrysophtharta agricola.

For *B. striatum,* as with *P. australis*, host instar had a significant effect on larval duration ($F = 7.89$, $df = 3$, $p = 0.0004$) with no significant interaction between sex and

host instar ($F = 0.65$, $df = 3$, $p = 0.59$) (Fig. 7.3a). However, *B. striatum* only had a significant developmental delay from first instars with development time from the other instars being equal. In contrast to larvae of *P. australis* where there was no difference between the sexes, male larvae of *B. striatum*, developed significantly faster than females ($F = 6.53$, $df = 1$, $p = 0.01$) (Fig. 7.3a) and were significantly smaller at pupation than females (Fig. 7.4). *B. striatum* developed faster from parasitism to pupation than *P. australis* for the first three instars, whereas when parasitising the fourth instar, the two species developed at approximately the same rate. As with *P. australis* irrespective of host instar parasitised, *B. striatum* larvae still emerged to pupate from fourth instar *C. agricola* larvae, although if parasitism occurred in first or second instar host larvae, emergence occurred before the host completed larval development, especially if the *B. striatum* parasitoids were males.

Unlike *P. australis*, host instar had no significant effect on pupation times of *B. striatum* ($F = 1.00$, $df = 3$, $p = 0.40$) though as with *P. australis*, male pupae developed significantly faster than females ($F = 21.11$, $df = 1$, $p < 0.001$) (Fig. 7.3b). There was a weak but significant interaction between host instar and sex on pupation times ($F = 3.02$, $df = 3$, $p = 0.045$).

For *B. striatum,* host instar also had a significant effect on overall developmental time ($F = 6.04$, $df = 3$, $p < 0.001$). As with *P. australis*, there was no significant correlation between an individual fly's larval and pupal developmental time ($\chi^2 = 0.12$, $df = 1$, $p = 0.72$). Sex of the emerging parasitoid significantly affected developmental times ($F = 32.05$, $df = 1$, $p < 0.001$), as it also did for *P. australis*, with males again

developing faster than females, on average eclosing up to five days earlier than females (Fig. 7.3.c).

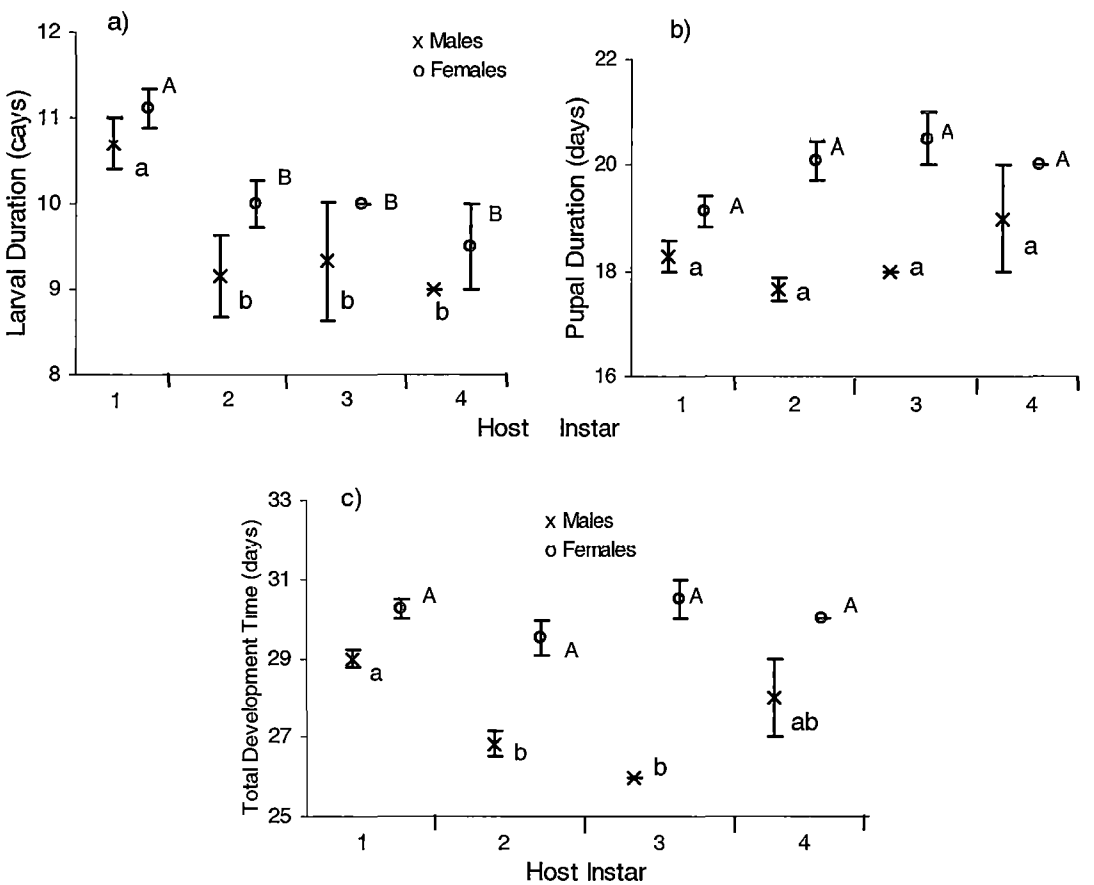

Figure 7.3 *Development times± SE vs. host instar for a) larvae and b) pupae, and c) total development time of* Balde striatum *reared through its host* Chrysophtharta agricola.

The interaction between host instar and sex approached significance (F =2.70, df = 3, p = 0.06), reflecting the difference in response of each sex to host instar. At their optimal developmental rate, female developmental times were around 17% longer in *B.*

striatum (30 days) than in *P. australis* (25 days) which is attributable to 25% longer pupal durations of *B. striatum* at 20 days compared to 15 – 16 days for *P. australis*.

B. striatum pupae averaged only around one-third of the weight of *P. australis* pupae, and pupal weights were not affected by host instar ($F = 0.86$, $df = 3$, $p = 0.47$) (Fig. 7.4). In contrast to *P. australis* but in agreement with body size measures (Chapter 2) females were significantly larger than males ($F = 33.52$, $df = 1$, $p < 0.001$). There was no significant interaction between host instar and the sex of the parasitoid on pupal weights ($F = 0.64$, $df = 3$, $p = 0.60$).

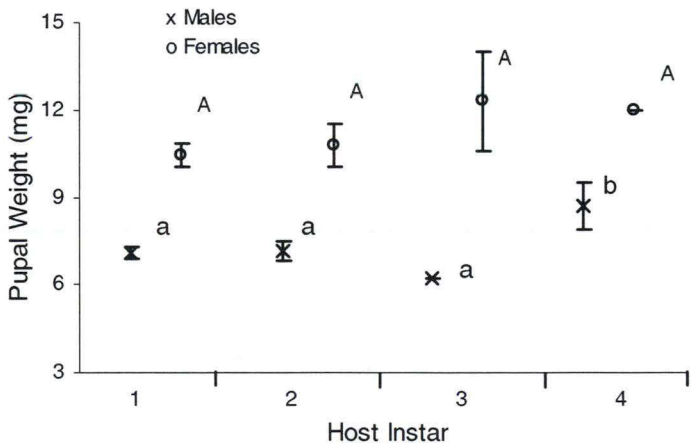

Figure 7.4 *Pupal weights± SE vs. host instar for* Balde striatum *reared through its host,* Chrysophtharta agricola.

7.2.3 Parasitoid Body Size and Fecundity. Both species of tachinid fly displayed a significant correlation between body size and fecundity (*P. australis*, Pearsons r = 0.70, Bartlett Chi-square statistic χ^2 24.02 df=1 p> 0.0001, *B. striatum*: Pearsons r = 0.66, χ^2 = 11.25, df = 1, p = 0.001). Mean fecundity of *B. striatum* (mean = 134.5, ± 6.2 SE, range = 69 to 198, n = 23) was higher than *P. australis* (mean = 90.4 - ± 6.3 SE, range = 11 to 174, n = 38). At almost equivalent body sizes, *B. striatum* was almost four-fold more fecund than *P. australis*.

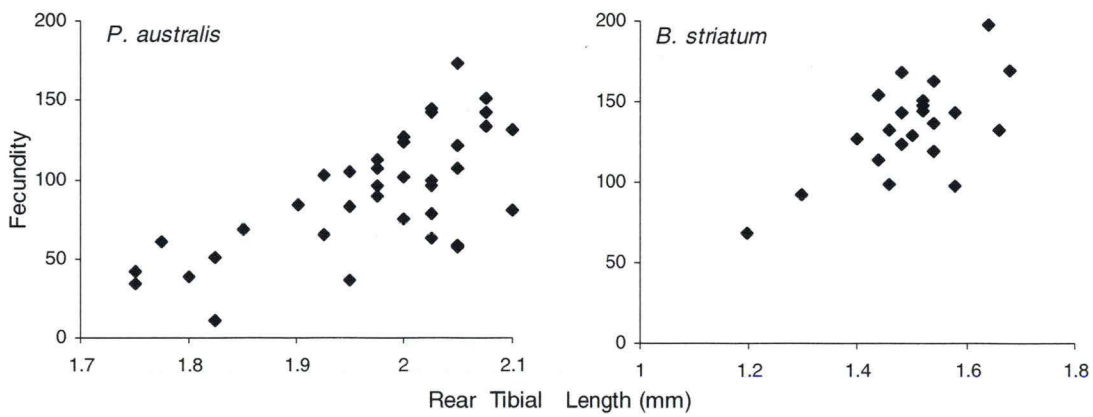

Figure 7.5 *Fecundity vs body size- estimated by length of right rear tibia- of the field collected tachinid flies* Paropsivora australis *and* Balde striatum.

7.4 DISCUSSION

To maximize body size from a small, immature host, a koinobiont parasitoid must delay development until the host contains sufficient resources to yield the optimally sized parasitoid (Mackauer and Sequeira 1993, Harvey *et al.* 2000, Harvey

and Strand 2002). In terms of development time, *P. australis* does exactly this, with every increase in instar resulting in a decrease in development time. However, although this strategy appears to maximize body size across all instars for the larger males, female *P. australis* are significantly smaller if reared from first or second instars. In contrast, *Balde striatum* exhibits a lag in development duration only from first instars, developing at an equal rate from the remaining three instars. Except for larger males from fourth instar hosts, this strategy again results in constant body size. This is a similar trend to that displayed by the braconid wasp *Eadya paropsidis* (Chapter 5) and indicates a trade-off between developmental rate and body size. The delay from first instar hosts for *B. striatum* suggests that first instars are not sufficiently large to support development but when they reach second instars they are, and development rate is maximised in all other instars.

Many authors have noted benefits to a parasitoid being large and we have shown here that larger females of both species are more fecund, which may be common among the Tachinidae as it has also been reported in other species (King *et al* 1976, Reitz and Adler 1995, Coombs 1997, Zhang *et al.* 2004). Therefore, we may expect selection pressure to maximise female body size in these two species. There are undoubtedly size-related selection pressures that we have not considered here and the evidence that overall *B. striatum* is favouring development rate over size appears compelling. However, *P. australis* is apparently maximising body size over developmental rate but can develop almost 20% faster than *B. striatum.* This suggests that there are constraints acting on *B. striatum* that we have not considered. It is well documented that both developmental rate and body size can differ in parasitoids reared through different host species (e.g., Nicol

and Mackauer 1999). We know that *P. australis* has other hosts, and may have a wide range of host species (Chapter 2). However, apart from a single rearing record of *B. striatum* from *Acacicola orphana* little else is known on the host range of *B. striatum*. *B. striatum* does not overwinter in the soil as the other larval parasitoids of *C. agricola* do but instead eclose from pupae even late in the season when there are no *C. agricola* larvae available to parasitise. If *B. striatum* does overwinter in another host *A. orphana* seems likely as it is the only winter active paropsine beetle in Tasmania (Simmul 2001). *A. orphana* is a smaller beetle than *C. agricola* with fourth instar larvae only up to around 11 mm length (Simmul 2001) compared to *C. agricola* at up 14 mm (de Little 1979), and the developmental strategy of *B. striatum* may also be shaped by selection pressures associated with this smaller beetle as a host.

The intraspecific preferences of these two tachinid flies place their immature stages at the opposite ends of the host mortality risk continuum. Their host, *C. agricola* fits in Price's (1975) type 'A' category of risk where most, in this case 71%, of mortality occurs by the half way point of development. *B. striatum* with a preference for early instars deposits its larvae at the high-risk end of host mortality, and *P. australis* with a strong preference for the final fourth instar of its host, oviposits at the low risk end. Both flies therefore, fit with Harvey and Strand's (2002) hypothesis on parasitoid developmental strategies and host risk. However, any savings in exposure to mortality via host risk gained by rapid parasitoid development by *B. striatum* is only realised at the end of parasitoid development, that is, by emerging early in fourth instar which itself is at the low-risk end of host development. Consequently, while the host-risk hypothesis may explain why *P. australis* can afford to maximise body size, it does not explain why

B. striatum cannot. Furthermore, the lower probability of survival of parasitised hosts containing immatures of *B. striatum* compared to *P. australis* is reflected in 1.5 fold higher fecundity of *B. striatum*, which follows the pattern predicted by Price (1974).

Sexual dimorphisms in insects are common, and reflect the different selection pressures that males and females are subject to (Mackauer *et al.* 1997). For males this is driven largely by mating success (e.g., Goldsmith and Alcock 1993, Gilburn and Day 1994, Tammaru *et al.* 1996) and consequently the biology and ecology of male insects may be linked strongly to their associated mating systems. Of the three reported types of mate location strategies used by the Tachinidae (described by Wood 1987), two are associated with morphological features in males. The first type relies largely on vision and is associated with larger eyes in males that occupy a greater proportion of the head which in turn, results in smaller frons widths in males (Thornhill and Alcock 1983, Wood 1987). The second type predominantly relies on the antennae to detect suitable females and males have eyes and frons widths that are essentially the same as females but third antennal segments that are longer than those of females with comparable body size (Wood 1987).

P. australis falls into Wood's (1987) first category, with males having conspicuously larger eyes and smaller frons widths than females. Aggregations of up to about ten males were commonly observed on the tops of trees within the plantation, and males were observed to fly out to meet many insects on the wing, including other males (ADR pers. obs.). We cannot say that these male aggregations were leks in the strictest sense (*sensu* Bradbury 1977) because it is not clear whether the males were actually

defending territory within the aggregation, nor whether females had the opportunity to choose among the males within the aggregation. Wood (1987) rejects the assumption that males tachinids observed chasing other males are acting territorially, citing lack of direct evidence that one male has succeeded in repelling another. However, Barraclough (1990) has since reported just such behaviour in the tachinids *Senostoma tessalatum* (Macquart), *S. longipes*, (Macquart), and *S. pallidihirtum,* (Malloch) as has Alcock and Smith (1995) in *Microtopesa sinuate* Donovan. Therefore, we assume here that there are sufficient similarities between the mating system of *P. australis* and true lekking species to be able to draw some comparisons between fitness characteristics of the two. For example, the protandrous eclosion of *P. australis* males would allow them to arrive at the site of aggregation early and be ready to mate with early eclosing females. Barraclough (1990) reports that large male tachinids are more likely to win competitive face-offs and posturing displays involved in maintaining territoriality within mating aggregations. Furthermore, Reitz and Adler, (1991) note that in the tachinid *Eucelatoria bryani* (Sabrosky) which has similar male aggregation behaviour as *P. australis*, it is a male's ability to grasp and hold a female that determines its mating success. It follows that larger males should be better able to grasp females during copulation which may select for larger males. The male's conspicuously larger body is emerging from pupae that weigh the same as the female's, which suggests that there may be greater selection pressure on males to maximise their body size than females.

B. striatum fits into the second of Wood's (1987) three types of mating system by having conspicuously longer third antennal segments in males than females and male frons widths of the same proportion to body size as females (A.D. Rice unpublished).

This character state is typical of tachinids that use their antennae to find mates and males that don't tend to form male aggregations, but rather are more dispersed and actively seek out females. Although we have no direct evidence of where mating occurs, we rarely observed males within the plantation, and unlike *P. australis* we never observed male aggregations. However, our Malaise traps yielded large numbers of male *B. striatum* relative to females, around the predicted time of emergence of a second generation of this species (Chapter 4), suggesting that males may have been actively seeking females within the plantation. The smaller size of *B. striatum* males compared to females further suggests a lack of direct competition between males for mating territory, or size related mate choice by females, but rather suggests a scramble competition type mating system in this species, as has been described for the host, *C. agricola* (Nahrung and Allen 2004).

In Chapter 6 we discussed some of the constraints on intraspecific host use by these two flies. *P. australis* has a preference for fourth instar hosts, suffers egg loss when utilising earlier instars, and by rapid oviposition avoids much interaction with the defensive behaviour of fourth instars. This study has shown that developmental rate and body size are favoured by *P. australis* utilizing third and fourth instars. However, our findings also raised further questions. Delaying development in small hosts is generally interpreted as allowing the host to continue growing to yield a larger parasitoid yet small females eclosed from instars one and two despite delaying development. Also it is not clear why host instar should impact on parasitoid pupal duration. Early emergence of adult *P. australis* males was facilitated by shorter pupation times, as was also noted by Harvey and Strand (2003) for *Microplitis mediator* Haliday (Hymenoptera: Braconidae).

In *P. australis* this would enable protandrous arrival at mating sites but Harvey and Strand (2003) in addition also propose that constraints on egg maturation rates, increased risk of mortality and metabolic costs associated with activity, may also lead to prolonged female pupation times.

By using its preferred second instar hosts *B. striatum* is less interfered with by host defensive behaviour during oviposition (Chapter 6). Furthermore, there were no body size or developmental rate costs to *B. striatum* using second instar hosts. When parasitising fourth instar hosts, especially late fourth instars some unconsumed host resources remain (ADR pers. obs). *B. striatum* usually pupates within the remains of the host, and there may be some disadvantages to pupating within an excess of host remains. For example, parasitoids are often incapable of pupating in the presence of moisture and die as a result, a phenomenon that is also common in Diptera as a whole (Denlinger and Zdarek 1994). Strand (2000) also reports that excess resources are commonly fatal to endoparasitoids. Therefore, using smaller hosts may provide the additional benefit of enabling *B. striatum* to avoid any detrimental effects of left-over host body mass.

In conclusion, although differences in developmental strategies and host risk do seem to conform to predictions with respect to host preference in these tachinid flies, the degree of current selection pressure is not evident. Other considerations in host selection must include life history, fecundity and the possibility that selection pressures may often differ between males and females. Nevertheless, it is evident that tachinids, with their diversity of reproductive strategies, mating systems and life histories when placed in an

evolutionary and ecological context have much to offer to the development and testing of parasitoid evolutionary theory.

CHAPTER 8

The Larval Parasitoid Guild of *Chrysophtharta agricola*: Ecology and Prospects as Biocontrol Agents.

8.1 INTRODUCTION

The leaf beetle *Chrysophtharta agricola* (Chapuis) (Coleoptera: Chrysomelidae) is an endemic pest of eucalypt plantation forestry in south-eastern Australia (de Little 1989, Elliott *et al.* 1998, Ramsden and Elek 1998, Collett 2001, Nahrung 2003). Larvae feed on the juvenile foliage of *Eucalyptus nitens* and *E. globulus* and adult beetles preferentially feed on the adult foliage (Nahrung 2004). Natural enemies include a suite of predators, parasites and pathogens, as well as parasitoids that attack both egg and larval stages (Nahrung 2004). Larval parasitoids attacking *C. agricola* in Tasmania include *Balde striatum* Rice, *Paropsivora australis* (Macquart) (Diptera: Tachinidae) and *Eadya paropsidis* Huddleston and Short (Hymenoptera: Braconidae) (Chapter 2). *B. striatum* is itself parasitised by the hyperparasitoid *Mesochorus* sp. (Hymenoptera: Ichneumonidae) with parasitism sometimes reaching high levels towards the end of the summer season (Chapter 4). *Perilampus tasmanicus* Cameron (Hymenoptera: Perilampidae) occasionally parasitises *E. paropsidis* and at least one of the two tachinids but in Tasmania is only found at low levels (Chapter 2).

Chrysomelid beetles are the most significant insect pests in Tasmanian plantation forestry, incurring costs through control measures, pest monitoring, and lost production. Around AU$ 378 000[1] is spent annually on pesticide applications alone to control

[1] estimated as 5 % of total estate requiring control @ $50 /ha (D. de Little 2005, pers. com.)

outbreaks of Chrysomelids, with this figure set to increase with the expansion of plantings of *E. nitens*. With a growing recognition that biological control may reduce the need for pesticide applications, there has been an increasing scientific interest in natural enemies of forestry pest insects (Short and Steinbauer 2004). In this thesis I have presented the results of several areas of study, and in this chapter, I discuss these findings and their implications for the management of *E. nitens* plantations in Tasmania. I firstly discuss the potential of these parasitoids as biocontrol agents, and then look at considerations for their inclusion in IPM strategies.

8.2 Larval Parasitoids as Biocontrol Agents

Parasitoids have been identified as key mortality agents in natural and forestry environments (e.g., Herz and Heitland 1999), and are proven population regulators of forestry pests such as *Malacosoma disstria* Hubner (Lepidoptera: Lasiocampidae) (Fitzgerald 1995). Compared to the Hymenoptera, biocontrol programs using tachinids have been less common but some successes have been spectacular (e.g., O'Conner 1950, Alam *et al.* 1971, Rao *et al.* 1971, Murdoch *et al.* 1985, Greathead 1986). Of the different approaches to biocontrol - classical, inundative and augmentative releases and conservation biocontrol – the use of larval parasitoids to reduce feeding damage by *C. agricola* would best fit into a conservation biocontrol framework. Conservation biocontrol involves modification to landscapes or practices to enhance the abundance and activity of natural enemies, and is appropriate here because both the pest and natural enemies are endemic to Tasmania, and parasitoid rearing and release is unlikely to be economically viable.

One of the drawbacks of using idiobiont larval parasitoids as biocontrol agents is that they allow the host to continue to feed and grow before killing it. However, the generational impact can still be significant and in this system parasitoid activity targeting early season pest activity from November to January is most important. This is because even though there is pest activity up until mid March, it is earlier in the season that the population of larval *C. agricola* peaks (Nahrung 2004, Chapter 4). Many of the eggs deposited late in the season will fail to reach maturity, and are unlikely to contribute to the following year's pest population. Furthermore, Elek (2005) concludes that adult feeding damage by *C. agricola*'s congener, *C. bimaculata,* contributes around one third of overall feeding damage by this pest. The two *Chrysophtharta* species have similar life histories, and adult *C. agricola* can also cause extensive damage to adult *E. nitens* foliage late in the season (Nahrung and Allen 2003), when foliage loss is most likely to inhibit tree growth (Elliott *et al.* 1993, Elek 1997). Consequently, the larval parasitoids that attack *C. agricola* have the potential to reduce the same-season damage by reducing the number of larvae reaching the adult feeding stage.

8.3 Prospects for Biological Control.

The three parasitoids together can achieve parasitism peaks of up to 50% of the larval *C. agricola* population with peaks at 30% being commonly achieved (Appendix 1). For the two species that we were able to quantify parasitism (*E. paropsidis* and *P. australis*), there is a large component of additional host mortality due to oviposition that is above that indicated by successful parasitism where neither host nor parasitoid pupated. For *E. paropsidis* about 20 % of parasitised hosts die prematurely, and for *P. australis,* when parasitising its preferred fourth instar hosts, around 32 % of hosts die

prematurely. Hence, successful parasitism for these two parasitoids represents only 65 - 80% of the actual host mortality cause by parasitism. If the rate of premature death caused by parasitism is similar for *B. striatum*, the combined larval mortality caused by these parasitoids may be up to 60-75%. This significant

Table 8.1 *Life history traits of each of the three larval parasitoids of* Chrysophtharta agricola.

	E. paropsidis	*B. striatum*	*P. australis*
abundance	high	high	low
max. parasitism[1]	31%	32%	3%
time of parasitism in host season	early	mid-late	mid to late
preferred host size	small	small	large
fecundity	c 1000	c 135	c 90
voltinism	univoltine	multivoltine	multivoltine
overwintering location	plantation	unknown	plantation
overwintering stage	pupa	unknown	pupa
attack *C. bimaculata*?	yes[2]	no	yes[2]
hyperparasitoid load	v. low	mid to high	v. low
flying strength	poor	good	good
max adult longevity[3]	24 days	37 days	47 days

[1] combined % parasitism of all four instars, [2] data from de Little 1982, Bashford, 1997, [3]longevity of field collected females in laboratory.

reduction in the number of *C. agricola* adults eclosing, pre-winter feeding and entering the overwintering population is likely to already be reducing the need for control measures to be implemented.

Eadya paropsidis. The high abundance, fecundity and longevity of *E. paropsidis* makes it a good prospect for a biocontrol agent. Furthermore, by parasitising hosts early in the season, *E. paropsidis* targets the first larval peak in the host population which is the population that is most likely to be able to complete development to the overwintering stage. However, by having an obligate diapause, *E. paropsidis* fails to realise the benefits, in terms of population size, that can be gained from having second and subsequent generations and by parasitising early host instars that suffer the highest mortality it is likely to lose much of its offspring in the larval stage. We also noted that *E. paropsidis* is a weak and sometimes reluctant flier in the laboratory and field, especially when compared to the tachinids, a trait that could make establishment in new host populations slow. Nevertheless, the contributions of *E. paropsidis* to pest management are significant, and are likely to be greater than reported here because it also parasitises *C. bimaculata* where successful parasitism can reach up to 80% towards the middle of the summer season, although around 30% is more common (de Little 1982). However, in field collections of around 1000 *C. bimaculata* larvae we only found parasitism by *Anagonia rufifacies* (Macquart) (Diptera: Tachinidae: Blondeliini). Our collections of *C. bimaculata* were made within eucalypt plantations at three Tasmanian field sites. The sites at Frankford (41°20S 146°45E), and Florentine Valley (42°38S 146°29E) are *E. nitens* plantations and described elsewhere, but most of the larvae were collected from an *E. regnans* site at Lower Coles Road in the Florentine Valley (42°29S

146°24E) (743 larvae from 25 cohorts) in Southern Tasmania, but yielded no *E. paropsidis.*

There are two possible explanations for the observed lack of *E. paropsidis* parasitism in our *C. bimaculata* collections. Firstly, *E. paropsidis* is generally low in abundance in Southern Tasmania. This is supported by this study which found that *E. paropsidis* was far more abundant at the northernmost site at Frankford, than at the Florentine Valley and also by de Little (1982) who reports parasitism levels up to 81% in collections at East Ridgley (41°10S 145°53E) in Northern Tasmania and low abundance of *E. paropsidis* at southern sites David de Little (then of Gunns Limited) (pers. com.). Secondly, *E. paropsidis* may have a preference for *C. agricola* over *C. bimaculata*, as our northern collections where *E. paropsidis* is abundant, were only made where *C. agricola* larvae were also available in high numbers. Early establishment of *E. paropsidis* on *C. agricola* on juvenile *E. nitens* foliage would have the additional benefit of enabling the parasitoid to move on to *C. bimaculata* as the plantation shifts to adult foliage as both larvae and adult *C. bimaculata* feed on adult foliage.

Balde striatum. Like *E. paropsidis* the tachinid fly, *B. striatum*, parasitises early instars of its host and can become abundant. Its fecundity, at 1.5 times that of *P. australis* means that it can potentially parasitise more hosts but its preference for high-risk early instars, like *E. paropsidis,* may reduce the survival of its offspring. From a plantation management point of view, *B. striatum,* has an advantage over the other parasitoids in that when it parasitises the host in its first instar, it kills the host before the host completes its most destructive feeding stage (Appendix 4). Nahrung (2004) reports

that feeding by fourth instars accounts for over half of the defoliation caused by *C. agricola* larvae. If a first instar host is parasitised by *B. striatum,* at 22 °C, the parasitoid can pupate two days before the host completes its feeding, a potential reduction of 25% of the defoliation by that host. Furthermore, the host dies at least a day before the fly pupates (ADR pers. obs) so that reduction in total larval defoliation may be up to almost 40%. By the same reasoning we may expect that male larvae of *B. striatum* , which develop faster than females (Chapter 6), can also reduce feeding in larvae parasitised as a second instar by around 25%, and female *B. striatum* larvae by around 12%.

Being multivoltine, and having an adult lifespan of up to 37 days under laboratory conditions, *B striatum* has a greater opportunity for population increase over the summer season than *E. paropsidis*. Unfortunately, this results in the population of *B. striatum* not usually becoming abundant until late in the season (Chapter 4) and consequently its main peak in parasitism levels do not coincide with the main peak of larval *C. agricola* population in the field. Furthermore, the hyperparasitoid, *Mesochorus* sp., is also abundant at this time and can also reach almost 30% parasitism of *B. striatum* (Chapter 4). While hyperparasitism does not affect the parasitism levels in that generation, it can impede population growth of *B. striatum.*

Paropsivora australis. The relatively low fecundity and abundance of *P. australis* means that of the three parasitoids it is least likely to exert population control pressures on *C. agricola*. However, under laboratory conditions *P. australis* has the greatest longevity of the three parasitoids of up to 47 days, and also due to its bet-hedging behaviour (Chapter 4) there are pupae in the soil for most of the year so that this

tachinid fly is least likely to be subject to great fluctuations in population abundance. Despite the low abundance in this study and relatively low fecundity of *P. australis,* this tachinid fly offers some advantages for pest management over the other parasitoids. Due to its preference for fourth instars, it is removing pests from the next generation of the host that have already survived the high mortality risk early instars. Fourth instar hosts suffer only around 10% of total mortality of *C. agricola. P. australis* also parasitises *C. bimaculata,* but de Little (1982) reports that parasitism of fourth instars (6.3%) was lower than in third instars (12.4%). This may be due to competitive loss to another tachinid fly *Anagonia rufifacies* (Macquart), which de Little (1982) reports as achieving 62.5% parasitism in fourth instars. Both tachinids have a preference for fourth instars (ADR, pers, obs.) but *A. rufifacies* deposits fully embryonated eggs onto the host that hatch immediately and burrow into the host (ADR, pers. obs.) whereas *P. australis* eggs may take some days to hatch. Anecdotal evidence from forestry field staff and earlier entomologists suggests that parasitism of *C. bimaculata* by *P. australis* was once much higher than we observed. *P. australis* may have a preference for *C. agricola* over *C. bimaculata*, if so with *C. agricola* becoming more abundant due to the expansion of *E. nitens* plantings it may now be parasitising *C. agricola* at the expense of *C. bimaculata.*

Paropsivora australis may have a wide host range (Chapter 2) and Nahrung and Allen (2004) report another eight species of paropsine chrysomelids in *E. nitens* plantations. If the host range of *P. australis* includes these other species, this tachinid fly may be having additional beneficial effects by parasitising other paropsines.

Competitive interactions. This study yielded no evidence to suggest that larvae of *C. agricola* could support more than one individual parasitoid. Hence, the three

parasitoid species may often be interacting within the host and competing with one another for available resources. Although host discrimination (detection and rejection of parasitised hosts) could prevent such interactions, both superparasitism (supernumerary self and conspecific larvae) and multiparasitism (supernumerary larvae of other species) are common in larval endoparasitoids (van Alphen and Visser 1990). In this study we observed instances of superparasitism in all three parasitoid species (ADR pers. obs.) but did not test for multiparasitism or discrimination, and assume the opportunity for interspecific larval competition frequently arises. In solitary endoparasitoids supernumerary larvae are eliminated through internal competition within the host (Hubbard *et al.* 1987). The larvae of many hymenopteran parasitoids have mandibles, and are thus adapted for fighting other larval parasitoids within the host (Salt 1961, cited in Godfray 1994, Kfir and van Hamburg 1988, McBrien and Mackauer 1990, Quicke 1997). Although some species of Tachinidae do engage in physical combat with rivals (Mellini and Baronio 1971, cited in Reitz 1995), they also rely on anoxia (oxygen depletion) (King *et al.* 1976) and physiological suppression (Edson and Vinson 1976, Mackauer 1986, Reitz 1995). Common to these strategies is that the oldest parasitoid larva is most likely to survive (Mackauer 1986, Mellini 1990, cited in Reitz 1996).

Of the three parasitoids studied here, *E. paropsidis* would be most likely to have the competitive advantage over the two tachinids, as it not only preferentially oviposits into early instar hosts, but has first instar larvae that possesses mandibles. Between the tachinids, *B. striatum,* which also mostly parasitises early instars, is likely to have a competitive advantage over *P. australis* which mainly parasitises fourth instar hosts, as its larvae are likely to be well established and better able to suppress the development of

any later arriving parasitoids. In Chapter 4 we report some evidence of temporal partitioning between *E. paropsidis* and *B. striatum* whereby *E. paropsidis* was abundant early in the season, and *B. striatum* later, which reduced direct competition between these two species. However, more work is required to determine whether this is a regular occurrence and whether it is a valid strategy.

8.4 Recommendations from this thesis.

Without any specific consideration for these parasitoids in plantation management plans, they can cause significant mortality of *C. agricola* larvae. However, the question arises 'how can we improve the efficacy of theses parasitoids to improve their contributions to pest management, and minimise the impact of any current management practices on their efficacy?'

Native vegetation. The value of native vegetation to natural enemy systems, in terms of refuges, resources, and improved access into the heart of the crop, has been demonstrated repeatedly. In hardwood plantation systems here in Tasmania, native vegetation can offer similar advantages, and provide a source of natural enemies (Strauss 2001). Furthermore, during this study, all three parasitoids were kept for extended periods in the laboratory on water and honey, whereas without honey the parasitoids rarely survived more than a day. Hence the life-spans reported in Table 8.1 represent up to a 24 to 47-fold increase in longevity for parasitoids with constant access to a food source. Native vegetation has a greater richness of trees and understorey species than eucalypt plantations (Elek 2005, ADR pers. obs.), and more likely to provide suitable floral sources of carbohydrates for parasitoids (e.g., Jervis *et al.* 1993, Idris and Gafius

1995, Jervis and Kidd 1996, Cappuccino *et al.* 1999, Irvin, *et al.* 2000). A preliminary study examining parasitoids for the presence of pollen on the parasitoids aimed at identifying floral nectar resources used yielded no result and was not continued further. We concluded that the tachinid flies probably removed any pollen by grooming, and that *E. paropsidis* lacked any external setae or structures suitable to retain pollen grains (but see future research directions below).

That native vegetation can act as a source of parasitoids is supported by data from Malaise traps where we found greater abundance and diversity of tachinids at a Florentine Valley site which is in close proximity to native vegetation compared to a Frankford site which is surrounded by pasture and plantations (Appendix 2). At the Florentine Valley we found the diversity in tachinid morphospecies to be nearly two fold more than that at Frankford, and abundance to be a third greater at the Florentine Valley than at Frankford. Moreover, the three species studied here have alternative hosts that are known to feed on eucalypt species other than *E. nitens*, and the benefit of alternative hosts adjacent to plantations is that it provides an opportunity for parasitoids to begin parasitising the pest population as soon as it appears in the plantation. Proximity may not be as important for the two tachinids as they are strong fliers, but for *E. paropsidis* which is a poor flier, alternative hosts in remnant native vegetation may aid their dispersal into the plantation system. *P. australis* emerged in Frankford 2001/02 in October and early November long before *C. agricola* were available for parasitism, but was only detected in malaise traps, in good numbers, towards the end of December which is before there were sufficient heat units to complete development (Chapters 3 and 4). We conclude that the malaise trap catches resulted from immigration into the

plantation and this species appears to leave the plantation and return when fourth instar *C. agricola* larvae are most abundant. A plantation that is surrounded only by pastureland and similar aged trees of the same species may not have as many *P. australis* returning. Level of forest fragmentation has been shown to affect the survival and thus parasitism levels of four tachinid parasitoids of forest tent caterpillar (*Malacosoma distra*) in Canada (Roland & Taylor 1997).

We have determined that the tachinid fly *B. striatum*, also parasitises the paropsine beetle *Acacicola orphana* (Erichson), a noted pest of *Acacia dealbata* (Elliott 1978, Simmul and Clarke 1999). While the extent to which *B. striatum* utilises *A. orphana* as a host is yet to be established, we note from Chapter 4 that *B. striatum* does not remain to overwinter as a pupa within the plantation, and also that *A. orphana* is winter active. Furthermore, the site that we monitored in the Florentine Valley in Chapter 4 lies adjacent to a six hectare block of *A. dealbata* which is heavily attacked by *A. orphana.* In both seasons that we monitored parasitism levels of *C. agricola*, *B. striatum* attacked the pest early in the season, from the time of first sampling date that hosts became available in November, at 12 and 17 % for 200/01 and 2001/02 respectively.

We have indicated the potential benefits to the guild of larval parasitoids of *C. agricola* by retaining native vegetation in plantation landscapes. There would undoubtedly be flow-on benefits, in terms of abundance and diversity, to other functional groups of natural enemies such as generalist predators of chrysomelids and

we conclude that it would be beneficial to maximise native vegetation retention during plantation establishment.

Larval parasitoids of C. agricola in IPM. Control measures based on pesticide applications can be carried out in such a way as to minimise their impact on natural enemies. This can be achieved by either using narrow spectrum sprays such as Success™ (Elek *et al.* 2004), or by timing pesticide applications to avoid susceptible stages of the parasitoids. At present, IPM spraying strategies target second instar hosts which allow natural enemies to target the eggs and first instars where the greatest mortality occurs (Elek 2005). As a result the two most abundant and beneficial parasitoids that target this stage, *E. paropsidis* and *B. striatum,* would also be vulnerable, especially to broad spectrum sprays such as organophosphates or pyrethroids, and since natural enemies appear to be less affected by some of the 'softer' insecticides such as Success™, (Elek *et al.* 2004) we would recommend their use. By targeting fourth instar hosts and bet-hedging with pupal diapause, *P. australis* is less likely to be affected by the current spray strategies, as it does not become abundant in plantations until after IPM spraying has occurred and also has some pupae in the soil almost throughout the year. Timing may also be used to ameliorate impacts on parasitoids and the adult, pupal and adult stages of the parasitoids shown in Table 8.2. For example, spraying in mid November 2000 and 2001 at Frankford would have targeted the pest population while it was still mainly comprised of first instars (Fig. 4.2), a stage more susceptible to the 'softer' Success™ (Elek *et al.*2004), and yet most of the *Eadya paropsidis* population would not have emerged from diapause (Fig. 4.3) so and would have been protected from the insecticide.

Table 8. 2 *Life cycle stages of the three larval parasitoids of* Chrysophtharta agricola *and the time of year that they are present in the plantation. (+- indicates may be present in some seasons at some sites). E.p* = Eadya paropsidis, *B.S* = Balde striatum, *P.a* = Paropsivora australis.

Stage present in field		Jul.	Aug.	Sep.	Oct	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.
adult	*E.p*	-	-	-	-	+	+	+	+/-	-	-	-	-
	B.s	?	?	?	-	+	+	+	+	+	?	?	?
	P.a	-	-	-	+	+	+	+	+	+	-	-	-
larva	*E.p*	-	-	-	-	+/-	+	+	+/-	-	-	-	-
	B.s	-	-	-	-	+	+	+	+	+	+	-	-
	P.a	-	-	-	-	+	+	+	+	+	+	-	-
pupa	*E.p*	+	+	+	+	+/-	+/-	-	+/-	+	+	+	+
	B.s	-	-	-	-	-	+/-	+	+	+	+	+/-	-
	P.a	+	+	+	+/-	-	+/-	+	+	+	+	+	+

8.6 Future Research Directions. There are several areas that research could potentially provide methods or management options for improving the abundance and therefore biocontrol efficacy of these three parasitoids.

For *Eadya paropsidis* determining the cues for diapause termination could produce well defined protocols for timing of early season pesticide applications. At this stage, only in hindsight can we say that mid November would have been suitable for spraying in 2000 and 2001, and on this basis we may have thought that mid December was too late to avoid eliminating the populations if Dominex ™ or similar broad spectrum pesticides were used. However, Elek *et al.* (2004) showed that in 2002 a mid December spray of either Success ™ or Dominex ™ was early enough to avoid significant loss of *E. paropsidis* populations, suggesting that a significant proportion of

the *E. paropsidis* population had not eclosed by this time. As the emergence traps (Chapter 4) show that at a similar time, there were only a few left to eclose, more research is required to be able to identify the optimum time for spraying to minimise impact on *E. paropsidis*.

The overwintering ecology of *B. striatum* is at this stage unknown. We have evidence that suggests that *B. striatum* leaves the plantation system to overwinter, and if so probably uses alternative hosts, possibly *Acacicola orphana*. If its alternative host could be determined, it may provide opportunities for improving its efficacy early in the season. In 2000/01 and 2001/02 *B. striatum* was more abundant, and parasitism levels were higher, in the Florentine Valley early in the season (Figs. 4.5a and 4.5b). This site is bordered by large tracts of native forest, and also by a large plantation of *Acacia dealbata,* a known host of the winter-active *A. orphana*, *B. stratum's* possible alternative host. Once its alternative host is confirmed, its timing of emergence can be determined and included in strategic planning of control operations, as well as considerations for retaining stands of native vegetation or planting of appropriate plant species to provide alternate hosts for *B. striatum*.

The slow growth-high mortality hypothesis proposes that in risky environments increased development time equates to increased mortality risk (Chapter 5 and 7). Breeders of plantation eucalypts may improve the trees resistance to insect herbivores thereby reducing the herbivores feeding damage, and also extend development time for *C. agricola*. Slower herbivore growth may in turn render them exposed to natural enemies for longer and also compromise the hosts ability to resist parasitism.

Parasitoids can live longer, have greater realised fecundity and search for hosts better if they have consistent access to food resources, and the dependence of all three species of parasitoid to a reliable food source has been demonstrated in the laboratory. Wasps have the advantage of mandibles that can enable them to feed on solids such as flower pollen, but tachinid flies can only imbibe liquids. This commonly takes the form of sugars from flower nectar or extra-floral nectaries or even honeydew excreted from homopterans. Research into whether these parasitoids are nectar feeding, and on which plant species as well as the phenology of flower availability could provide data on what may be economically provided as food sources which may in turn significantly improve the efficacy of all three parasitoid species. However, as indicated earlier, pollen tracing, using pollen lodged on the body surface may not be viable but instead it may be possible to examine gut contents to look for evidence of pollen or carbohydrates. The relationship between parasitism rates and proximity to food sources (once they are known) could further reveal their importance, and the addition of studies on distribution of parasitoid activity throughout the plantation edge effects, and parasitoid dispersal ability could be used to identify the ways that native vegetation can best be incorporated in plantation management.

Host preference and host range testing could greatly improve our appreciation of the value of these parasitoids, especially in *E. paropsidis* and *P. australis*. An understanding of the parasitoids' preferences between *C. agricola* and *C. bimaculata* would allow us to more effectively incorporate these parasitoids into strategic planning. For example, will *E. paropsidis* move onto *C. bimaculata* as the plantations gains adult

foliage if the population originated in *C. agricola*, or will it move out to find more *C. agricola*? Also, which host species will it preferentially parasitise if both are present and which host stage of *C. bimaculata* does it prefer? Other unknowns that may help us gauge their value are their dispersal potential, and the effects of parasitism on feeding rates, i.e., are the parasitoids making the host feed more, or are there additional benefits in terms of reduced feeding rates?

Elek (2005) discussed the merits of incorporating natural enemies into IPM by targeting second instar hosts by which time natural enemies have done most of their work. In addition natural enemy abundance could also be incorporated into action thresholds and other decision making.

Longer term studies could yield annual trends in parasitoid activity that could be used to predict current year activity. For example, we could test whether high levels of parasitism in one year mean the same for the next, and whether this is affected by proximity to different aged stands, and also whether parasitoid activity can be relied upon in the longer term, perhaps into the stage when the plantation becomes susceptible to *C. bimaculata* attack. Appendix 2 shows that parasitism rates at both sites were higher in the second year of this study, and the starting at the Florentine Valley rose from 10% in 2000/01 to 16% in 2001/02. These type of data could be used in conjunction with data on chrysomelid outbreaks to investigate whether particular coups, or parts thereof, consistently suffer high rates of defoliation which may be exacerbated by spraying, and whether a larger long term parasitoid population would be more valuable in terms of tree growth than short term growth gains achieved by spraying.

Lastly, investigation into parasitoid activity in native forests may illuminate new key features of undisturbed landscapes that can be incorporated into plantation design and management to reduce pest damage. For example, these parasitoids may not constitute the entire guild that attacks *Chrysophtharta* spp. and further research may yield additional parasitoid species that may be encouraged into the plantation systems.

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Appendix 1. Lower developmental thresholds and thermal constants for parasitoids and their hosts used in figure 3.3

Parasitoid	*t* (°C)	DD	Reference	Host	*t* (°C)	DD	Reference	Highest *t*	Highest DD
Hymenoptera									
Aphelinidae									
Aphelinus albipodus	8.5	208.2	Bernal & Gonzalez 1996	*Diuraphis noxia* (aphid)	0.5	158.7	Aalbersberg *et al.*	Parasitoid	Parasitoid
Aphelinus asychis	8.2	229	Lee *et al.* 1998	*Diuraphis noxia* (aphid)	0.5	158.7	Aalbersberg *et al.*	Parasitoid	Parasitoid
Aphelinus gossypii	6.7	312	Tang and Yokomi 1995	*Toxoptera aurantii* (aphid)	3.8	129.9	Wang and Tsai. 2001	Parasitoid	Parasitoid
Aphelinus spiraecolae	7.9	294.1	Tang and Yokomi 1995	*Toxoptera aurantii* (aphid)	3.8	129.9	Wang and Tsai. 2001	Parasitoid	Parasitoid
Aphelinus sp. nr *varipes*	9.7		Lajeunesse *et al.*1992	*Diuraphis noxia* (aphid)	0.5	158.7	Aalbersberg *et al.*	Parasitoid	
Encarsia formosa	13.3	207	Enkegaard 1993a	*Bemesia tabaci* (whitefly)	14.0	327	Enkegaard 1993b	Host	Host
Eretmocerus eremicus	5.4	417.3	Greenberg *et al.*2000	*Trialeurodes vaporariorum* (whitefly)	2.9	483.4	Greenberg *et al.*2000	Parasitoid	Host
Eretmocerus eremicus	8.7	314.4	Greenberg *et al.*2000	*Bemisia argentifolia* (whitefly)	10.3	319.7	Greenberg *et al.*2000	Host	Host
Bethylidae									
Cephalonomia stephanoderis	13.8	242.5	Infante *et al.* 1992	*Hypothenemus hampei* (Coleoptera)	11.2	386.9	Costa *et al.*1989	Parasitoid	Host
Parops nasuta	11.4	297.0	Infante 2000	*Hypothenemus hampei* (Coleoptera)	11.2	386.9	Costa *et al.*1989	Parasitoid	Host
Braconidae									
Apanteles glomeratus [1 e.1]	9.6	196	Nealis *et al.* 1984	*Pieris rapae*[1] (Lepidoptera)	10.2[+]	323.8[+]	Nealis *et al.* 1984	Host	Host
Apanteles glomeratus [2]	11.0[+]	272.4[+]	Nealis *et al.* 1984	*Pieris rapae*[2] (Lepidoptera)	8.2[+]	355.1[+]	Nealis *et al.* 1984	Parasitoid	Host
Apanteles rebecula[1]	11.0[+]	198.4	Nealis *et al.* 1984	*Pieris rapae*[1] (Lepidoptera)	10.2[+]	323.8[+]	Nealis *et al.* 1984	Parasitoid	Host
Apanteles rebecula[2]	11.6	219.1	Nealis *et al.* 1984	*Pieris rapae*[2] (Lepidoptera)	8.2[+]	355.1[+]	Nealis *et al.* 1984	Parasitoid	Host
Aphidius colemani	2.8	301	Elliot *et al.* 1995	*Diuraphis noxia* (aphid)	0.5	158.7	Aalbersberg *et al.*	Parasitoid	Parasitoid
Aphidius matricariae	7.6		Makarova 1997	*Schizaphis graminum* (aphid)	5.0		Kirkland *et al.* 1981	Parasitoid	
Aphidius matricariae	4.5	273.1	Miller & Gerth 1994	*Diuraphis noxia* (aphid)	0.5	158.7	Aalbersberg *et al.*	Parasitoid	Parasitoid
Aphidius ervi ervi	6.0	197	Campbell *et al.* 1974	*Acyrthosiphon pisum* (aphid)	5.1	105	Campbell *et al.* 1974	Parasitoid	Parasitoid
Aphidius ervi pulcher	6.1	188	Campbell *et al.* 1974	*Acyrthosiphon pisum* (aphid)	5.1	105	Campbell *et al.* 1974	Parasitoid	Parasitoid
Aphidius rubifolii	5.3	176	Campbell *et al.* 1974	*Masonaphis maxima* (aphid)	3.9	125	Campbell *et al.* 1974	Parasitoid	Parasitoid
Aphidius smithii [3]	4.8	172	Campbell *et al.* 1974	*Acyrthosiphon pisum* (aphid)	5.1	102	Campbell *et al.* 1974	Host	Parasitoid

Appendix 1 *cont*

Parasitoid	*t* (°C)	DD	Reference	Host	*t* (°C)	DD	Reference	Highest *t*	Highest DD
Braconidae *cont...*									
Aphidius smithii [4]	6.1	180	Campbell *et al.* 1974	*Acyrthosiphon pisum* (aphid)	5.1	105	Campbell *et al.* 1974	Parasitoid	Parasitoid
Chelonus sp	12.9	359.5	Hentz *et al.* 1998	*Pectinophora gossypiella* (Lepidoptera)	13.9	492	Beasley & Adams 1996	Host	Host
Cortesia urabae small hosts	10.5+	441+	Allen and Keller 1991	*Uraba lugens* (Lepidoptera)	9.3+	1132+	Allen and Keller 1991	Parasitoid	Host
Cortesia urabae mid-sized hosts	8.0+	424	Allen and Keller 1991	*Uraba lugens* (Lepidoptera)	9.3+	1132+	Allen and Keller 1991	Host	Host
Dolichogenidea eucalypti	9.3+	428	Allen and Keller 1991	*Uraba lugens* (Lepidoptera)	9.3+	1132+	Allen and Keller 1991	equal	Host
Diaeretiella rapae	3.6		Bernal *et al.* 1995	*Diuraphis noxia* (aphid)	0.5		Aalbersberg *et al.*	Parasitoid	
Diaeretiella rapae [3]	3.5	241	Campbell *et al.* 1974	*Brevicoryne brassicae* (aphid)	7.1	136	Campbell *et al.* 1974	Host	Host
Diaeretiella rapae [2]	7.0	97	Campbell *et al.* 1974	*Brevicoryne brassicae* (aphid)	5.0	127	Campbell *et al.* 1974	Parasitoid	Host
Diaeretiella rapae [5]	6.5	188	Campbell *et al.* 1974	*Brevicoryne brassicae* (aphid)	6.5	182	Campbell *et al.* 1974	Equal	Parasitoid
Eadya paropsidis [e,l]	5.9	294.1	this study	*Chrysophtharta agricola* (coleoptera)	7.8	400	Nahrung *et al.* 2003	Host	Host
Lysiphlebia japonica	2.9	223.5	Deng & Tsai 1998	*Toxoptera citricida* (aphid)	6.3		Tsai and Wang 1999		Host
Lysiphlebia mirzai	6.2	181.2	Liu & Tsai 2002	*Toxoptera citricida* (aphid)	6.3		Tsai and Wang 1999		Host
Lysiphlebus testaceipes	6.2		Royer *et al.* 2001	*Schizaphis graminum* (aphid)	5.0		Kirkland *et al.* 1981	Parasitoid	
Lysiphlebus testaceipes	7.5		Tang *et al.* 1995	*Toxoptera aurantii* (aphid)	3.8		Wang and Tsai. 2001	Parasitoid	
Meteorus communis	9.5	274	Miller 1996	*Peridroma saucia* (lepidoptera)	7.2	676	Simonet *et al.* 1981	Parasitoid	Host
Meteorus trachynotus	5.9	348	Thireau *et al.* 1995	*Cornistoneura fumerana* (lepidoptera)	8.0	452+	Lysyk 1989	Host	Host
Opius concolor	11.8		Loni 1997	*Dacus (Bactrocera) oleae* (Diptera)	9.5#		Crovetti *et al*	Parasitoid	
Praon pequodorum	6.9	199	Campbell *et al.* 1974	*Acyrthosiphon pisum* (aphid)	5.6	104	(Campbell *et al.* 1974)	Parasitoid	Parasitoid
Ceraphronidae									
Dendrocerus niger[4]	6.5		Campbell *et al.* 1974	*Aphidius smithii* [4] (aphid)	6.1		Campbell *et al.* 1974	Parasitoid	
Encyrtidae									
Aphelinus spiraccolae	7.9		Tang *et al.* 1995	*Toxoptera aurantii* (aphid)	3.8		Wang and Tsai. 2001	Parasitoid	
Aphelinus gossypii	6.7		Tang *et al.* 1995	*Toxoptera aurantii* (aphid)	3.8		Wang and Tsai. 2001	Parasitoid	

Appendix 1 *cont*

Parasitoid	*t* (°C)	DD	Reference	Host	*t* (°C)	DD	Reference	Highest *t*	Highest DD
Eulophidae									
Citrostichus phyllocnistoides	9.8	212.0	Urbaneja *et al.* 2003	*Phyllocnistis citrella* (aphid)	10.6		Liu 1993	Host	
Cirrospilus vittatus	8.2		Urbaneja *et al.* 2002	*Phyllocnistis citrella* (aphid)	10.6		Liu 1993	Host	
Cirrospilus sp. nr. *lyncus*	8.8		Urbaneja *et al.* 1999	*Phyllocnistis citrella* (aphid)	10.6		Liu 1993	Host	
Diglyphus isaea	9.2	161.8	Bazzocchi *etal.* 2003	*Liriomyza trifolii* (Diptera)	10.5		Lanzoni *et al.* 2002	Host	
Diglyphus isaea	9.3	165.0	Bazzocchi *etal.* 2003	*Liriomyza huidobrensis* (Diptera)	8.1		Lanzoni *et al.* 2002	Parasitoid	
Hyssopus pallidus	10.0		Zaviezo & Mills 1999	*Cydia pomonella* (Lepidoptera)	11.8	529.4	Pitcairn *et al.* 1991	Host	
Oomyzus sokolowskii	9.5		Wang *et al.* 1999	*Plutella xylostella* (Lepidoptera)	7.7		Lu *et al.* 1988	Parasitoid	
Testrastrichus howardi[b]	12.8	239.8	Kfir *etal.* 1993	*Xanthopimpla stemmator* (Hymenoptera)	11.4		Moore and Kfir 1996	Parasitoid	
Ichneumonidae									
Diadromus collaris	7.4	225.1	Liu *et al.* 2001	*Plutella xylostella* (Lepidoptera)	10.6[+]		Chung *et al.* 1989	Host	
Mesochorus sp.[4]	9.9	333.3	this study	*Balde striatum* (*C.agricola*) (Diptera)	6.4		this study	Parasitoid	
Xanthopimpla stemmator	15.5	187	Hailemichael *et al.*1994	*Diatraea saccharalis* (Lepidoptera)	9.7[+]		de Melo *et al.*1983	Parasitoid	
Platygasteridae									
Amitus bennetti	10.0	400.0	Drost *et al.* 1999	*Bemisia argentifolia* (whitefly)	10.3	319.7	Greenberg *et al.*2000	Host	Parasitoid
Pteromalidae									
Asaphes lucens[b]	8.1	233	Campbell *et al.* 1974	*Aphidius smithii*[4] (aphid)	6.1	150	Campbell *et al.* 1974	Parasitoid	Parasitoid
Muscidifurax raptor	10.2	285.7	Mann *et al.*1990	*Musca domestica* (Diptera)	12.3	250.6[e-e]	Ghizdavu 1975	Host	Parasitoid
Muscidifurax zaraptor	12.2	263.2	Mann *et al.*1990	*Musca domestica* (Diptera)	12.3	250.6[e-e]	Ghizdavu 1975	Host	Parasitoid
Nasonia vitripennis	9.8	224.3	Grassberger *et al.*2003	*Protophormia terraenovae* (Diptera)	8.9		Grassberger *et al.*2002	Parasitoid	
Pteromalus puparium[1]	11.0	188.7	Nealis *et al.* 1984	*Pieris rapae*[1] (Lepidoptera)	10.2[+]	323.8	Nealis *et al.* 1984	Parasitoid	Host
Spalangia cameroni	13.9	357.1	Mann *et al.*1990	*Musca domestica* (Diptera)	12.3	250.6[e-e]	Ghizdavu 1975	Parasitoid	Host

Appendix 1 *cont*

Parasitoid	*t* (°C)	DD	Reference	Host	*t* (°C)	DD	Reference	Highest *t*	Highest DD
Pteromalidae *cont.*									
Trichomalopsis sarcophagidae	12.5	197	Lysyk 1998	*Stomoxys calcitrans* (Diptera)	11.8	238.1	A.-Valgode *et al.* 1992	Parasitoid	Host
Trichomalopsis sarcophagidae	12.4	197	Lysyk 1998	*Musca domestica* (Diptera)	12.3	260.5[e-e]	Ghizdavu 1975	Parasitoid	Host
Trichogrammatidae									
Trichogramma pretiosum	13.0	131.3	Pratissoli *et al.* 2000	*Tuta absoluta* (Lepidoptera)	8.3[+]	470.0[+]	Bentancourt *et al.* 1996	Parasitoid	Host
Trichogramma pretiosum	13.5	120.9	Pratissoli *et al.* 2000	*Phthorimaea operculla* (Lepidoptera)			Horne *et al.* 1991		
Scelionidae									
Telenomus busseolae	13.7		Olaye *et al.* 1997	*Sesamia calamistis* (Lepidoptera)	10.7[+]	709[+]	Shanower *et al.* 1993	Parasite	
Telenomus isis	13.1		Chabi-Olaye *et al.* 2001	*Sesamia calamistis* (Lepidoptera)	10.7[+]	709[+]	Shanower *et al.* 1993	Parasite	
Diptera									
Tachinidae									
Balde striatum [1, p]	6.4	344.0	this study	*Chrysophtharta agricola* (Coleoptera)	7.8	400	Nahrung *et al.* 2003	Host	Host
Paropsivora australis	6.2	384.6	this study	*Chrysophtharta agricola* (Coleoptera)	7.8	400	Nahrung *et al.* 2003	Host	Host
Lixophaga diatraeae	10.7	116.3	King *et.al* 1975	*Diatraea saccharalis* (Lepidoptera)	9.7[+]	710[+]	de Melo *et al.* 1988	Parasitoid	
Lydelia thompsoni [1, p]	3.8[+]	424.0[+]	Cagan *et al.* 1999	*Ostrinia nubilalis* (Lepidoptera)	10.0		Got & Rodolphie 1989	Host	
Lydella jalisca [1, p]	14.2		Lauziere *et al.* 2002	*Eoreuma loftini* (Lepidoptera)					
Myiopharus doryphorae [1, p]	12.8		Lopez *etal.*1997	*Leptinotarsa decemlinata* (Coleoptera)	9.7		Tauber *et al.* 1988	Parasitoid	
Trichopoda giacomelli [p]	7.9	235.6	Liljesthrom 1996	*Nezara viridula* (Hemiptera)	15		Egwuatu *et al.* 1986	Host	
Winthemia fumiferani [e, l]	6.0		Hebert *et al.* 1990	*Cornistoneura fumerana* (lepidoptera)	8.0	452[+]	Lysyk 1989	Host	

1 = Canada, 2 = Australia, 3 = Berkeley, California, 4 = Kamloops, British Colombia, 7 = Pingluo, China, 8 = Sette, Morocco,
* = Hyperparasitoid, [+] = Estimated by means of thresholds of different life stages ^ = means from more than one location and reported together . If males and females reported separately, data was pooled. If no life cycle stages were mentioned it was assumed that the data referred to complete immature life stages ie egg to adult.

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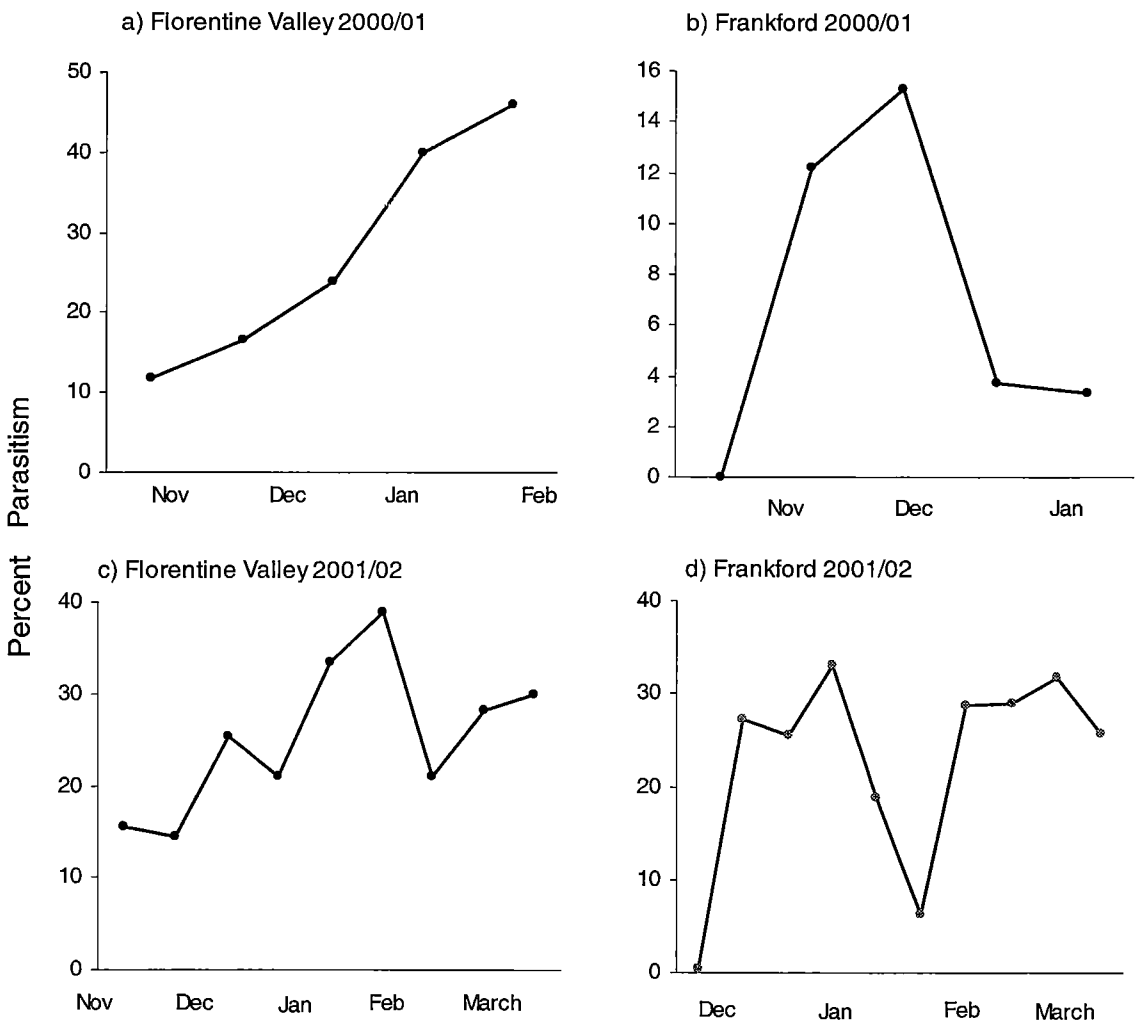
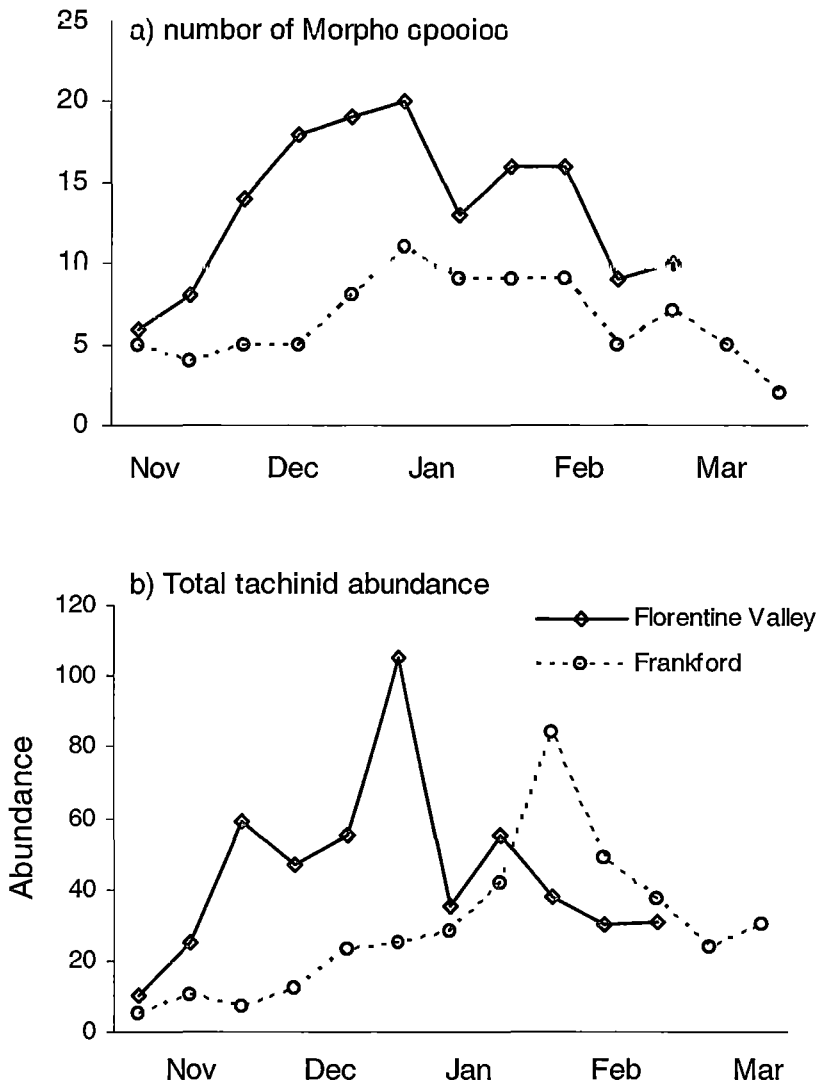
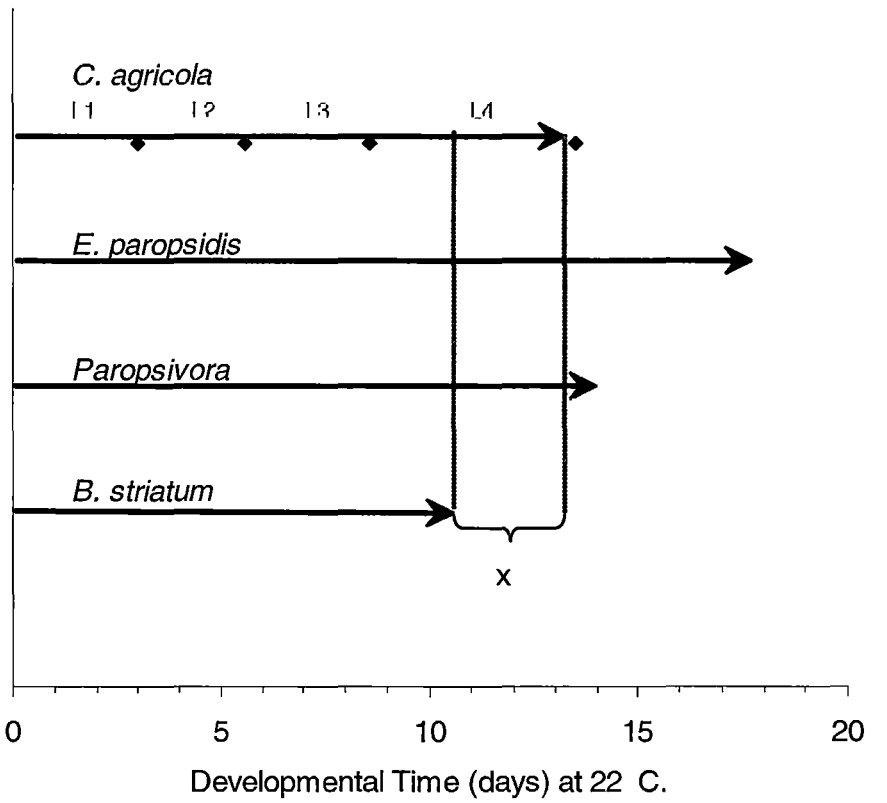
Appendix 2. Combined percent parasitism by the three larval parasitoids *Eadya paropsidis, B. striatum* and *P. australis* in their host *Chrysophtharta agricola.*



Appendix 3. Abundance of the Tachinidae at two sites in Tasmania over the summer of 2001/2002. a) Number of morphospecies and b) tachinid abundance.



Appendix 4. Comparison of larval development time of the three larval parasitoids, *E. paropsidis, P. australis* and *B. striatum* and their host, *C. agricola.* 'x' denotes potential saving in feeding damage by *B. striatum* parasitising first instar hosts.



Note – *Paropsivora australis* can potentially develop faster than *B. striatum* but because it delays development from early instars, it does not kill the host before it finishes its fourth instar.

Appendix 5. Conference presentations and publications arising from this work

Rice, A.D. 2005. The parasitoid guild of larvae of *Chrysophtharta agricola* Chapuis (Coleoptera: Chrysomelidae) in Tasmania, with notes on biology and a description of a new genus and species of tachinid fly. Australian Journal of Entomology (in press).

Rice, A.D. and Allen, G.R. 2004. Host defense and parasitoid reproductive strategies limit niche breadth in a host-parasitoid system. Oral presentation. 22nd International Congress of Entomology. Brisbane Qld.

Rice, A.D. and Allen, G.R. 2003. Host quality and changes in host mortality risks in relation to the developmental strategies of the tachinid larval parasitoid guild of *Chrysophtharta agricola* Chapuis (Coleoptera: Chrysomelidae). Oral presentation. 34th Australian Entomological Society and 6th Invertebrate Biodiversity and Conservation Conference. Hobart, Tasmania.

Rice, A.D. and Allen, G.R. 2001a. Phenology and rates of population increase of *Eadya paropsidis* and *Lixophaga* sp. – Two of the primary parasitoids associated with *Chrysophtharta agricola.* Poster presentation. CRC for Sustainable Production Forestry Annual Meeting. Caloundra, Qld.

Rice, A.D. and Allen, G.R. 2001b. Reproductive biology of the parasitoid guild associated with *Chrysophtharta agricola* (Coleoptera: Chrysomelidae) in Tasmania. Oral presentation. Australian Entomological Society 32nd AGM and Scientific Conference. Sydney, NSW.

Rice, A.D. and Allen, G.R. 1999. Host interactions and impact of parasitoids upon the eucalypt-defoliating beetles *Chrysophtharta agricola* and *C. bimaculata.* Poster presentation. Australian Entomological Society 30th AGM and scientific conference. Canberra ACT.

Phenology and Rates of Population Increase of *Eadya paropsidis* and *Lixophaga* sp. -- Two of the Primary Larval Parasitoids Associated with *Chrysophtharta agricola*.

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Introduction.

Heavy outbreaks of the paropsine leaf beetle *Chrysoptharta agricola* Chapuis (Coleoptera: Chrysomelidae) are known cause serious defoliation of two-to-five year old *Eucalyptus nitens* plantations throughout Tasmania (Ref). Both larvae and adults feed on juvenile foliage resulting in a reduced leaf area which inhibits plant growth and hence reduces productivity. A growing awareness of the need for sustainability in forestry has put the long term future of present chemical control practices in doubt. Natural enemies can be effective regulators of pest populations and artificial manipulations of these beneficial insects have been used successfully in forestry environments (ref). Immature *C. agricola* are subject to predation and parasitism by many species of natural enemies. Among these are the solitary larval parasitoids *Eadya paropsidis* Huddleston and Short (Hymenoptera: Braconidae) and *Lixophaga* sp. (Diptera: Tachinidae).

Aims

The aim of this project was to identify larval parasitoids most likely to be able to impact on eruptive populations of *C. agricola*. Here we aimed to:

- investigate the life history strategies of *Eadya paropsidis* and *Lixophaga* sp.
- detect any thermal constraints on the phenologies of parasitoids or host.
- determine parasitoid phenologies.
- estimate field parasitism levels achieved achieved by these two parasitoids.

Materials and methods

- Life-history strategies were determine by dissections, laboratory experiments and field observations. Fecundity was estimated as instantaneous egg load.
- Phenologies were investigated by the use of two types of emergence traps: the 'tent' trap and 'bucket' trap (Figures 1 & 2).

Figure 1. 'Tent' type emergence trap, designed to show when the parasitoids first emerge from the soil and the temporal spread of emergence.

Figure 2. 'Bucket' type emergence trap designed to show second generations and bet-hedging at the population level.

The tent trap was placed over a m^2 of soil and emerging insects were collected fortnightly. Parasitised larvae were placed in the bucket trap and emerging parasitoids were collected.

- Thermal constraints on phenology were investigated by comparison of Day Degrees (DDs) required for insect development with the DDs available in the field. DDs required for development were calculated by the formula:

DD = (Time in days) x (Rearing Temp - Threshold Temp)

Developmental thresholds were estimated by the constant temperature rearing method described in Dent 1991. Field DDs were calculated using output from 'I button' ™ data loggers.

- Field parasitism rates were estimated by brining cohorts of *C. agricola* larvae back to the laboratory from the field and rearing them through to identify parasitised individuals.

Results

Eadya paropsidis deposits eggs early in their embryonic development directly into the haemocoel of the host. *Lixophaga* sp. matures her offspring to the point of hatching before oviposition and therefor appears to be ovolarviperous.

E. paropsidis invests less resources per egg (size=0.16mm) than *Lixophaga* sp. does (egg size = 1.5mm) but can therefor produce many more of them (mean fecundity=899, n=6, SE=207) compared to *Lixophaga* sp. (mean fecundity=100, n=5, SE=38) (Figure 3).

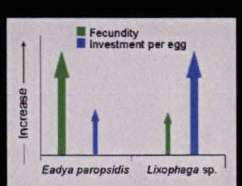

Figure 3. Fecundity / investment-per-egg tradeoff strategies for *E. paropsidis* and *Lixophaga* sp.

Results *cont*

Table 1. Day Degree developmental requirements for one and two generations of the parasitoids *E. paropsidis*, *Lixophaga* sp. and their host *C. agricola*.

	C. agricola	*E. paropsidis*	*Lixophaga* sp.
DD required for one generation	582	794??	450
DD required for two generations	984		794
DD available in field	853	1085	1034

Chrysophtharta agricola has sufficient heat units in the field for only one generation per year at Florentine Valley. *E. paropsidis* undergoes an obligatory pupal diapause and therefor also only has one generation per year. *Lixophaga* sp. has sufficient field DDs for at least two generations per year (Table 1).

Tent trap catches indicate that *C. agricola* appears in the field before either of the two parasitoids which concurs with field observations. However, *Lixophaga* sp. also appears in the field in late October but was not collected from the traps until November and December. *E. paropsidis* had a narrow window of emergence appearing only in November (Figure 4) which agrees with field observations.

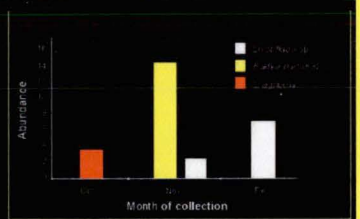

Figure 4. Tent Trap Catches at Frankford and Florentine Valley, Tasmania (Summer 00/01).

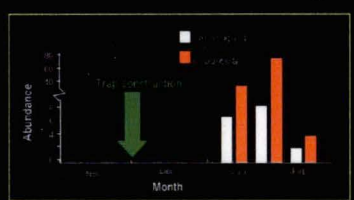

Figure 5. Bucket trap catches from larval *C. agricola* collected at sites in southern Tasmania (Summer 00/01).

Catches from the bucket traps show the host *C. agricola* emerging from the soil and also indicate the emergence of a second generation of the tachinid *Lixophaga* sp. There is no evidence of a second generation of the braconid *E. paropsidis* (Figure 5).

Field rates of parasitism by *Lixophaga* sp. continued to increase throughout the season and were consistently higher than those achieved by *E. paropsidis*. Peak parasitism achieved by *Lixophaga* sp. was nearly threefold (46%) that of *E. paropsidis* (16%) (Figure 6).

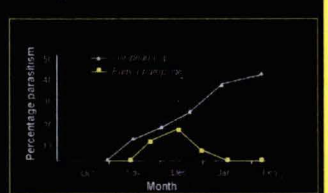

Figure 6. Field parasitism levels for larval *C. agricola* at Frankford and Florentine Valley, Tasmania (Summer 00/01).

Discussion/Conclusion

Superficially, *Eadya paropsidis* with its much greater fecundity looks the better prospect of these two parasitoids for a biocontrol agent, but its obligatory pupal diapause means it can manage only one generation per year. This restricts the braconid's intrinsic rate of increase and hence inhibits its ability to build up the large populations required to effectively impact upon *C. agricola* outbreaks. On the other hand, the tachinid *Lixophaga* sp. does not have an obligatory diapause or any thermal constraints on a second generation. Evidence from the bucket type emergence traps confirms a second emergence of *Lixophaga* sp. and the tachinid's steady rise in parasitism rates in the field suggests that this emerging generation is indeed ovipositing. Thus *Lixophaga* sp. can achieve parasitism rates of nearly 50% of it's host by the end of the summer season, and of the two parasitoids studied here, looks the most likely to be able to significantly impact upon eruptive populations of the pest *C. agricola*.

Acknowledgements:
We would like to thank Vin Patel for technical support, temperature data and assistance with photography and slides, Helen Nahrung for temperature data, Mamoru Matsuki for statistical advice, and CRC - SPF and industry partners for financial and resource support as well as field site access.

References

Appendix 7. Tips for collecting and handling the larval parasitoids of *C. agricola.*

I have included this section as a guide for anyone wishing to collect these parasitoids for use in experiments, as I have found by experience that a day's collecting can be wasted if all the parasitoids are dead the following day.

Collection. To obtain parasitised hosts it is easiest to use gravid field collected females of all three parasitoids, but especially for *B. striatum.* I did have limited success in getting all three parasitoids to mate in the laboratory, but *B. striatum* lacked vigour and rarely achieved parasitism in the lab if they were reared. Parasitoids can be collected by net whilst ovipositing onto hosts, and held in ventilated specimen tubes. I found the best to be 100 x 25 mm, available from Australian Entomological Supplies (E 75). On hot days the parasitoids need to be kept cool; I used a padded camera bag with a freezer brick to keep them cool while collecting in the plantation which left ones hands free. The insects can be transferred to an esky later. They must also be fed honey before leaving the field, which can easily be smeared through the gauze top of the vials but not too much as the insects can easily become stuck in it. On return to the laboratory, they must be housed and given water, in the form of a wet paper towel, as with free water they still find a way to drown.

Identification. Using the keys provided in Chapter 2 and with a little experience identification of these parasitoids is relatively straight-forward. However, when *C. bimaculata* is present along with *C. agricola*, another Blondeliine tachinid, *Anagonia rufifacies* may also be present, and is difficult to distinguish from *P. australis.* Therefore, I have provided a 'field key' which is not designed to be

exhaustive but merely to aid in day to day identifications of parasitoids of *C. agricola*, and *C. bimaculata.*

1. - Two pairs of wings (wasp).....2

   - One pair of wings (fly).....3

2.- Body black, head bright orange/red. .....*Eadya paropsidis.*

Female *E. paropsidis* have a globular shaped abdomen and a (just) visible ovipositor, whereas the abdomen in males is slightly smaller, and dorso-ventrally flattened, and they lack an ovipositor.

- Body orange –brown, abdomen sometimes darker.....*Mesochorus* sp.

Two species were noted, both hyperparasitoids of tachinids, one (smaller species) parasitises *B. striatum* in *C. agricola* and the other (much larger) parasitises *A. rufifacies* in *C. bimaculata.*

3.- Smallish fly, often very active, can be seen circling *E. nitens* shoots with early instar *C. agricola* larvae. Closer inspection reveals transverse bands on body giving 'stripey' appearance .....*Balde striatum.*

Male *B. striatum* are only rarely seen in the field but can be distinguished from females by possessing a yellow lateral patch towards the front of the abdomen.

- Larger fly, lacks transverse bands and is often less active...*P. australis* or *A. rufifacies.*

Males of *P. australis* and *A. rufifacies* can be distinguished from their respective females by being larger and darker. Male *A. rufifacies* can be separated from male *P. australis* by having an orange-brown scutellum, visible as an inverted

orange-brown triangle between the thorax and abdomen when viewed from above. Male *P. australis* can often be seen in aggregations in the tips of young trees, flying out to meet any insects that fly past, whereas male *A. rufifacies* congregate lower down on the tree.

Females of these two species can only be reliably separated by examination of the post sutural dorso-central bristles (see Crosskey 1973, p 195) under a microscope: *Paropsivora* spp. have three and *Anagonia* spp. have four. I recommend placing the live females in a clean petri dish and holding them at around 5° C for about 15 minutes to slow them down for examination under a dissecting microscope.

I have described in the materials and methods sections of Chapters 5, 6 and 7 the easiest methods I have found for obtaining parasitised larvae from these parasitoids. Each species shows varying levels of idiosyncrasies in terms of conditions required for oviposition, with *E. paropsidis* the easiest to propagate. However, *E. paropsidis* pupae should be held over a saturated NaCl solution to protect them from desiccation and they should then successfully emerge the following spring.