The significance of genetic and ecological diversity in a wide-ranging insect pest, *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae)

Mark Kurt Schutze

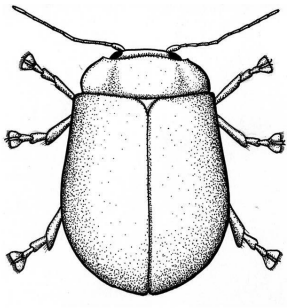
B.Sc. (Hons), University of Queensland

School of Natural Resource Sciences

Queensland University of Technology

Gardens Point Campus

Brisbane, Australia



This thesis is submitted as a requirement for the degree of Doctor of Philosophy

Keywords: cryptic species, local adaptation, phenotypic plasticity, seasonal plasticity, host specialisation, population genetics, *Eucalyptus*, forestry, predictive modelling, body size, Bergmann's Rule.

Abstract to thesis

Paropsis atomaria (Coleoptera; Chrysomelidae) is a eucalypt feeding leaf beetle endemic to southern and east coast Australia, and it is an emergent pest of the eucalypt hardwood industry. *Paropsis atomaria* was suspected to be a cryptic species complex based on apparent differences in life history characteristics between populations, its wide geographical distribution, and extensive host range within *Eucalyptus.* In this study genetic and ecological characters of *P. atomaria* were examined to determine the likelihood of a cryptic complex, and to identify the nature and causes of ecological variation within the taxon.

Mitochondrial sequence variation of the gene COI was compared between populations from the east coast of Australia (South Australia to central Queensland) to assess genetic divergence between individuals from different localities and host plant of origin. Individuals from four collection localities used for the molecular analysis were then compared in a morphometric study to determine if observed genetic divergence was reflected by morphology, and common-garden trials using individuals from Lowmead (central Qld) and Canberra (ACT) were conducted to determine if morphological (body size) variation had a genetic component. Host plant utilisation (larval survival, development time, and pupal weight) by individuals from Lowmead and Canberra were then compared to determine whether differential host plant use had occurred between populations of *P. atomaria*; individuals from each population were reared on an allopatric and sympatric host eucalypt species (*E. cloeziana* and *E. pilularis*). Finally, developmental data from each population was compared and incorporated into a phenology modelling program (Dymex™) using temperature as the principle factor explaining and predicting population phenology under field conditions.

Molecular results demonstrated relatively low genetic divergence between populations of *P. atomaria* which is concomitant with the single species hypothesis, however, there is reduced gene flow between northern and southern populations, but no host plant related genetic structuring. Morphometric data revealed insufficient evidence to separate populations into different taxa; however a correlation between latitude and size of adults was discovered, with larger beetles found at lower latitudes (*i.e.,* adhering to a converse Bergmann cline). Common garden experiments revealed

body size to be driven by both genetic and environmental components. Host plant utilisation trials showed one host plant, *E. cloeziana*, to be superior for both northern and southern *P. atomaria* populations (increased larval survival and reduced larval development time). *Eucalyptus pilularis* had a negative effect on pupal weight for Lowmead (northern) individuals (to which it is allopatric), but not so for Canberra (southern) individuals. DYMEX™ modelling showed voltinism to be a highly plastic trait driven largely by temperature.

Results from across all trials suggest that *P. atomaria* represents a single species with populations locally adapted to season length, with no evidence of differential host plant utilisation between populations. Further, voltinism is a seasonally plastic trait driven by temperature, but with secondary influential factors such as host plant quality. These data, taken combined, reveal phenotypic variability within *P. atomaria* as the product of multiple abiotic and biotic factors and representing a complex interplay between local adaptation, phenotypic plasticity, and seasonal plasticity. Implications for pest management include an understanding of population structure, nature of local adaptation and host use characteristics, and predictive models for development of seasonal control regimens.

Table of contents

List of Figures

Chapter 2

Chapter 3

Chapter 4

Chapter 6

Appendix 1

List of Tables

Chapter 4

Chapter 5

Chapter 6

Appendix 1

Appendix 2

List of publications

1. CAB International (2005) *Forestry Compendium*. CAB International, U.K.

2. Schutze, M.K., Mather, P.B. & Clarke, A.R. (2006) Species status and population structure of the Australian *Eucalyptus* pest *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae). *Agricultural and Forest Entomology*, 8, 323-332.

3. Schutze, M.K. and Clarke, A.R. Converse Bergmann cline in a *Eucalyptus* herbivore, *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae): phenotypic plasticity or local adaptation? *Global Ecology and Biogeography*, 17 (3), 424-431.

4. Schutze, M.K. and Clarke, A.R. Larval development by two geographically isolated populations of *Paropsis atomaria* on two species of *Eucalyptus*. In prep for submission to: *Australian Journal of Entomology*.

5. Nahrung, H.F., Schutze, M.K., Clarke, A.R., Duffy, M.P., Dunlop, E.A. and Lawson, S.A. (2008) Thermal requirements, field mortality and population phenology modelling of *Paropsis atomaria* Olivier, an emergent pest in subtropical hardwood plantations. *Forest Ecology and Management*, 255 (8), 3515-3523.

Statement of original authorship

This work has not previously been submitted for a degree or diploma at any other educational institution. To the best of my knowledge, this thesis contains no material from any other source, except where due reference is made

Mark K. Schutze

Acknowledgements

First and foremost I extend my greatest thanks to my principal supervisor, Anthony R. Clarke. Without Tony's sagely guidance, consistent support, and unending patience, I would never have made it this far – Tony is a mentor in every sense of the word.

Thanks also to Peter B. Mather, my associate supervisor. Peter has always been there to provide another view, perspective, and opinion, which is something that has always been valued and appreciated.

My parents, Kurt, Maureen, and Len, all deserve special mention. While my father, Kurt, is sadly now gone, he always encouraged me to pursue my interests and to do my best at whatever I undertook. I hope I would have made him proud. My mum, Maureen, and stepfather, Len, have been an unending source of support throughout the entire PhD process, always helping me see the brighter side during darker times, and reminding me of the important things – even when I insisted that they 'didn't understand'; I now know that they did.

Without my friends, too, I would be nowhere. Those who have supported and helped me, even when they didn't know they were doing so, are too numerous to mention, but particular thanks must go to (in no particular order) Katarina Mikac, Helen Nahrung, J. Paul Cunningham, Angela Duffy, Andrew Ridley, Luis Fernando Vargas, Amanda Mergler, Stephen Montieth, Mike Duffy, Jo Kent, Alexsis Wilson, Ana Pavasovic, Peter Prentis, Corinna Lange, and Daniel Jackson.

Thank you also to the following colleagues and professional organisations that contributed to this project: Gunter Maywald (CSIRO), Simon Lawson (QDPI&F), Richard Lunney (ITC Plantations), David De Little, Mamoru Matsuki, ANIC, Orange Agricultural Institute, University of Sydney, State Forests of NSW, Forestry SA, WA Museum, and of course, QUT, for funding me through a QUT Postgraduate Research Award.

Chapter 1

General introduction and literature review

Variation within and among individual organisms can result from a number of influences. Such variation may be due to individuals belonging to two or more 'good' biological species, even if they are not currently recognised by taxonomists. Alternatively, differences may be due to intraspecific variation within a single genome, differential phenotypic expression of the same genome (phenotypic plasticity), or a combination of the two. The current study addresses these issues as they apply to a widely dispersed Australian eucalypt leaf beetle, *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae).

This introduction provides a background to the importance of accurate species level identification, regardless of whether the direction of the research is theoretical or applied. Initially, it focuses on identifying possible cryptic species complexes, followed by a brief appraisal of abiotic and biotic environmental influences on geographic variation in phytophagous ectotherms. There follows a brief overview of the study organism, the chrysomelid beetle *P. atomaria*, and a final section that outlines the thesis objectives and flow of research chapters.

Patterns and processes of genotypic and phenotypic variation

Variation and cryptic species complexes

For more than 150 years, biologists have been interested in how and why species vary over their geographical distributions. To this end, physiological, morphological, developmental, or behavioural studies have the potential to address questions of local adaptation and speciation, and hence provide the foundation from which to explain such variation. Geographical variation is widespread across many phytophagous insects, with high diversity often explained by specialisation on one or a few species of host plant (Ballabeni et al., 2003). Indeed, after closer inspection, many 'generalist' herbivores are often found to consist of host adapted populations (= host races) or even cryptic species (Jaenike, 1990). While adaptation to host plants can be a driver of variation in herbivore taxa, other factors can also play a role in influencing diversity across a species' range. Climatic conditions, for example, can

shape the characteristics of individuals in a population, especially for ectotherms that have rates of development closely correlated with temperature (Chown and Gaston, 1999).

The first step toward explaining variation, however, is to *identify* the biological system under study. That is, are we observing a single biological species, or a complex of previously unrecognised species masquerading under a single taxonomic identity, *i.e.*, a cryptic species complex? This question is paramount, as future research must be based on a confident appraisal of the biological unit (or units) concerned. While many studies have been undertaken without initial recourse to rigorous species confirmation, the absence of fundamental taxonomic groundwork has the potential to result in many years of wasted effort and resources (Walter, 2003). This is especially relevant for taxa of economic or conservation significance. In 1982, for example, isoenzyme analysis revealed a Western Australian moth, the Jarrah Leaf Miner (*Perthida glyphopa*), to consist of up to three genetically distinct, but morphologically indistinguishable taxa (Mahon et al., 1982). This rendered the previous 50 years of related ecological research largely uninterpretable. The biological control of Karoo caterpillar, *Loxostege frustalis*, in South Africa provides a more dramatic example. Between 1942 and 1952 large numbers (up to 6 million) of the exotic parasitoid wasp, *Chelonus texanus*, were released to control the pest caterpillar. In 1951, however, researchers discovered that all parasitism-related caterpillar deaths were not caused by the introduced *C. texanus*, but by the morphologically similar native wasp, *C. curvimaculatus* (Annecke and Moran, 1977). Clearly, the above cases would have benefited from accurate, meaningful species identification at the beginning of the study, and they consequently serve as a salient reminder to initiate such research prior to undertaking any larger programme.

Cryptic species complexes were once thought uncommon, as morphologically defined species were assumed to closely match the biological reality (Dobzhansky, 1941). Today, however, it is widely recognised that previously defined strains, host races, or biotypes often constitute ‘good’ species (Clarke and Walter, 1995), with examples of cryptic complexes now common throughout the literature (Bickford et al., 2007). But what defines a ‘good’ species? There is perhaps no more vexed question in evolutionary biology than how to define species. With regularity, new books or proceedings appear debating existing species concepts and may even

suggest new ones (Hey, 2001). Allopatric versus sympatric speciation, the relative importance of pre- and post-mating isolating mechanisms, and the role of selection versus genetic drift are just some of the issues that are revisited continually (see individual chapters in Hey (2001) for examples). In spite of the intensity of the debate, most biologists accept a genetic view, in which a species is regarded as "a field for gene recombination" (Carson, 1957). That is, individuals within a species (under normal conditions) freely exchange and recombine genes with other members of the same gene pool. What limits the "field", *e.g.,* isolation (*sensu* Mayr) versus recognition (*sensu* Paterson) versus cohesion (*sensu* Templeton), is a related, yet another, critical and ongoing debate (Harrison, 1998). Given the lack of resolution as to how to define biological species, and the potential conflict between definitions, it is important to state the definition being used in any particular study. For the current study, Paterson's (1993) Recognition Concept of species will be the reference point because it focuses on critical factors that unite individuals within a species without the need to relate to other species (unlike the Biological / Isolation Species Concept) and hence has value in helping to understand variation within and among species (Lambert & Spencer 1995).

According to the Recognition Concept, species are, "*that most inclusive population of individual biparental organisms which share a common fertilisation system*" (Paterson, 1982). In theory, cryptic species represent such populations, but whose identification and separation from other groups (with different fertilisation systems) is confounded by high levels of among taxon morphological similarity. Hence, they cannot be easily identified using traditional taxonomic (*i.e.,* morphological) techniques (Paterson, 1991). Furthermore, the discovery of cryptic species complexes is likely to be non-random, and we may expect to find them in some groups more so than in others (Bickford et al., 2007). For instance, taxa with a wide host range across a large distribution, varying life history traits from one region to another (*e.g*., obligate diapause in one region, facultative in the other), or those that possess critical non-visual components in the mate recognition system (*e.g*., auditory, chemical, or tactile) may represent groups that we may *a priori* suspect of more likely constituting a cryptic species complex (Paterson, 1991; Bickford et al., 2007).

In line with the Recognition Concept of species, cryptic species – like any other – should be identifiable by elements of their fertilisation system which are discrete

within a specific gene pool and different from those among other gene pools. Furthermore, as a critical component of the fertilisation system is the specific mate recognition system (the series of signals and reciprocal responses between potential mates), mating trials under controlled conditions should therefore, in theory, aid in species identification. Mating trials are, however, time consuming, logistically challenging, and results may be inconclusive (due to inappropriate experimental design, the confounding effects of unnatural laboratory conditions, or uninterpretable results) (Walter, 2003). Indirect methods, including molecular approaches, may provide a more expedient pathway for estimating the extent of a gene pool, as members of the same species should possess relatively high levels of genetic similarity (compared with other taxa) due to sharing a common fertilisation system. Additionally, molecular approaches glean information from individuals collected directly from the wild, providing a snap-shot of the natural situation, and thus eliminating the potential confounding influences of mating trials conducted under artificial conditions.

There are several molecular marker approaches that have been used for species identification, each providing indirect measures of gene flow within and among populations. Allozyme gel electrophoresis, for example, has been in use since the late 1960's (Hsiao, 1989) and has been applied extensively for cryptic species studies (see, for example, Krafsur and Obrycki, 2000; Navajas et al., 2000; Aguin-Pombo, 2002; Mutebi et al., 2002; Zhu et al., 2002; Mattiucci et al., 2003; Martin-Sanchez et al., 2003; Naumova et al., 2003). This method documents variation in a diverse array of soluble enzyme products, determining relative frequencies of alleles in sample populations, thus estimating levels of contemporary gene flow within and among populations. There are limitations to this technique, however, as it is time consuming and can produce ambiguous results if populations do not exist in sympatry, as geographical barriers between populations may explain any lack of gene flow rather than existence of discrete mating systems (Walter, 2003).

Analysis of historical gene flow using a direct sequence analysis of DNA (especially of the mitochondrial genome) is now a frequently used method for assisting cryptic species identification, largely due to its recent reduction in cost, and the rapidity and ease of its execution (Bickford et al., 2007). Mitochondrial DNA (mtDNA) is maternally inherited, it is not altered by recombination, there are many copies per

cell, and its mutation rates are often up to 20 times faster than nuclear DNA. Furthermore, well studied mtDNA genes (*e.g.,* cytochrome *c* oxidase I) consist of variably constrained regions, thus permitting their use across a range of taxonomic levels (Lunt et al., 1996; Loxdale and Lushai, 1998). Such characteristics allow questions of maternal ancestry, population genetic structure, and gene flow to be addressed at the species level (Avise, 1986; Avise et al., 1987; Simon et al., 1994). However, as mtDNA is maternally inherited, the degree of paternal gene flow remains unknown, and may have consequences in studies of species exhibiting sex-biased dispersal patterns which can generate results similar to those expected for a cryptic species complex. Additionally, due to the reduced variability of mtDNA sequence data compared with enzyme data (in certain cases), fewer numbers of individuals can substantiate a cryptic complex study. For example, in the case of the Sugarcane Weevil, *Rhabdoscelus obscurus*, two cryptic species were confirmed from sequence data obtained from only six individuals, which was subsequently corroborated with ecological data (Giblin-Davis et al., 2000). Consequently, many recent cryptic complex studies have, and continue to assess, mtDNA sequence data (Mattiucci et al., 2003; Hebert et al., 2004; Quicke et al., 2006).

One of the principle cautions when using mtDNA to assess intraspecific and interspecific relationships is that there is no standard level of divergence that allows certainty with regard to species identification. Relative levels of divergence between populations remains the most common – albeit arbitrary – measure used, with mtDNA distances between species considered to range upwards of 3%. For example, the earwig, *Forficula auricularia*, was considered a single species consisting of a mosaic of populations that differed in reproductive biology (unlikely considering the recognition concept as outlined above). Mitochondrial DNA sequence data, however, revealed inter-population sequence divergence at 5.82%, approximately six times greater than intra-population divergence (1.07% and 0.66% among each of two groups). When coupled with the data on reproductive biology, mtDNA analysis provided indirect support for the presence of two cryptic species (Wirth et al. 1998). Therefore, sequence distance data used in isolation can not define species, but rather it provides a level of relatively easily acquired information that can contributes to their identification. The coupling of sequence data with other information (*e.g.,* ecological, behavioural, or physiological data) is desirable and has been

demonstrated across a range of studies incorporating molecular data with information on host utilisation (Kaneshiro and Kambysellis, 1999; Sembene and Delobel, 1998), variable life history and behavioural traits (Morrow et al., 2000; Knio et al., 2001; Willmott et al., 2001), symbiont associations (Six and Paine, 1997), elements of specific mate recognition systems (Jeraj and Walter, 1998; Mousseau and Howard, 1998; Schul, 1999; Kimura et al., 2002; Henry et al., 2003), and traditional taxonomic appraisals (Palmer, 2002; Maingon et al., 2003).

Causes of intraspecific geographic variation

Assuming appropriate studies have addressed questions on gene flow, population structure, and species limits, and there is confidence that a variable taxon constitutes a single biological species (versus a cryptic complex of species), then it would be appropriate to investigate factors that contribute to intra-specific variation. For phytophagous insects – as in other species – phenotypic variation is determined by either genetic factors, environmental influences, or their interaction. Furthermore, expression of specific phenotypes may be due to either local adaptation of specific traits or phenotypic plasticity.

Local adaptation is generally reserved for patterns and processes observed among populations of a single species connected by dispersal and gene flow; it is defined as the acquisition of a suite of locally suited traits resulting from the *genetic differentiation* of a population relative to other populations (Kawecki and Ebert, 2004). Phenotypic plasticity, on the other hand, is the result of a *single genotype* producing multiple phenotypes as a direct response to environmental conditions experienced by an individual (West-Eberhard, 1989). Additionally, phenotypic plasticity *itself* may be adaptive and selected for by species inhabiting highly heterogeneous environments subject to predictable change (Via et al., 1995). A species of frog in Sweden, *Rana temporaria*, for example, experiences variable pool-drying regimens across different islands and has evolved phenotypic plasticity suited to changing conditions (Lind and Johansson, 2007). Therefore, a study investigating variation within a species must seek to understand the underlying mechanisms driving phenotypic diversity; namely, are geographically related differences the product of local adaptation or phenotypic plasticity?

Local adaptation can be hindered by temporal variation in selective forces or habitat quality, together with the homogenising effects of high levels of gene flow among populations (Kawecki and Ebert, 2004). Consequently, reduced temporal but greater spatial heterogeneity in habitat quality and low levels of gene flow between phenotypically distinct populations may present an *a priori* reason for suspecting local adaptation (Kawecki and Ebert, 2004). Phenotypic plasticity can, however, occur in species with populations that experience high levels of gene flow and be more likely if: (i) there is a strong match between individual phenotypes and respective local environments; (ii) there is an associated low cost of plasticity; (iii) there are equal frequencies of alternative environments; and (iv) environments vary temporally rather than spatially (Moran, 1992). With this background in mind, the relative contribution of either local adaptation or phenotypic plasticity can be determined relative to abiotic and biotic influences affecting geographic variation within taxa

Abiotic factors can play a significant role toward influencing variation in insects. Temperature, for example, often directly influences rates of metabolism and hence larval development in insects and other ectothermic taxa (Sibly and Atkinson, 1994; Van der Have and De Jong, 1996). The observed strength of this relationship has resulted in the formulation of theories such as the Temperature-Size Rule, whereby lower developmental temperatures produce larger individuals (Carleton, 1960; Vannote and Sweeny, 1980; Lonsdale and Levinton, 1985; Atkinson, 1994; Partridge et al., 1994; Van der Have and De Jong, 1996; Atkinson and Sibly, 1997; Chown and Gaston, 1999; Ramsden and Elek, 1998; Reeve et al., 2000). Large body size for certain species can confer an advantage, and hence is a possible source of local adaptation. Advantages associated with large body size may include greater potential fecundity for females (Carne, 1966), increased reproductive success in males (Reeve et al., 2000), or improved starvation resistance for individuals that experience frequent adverse environmental conditions (Arnett and Gotelli, 2003).

Season length can also play a pivotal role in influencing body size in ectotherms. Extended season length provides increased time for individuals to reach a larger body size at maturation, and hence may increase their potential fecundity. Shorter seasons, however, provide less time for individuals to reach maturity, which may produce smaller adult body sizes (Roff, 1980). As both season length and

temperature are usually highly correlated with latitude, they can produce large-scale geographical trends in ectotherm variation. Indeed, body size of both endotherms and ectotherms over latitudinal gradients has been documented widely in the literature (see Blanckenhorn and Demont (2004) for a list of arthropod examples) and has resulted in the formulation of numerous rules to explain such variation, the most famous of which was proposed by Carl Bergmann, and is known as Bergmann's Rule (Bergmann, 1847).

Bergmann's Rule states that members of a species tend to be larger at higher latitudes (Blanckenhorn and Demont, 2004). The original mechanism proposed to explain this observation was that large size conferred greater heat retention in mammals found in colder climates (*i.e.,* higher latitudes) due to a reduction in the surface area-volume ratio (Bergmann, 1847). This mechanism is now largely dismissed for many endothermic species exhibiting such a cline, and is even less probable for ectotherms. As the body temperature of ectothermic animals fluctuates relative to ambient conditions, heat conservation, regardless of ectotherm size, is therefore not usually possible (McNab, 1971; Atkinson and Sibly, 1997). Additionally, the reverse of a classical Bergmann cline, a *converse* Bergmann cline (Park, 1949) (*i.e.,* larger sizes at lower latitudes), is equally as common amongst arthropod taxa and is often considered to be under genetic control (Masaki, 1978; Mousseau and Roff, 1989; Blanckenhorn and Demont, 2004). Indeed, it is often for the reasons outlined above (*i.e.,* shorter season lengths providing less time to mature and hence smaller adults) that converse Bergmann clines in ectothermic species are thought to evolve, and is often hypothesised to result from local adaptation of individuals to local season length (Roff, 1980; Blanckenhorn and Demont, 2004),

Biotic influences may also play a strong role in shaping variation, as host plant specialisation in phytophagous insects is widespread, with many herbivores having a close association with one or few host plant species (Fox and Morrow, 1981; Joshi and Thompson, 1995). This contributes to increased diversity amongst plant-feeding clades relative to their non-phytophagous sister groups (Jaenike, 1990). Consequently, host plant quality, distribution, and composition can have profound effects on variation in herbivorous species, as specialisation on host plants, if given sufficient time and isolation between populations, may lead to the evolution of host races, and potentially new species (Ballabeni et al., 2003).

As outlined above, commencing a study of biological variation requires careful consideration of several key components. To begin, the very nature of the taxon under study requires clarification: does it likely consist of single biological species, or is it probably a complex of morphologically indistinquishable cryptic taxa? This question may be addressed via multiple approaches, ranging from molecular characterisation to rigorous morphological assessments, or investigating ecological aspects of the study group. Only from this foundation can studies of patterns and processes relating to geographic and phenotypic variation proceed, regardless of whether they are driven by biotic influences, abiotic factors, or a complex interaction of the two.

Study System

Paropsis atomaria is a leaf beetle endemic to Australia. This species feeds principally on plants that belong to the genus *Eucalyptus* and has been recorded from at least 20 host tree species (CABInternational, 2005), a degree of polyphagy rarely seen for herbivorous insects (Fox and Morrow, 1981; Jermy, 1984; Claridge et al., 1997). The geographical distribution of *P. atomaria* extends from the temperate south-east of Australia to the tropical north of coastal Queensland (Schutze et al., 2006), covering a wide latitudinal gradient across varied environmental conditions. Existing information on *P. atomaria* (detailed in Chapter 2 of this thesis) identifies biological attributes such as wide host range, variable voltinism (*e.g.,* increased number of generations per season in northern, lower latitudes) and developmental physiology. Important differences in the biology of *P. atomaria* populations from different geographical locations have also been reported. Carne (1966) measured growth rates of *P. atomaria* larvae collected from Canberra and found optimum developmental time was achieved between 21ºC and 24ºC, whereas Bailey (2001) determined 27.5ºC to be the optimum developmental temperature for larvae from sub-tropical Queensland. Furthermore, populations in Canberra undergo diapause during the colder months of the year (May – August) (Carne, 1966), a behaviour not clearly demonstrated for individuals from warmer climates (such as Queensland) (S. Lawson, [Qld] Department of Primary Industries & Fisheries, pers. comm.).

These biological differences, when coupled with the wide host range, extensive geographical distribution, and unresolved taxonomic history of *P. atomaria*, give

strong *a priori* reasoning for suspecting a cryptic species complex within the taxon. Furthermore, as *P. atomaria* is an emerging pest of plantation forestry in Australia, it is likely to be of growing interest in the future and it is therefore timely to review both the extent and underlying causes of its biological variation.

Objectives of the study

Given that previous studies had identified different life history characteristics between southern (temperate) and northern (sub-tropical) populations, the principle aim was to address the question as to whether *P. atomaria* constitutes a cryptic species complex. To this end, the current state of knowledge about the taxonomy and biology of *P. atomaria* was examined (Chapter 2), to form a basis for subsequent molecular and ecological studies. Subsequently, beetles were collected from multiple locations across the natural distribution, ranging from temperate south-eastern Australia (Victoria, Canberra, and South Australia), to the sub-tropics of central Queensland, for a population genetic study of the species (Chapter 3). Due to its utility, the COI mtDNA gene was selected for sequence analysis to assess haplotype diversity within and among collection localities. From these data, gene flow was measured among populations and an assessment made as to whether *P. atomaria*, as currently recognised, constitutes a cryptic species complex. Results of this analysis showed that populations were sufficiently homogeneous to argue that *P. atomaria* constitutes a single taxon, but that gene flow was reduced between southern (Canberra, A.C.T.) and northern (Bangalow, N.S.W. to Lowmead, Qld) populations.

Following the genetic study, individuals from four of the locations used in the molecular analysis were examined in a morphometric analysis (Chapter 4). This was conducted to determine if morphometric data supported the single species hypothesis proposed by the molecular study. Initial morphometric results of field collected beetles demonstrated insufficient differentiation between populations to render them different species, however there was a clear trend of increasing body size at lower, northern latitudes (= a converse Bergmann cline). To investigate the importance and nature of the cline, further historical *P. atomaria* collections were included to assess the correlation between body size and latitude over time. In addition, common garden experiments using beetles collected from the full extent of the natural range (Canberra, A.C.T. and Lowmead, Qld) were conducted with individuals reared under four constant temperature conditions. Results demonstrated the converse Bergmann

cline was also evident in historical collection material, that *P. atomaria* populations conformed to the Temperature-Size rule (*i.e.,* inverse relationship between developmental temperature and adult body size), and that body size variation across the latitudinal gradient was under genetic control, and not the product of phenotypic plasticity (reflecting the results of the initial population genetic analysis).

Given that genetic variation among populations also influenced morphology in the form of a converse Bergmann cline, physiological differences among populations were examined focussing on larval host plant utilisation on different host plants (Chapter 5). Additional common garden experiments were conducted, also using Canberra and Lowmead individuals, with larvae from both populations reared on two eucalypt host species, *E. cloeziana* and *E. pilularis*. Host species were chosen as each occurs sympatrically with one or the other beetle population while being allopatric with the other: *E. cloeziana* occurs in sympatry with the northern Lowmead population whilst *E. pilularis* occurs in sympatry with the southern Canberra population. The nature of host plant distribution allowed larval fitness on the sympatric host plant to be assessed relative to that for the allopatric host species. Results of this study reveal that *E. cloeziana* is a better host plant compared to *E. pilularis*, as it resulted in increased survival and reduced development time for both populations, and increased pupal mass for individuals from Lowmead – further supporting the single species hypothesis.

Finally, constant temperature development data generated in Chapter 4 was incorporated into a DYMEX^{TM} based cohort phenology model for *P. atomaria* developed in collaboration with colleagues (Chapter 6). This model uses different biological data sets, including laboratory development data, field mortality, fecundity, and diapause characteristics, to predict *P. atomaria* field phenology based on local field temperatures. Output of the final model closely matched field observation data not used in its development and is thus potentially a valid tool for testing different hypotheses concerning phenological variation across *P. atomaria*'s geographical range. The model shows reduced voltinism for southern temperate populations (due to reduced season length) when compared with northern populations, suggesting disjunct seasonal phenologies among *P. atomaria* populations are a plastic response largely driven by temperature.

In the final discussion (Chapter 7), findings of previous chapters are reviewed and the broader significance discussed, particularly relating to determining species limits, causes and influences of intraspecific variation in ectotherm species such as insects, and how the information can be applied for practical management strategies.

Thesis style

The structure of this thesis follows QUT rules for a PhD by publication, which allows thesis examination to be based on the presentation of a body of related published or submitted works, linked together by introduction and discussion chapters. Consequently, all figures and tables are reinitialised for each chapter.

References

Aguin-Pombo, D. (2002). Genetic differentiation among host-related *Alebra* leafhoppers (Hemiptera: Cicadellidae). *Heredity* **88**, 415-422.

Annecke, D. P., and Moran, V. C. (1977). Critical reviews of biological control in South Africa. *Journal of the Entomological Society of Southern Africa* **40**, 127-145.

Arnett, A. E., and Gotelli, N. J. (2003). Bergmann's rule in larval ant lions: testing the starvation resistance hypothesis. *Ecological Entomology* **28**, 645-650.

Atkinson, D. (1994). Temperature and organism size - a biological law for ectotherms. *Advances in Ecological Research* **25**, 1-58.

Atkinson, D., and Sibly, R. M. (1997). Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology and Evolution* **12**, 235-239.

Avise, J. C. (1986). Mitochondrial DNA and the evolutionary genetics of higher animals. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences.* **312**, 325-342.

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A., and Saunders, N. C. (1987). Intraspecific phylogeny: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics.* **18**, 489-552.

Bailey, K. E. (2001). The effect of temperature on development and growth of *Paropsis atomaria*: Using Dymex to predict population trends of *P. atomaria* for future management, Queensland University of Technology.

Ballabeni, P., Gotthard, K., Kayumba, A., and Rahier, M. (2003). Local adaptation and ecological genetics of host-plant specialization in a leaf beetle. *OIKOS* **101**, 70-78.

Bergmann, C. (1847). Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Gottinger studien* **3**, 595-708.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winkler, K., Ingram, K. K., and Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22**, 148-155.

Blanckenhorn, W. U., and Demont, M. (2004). Bergmann and converse Bergmann latitudinal clines in arthropods: Two ends of a continuum? *Integrative and Comparative Biology* **44**, 413-424.

CABInternational (2005). Forestry Compendium. CAB International, Wallingford UK.

Carleton, R. (1960). The application of Bergmann's and Allen's rules to the poikilotherms. *Journal of Morphology* **106**, 85-108.

Carne, P. B. (1966). Ecological characteristics of the eucalypt defoliating chrysomelid *Paropsis atomaria* Ol. *Australian Journal of Zoology* **14**, 647-672.

Carson, H. L. (1957). The species as a field for gene recombination. *In* "The Species Problem" (E. Mayr, ed.), pp. 23-38. American Association for the Advancement of Science, Pub. no 50, Washington, DC.

Chown, S. L., and Gaston, K. J. (1999). Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews of the Cambridge Philosophical Society* **74**, 87-120.

Claridge, M. F., Dawah, H. A., and Wilson, M. R. (1997). Species in insect herbivores and parasitoids - sibling species, host races and biotypes. *In* "Species: The Units of Biodiversity" (M. F. Claridge, Dawah, H.A., and Wilson, M.R, ed.), pp. 247-272. Chapman & Hall, London.

Clarke, A. R., and Walter, G. H. (1995). "Strains" and the classical biological control of insect pests. *Canadian Journal of Zoology* **73**, 1777-1790.

Dobzhansky, T. (1941). "Genetics and the Origin of Species," Colombia University Press, New York.

Fox, L. R., and Morrow, P. A. (1981). Specialization: Species property or local phenomenon? *Science* **211**, 887-893.

Giblin-Davis, R. M., Gries, R., Crespi, B., Robertson, L. N., Hara, A. H., Gries, G., O'Brien, C. W., and Pierce, H. D. J. (2000). Aggregation pheromones of two geographical isolates of the New Guinea sugarcane weevil, *Rhabdoscelus obscurus*. *Journal of Chemical Ecology* **26**, 2763-2780.

Harrison, R.G. (1998) Linking evolutionary pattern and process: The relevance of species concepts for the study of speciation. Pp 19-31 (Chp 2) in: *Endless Forms: species and speciation* (Howard, D.J. & Berlocher, S.H. eds). Oxford University Press, Oxford.

Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H., and Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Science of the United States of America* **101**, 14812-14817.

Henry, C. S., Brooks, S. J., Duelli, P., and Johnson, J. B. (2003). A lacewing with the wanderlust: The European song species 'Maltese', *Chrysoperla agilis*, sp.n., of the carnea group of *Chrysoperla* (Neuroptera: Chrysopidae). *Systematic Entomology* **28**, 131-147.

Hey, J. (2001). "Genes, catgegories and species: The evolutionary and cognitive cause of the species problem," Oxford University Press, Oxford.

Hsiao, T. H. (1989). Estimation of genetic variability amongst Coleoptera. *In* "Electrophoretic Studies on Agricultural Pests" (H. D. Loxdale and J. den Hollander, eds.), Vol. 39, pp. 143-180. Clarendon Press, Oxford.

Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics.* **21**, 243-273.

Jeraj, M., and Walter, G. H. (1998). Vibrational communication in *Nezara viridula*: Response of Slovenian and Australian bugs to one another. *Behavioural Processes* **44**, 51-58.

Jermy, T. (1984). Evolution of insect/host plant relationships. *American Naturalist* **124**, 609-630.

Joshi, A., and Thompson, J. N. (1995). Trade-offs and the evolution of host specialization. *Evolutionary Ecology* **9**, 82-92.

Kaneshiro, K. Y., and Kambysellis, M. P. (1999). Description of a new allopatric sibling species of Hawaiian picture-winged *Drosophila*. *Pacific Science* **53**, 208-213.

Kawecki, T. J., and Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters* **7**, 1225-1241.

Kimura, T., Sakai, J., and Honda, H. (2002). Microstructure and pheromone producing function of male hair-pencils in the yellow peach moth, *Conogethes punctiferalis* (Lepidoptera: Pyralidae). *Entomological Science* **5**, 237-247.

Knio, K. M., Goeden, R. D., and Headrick, D. H. (2001). Resource utilisation in the sibling species *Trupanea nigricornis* (Coquillett), a polyphage, and the

narrowly oligophagous *T. bisetosa* (Coquillett) (Diptera: Tephritidae) in southern California. *Proceedings of the Entomological Society of Washington* **103**, 946-961.

Krafsur, E. S., and Obrycki, J. J. (2000). *Coleomegilla maculata* (Coleoptera: Coccinellidae) is a species complex. *Annals of the Entomological Society of America* **93**, 1156-1163.

Lambert, D. M., and Spencer, H. G. (eds.) (1995). *Speciation and the Recognition Concept: Theory and Application.* John Hopkins University Press, Baltimore, Maryland U.S.A.

Lind, M. I., and Johansson, F. (2007). The degree of adaptive phenotypic plasticity is correlated with the spatial environmental heterogeneity experienced by island populations of *Rana temporaria*. *Journal of Evolutionary Biology* **20**, 1288-1297.

Lonsdale, D. J., and Levinton, J. S. (1985). Latitudinal differentiation in copepod growth: An adaptation to temperature. *Ecology* **66**, 1397-1407.

Loxdale, H. D., and Lushai, G. (1998). Molecular markers in entomology. *Bulletin of Entomological Research* **88**, 577-600.

Lunt, D. H., Zhang, D.-X., Szymura, J. M., and Hewitt, G. M. (1996). The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology* **5**, 153-165.

Mahon, R. J., Miethke, P. M., and Mahon, J. A. (1982). The evolutionary relationships of three forms of the Jarrah Leaf Miner, *Perthida glyphopa* (Common) (Lepidoptera: Incurvariidae). *Australian Journal of Zoology* **30**, 243-249.

Maingon, R. D. C., Ward, R. D., Hamilton, J. G. C., Noyes, H. A., Souza, N., Kemp, S. J., and Watts, P. C. (2003). Genetic identification of two sibling species of *Lutzomyia longipalpis* (Diptera: Psychodidae) that produce distinct male sex pheromones in Sobral, Ceara State, Brazil. *Molecular Ecology* **12,** 1879-1894.

Martin-Sanchez, J., Diaz, M., Artacho, M. E., and Valero, A. (2003). Molecular arguments for considering *Hysterothylacium fabri* (Nematoda: Anisakidae) a complex of sibling species. *Parasitology Research* **89**, 214-220.

Masaki, S. (1978). Climatic adaptation and species status in the lawn ground cricket. *Oecologia* **35**, 343-356.

Mattiucci, S., Cianchi, R., Nascetti, G., Paggi, L., Sardella, N., Timi, J., Webb, S. C., Bastida, R., Rodriguez, D., and Bullini, L. (2003). Genetic evidence for two sibling species within *Contracaecum ogmorhini* Johnston & Mawson, 1941 (Nematoda: Anisakidae) from otariid seals of boreal and austral regions. *Systematic parasitology* **54**, 13-23.

McNab, B. K. (1971). On the ecological significance of Bergmann's Rule. *Ecology* **52**, 845-854.

Moran, N. (1992). The evolutionary maintenance of alternative phenotypes. *The American Naturalist* **139**, 971-989.

Morrow, J., Scott, L., Congdon, B., Yeates, D., Frommer, M., and Sved, J. (2000). Close genetic similarity between two sympatric species of tephritid fruit fly reproductively isolated by mating time. *Evolution* **54**, 899-910.

Mousseau, T. A., and Howard, D. J. (1998). Genetic variation in cricket calling song across a hybrid zone between two sibling species. *Evolution* **52**, 1104-1110.

Mousseau, T. A., and Roff, D. A. (1989). Adaptation to seasonality in a cricket: patterns of phenotypic and genotypic variation in body size and diapause length along a cline in season length. *Evolution* **43**, 1483-1496.

Mutebi, J. P., Tripet, F., Alexander, J. B., and Lanzaro, G. C. (2002). Genetic differentiation among populations of *Lutzomyia longipalpis* (Diptera: Psychodidae) in Central and South America. *Annals of the Entomological Society of America* **95**, 740-752.

Naumova, E. S., Bulat, S. A., Mironenko, N. V., and Naumov, G. I. (2003). Differentiation of six sibling species in the *Saccharomyces sensu stricto* complex by multilocus enzyme electrophoresis and UP-PCR analysis. *Antonie van Leeuwenhoek* **83**, 155-166.

Navajas, M., Tsagkarakov, A., Lagnel, J., and Perrot, M. J. M. (2000). Genetic differentiation in *Tetranychus urticae* (Acari: Tetranychidae): Polymorphism, host races or sibling species? *Experimental and Applied Acarology* **24**, 365-376.

Palmer, M. (2002). Landmark-based morphometric analysis of two sibling species of the genus *Asida* (Coleoptera, Tenebrionidae). *Contributions to Zoology* **70**, 213-220.

Park, O. (1949). Application of the converse Bergmann principle to the carabid beetle, *Dicaelus purpuratus*. *Physiological Zoology* **22**, 359-372.

Partridge, L., Barrie, B., Fowler, K., and French, V. (1994). Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* **48**, 1269-1276.

Paterson, H. E. H. (1982). Perspectives on speciation by reinforcement. *South African Journal of Science* **78**, 53-57.

Paterson, H. E. H. (1991). The recognition concept of cryptic species among economically important insects. *In* "Research methods and prospects" (M. P. Zalucki, ed.), pp. 1-10. Springer-Verlag, New York.

Paterson, H. E. H. (1993). *Evolution and the recognition concept of species. Collected writings*. The Johns Hopkins University Press. Baltimore, U.S.A.

Quicke, D. L. J., Mori, M., Zaldivar-Riverson, A., Laurenne, N. M., and Shaw, M. R. (2006). Suspended mummies in *Aleiodes* species (Hymenoptera : Braconidae : Rogadinae) with descriptions of six new species from western Uganda based largely on DNA sequence data. *Journal of Natural History* **40**, 2663-2680.

Ramsden, N., and Elek, J. A. (1998). Life cycle and development rates of the leaf beetle *Chrysophtharta agricola* (Chapuis) (Coleoptera: Chrysomelidae) on *Eucalyptus nitens* at two temperature regimens. *Australian Journal of Entomology* **37**, 238-242.

Reeve, M. W., Fowler, K., and Partridge, L. (2000). Increased body size confers greater fitness at lower experimental temperature in male *Drosophila melanogaster*. *Journal of Evolutionary Biology* **13**, 836-844.

Roff, D. A. (1980). Optimizing development time in a seasonal environment: The ups and downs of clinal variation. *Oecologia* **45**, 202-208.

Schul, J. (1999). Neuronal basis for spectral song discrimination in the bushcricket *Tettigonia cantans*. *Journal of Comparative Physiology A* **184**, 457-461.

Schutze, M. K., Mather, P. B., and Clarke, A. R. (2006). Species status and population structure of the Australian Eucalyptus pest *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae). *Agricultural and Forest Entomology* **8**, 323-332.

Sembene, M., and Delobel, A. (1998). Genetic differentiation of groundnut seed-beetle populations in Senegal. *Entomologia Experimentalis et Applicata* **87**, 171-180.

Sibly, R. M., and Atkinson, D. (1994). How rearing temperature affects optimal adult size in ectotherms. *Functional Ecology* **8**, 486-493.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87**, 651-701.

Six, D. L., and Paine, T. D. (1997). *Ophiostoma clavigerum* is the mycangial fungus of the Jeffrey pine beetle, *Dendroctonus jeffreyi*. *Mycologia* **89**, 858-866.

Van der Have, T. M., and De Jong, G. (1996). Adult size in ectotherms: temperature effects on growth and differentiation. *Journal of Theoretical Biology* **183**, 329-340.

Vannote, R. L., and Sweeny, B. W. (1980). Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. *American Naturalist* **115**, 667-695.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S. M., Schlichting, C. D., and Van Tienderen, P. H. (1995). Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology and Evolution* **10**, 212-217.

Walter, G. H. (2003). "Insect Pest Management and Ecological Research," Cambridge University Press, Cambridge, UK.

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics.* **20**, 249-278.

Willmott, K. R., Constantino, L. M., and Hall, J. P. W. (2001). A review of *Colobura* (Lepidoptera: Nymphalidae) with comments on larval and adult ecology and description of a sibling species. *Annals of the Entomological Society of America* **94**, 185-196.

Wirth, T., Le Guellec, R., Van Cassel, M., and Veuille, M. (1998). Molecular and reproductive characterization of sibling species in the European earwig (*Forficula auricularia*). Evolution 52, 260-265.

Zhu, X. Q., D'Amelio, S., Palm, H. W., Paggi, L., George, N. M., and Gasser, R. B. (2002). SSCP-based identification of members within the *Pseudoterranova decipiens* complex (Nematoda: Ascaridoidea: Anisakidae) using genetic markers in the internal transcribed spacers of ribosomal DNA. *Parasitology* **124**, 615-623.

Chapter 2

Literature Review on *Paropsis atomaria*

This chapter has been published as the 'Datasheet for *Paropsis atomaria*' within:

CAB International (2005) *Forestry Compendium*. CAB International, U.K.

The purpose of the Compendium is to provide forestry workers with a detailed background on the current state of knowledge regarding important pests to the industry. Relevant information covers taxonomy, biology, morphology, similar taxa, diagnostics, natural enemies, risk assessment, and control measures. This chapter is, therefore, a comprehensive appraisal of the literature specific to *Paropsis atomaria* up to the commencement of this thesis and I am the sole author.

Literature review on *Paropsis atomaria*

Notes on taxonomy and nomenclature

The family Chrysomelidae contains an extreme variety of beetles, with the Chrysomelinae representing the largest subfamily in Australia, with more than 50 described genera and over 600 species (Lawrence and Britton, 1996). The most important group, Paropsina, contains the genus *Paropsis*, erected by Oliver (1807) when he described 15 new species of chrysomelid within this new genus, of which *P. atomaria* was one. Upon re-examination of the genus by Selman (1963), *P. obsoleta* was selected as the type species since a number of Olivier's original *Paropsis* species had subsequently been reassigned to different genera.

Paropsis atomaria was first described by Olivier (1807). Marsham (1808) also described a species under the name *P. atomaria*, that was subsequently synonymised with *P. charybdis* Stål, now a significant pest in New Zealand, where it was accidentally introduced from Australia. Furthermore, Marsham described another species, *P. reticulata*, which is now synonymised with *P. atomaria* Olivier (Selman, 1963). Other synonyms of *P. atomaria* include *P. granulosa* Boisd., *P. sanguinipennis* Germar. and *P. incarnata* Erich. *Paropsis incarnata* has since been recognized as a different species (Blackburn, 1901), and has been renamed *P. deboeri* Selman (Selman, 1983).

Additionally, *P. elytrura* Blackburn and *P. deboeri* Selman, found in Western Australia and Tasmania, respectively, are morphologically very similar to *P. atomaria* and their taxonomic status regarding their relationship with *P. atomaria* remains to be fully resolved.

Biology and ecology

Paropsis atomaria has been studied most comprehensively in the Australian Capital Territory (ACT) and it is from here that most of its biological traits have been characterized.

In the ACT, *P. atomaria* is bivoltine, with adults actively flying, feeding and mating over the periods October to December and mid-late January to March (Carne, 1966). The period between April and September sees a hibernation stage, where sexually mature adults overwinter. Diapause commences in response to a reduction in day

length, whilst the exhaustion of fat bodies stimulates its termination (Carne, 1966). The egg, larval and pupal stages continue beyond March, however, fourth instar larvae are not encountered after the beginning of May.

Females lay eggs in a distinctive fashion. Eggs are deposited upright around the stem of a young eucalypt shoot, forming a ringed cluster with each of the eggs projecting radially (Fig. 1a,b) (Cumpston, 1939). The number of eggs per cluster varies between 20-100 (Elliott et al., 1998), with the diameter of the stem of high importance with regard to selection of the oviposition site (Tanton and Khan, 1978a). Eggs are also occasionally deposited on the apex of leaves (Fig 1b). Larvae hatch after 10-14 days (Carne, 1966) by means of concentrated pressure in the thorax expanding hatching spines on each side of the body (Cumpston, 1939). Newly-emerged larvae consume their egg shells before moving to suitable foliage to feed.

One of the key factors determining suitable larval food is leaf toughness. This has been demonstrated in a number of studies where leaf toughness versus larval development was measured (Larsson and Ohmart, 1988; Ohmart et al., 1987). Previous ideas that nitrogen and secondary plant compounds such as tannins, phenols and oils may also be critical factors in choice of leaf have been largely dispelled after studies demonstrated these to be much less important factors with regard to feeding and development than leaf toughness (Fox and Macauley, 1977). Some work has also looked at the ways in which *P. atomaria* sequesters secondary plant compounds (Morrow and Fox, 1980).

The gregarious larval stage lasts for a period of 3-4 weeks, with a total of four instars. Optimum larval developmental time occurs between 21°C-24°C. A notable behaviour of the larvae is their defence mechanism. When disturbed, they elevate their posterior end and evert defensive glands from between terminal segments. Attacking insects, *e.g.,* ants, have been observed to die within a few minutes of contact with these glands (Carne, 1966). The defensive chemical secreted contains hydrogen cyanide, benzaldeyhyde and glucose (Moore, 1967).

During the fourth instar and towards the end of the larval phase, individuals cling less tenaciously to the leaves, and ultimately drop to the ground, where they form cells several inches below the surface. About five days later, cell pupation commences, and about ten days later the adult emerges. Three weeks following adult emergence, females are competent to oviposit (Carne, 1966).

Notes on host range

Paropsis atomaria occurs on multiple species of the genus *Eucalyptus* L'Her (Myrtaceae) (Table 1). It generally occurs in low numbers under natural conditions; however, beetle populations occasionally outbreak in plantations, especially in susceptible eucalypt species such as *E. cloeziana* (Shepherd, 2001), which is native to the sub-tropics of Australia and is utilized by forestry. In the ACT, however, the preferred host species of *P. atomaria* is *E. blakelyi* (Ohmart *et al.*, 1985). Because *E. blakelyi* does not extend far into the distribution of *E. cloeziana*, it remains unclear which of these two hosts *P. atomaria* would preferentially use given the opportunity to select one over the other.

Other commercial plantation eucalypts on which this species is found include *E. pilularis, E. grandis, E. dunnii* and *E. camaldulensis.* Of these, *E. grandis* can be subjected to intensive infestation, and occurs from within the distribution of *E. blakelyi*, up into the tropics, within the distribution of *E. cloeziana*.

Table 1. Host plants from which *Paropsis atomaria* has been collected.

HOST PLANTS or CROPS AFFECTED (Please write scientific name)	MAIN HOST (main host on which pest causes economic damage)	OTHER HOSTS
Eucalyptus cloeziana	X	
Eucalyptus pilularis	X	
Eucalyptus grandis	X	
Eucalyptus dunnii	X	
Eucalyptus cladocalyx		X
Eucalyptus blakelyi		X
Eucalyptus melliodora		X
Eucalyptus polyanthemos		X
Eucalyptus leucoxylon		X
Eucalyptus conica		X
Eucalyptus fastigata		X
Eucalyptus rossi		X
Eucalyptus macrorhyncha		X
Eucalyptus radiata		X
Angophora floribunda		X
Eucalyptus pauciflora		X
Eucalyptus divei		X
Eucalyptus camaldulensis	X	

Symptoms - description

Larval aggregations may be seen on leaves of varying ages on affected trees. Young larvae, particularly 1st and 2nd instar are usually located on the newest foliage, as they are incapable of consuming the tougher, older leaves. Aggregations may comprise individuals of varying instars. Heavy infestations are readily identifiable by the loss of foliage and high numbers of larvae and adults. Lower level infestations may be detected by the characteristic 'scalloping' of leaves caused by adult feeding, and/or the loss of young foliage in the growth areas of the tree caused by larval attack.

Whilst tree death occurs under heavy infestation, the direct effect on the tree from heavy infestation is more likely to be reduction in growth rate and wood quality. Tree death is more likely in younger trees, which are more susceptible to infestation.

Morphology

Eggs

Eggs are elongate and laid upright with their longitudinal axis perpendicular to the substrate, in a ringed cluster around the stem of a young shoot, or occasionally on the leaf-tip (Fig 1a,b). They are distinctive in that they possess external ornamentation, comprising four apical projecting horns and four longitudinal ridges. Colour varies from almost white to mauve, with ornamentation tending to be more golden or purplish in colouration. The number of eggs per egg cluster varies from 40-100, but usually consists of around 60-80 individual eggs (Cumpston, 1939).

a)

b)

Figure 1. *Paropsis atomaria* egg batches laid on a) branchlet of *Eucalyptus* sp., and b) leaf tip. Photos by Mark Schutze (not included in CABI datasheet)

Larvae

First instar larvae (Fig. 2) possess large, kidney-shaped tubercles on the meso- and meta-thorax. General body colour is shiny yellow, with contrasting black pigmented areas.

Figure 2. First instar *Paropsis atomaria* larvae. Photo by Mark Schutze.

Second and third instar (Fig. 3) larvae have lost pigmentation of the body tubercles and prothoracic shield, so that the body is glistening yellow, with black head capsule and terminal abdominal segments. Legs and spiracles are brown. Larvae of the closely related species *Chrysophtharta variicollis* may be distinguished from *P. atomaria* in that the former are dull cream in colour with black spiracles.

Figure 3. Third instar *Paropsis atomaria* larvae. Photo by Amy Carmichael

The fourth (final) instar is very distinctive (Fig. 4). Pigmented areas show increased intensity, in stark contrast to the general yellow colouration of the body. The

prothoracic shield is black, and legs brown. A black median longitudinal line extends from the prothoracic shield to the 7th abdominal segment, and large lateral black areas on abdominal segments 1-6, with each partially enclosing a white spot. Lateral tubercles enclosed in these areas are prominent and bear numerous setae (Cumpston, 1939).

Figure 4. Fourth instar *Paropsis atomaria* larvae showing distinctive black lateral and dorsal markings. Photo by Amy Carmichael

Pupae

Pupae are pale to bright yellow in colouration, with light brown pubescence (Fig. 5). Male pupae are generally smaller than females, with male body length averaging 13.9mm and females 15mm (Reid and Ohmart, 1989). The terminal portion carries a ventral bilobed dark brown shield and two rows of small brown tubercles. Legs and wing sheaths are pallid and translucent. Just prior to emergence, the hindwings appear black, and the elytra pink (Cumpston, 1939). Pupae can be sexed via examination of the ventral part of the abdominal apex (Reid and Ohmart, 1989). The hind margin of sternite VIII is with a small median incision in males, and with a deep, median cleft to the base in females. Also, lobes of sternite IX are ovate and separated by their diameter in males, whereas in females they are transverse and contiguous.

Figure 5. *Paropsis atomaria* pupa. Photo by Amy Carmichael

Adults

Strongly convex body (Fig. 6). Antennae moderately robust and filiform, consisting of 11 segments (including scape and pedicel). Dorsal colouration: yellow with orange/pale sanguineous markings, more intense on elytra. Elytra may also possess darker markings, consisting of peripheral longitudinal line on either side and one-two dots per elytra. Ventral colouration: pale fulvous yellow. Legs pale fulvous yellow.

Males: 10mm long, approximately 7-8 mm wide. Fore and mid basitarsi possess uniform ventral discs of setae, which have an adhesive quality and are used for gripping the elytra of the female during mating. Hind basitarsi do not possess such setae, but rather a narrow glabrous line.

Females: Generally larger than males, 12-13mm long, and 8-9mm wide. All basitarsi lack ventral disc of setae as seen in fore and mid basitarsi of males.

Figure 6. Pair of *Paropsis atomaria* adults. Photo by Mark Schutze

Similarities to other species

Other paropsine species may exist within eucalypt plantations; however their morphology is sufficiently disparate to prevent misidentification. There exist two closely related sibling species of *P. atomaria*: *P. elytrura* Blackburn (Fig. 7a) and *P. deboeri* Selman (Fig. 7b). The former is restricted to south-west Western Australia, the latter to Tasmania. These species are very similar morphologically, however due to their allopatric distribution they are unlikely to cause confusion in the field.

a)  b) 

Figure 7. Species morphologically similar to *Paropsis atomaria*: a) *P. elytrura* from south-west Western Australia, and b) *P. deboeri* from Tasmania. Photos by a) Mark Schutze and b) David de Little. (not included in CABI datasheet)

Second and third instar larvae of *P. atomaria* may be confused with those of *Chrysophtharta variicollis* Chap., however *P. atomaria* larvae are much brighter and possess brown spiracles, whereas *C. variicollis* larvae are dull cream in colour and have black spiracles.

Detection and inspection methods

Paropsis atomaria infestation is typified by extensive damage to young and coppicing leaf-growth. This is principally caused by the larvae (especially early instars) preferentially feeding upon softer, younger leaves. Adults and later instars are capable of consuming older, tougher material, and may therefore be located on other parts of affected trees.

Leaf damage is particular to life-stage, and it is possible to discern larval from adult attack. For example, adult damage generally has the appearance of multiple semi-circular ‘bite-marks’ along the perimeter of leaves, known as ‘scalloping’ (Fig. 8). Larval attack is gregarious and nocturnal, consisting of consuming one entire leaf before moving onto the next, leaving nothing but the bare twig (Cumpston, 1939).

Figure 8. Characteristic leaf 'scalloping' damage of eucalypt foliage by adults of *P. atomaria*. Photo by Mark Schutze

Adults and larvae are typically observed over the warmer months, active from October to March in the colder climates of Australia (*e.g.,* ACT), however they may be observed in the field a little beyond March in warmer, more northerly regions such as south-east Queensland.

Overwintering adults conceal themselves between crevices and any leaves that may be bound together (*e.g.,* from spider silk), and may be found outside their active months.

The distinctive egg batches are usually located on young branchlets, typically around 1-1.5mm in diameter. Egg clusters are occasionally laid on leaf tips.

Pupae are located in the soil under affected trees, and can only be detected by extensive soil sampling.

Geographical distribution

Paropsis atomaria has a wide distribution within Australia. Records place it from eastern central Queensland, along the east coast of Australia, to southern Victoria, extending west to South Australia. There is one record of this species existing as far north as Townsville. It extends as far west inland as Orange, NSW, however significant populations are usually located in more coastal regions.

Invasiveness

In some countries this species is considered a potential invasive pest. See 'Phytosanitary Risk' for details.

Phytosanitary risk

This is an endemic pest to Australia, and has not been recorded as impacting on any other countries. Any concern regarding *P. atomaria* should be restricted to industries where *Eucalyptus* species are the commodity in question. Other species of paropsine beetles have been accidentally introduced to New Zealand and South Africa from Australia where they cause considerable defoliation to commercial hardwood forests.

Paropsis atomaria is listed as of high risk potential for importation of unprocessed logs into the United States (Kleinjunas *et al.,* 2003). In New Zealand, where the accidental introduction of paropsine beetles has caused considerable damage to eucalypt plantation productivity (Withers, 2001), *P. atomaria* is listed as a regulated pest for imports from Australia, including pests potentially associated with bark, wood packing and sawn wood (Ormsby, 2001). Phytosanitary treatment options include fumigations, heat treatment, reshipment, or destruction (Ormsby, 2001).

Means of movement and dispersal

Natural dispersal (non-biotic)

There remains a risk for *P. atomaria* eggs and larvae to be transported if plant material from an infested area is moved to a new, un-infested area. Likewise, there is a small risk of pupae being relocated if soil from an infested area is also moved. Adults and pre-pupae may be transported inadvertently in camping gear.

Silvicultural practices

It is possible that overwintering adults may be transported in logs, as they may be located beneath bark or in splits and cracks in the wood (Simmul & deLittle, 1999)

Movement in trade

There is potential for movement of individuals through trade if untreated logs are transported containing overwintering adults beneath bark.

Notes on natural enemies

Paropsis atomaria is attacked by numerous hymenopteran and dipteran parasitoids and hyperparasitoids, as well as several predators. Tanton and Khan (1978a) highlighted numerous such species.

Eggs are primarily parasitized by hymenopteran species, including *Aphanomerella ovi* Dodd (Platygasteridae), *Neopolycystus insectifurax* Gir. (Pteromalidae), and *Enoggera* sp. (Pteromalidae). *Baeoanusia albifunicle* (Girault) (Platygasteridae) is a hyperparasitoid of *Enoggera* sp. (Jones & Withers, 2003). Additionally, unidentified species of *Epiencyrtus* (Encyrtidae) and *Trissolcus* (Scelionidae) emerged from eggs. Parasitism causes up to 20% mortality, with parasitized eggs possessing a dull brown appearance rather than the typically glossy, yellow, unaffected eggs.

Larvae are parasitised by hymenopteran and dipteran species. Dipteran species include members of the Tachinidae: *Froggattimyia anguliventris* Mall., *F. tillyardi* Mall., and *Paropsivora* sp. These species are common each year. Hymenopteran parasitoids include *Eadya paropsidis* (Huddleston & Short) (Braconidae), *Bracon* sp. (Braconidae) and *Tetrastichus* sp. (Eulophidae), with *E. paropsidis* representing the dominant larval parasitoid (Simmul & deLittle, 1999), usually laying six eggs per host and causing up to 93% parasitisation (Tanton & Epila, 1984). Furthermore, two hyperparasitoids, *Mesochorus* sp. (Ichneumonidae), and *Perilampus tasmanicus* (Cameron) (Pteromalidae), hyperparasitise tachinid puparia. Parasitised larvae show symptoms in the pre-pupal stages, taking on a darker colouration, with tachinid larvae boring a hole out of the body to pupate. Tachinid larvae that remain within the larvae during pupation are attacked by hyperparasitoids. High levels of parasitiasation of larvae occur in Febuary-March. Rates of parasitisation range from 0%-20% for tachinids, and 0%-25% for hymenopterans. Combined parasitisation rates range from 0%-41%.

Adult parasitisation is less common, and in cases where it has been observed, a protozoan, *Pleistophora* sp. is found to have been the cause. Adults parasitized by this species exhibit lack of coordination and ability to maintain adequate contact to the substrate.

Predation is typically seen on eggs and larvae. Principle insect predators include coleopterans and hemipterans. Coleopteran predators consist of coccinellids, such as

Cryptolaemus montrouzieri Muls., *Harmonia* (*Leis*) *conformis* (Boisd.), *Rhyzobius discolor* Er., *R. ventralis* Er, and *Cleobora mellyi* (Mulsant). Only first and second instar larvae are particularly susceptible to attack. Hemipteran predators include species from the family Pentatomidae, *Cermatulus nasalis* (West.) and *Oechalia schellenbergii* (Guer.-Men.). The former species, *C. nasalis* feeds on sluggish, fourth-instar larvae, whist *O. schellenbergii* feeds on eggs as well as larvae. A possible predator, *Rayieria basifer* (Walk.) (Miridae), has been observed on *P. atomaria* larvae, but not attacking them.

Control

Biological control

No biological control programmes have been developed for this pest species.

Host-Plant Resistance

Even though *P. atomaria* exists on multiple eucalypt species, certain eucalypts are more susceptible to attack than others. Where possible, less susceptible alternatives should be considered for forestry programs. See 'Notes on Host Range' for details.

Pheromonal control

Little is known concerning pheromonal attractants in this species, so as a result no such means of control have been developed.

Chemical control

Two chemicals were assessed by Tanton and Khan (1978b,c,d): fenitrothion and aminocarb. With regard to egg mortality, fenitrothion proved more effective at lower does, being about 30 times more effective than aminocarb. It was found that a 20ppm concentration of fenitrothion achieved 100% mortality, with concentrations as low as 0.625ppm achieving high levels of mortality. In contrast, aminocarb needed applications of at least 62.5ppm in order to achieve comparable effectiveness. Similarly, fenitrothion had an increased effect on larval mortality over aminocarb. However, as Tanton and Khan (1978b) outlined, aminocarb was more effective than fenitrothion when considering growth rate of 2^{nd} instar larvae. In contrast, 4^{th} instar larval growth rate was more severely affected by fenitrothion than aminocarb.

Insecticide application did not affect food consumption by larvae of any given weight, but rather the effect was a disruption of digestion and utilization of food. In treated larvae, there was disintegration of cellular structures of the digestive system, curtailing digestion and absorption (Tanton and Khan, 1978c). Consequently, there was increased utilization of fat bodies. Fourth instar larvae were the most capable of recovery after insecticide application.

Adults produced from treated larvae showed increased levels of deformity; however they fed and excreted normally. Fecundity was reduced in all adults whose larval stage had been treated, with aminocarb-treated individuals demonstrating lower fecundity than fenitrothion-treated individuals. There were no adverse effects on parasitoids in treated individuals. Furthermore, a later study demonstrated that parasitized larvae were more susceptible to fenitrothion or DDT had higher mortality rates if they had been parasitized (Tanton and Epila, 1984).

Paropsine populations can also be controlled using a pyrethroid-based insecticide (α-cypermethrin) (Elliott *et al.* 1998), which has been used against Tasmanian eucalyptus leaf beetles in quantities of 100g/L, and applied at a rate of 250mL/ha. Additionally, maldison (500g/L) has been used against leaf beetles, amongst other pests, in eucalypt and native plant situations in Western Australia. Maldison is effective on contact or after digestion. Carbaryl is also effective.

In Queensland, two insecticides are used against leaf-beetle populations, namely Dominex 100 (α-cypermethrin) and Saboteur 400 (systemic insecticide dimethoate). Application of these chemicals is only recommended when levels of infestation are severe (>50%), as infestation rates below this level are unlikely to cause significant loss of growth to the tree.

Application of any of these insecticides should coincide with presence of the first two instars, as it is at this stage that the pest is noticeable without having caused serious damage.

Economic impact

Because the intensive cultivation of eucalypts is a fairly recent forestry initiative, there exists little to no information on the precise economic impact *P. atomaria* has on the industry.

Environmental impact

Environmental impact is negligible as this is an endemic species to Australia, and adverse effects are only demonstrated within the forestry industry.

References

Baly JS, 1862. Descriptions of the species belonging to the genus Paropsis Journal of Entomology, 2:291-310.

Blackburn T, 1901. Revision of the genus Paropsis Part VI Proceedings of the Linnean Society of NSW, 26:159-196.

Carne PB, 1966. Ecological characteristics of the eucalypt defoliating chrysomelid Paropsis atomaria Ol. Australian Journal of Zoology, 14:647-672.

Cumpston M, 1939. Observations on the bionomics and morphology of seven of the tribe Paropsini (Chrysomelidae). Proceedings of the Linnean Society of NSW, 64:353-366.

Elliott HJ, Ohmart CP, Wylie FR, 1998. Insect pests of Australian forests: ecology and management Inkata Press, Melbourne.

Fox LR, Macauley BJ, 1977. Insect grazing on Eucalyptus in response to variation in leaf tannins and nitrogen. Oecologia, 29:145-162.

Jones DC, Withers TM, 2003. The seasonal abundance of the newly established parasitoid complex of the Eucalyptus Tortoise Beetle (*Paropsis charybdis*). New Zealand Plant Protection, 56:51-55.

Kliejunas JT, Burdsall HH, DeNitto GA, Eglitis A, Haugen DA, Harverty MI, Micales JA, Tkacz BM, Powell MR, 2003. Pest risk assessment of the importation into the United States of unprocessed logs and chips of eighteen Eucalypt Species from Australia. General Technical Report FPL-GTR-137. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 206 p.

Larsson S, Ohmart CP, 1988. Leaf age and larval performance of the leaf beetle Paropsis atomaria. Ecological Entomology, 13:19-24.

Lawrence JF, Britton EB, 1996. Coleoptera (Beetles). In: The Insects of Australia: A textbook for students and research workers. Melbourne University Press, Melbourne.

Moore BP, 1967. Hydrogen cyanide in the defensive secretions of larval Paropsini (Coleoptera: Chrysomelidae). Journal of the Australian Entomological Society, 6:36-38.

Morrow PA, Fox LR, 1980. Effects of variation in Eucalyptus essential oil yield on insect growth and grazing damage. Oecologia, 45:209-219.

Ohmart CP, Stewart LG, Thomas JR, 1985. Effects of food quality, particularly nitrogen concentrations, of Eucalyptus blakelyi foliage on the growth of Paropsis atomaria larvae (Coleoptera: Chrysomelidae). Oecologia, 65:543-549.

Ohmart CP, Thomas JR, Stewart LG, 1987. Nitrogen, leaf toughness and the population dynamics of Paropsis atomaria Olivier (Coleoptera: Chrysomelidae) - A hypothesis. Journal of the Australian Entomological Society, 26:203-207.

Olivier GA, 1807. Paropside. Hist. Nat. Mus., 92:596-598.

Ormsby M, 2001. Import Health Standard (draft). Ministry of Agriculture and Forestry Biosecurity Authority, Wellington, NZ. 13 pp.

Phillips C, 1996. Insects, diseases and deficiencies associated with eucalypts in South Australia. 1st edn. Primary Industries, SA Forests.

Reid CAM, Ohmart CP, 1989. Determination of the sex of pupae of Paropsis atomaria Olivier, and the related paropsina (Coleoptera: Chrysomelidae). Journal of the Australian Entomological Society, 28:29-30.

Selman BJ, 1963. A reappraisal of the genus Paropsis Ol. (Chrysomelidae, Coleoptera), with particular reference to the species introduced into New Zealand. The Annals and Magazine of Natural History, 13:43-47.

Selman BJ, 1983. The naming of the Tasmanian species of Paropsis Olivier (Coleoptera: Chrysomelidae) Journal of the Australian Entomological Society, 22:333-339.

Shepherd B, 2001. Chrysomelid leaf-eating beetle Paropsis atomaria (Olivier): its damage to Eucalyptus cloeziana (F. Muell), with identification of potential parasitic control agents in recently established southeast Queensland commercial hardwood forests, Sunshine Coast University.

Simmul TL, de Little DW, 1999. Biology of the Paropsini (Chrysomelidae: Chrysomelinae) Advances in Chrysomelidae Biology ML Cox Leiden, Backhuys Publ. 463-477.

Tanton MT, Epila JSO, 1984a. Parasitization of larvae of Paropsis atomaria Ol. (Coleoptera: Chrysomelidae) in the Australian Capital Territory Australian Journal of Zoology, 32:251-259.

Tanton MT, Epila JSO, 1984b. Effects of DDT and Fenitrothion on field collected larvae of a eucalypt-defoliating beetle, Paropsis atomaria Ol.: II. Effects of

treatment of the host host on the parasitoids. Australian Journal of Zoology, 32:337-344.

Tanton MT, Khan SM, 1978a. Aspects of the biology of the eucalypt-defoliating chrysomelid beetle Paropsis atomaria Ol. in the Australian Capital Territory. Australian Journal of Zoology, 26:113-120.

Tanton MT, Khan SM, 1978b. Effects of Fenitrothion and Aminocarb, at doses giving low mortailty, on surviving eggs and larvae of the eucalypt-defoliating chrysomelid beetle Paropsis atomaria Ol.: I. Methods, mortality and relative toxicity. Australian Journal of Zoology, 26:121-126.

Tanton MT, Khan SM, 1978c. Effects of Fenitrothion and Aminocarb, at doses giving low mortailty, on surviving eggs and larvae of the eucalypt-defoliating chrysomelid beetle Paropsis atomaria Ol.: II. Biology of survivors. Australian Journal of Zoology, 26:127-138.

Tanton MT, Khan SM, 1978d. Effects of Fenitrothion and Aminocarb, at doses giving low mortailty, on surviving eggs and larvae of the eucalypt-defoliating chrysomelid beetle Paropsis atomaria Ol.: III. Histological changes in treated larvae. Australian Journal of Zoology, 26:139-146.

Withers TM, 2001. Colonisation of eucalypts in New Zealand by Australian insects. Austral Ecology, 26:467-476.

Chapter 3

Species status and population structure of the Australian *Eucalyptus* pest *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae)

This chapter has been published as:

Schutze, M.K., Mather, P.B. & Clarke, A.R. (2006) Species status and population structure of the Australian *Eucalyptus* pest *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae). *Agricultural and Forest Entomology*, **8**, 323-332.

Statement of Joint Authorship

Schutze, M.K.

Designed and developed experimental protocol. Carried out field and laboratory work, and analysed data. Wrote manuscript and acted as corresponding author

Mather, P.B.

P.B. Mather co-supervised the study design and experimental protocols, assisted in the interpretation of data, and contributed to editing and structure of the manuscript.

Clarke, A.R.

A.R. Clarke was the principal supervisor of study design and experimental protocols, and assisted in the interpretation of data and the construction of the manuscript.

Species status and population structure of the Australian *Eucalyptus* pest *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae)

Abstract

1 *Paropsis atomaria* Olivier represents an emergent pest of *Eucalyptus* plantations in Queensland and New South Wales, Australia. Most prior studies on the biology and control of *P. atomaria* have centred on populations from Canberra in the Australian Capital Territory, but the biological relationship between beetles from Canberra and those from up to 1500 km further north are unknown.

2 DNA markers were used to determine whether *P. atomaria* from Canberra are the same biological species as those from *Eucalyptus* forestry plantations in northern New South Wales and Queensland, where the beetle has become an important pest. Using the mitochondrial gene, cytochrome *c* oxidase I (COI), individuals collected from across the distribution of *P. atomaria* were investigated for haplotype diversity and levels of mitochondrial divergence.

3 Within *P. atomaria*, genetic distance averaged 0.5% across 23 unique haplotypes for 93 individuals, with an average of 14% difference between *P. atomaria* and the outgroup species, *Paropsis obsoleta*. Significant genetic structure was observed relative to geographical distribution, but not with respect to host plant species of origin. Greatest divergence was between the southern-most sample site (Canberra) and northern sites in New South Wales and Queensland, indicating reduced gene flow between these regions.

4 Individuals from across eastern Australia belong to the same genetic species with population substructuring evident. Consequently, there is no evidence to suggest cryptic species complexes exist within the currently defined taxon. Continued implementation of control strategies for *P. atomaria* across its distribution is appropriate.

Keywords Cryptic species, cytochrome *c* oxidase I, forestry, leaf beetle, population structure

Introduction

The implementation of control measures and management programmes for pest species relies on accurate, biologically meaningful identification. However, this process rests upon original taxonomic designations from which identifications are based. If the link between taxonomically defined and biological species (*sensu* Paterson, 1991) is incongruous, management efforts may be misdirected, resulting in wastage of time, effort and resources (Annecke & Moran, 1977; Mahon *et al.*, 1982; Walter, 2003). Unlike some historical beliefs that biological species would nearly always match their taxonomically defined counterparts (Dobzhansky, 1941), disparity between the two is now commonplace, with many previously identified groups such as 'strains', 'biotypes' or 'populations' of one species probably representing separate 'good species' (Clarke & Walter, 1995). Therefore, it is of paramount importance that economically important species be investigated at the outset of a control programme to ensure control measures are directed towards a cohesive biological entity, rather than a collection of different biological species masquerading under a single taxonomic identity, the so-called cryptic species complex (Walter, 2003). Molecular studies over the last 5 years have demonstrated that cryptic species occur much more frequently than previously thought, whereas theory has predicted for many years that cryptic species may exist where courtship relies on transitory signals (e.g. pheromones, calls, optical signals), rather than fixed morphological features (Paterson, 1991).

Suspected species complexes are expensive to investigate in time and resources. It is therefore desirable first to determine the likelihood of a cryptic complex and then make informed decisions as to whether further investigation is a priority. A number of clues may indicate if a cryptic complex exists, usually centred on disparate biological observations within a taxonomically defined species. Broad polyphagy, for example, is considered rare, because most insect species are host specific (Fox & Morrow, 1981; Jermy, 1984; Claridge *et al.*, 1997), particularly in the Lepidoptera, Coleoptera (Singer, 2001) and Chrysomelidae (Mardulyn & Mililkovitch, 2005). Therefore, highly polyphagous systems may consist of complexes of multiple specialist species, rather than a single generalist species (Walter & Benfield, 1994; Milne & Walter, 1998, 2000). Disparity in life history characteristics may also suggest complexes, as demonstrated by variation in insecticide resistance (Subbarao

et al., 1988; Hemingway *et al.*, 1999; Umina & Hoffman, 1999), reproductive strategies (Wirth *et al.*, 1998; Guillet *et al.*, 2000a, b), prey suitability (Krafsur & Obrycki, 2000), diapause characteristics (Kenis & Mills, 1998) or fecundity (Frohlich *et al.,* 1999).

Using the above criteria, the *Eucalyptus* leaf beetle, *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae), is a candidate for *a priori* suspicion of a cryptic species complex. The beetle is endemic to eastern Australia and is widely distributed from the temperate south (Victoria and South Australia) to the tropical north (Queensland), a distance greater than 2000 km. *Paropsis atomaria* has been studied predominantly in the Australian Capital Territory (towards the southern part of its range) and, as such, most data describing life history characteristics and other biological attributes have been based on individuals sourced from this region. Such studies include early natural history work under the synonym *Paropsis reticulata* (Cumpston, 1939), assessment of ecological characteristics (Carne, 1966a, b), research into causes of mortality and effect of control measures (Tanton & Khan, 1978a, b, c, d; Tanton & Epila, 1984a, b, c, d), and investigation of growth rate and fecundity responses to variable plant compounds (Ohmart *et al.,* 1985a, b; Ohmart *et al.,* 1987; Larsson & Ohmart, 1988; Ohmart & Larsson, 1989). Studies on *P. atomaria* outside of the Australian Capital Territory are scant, particularly in northern parts of its natural range, due to its historical nonpest status in the subtropics. For example, two studies of emergent eucalypt pests, one from New South Wales (Stone, 1993) and the other from Queensland (Wylie & Peters, 1993), demonstrate that although *P. atomaria* was an established pest in New South Wales (which entirely surrounds the Australian Capital Territory), the beetle did not rate a mention in the Queensland appraisal. It is only after recent large-scale establishment of eucalypt plantations in the Australian subtropics, and the consequent emergence of *P. atomaria* as a pest in these plantations, that studies into biological characteristics of *P. atomaria* from regions outside the Australian Capital Territory have been undertaken (Bailey, 2001, unpublished Honours thesis). This increased pest status is probably due to the emergence of *P. atomaria* from adjacent natural forests into newly-established forestry stands, rather than a range expansion from southern populations. These investigations have yielded differences in life-history characteristics between populations collected from south-east Queensland compared

with previous studies from the Australian Capital Territory (Carne, 1966a), particularly with regard to the optimal developmental temperatures of larvae.

Other traits also suggest that *P. atomaria* may be polytypic. The beetle possesses a wide host range and utilizes at least 21 *Eucalyptus* species across its current distribution (CAB International, 2005), in spite of it belonging to a taxonomic group that typically possesses much narrower host affinities (Edwards & Wanjura, 1990). Additionally, across the broad geographical distribution of *P. atomaria,* there is a marked change in climatic conditions, from the temperate south to the tropical north, and the eucalypts on which *P. atomaria* feed rarely occur naturally across this entire range, with nearly all possessing limited regional distributions (Brooker & Kleinig, 1983, 1994). *Paropsis atomaria* therefore may represent either a single biological species, tolerant of wide ranging environmental variables and possessing a large host range, or multiple independently evolving populations, which may include cryptic species, adapted to local environmental conditions and hosts.

Molecular studies utilizing the mitochondrial genome have demonstrated effectiveness when assessing potential cryptic species complexes for groups from varied taxa (Yeh *et al.,* 1997; Danforth *et al.,* 1998; Funk, 1998; Wirth *et al.,* 1998; Frohlich *et al.,* 1999; Giblin *et al.,* 2000; Guillet *et al.,* 2000b; Uribe *et al.,* 2001; Salvato *et al.,* 2002; Mattiucci *et al.,* 2003). Calculation of F_{ST} values from sequence data and subsequent determination of gene flow estimates enable a relatively straightforward assessment of potential evolutionary divergence between sampled populations. The protein coding mtDNA gene cytochrome *c* oxidase I (COI) is commonly used in such studies due to its versatility across multiple taxa because both its structure and function are well understood (Simon *et al.,* 1994; Lunt *et al.,* 1996). For cryptic species studies, the highly variable regions of the gene are often targeted because they are more likely to reveal differences between recently evolved sibling species should they occur.

The present study aimed to utilize the highly variable region of the molecular marker COI to determine: (i) whether there is evidence for cryptic species or locally adapted populations within *P. atomaria* and (ii) the level of genetic structure within *P. atomaria* and assess potential causes for such intraspecific variation if such cryptic complexes are not apparent.

Materials and methods

Insect collection and identification

Adult and larval beetles were hand collected into > 70% alcohol over two seasons (2003 and 2005) from eucalypt plantation and revegetation sites across the east coast of Australia (Table 1, Fig. 1). Due to the gregarious nature of *P. atomaria,* and the tendency of individuals to remain sedentary in the presence of abundant resources (Carne, 1966a), samples were collected from multiple locations within a field site to reduce the chance of collecting related individuals from the same broods. Because of the lack of reliable systematic keys, field identification of *P. atomaria* by forestry researchers is currently based on experience, gross morphological comparison to illustrations from handbooks (Waterson & Urquhart, 1995), and by the presence of highly distinctive egg masses (Cumpston, 1939). Consequently, material for this study was collected in the same manner. The outgroup species for genetic analysis was *Paropsis obsoleta* Olivier, collected from Rosedale, central Queensland (24° 38′ S, 151° 55 ′ E).

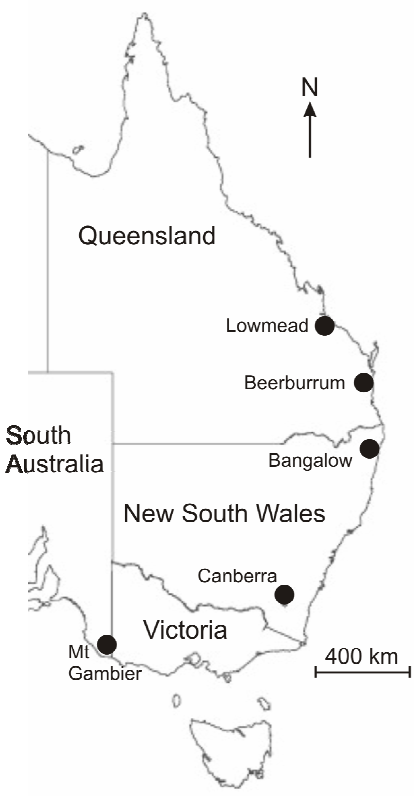


Figure 1: Map of eastern Australia showing the five sampling locations for *Paropsis atomaria* investigated in this study.

Table 1. Location, date, sample size (n) and host species of *P. atomaria* investigated in this study.

Location and date	n	*Eucalyptus* host
Lowmead Queensland, 24º29'22"S, 151 º42'14"E February 2005	14	*E. grandis* X *E. camaldulensis*
Beerburrum Queensland, 26 º58'02"S, 153 º03'06"E March 2003	24	*E. cloeziana* and *E. pilularis*
Bangalow N.S.W., 28 º43'11"S, 153 º31'07"E April 2003	21	*E. grandis* and *E. pilularis*
Canberra A.C.T., 35 º18'51"S, 149 º09'16"E March 2003	28	*E.* spp.
Mount Gambier S.A., 37 º 54'42"S, 140 º 53'13"E March 2003	6	*E. cladocalyx*

DNA sequencing

Genomic DNA was extracted from larvae using a Chelex extraction technique (Walsh *et al.,* 1991). DNA of adults was extracted from three legs in a standard proteinase K phenol : chloroform extraction method (Fukatsu, 1999).

A highly variable fragment of COI was polymerase chain reaction (PCR) amplified using primers UEA7 (5 ′ -TAC AGT TGG AAT AGA CGT TGA TAC-3 ′) and UEA10 (5 ′ -TCC ATG CAC TAA TCT GCC ATA TTA-3 ′) (Lunt *et al.,* 1996). PCR amplification was carried out in a 25-µL final volume reaction containing 3.1 µL Biotech 10 × PCR buffer, 3 mM $MgCl_2$, 0.4 mM dNTPs, 0.4 µM of each primer, 1.5 U Biotech *Taq* polymerase, 1 µL tDNA and 15.6 µL ddH_2O. PCRs were run on an Eppendorf mastercycler gradient thermocycler, with the profile: 94 °C for 5 min; 39 cycles of 95 °C for 40 s, 48 – 56 °C for 1 min, 72 °C for 40 s; 72 °C for 8 min; held at 4 °C.

PCR products were visualized on 1.5% agarose gels run in TBE buffer and stained with ethidium bromide prior to DNA purification using the Roche High PCR product purification kit (Roche, Germany). Gel verification and spectrophometer quantification were conducted prior to sequencing. Sequencing PCR was conducted according to manufacturer's specifications using the BigDye Terminator mix version 3.1 and the UEA7 primer. Sequencing was carried out in 12-μL final volume reactions, consisting of 1 μL BigDye Terminator (PE Applied Biosystems, Foster City, California), 3 μL dilution buffer, 0.27 μM primer and 4 μL of tDNA (at 5 – 20 ng / μL). The sequencing profile was: 94 °C for 5 min; 29 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min; held at 4 °C. Purification of sequencing reactions was accomplished via the salt method, and samples were analysed on an ABI 3730xl automated DNA sequencer by the Australian Genome Research Facility Ltd.

Statistical analysis

Sequence data were aligned and edited in Biomanager, version 2.0 (Australian National Genomic Information Service; http://biomanager.angis.org.au) after confirmation via National Centre for Biotechnology Information GenBank (http://www.ncbi.nlm.nih.gov/). Sequence alignment was achieved using CLUSTAL W (Thompson *et al.,* 1994), followed by manual cross-verification. Unique haplotypes were determined in the program COLLAPSE, version 1.2 (D. Posada, available at http://darwin.uvigo.es/) and imported into MEGA, version 2.1 (Kumar *et al.,* 2001), where the number of variable sites and nucleotide and amino acid composition were calculated. Sequences of each *P. atomaria* haplotype are available in GenBank under accession numbers DQ335220 – 42, and the sequenced fragment for outgroup *P. obsoleta* under accession number DQ338533.

The computer program TCS version 1.2 (Clement *et al.,* 2000) was used to construct a statistical parsimony haplotype network using the 95% parsimony criterion. This procedure provides an overall visual impression of how divergent haplotypes are from each other with regard to number of base pair changes, allowing for a qualitative assessment of haplotype distribution with regard to sample site and host plant of origin.

Genetic differentiation within and among groups incorporating haplotype frequencies together with evolutionary divergences among haplotypes was determined by

analysis of molecular variance (Excoffier *et al.,* 1992) using ARLEQUIN, version 2.000 (Schneider *et al.,* 2000). Distances among haplotypes were estimated using the Kimura 2 parameter distance method that accounts for differing rates of transition versus transversion mutations (Kimura, 1980). The significance of resulting global *F* statistics and corresponding pairwise F_{ST} were determined using a nonparametric permutation procedure incorporation 1000 permutations (Excoffier *et al.,* 1992). Estimation of the number of migrants between populations (*N*m) were calculated from F_{ST} but the results are not presented due to the inherent unreliability of such estimates due to the likely violation of key assumptions (Whitlock & McCauley, 1999). Two separate analyses were undertaken: one to determine geographical substructure and the other to assess substructure based on host plant of origin.

For analysis based on geography, individuals collected from a district were grouped together for: Lowmead, Queensland; Beerburrum, Queensland; Bangalow, New South Wales; and Canberra, Australian Capital Territory (Fig. 1). Samples from Mount Gambier were excluded from geographical analysis due to low sample size. The second analysis, based on host plant of origin, consisted of grouping individuals based on the species of eucalypt from which they were sampled. In some cases, the host plant species of origin was unknown, especially for material collected from Canberra. Additionally, material collected from Lowmead was sourced from a single species of eucalypt, a hybrid of *E. grandis* and *E. camaldulensis*. To avoid confounding host plant effect by geography, only those sample sites where at least two host species occur sympatrically were included. The host plant species assessed for this analysis were *E. cloeziana, E. pilularis,* and *E. grandis*.

Results

Sequence variation

Sequences of a 508-bp fragment of the COI gene were obtained for 93 *P. atomaria* individuals, representing 23 unique haplotypes. Of the 508 sites, 26 were variable. A single homologous fragment was amplified for the outgroup species *P. obsoleta*. Pairwise distances demonstrated reduced intraspecific distance compared with interspecific distance. Pairwise distances among *P. atomaria* populations were in the range 0 – 1.4% (average 0.5%), whereas those between *P. atomaria* and *P. obsoleta* were in the range 13.7 – 14.7% (average 14%). The amplified region was AT rich

(A, 31%; C, 16.1%; G, 13.7%; T, 39.2%), with a larger number of transition to transversion mutations present. Translation into amino acid identity revealed four *P. atomaria* individuals that each had a single unique amino acid substitution with respect to the remaining identical 89 individuals, compared with eight amino acid substitutions between *P. atomaria* and the single *P. obsoleta* sequence.

The 95% haplotype network generated in TCS (Figs 2 and 3) exhibited no evidence for homoplasy due to the absence of any loops (Posada & Crandall, 2001) and the most common haplotypes in the network were Ha 1 and Ha 6 (Table 2, Figs 2 and 3). When collection site was mapped onto the network, no haplotypes clustered with any specific site; instead well-represented haplotypes occurred at multiple collection sites (Fig. 2). Similarly, when host plant data were mapped onto the network, most haplotypes representing more than a single individual did not associate exclusively with a single host species, but consisted of individuals collected from multiple eucalypt hosts (Fig. 3).

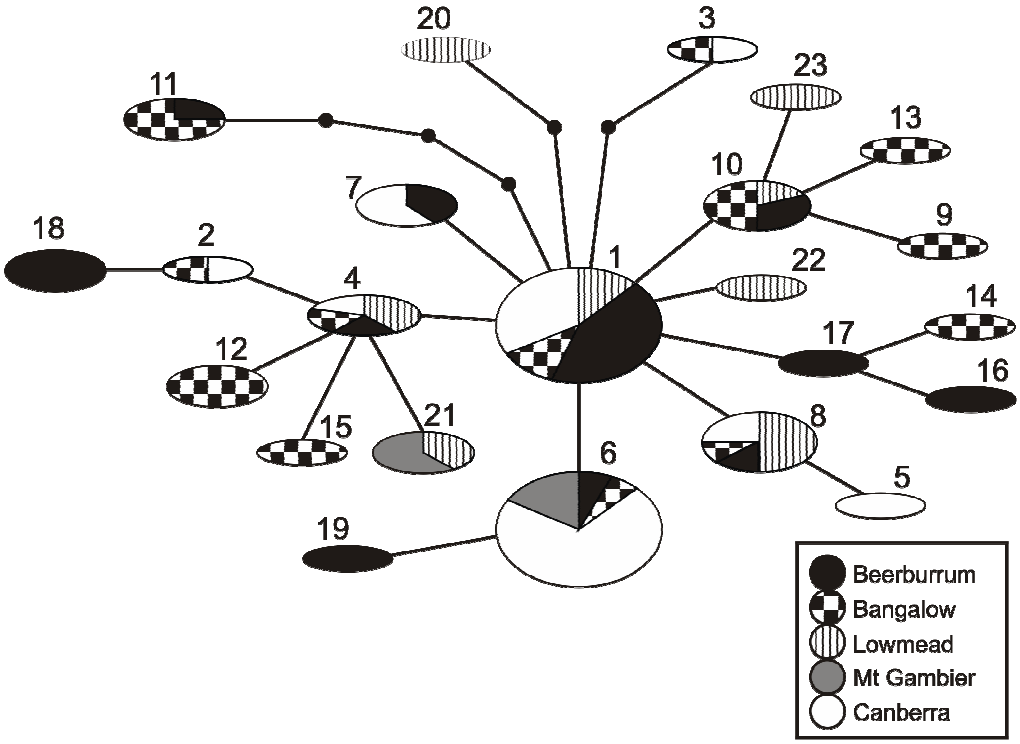


Figure 2: 95% parsimony network of 23 haplotypes obtained by sequencing a 508 bp fragment of the mtDNA COI gene for 93 individuals of *Paropsis atomaria* generated by TCS. Arbitrary haplotype numbers refer to haplotypes listed in Table 2. Size of oval represents relative numbers of individuals possessing the haplotype. Small filled circles represent hypothetical intermediate haplotypes. Shading denotes what proportion of each haplotype is represented by individuals from a specific collection site.

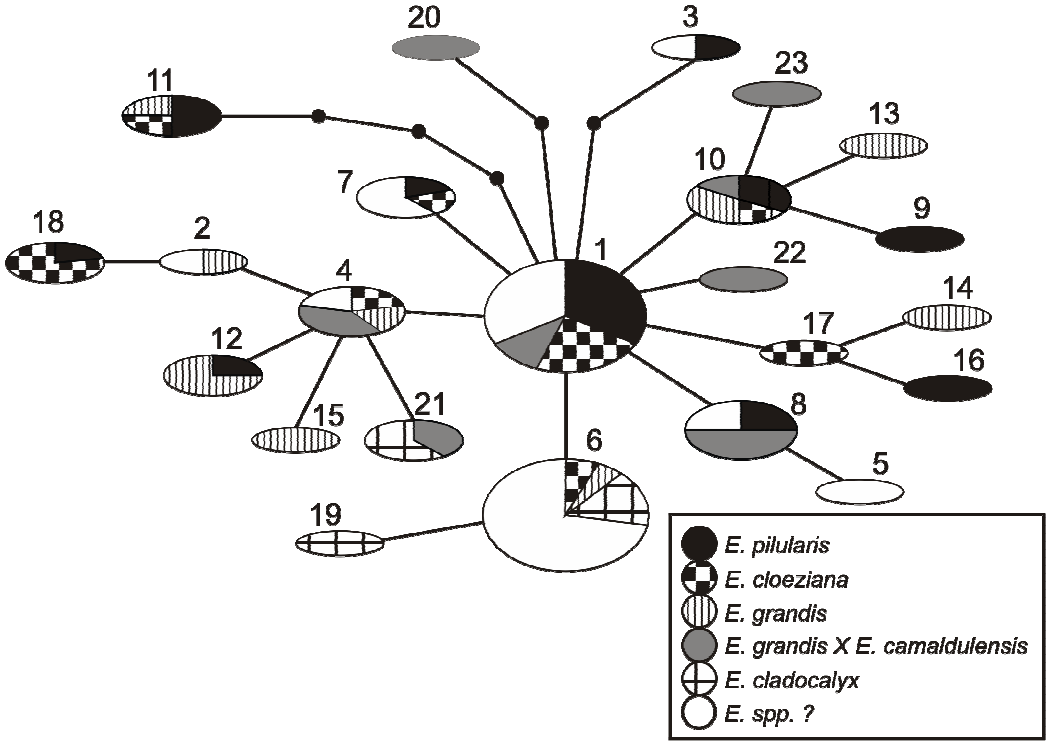


Figure 3: 95% parsimony network of 23 haplotypes obtained by sequencing a 508 bp fragment of the mtDNA COI gene for 93 individuals of *Paropsis atomaria* as generated by TCS. Arbitrary haplotype numbers refer to haplotypes listed in Table 2. Size of oval represents relative numbers of individuals possessing the haplotype. Small filled circles represent hypothetical intermediate haplotypes. Shading denotes what proportion of each haplotype is represented by individuals collected from a specific species of *Eucalyptus*.

Variation between sites within P. atomaria

An assessment of relative haplotype frequencies revealed ten common haplotypes at two sample sites, with 54% of haplotypes unique to a specific site (Table 2). However, unique haplotypes were represented predominantly by single individuals (85%), with haplotypes Ha 12, Ha 18 and Ha 21 represented by multiple individuals from Bangalow (n = 4), Beerburrum (n = 5) and Lowmead (n = 2), respectively.

Haplotypes Ha 1 and Ha 6 were most frequently represented, being the common haplotypes in Beerburrum (0.333) and Canberra (0.464), respectively. The two common haplotypes were not unique to any site, but were sampled from across the distribution, with Ha 1 found in all sites except Mount Gambier and Ha 6 found in all sites except Lowmead.

	LOCATION					HOST			
Site/Host	Lowmead	Beerburrum	Bangalow	Canberra	Mount Gambier	*E. pilularis*	*E. cloeziana*	*E. grandis*	*E.g. X E.c.*
n	14	24	21	28	6	18	15	12	14
Haplotype									
Ha 1	0.14 (2)	0.33 (8)	0.10 (2)	0.21 (6)		0.33 (6)	0.27 (4)		0.14 (2)
Ha 2			0.05 (1)	0.04 (1)				0.08 (1)	
Ha 3			0.05 (1)	0.04 (1)		0.06 (1)			
Ha 4	0.14 (2)	0.04 (1)	0.05 (1)	0.04 (1)			0.07 (1)	0.08 (1)	0.14 (2)
Ha 5				0.04 (1)					
Ha 6		0.04 (1)	0.05 (1)	0.46 (13)	0.50 (3)		0.07 (1)	0.08 (1)	
Ha 7		0.08 (2)		0.11 (3)		0.06 (1)	0.07 (1)		
Ha 8	0.29 (4)	0.04 (1)	0.05 (1)	0.07 (2)		0.11 (2)			0.29 (4)
Ha 9			0.05 (1)			0.06 (1)			
Ha 10	0.07 (1)	0.08 (2)	0.14 (3)			0.11 (2)	0.07 (1)	0.15 (2)	0.07 (1)
Ha 11		0.04 (1)	0.14 (3)			0.11 (2)	0.07 (1)	0.08 (1)	
Ha 12			0.19 (4)			0.06 (1)		0.23 (3)	
Ha 13			0.05 (1)					0.08 (1)	
Ha 14			0.05 (1)					0.08 (1)	
Ha 15			0.05 (1)					0.08 (1)	
Ha 16		0.04 (1)				0.06 (1)			
Ha 17		0.04 (1)					0.07 (1)		
Ha 18		0.21 (5)				0.06 (1)	0.27 (4)		
Ha 19		0.04 (1)					0.07 (1)		
Ha 20	0.07 (1)								0.07 (1)
Ha 21	0.14 (2)				0.50 (3)				0.14 (2)
Ha 22	0.07 (1)								0.07 (1)
Ha 23	0.07 (1)								0.07 (1)

TABLE 2: Number of *Paropsis atomaria* screened for each study with relative haplotype frequencies and absolute number of individuals sampled for each haplotype (in parenthesis). Left-hand of table denotes which haplotypes are associated with a particular sampling location and in what proportion. Right-hand of table denotes which haplotypes are associated with a particular *Eucalyptus* host and in what proportion. *E.g. X E.c.* = *Eucalyptus grandis X E. camaldulensis* hybrid.

Individuals from Bangalow possessed the highest nucleotide (0.007) and haplotype diversity (0.938) (Table 3). Conversely, individuals from Canberra possessed the lowest nucleotide diversity (0.003) and Mount Gambier the lowest haplotype diversity (0.600), albeit with high standard deviation (due to low sample size).

Table 3: Number of *Paropsis atomaria* individuals screened for location and *Eucalyptus* host molecular analyses with number of haplotypes, nucleotide diversity and haplotype diversity. Standard deviations (SD) are provided for nucleotide and haplotype diversity. (*E.g. X E.c.* = *Eucalyptus grandis X E. camaldulensis* hybrid). n = sample size.

Populations/ host species	n	Nucleotide diversity ± SD	Number of haplotypes	Haplotype diversity ± SD
Lowmead	14	0.004 ± 0.003	8	0.901 ± 0.058
Beerburrum	24	0.005 ± 0.003	11	0.855 ± 0.054
Bangalow	21	0.007 ± 0.004	13	0.938 ± 0.032
Canberra	28	0.003 ± 0.002	8	0.743 ± 0.070
Mt Gambier	6	0.004 ± 0.003	2	0.600 ± 0.129
E. pilularis	18	0.006 ± 0.003	10	0.882 ± 0.063
E. cloeziana	15	0.005 ± 0.003	9	0.886 ± 0.061
E. grandis	13	0.007 ± 0.004	10	0.949 ± 0.051
E. cladocalyx	6	0.004 ± 0.003	2	0.600 ± 0.129
E.g. X E.c.	14	0.004 ± 0.003	8	0.901 ± 0.058

Significant population differentiation was found among the four sites examined (F_{ST} = 0.0853, $P < 0.05$). The majority of variation was partitioned within sites (91.47%, d.f. = 83, $P < 0.05$), rather than among sites (8.53%, d.f. = 3, $P < 0.05$). F_{ST} estimates were highest for all pairwise Canberra comparisons (mean = 0.144, $P <$ 0.05 for all comparisons), suggesting reduced gene flow between Canberra and northern sample sites relative to the level of gene flow observed amongst northern sites Beerburrum, Bangalow, and Lowmead (mean pairwise F_{ST} = 0.022, $P > 0.05$ for all comparisons) (Table 4).

Table 4: Pairwise genetic differentiation between populations of *Paropsis atomaria* from four sample locations in eastern Australia. Below diagonal: pairwise estimates of *F*st calculated by AMOVA employing Kimura 2 parameter distances among haplotypes. Asterisk denotes statistical significance ($p < 0.05$). Above diagonal: distance in kilometres (km) between sample locations.

	Beerburrum	Bangalow	Canberra	Lowmead
Beerburrum	-	200 km	1000 km	320 km
Bangalow	0.012	-	800 km	520 km
Canberra	0.123*	0.153*	-	1,320 km
Lowmead	0.029	0.026	0.157*	-

Variation between host plants within P. atomaria

Genetic differentiation based on host plant data was not significant ($F_{ST} = 0.00197$, $P > 0.05$). Pairwise F_{ST} values were generally low (mean = 0.009, $P > 0.05$ for all comparisons), indicating an absence of genetic structuring related to host plant of origin (Table 5) with a low percentage of variation explained by the among-group comparison (0.2%, d.f. = 2, $P > 0.05$) compared with within-site comparisons (99.8%, d.f. = 42, $P < 0.05$).

Table 5: Estimation of genetic differentiation of populations of *Paropsis atomaria* from three different *Eucalyptus* host plants. Pairwise estimates of F_{st} calculated by AMOVA employing Kimura 2 parameter distances among haplotypes.

	E. pilularis	*E. cloeziana*
E. cloeziana	0.002	
E. grandis	0.011	-0.014

Discussion

Observed levels of intraspecific variation within *P. atomaria* were low (mean = 0.5%) compared with outgroup, *P. obsoleta* (mean = 14%), supporting the hypothesis that the *P. atomaria* populations sampled constitute a single species. Comparable levels of intra- and interspecific variation of the COI fragment used in the present study were found compared with studies of other insect taxa that have examined the same region (Jamnongluk *et al.,* 2003; Otranto *et al.,* 2003).

The parsimony haplotype network revealed no evidence for divergent haplotypes strictly associated with either collection locality or host plant of origin, supporting the contention that there were no geographically or host plant restricted races within

the taxon. The implications of these findings are that investigations carried out on *P. atomaria* in the Australian Capital Territory can be applied to populations elsewhere in the north of the range, particularly from northeast New South Wales to central Queensland, because individuals from the northern extent of the range are expected to possess similar biological attributes to those found in Canberra because they belong to the same gene pool. Local differentiation, if observed, may therefore be the result of phenotypic plasticity responding to local environmental conditions, rather than underlying genetic differences between regions or host plants, as would be expected in a case of divergent evolutionary lineages such as cryptic species complexes. However, it is important to note that the gene under study (COI) represents a potentially unlinked neutral marker, which may not reveal modes of local adaptation to prevailing conditions should they be apparent.

Assessment of gene flow among regions reveals historical dispersal between the northern sites, Lowmead, Beerburrum, and Bangalow. However, pairwise F_{ST} values were one order of magnitude higher (combined with corresponding low estimated migration rate, data not shown) between Canberra and the northern sites, suggesting reduced gene flow between the southern site and its northern counterparts. Specific isolation-by-distance (IBD) tests (e.g. Mantel tests) were not used due to inadequate number of populations sampled, resulting in an unacceptable risk of Type II error (Peterson & Denno, 1998). However, IBD is a potential explanation for this system because, similar to most leaf beetle species (Mardulyn & Mililkovitch, 2005), *P. atomaria* in the field is assumed to lead a moderately sedentary existence, with adults rarely leaving an area if resources are locally abundant (Carne, 1966a), and larvae completing their entire development on or very near to the host plant where they were deposited as eggs. As a consequence, long-distance migration is probably only likely when local resources are depleted, necessitating dispersal to new areas. Considering the broad host range of *P. atomaria* on such abundant hosts as eucalypts, we consider that populations in any one area will rarely encounter such a reduction in available resources that there would be a resulting need to disperse large distances to find new host plants. An alternative explanation is provided by population expansion. As with the case in testing for IBD, low numbers of populations sampled resulted in the inability to conduct rigorous statistical analyses to test this theory. Regardless, IBD is proposed as being more probable due to the

life-history characteristics of *P. atomaria* and the low likelihood that this species has expanded into new areas due to long historical associations between *P. atomaria* and its many host tree species over much of the Australian landscape.

No differentiation was revealed between host plant species, with populations on *E. cloeziana, E. pilularis* and *E. grandis,* essentially the same genetically. Although these three species encompass only a small number of the known host plants for *P. atomaria,* the results support the hypothesis that *P. atomaria* represents an oligophagous species on *Eucalyptus* species, with no strict host-associated races or sibling-species. *Paropsis atomaria* possesses a high tolerance for secondary eucalypt metabolites, such as variable concentrations in phenols (Fox & Macauley, 1977) and the ability of larvae to readily absorb a major proportion of ingested terpenoids (Ohmart & Larsson, 1989) may assist in explaining its capacity for oligophagy, with the predominant factors affecting fecundity and larval growth being leaf nitrogen levels and leaf toughness (Ohmart *et al.,* 1985a). Nitrogen levels are negatively correlated with leaf toughness and although a decrease in nitrogen results in a decrease in *P. atomaria* fecundity, the corresponding increase in leaf toughness results in increased mortality in early instars due to the inability of the larvae to physically chew the leaves (Ohmart *et al.,* 1987). Principle factors deterring feeding may therefore include surface chemicals (i.e. waxes) or volatiles (i.e. essential oils) (Ohmart *et al.,* 1987). However, if such deterrents are not present, or their impact on host selection is low [as is the case for the related paropsine *Chrysophtharta bimaculata* (Olivier) (Steinbauer *et al.,* 1998)], then it is unlikely *P. atomaria* would discriminate between host species, therefore resulting in a polyphagous herbivore.

This investigation into the potential existence of a cryptic complex within *P. atomaria* has revealed that the current taxonomic definition of *P. atomaria* remains sound. Although population analyses have revealed intraspecific structuring, suggestive of IBD, this intraspecific variation remains minor. Further sampling, especially for intermediate populations, is recommended to resolve the likely mechanism responsible for observed population structuring. Individuals collected from within the sampled distribution, and identified using gross external morphology, do represent the same biological species. Therefore, new work on *P. atomaria*, undertaken in the northern limits of its distribution where it is a serious

threat to the forestry industry, may reliably build upon studies previously undertaken in the southern part of the species' range.

Acknowledgements

We thank Stephen Monteith, Helen Nahrung, Martin Henery and Angela Duffy for assistance with field collection, Simon Lawson, Richard Lunney, QDPI-Forestry and New South Wales State Forests for access to plantation sites, David Hurwood for assistance with molecular analysis, and Katarina Mikac for useful comments on the manuscript.

References

Annecke, D. P. & Moran, V. C. (1977) Critical reviews of biological control in South Africa. *Journal of the Entomological Society of Southern Africa,* **40,** 127 – 145.

Brooker, M. I. H. & Kleinig, D. (1983) *F ield Guide to Eucalypts: South-Eastern Australia.* Inkata Press, Australia.

Brooker, M. I. H. & Kleinig, D. (1994) *F ield Guide to Eucalypts: Northern Australia.* Inkata Press, Australia.

CAB International (2005) *Forestry Compendium.* CAB International, U.K.

Carne, P. B. (1966a) Ecological characteristics of the eucalypt defoliating chrysomelid *Paropsis atomaria* Ol. *Australian Journal of Zoology,* **14,** 647 – 672.

Carne, P. B. (1966b) Growth and food consumption during the larval stages of *Paropsis atomaria* (Coleoptera: Chrysomelidae). *Entomologia Experimentalis et Applicata,* **9,** 105 – 112.

Claridge, M. F., Dawah, H. A. & Wilson, M. R. (1997) Species in insect herbivores and parasitoids – sibling species, host races and biotypes. *Species: The Units of Biodiversity* (ed. by M. F. Claridge, H. A. Dawah and M. R. Wilson), pp. 247 – 272. Chapman & Hall, U.K.

Clarke, A. R. & Walter, G. H. (1995) 'Strains' and the classical biological control of insect pests. *Canadian Journal of Zoology,* **73,** 1777 – 1790.

Clement, M., Posada, D. & Crandall, K. A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology,* **9,** 1657 – 1659.

Cumpston, M. (1939) Observations on the bionomics and morphology of seven of the tribe Paropsini (Chrysomelidae). *Proceedings of the Linnean Society of New South Wales,* **64,** 353 – 366.

Danforth, B. N., Mitchel, P. L. & Packer, L. (1998) Mitochondrial DNA differentiation between two cryptic *Halictus* (Hymenoptera: Halictidae) species. *Annals of the Entomological Society of America,* **91,** 387 – 391.

Dobzhansky, T. (1941) *Genetics and the Origin of Species.* Colombia University Press, New York, New York.

Edwards, P. B. & Wanjura, W. J. (1990) Physical attributes of eucalypt leaves and the host range of chrysomelid beetles. *Symposia Biologica Hungarica,* **39,** 227 – 236.

Excoffier, L., Smouse, P. E. & Quattro, L. M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics,* **131,** 479 – 491.

Fox, L. R. & Macauley, B. J. (1977) Insect grazing on *Eucalyptus* in response to variation in leaf tannins and nitrogen. *Oecologia,* **29,** 145 – 162.

Fox, L. R. & Morrow, P. A. (1981) Specialization: species property or local phenomenon? *Science,* **211,** 887 – 893.

Frohlich, D. R., Torres, J. I., Bedford, I. D., Markham, P. G. & Brown, J. K. (1999) A phylogeographical analysis of the *B emisia tabaci* species complex based on mitochondrial DNA markers. *Molecular Ecology,* **8,** 1683 – 1691.

Fukatsu, T. (1999) Acetone preservation: a practical technique for molecular analysis. *Molecular Ecology,* **8,** 1935 – 1945.

Funk, D. J. (1998) Isolating a role for natural selection in speciation: host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. *Evolution,* **52,** 1744 – 1759.

Giblin, D. R. M., Gries, R., Crespi, B. *et al.* (2000) Aggregation pheromones of two geographical isolates of the New Guinea sugarcane weevil, *Rhabdoscelus obscurus. Journal of Chemical Ecology,* **26,** 2763 – 2780.

Guillet, S., Guiller, A., Deunff, J. & Vancassel, M. (2000b) Analysis of a contact zone in the *Forficula auricularia* L. (Dermaptera: Forficulidae) species complex in the Pyrenean Mountains. *Heredity,* **85,** 444 – 449.

Guillet, S., Josselin, N. & Vancassel, M. (2000a) Multiple introductions of *Forficula auricularia* L. species complex (Dermaptera: Forficulidae) in eastern North America. *Canadian Entomologist,* **132,** 49 – 57.

Hemingway, J., Karunaratne, S. H. P. P. & Claridge, M. F. (1999) Insecticide resistance spectrum and underlying resistance mechanisms in tropical populations of the brown planthopper (*Nilaparvata lugens)* collected from rice and the wild grass *Leersia hexandra. International Journal of Pest Management,* **45,** 15 – 223.

Jamnongluk, W., Baimai, V. & Kittayapong, P. (2003) Molecular evolution of tephritid fruit flies in the genus *Bactrocera* based on the cytochrome oxidase I gene. *Genetica,* **119,** 19 – 25.

Jermy, T. (1984) Evolution of insect/host plant relationships. *American Naturalist,* **124,** 609 – 630.

Kenis, M. & Mills, N. J. (1998) Evidence for the occurrence of sibling species in *Eubazus spp.* (Hymenoptera: Braconidae), parasitoids of *Pissodes spp.* weevils (Coleoptera: Curculionidae). *Bulletin of Entomological Research,* **88,** 149 – 163.

Kimura, T. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution,* **16,** 111 – 120.

Krafsur, E. S. & Obrycki, J. J. (2000) *Coleomegilla maculata* (Coleoptera: Coccinellidae) is a species complex. *Annals of the Entomological Society of America,* **93,** 1156 – 1163.

Kumar, S., Tamura, K., Jakobson, I. B. & Nei, M. (2001) MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics,* **17,** 1244 – 1245.

Larsson, S. & Ohmart, C. P. (1988) Leaf age and larval performance of the leaf beetle *Paropsis atomaria. Ecological Entomology,* **13,** 19 – 24.

Lunt, D. H., Zhang, D.- X., Szymura, J. M. & Hewitt, G. M. (1996) The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology,* **5,** 153 – 165.

Mahon, R. J., Miethke, P. M. & Mahon, J. A. (1982) The evolutionary relationships of three forms of the Jarrah Leaf Miner, *Perthida glyphopa* (Common) (Lepidoptera: Incurvariidae). *Australian Journal of Zoology,* **30,** 243 – 249.

Mardulyn, P. & Mililkovitch, M. C. (2005) Inferring contemporary levels of gene flow and demographic history in a local population of the leaf beetle *Gonioctena olivacea* from mitochondrial DNA sequence variation. *Molecular Ecology,* **14,** 1641 – 1653.

Mattiucci, S., Cianchi, R., Nascetti, G. *e t al.* (2003) Genetic evidence for two sibling species within *Contracaecum ogmorhini* Johnston & Mawson, 1941 (Nematoda: Anisakidae) from otariid seals of boreal and austral regions. *Systematic Parasitology,* **54,** 13 – 23.

Milne, M. & Walter, G. H. (1998) Host species and plant part specificity of the polyphagous onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), in an Australian cotton-growing area. *Australian Journal of Entomology,* **37,** 115 – 119.

Milne, M. & Walter, G. H. (2000) Feeding and breeding across host plants within a locality by the widespread thrips *Frankliniella schultzei,* and the invasive potential of polyphagous insects. *Diversity and Distributions,* **6,** 243 – 257.

Ohmart, C. P. & Larsson, S. (1989) Evidence for absorption of eucalypt essential oils by *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae). *Journal of the Australian Entomological Society,* **28,** 201 – 205.

Ohmart, C. P., Stewart, L. G. & Thomas, J. R. (1985a) Effects of food quality, particularly nitrogen concentrations, of *Eucalyptus blakelyi* foliage on the growth of *Paropsis atomaria* larvae (Coleoptera: Chrysomelidae). *O ecologia,* **6 5,** 543 – 549.

Ohmart, C. P., Stewart, L. G. & Thomas, J. R. (1985b) Effects of nitrogen concentrations of *Eucalyptus blakelyi* foliage on the fecundity of *Paropsis atomaria* (Coleoptera: Chrysomelidae). *Oecologia,* **68,** 41 – 44.

Ohmart, C. P., Thomas, J. R. & Stewart, L. G. (1987) Nitrogen, leaf toughness and the population dynamics of *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae) – a hypothesis. *Journal of the Australian Entomological Society,* **26,** 203 – 207.

Otranto, D., Colwell, D. D., Traversa, D. & Stevens, R. (2003) Species identification of *Hypoderma* affecting domestic and wild ruminants by morphological and

molecular characterization. *Medical and Veterinary Entomology,* **17,** 316 – 325.

Paterson, H. E. H. (1991) The recognition concept of cryptic species among economically important insects. *Research Methods and Prospects* (ed. by M. P. Zalucki), pp. 1 – 10. Springer-Verlag, New York, New York.

Peterson, M. A. & Denno, R. F. (1998) The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. *American Naturalist,* **152,** 428 – 446.

Posada, D. & Crandall, K. A. (2001) Intraspecific gene geneologies: trees grafting onto networks. *Trends in Ecology and Evolution,* **16,** 37 – 44.

Salvato, P., Battisti, A., Concato, S., Masutti, L., Patarnello, T. & Zane, L. (2002) Genetic differentiation in the winter pine processionary moth (*Thaumetopoea pityocampa – wilkinsoni* complex), inferred by AFLP and mitochondrial DNA markers. *Molecular Ecology,* **11,** 2435 – 2444.

Schneider, S., Roessli, D. & Excoffier, L. (2000) *ARLEQUIN Computer Program,* Version 2.000. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America,* **87,** 651 – 701.

Singer, M. S. (2001) Determinants of polyphagy by a wooly bear caterpillar: a test of the physiological efficiency hypothesis. *Oikos,* **93,** 194 – 204.

Steinbauer, M. J., Clarke, A. R. & Madden, J. L. (1998) Oviposition preference of a *Eucalyptus* herbivore and the importance of leaf age on interspecific host choice. *Ecological Entomology,* **23,** 201 – 206.

Stone, C. (1993) Insect pest problems of eucalypt plantations in Australia: 2. New South Wales. *Australian Forestry,* **56,** 363 – 369.

Subbarao, S. K., Vasantha, K. & Sharma, V. P. (1988) Responses of *Anopheles culicifacies* sibling species A and B to DDT and HCH in India: implications in malaria control. *Medical and Veterinary Entomology,* **2,** 219 – 223.

Tanton, M. T. & Epila, J. S. O. (1984a) Parasitization of larvae of *Paropsis atomaria* Ol. (Coleoptera: Chrysomelidae) in the Australian Capital Territory. *Australian Journal of Zoology,* **32,** 251 – 259.

Tanton, M. T. & Epila, J. S. O. (1984b) Effects of DDT and Fenitrothion on field collected larvae of a eucalypt-defoliating beetle, *Paropsis atomaria* Ol. I. Mortality, relative toxicity, development of treated larvae, and effect of the primary parasitoids. *Australian Journal of Zoology,* **32,** 325 – 336.

Tanton, M. T. & Epila, J. S. O. (1984c) Effects of DDT and Fenitrothion on field collected larvae of a eucalypt-defoliating beetle, *Paropsis atomaria* Ol. II. Effects of treatment of the host host on the parasitoids. *Australian Journal of Zoology,* **32,** 337 – 344.

Tanton, M. T. & Epila, J. S. O. (1984d) Description of the planidium of *Perilampus tasmanicus* Cameron (Hymenoptera: Chalcidoidea), a hyperparasitoid of larvae of *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae). *J ournal of the Australian Entomological Society,* **23,** 149 – 152.

Tanton, M. T. & Khan, S. M. (1978a) Aspects of the biology of the eucalypt-defoliating chrysomelid beetle *Paropsis atomaria* Ol. in the Australian Capital Territory. *Australian Journal of Zoology,* **26,** 113 – 120.

Tanton, M. T. & Khan, S. M. (1978b) Effects of Fenitrothion and Aminocarb, at doses giving low mortailty, on surviving eggs and larvae of the eucalypt-defoliating chrysomelid beetle *Paropsis atomaria* Ol. I. Methods, mortality and relative toxicity. *Australian Journal of Zoology,* **26,** 121 – 126.

Tanton, M. T. & Khan, S. M. (1978c) Effects of Fenitrothion and Aminocarb, at doses giving low mortailty, on surviving eggs and larvae of the eucalypt-defoliating chrysomelid beetle *Paropsis atomaria* Ol. II. Biology of survivors. *Australian Journal of Zoology,* **26,** 127 – 138.

Tanton, M. T. & Khan, S. M. (1978d) Effects of Fenitrothion and Aminocarb, at doses giving low mortailty, on surviving eggs and larvae of the eucalypt-defoliating chrysomelid beetle *Paropsis atomaria* Ol. III. Histological changes in treated larvae. *Australian Journal of Zoology,* **26,** 139 – 146.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research,* **22,** 4673 – 4680.

Umina, P. A. & Hoffman, A. A. (1999) Tolerance of cryptic species of blue oat mites (*Penthalus spp.*) and the redlegged earth mite (*Halotydeus destructor)* to pesticides. *Australian Journal of Experimental Agriculture,* **39,** 621 – 628.

Uribe, S. S. I., Lehmann, T., Rowton, E. D., Velez, B. I. D. & Porter, C. H. (2001) Speciation and population structure in the morphospecies *Lutzomyia longipalpis* (LutZ. and Neiva) as derived from the mitochondrial ND4 gene. *Molecular Phylogenetics and Evolution,* **18,** 84 – 93.

Walsh, P. S., Metzger, D. A. & Higuchi, R. (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing of forensic material. *Biotechniques,* **10,** 506 – 513.

Walter, G. H. (2003) *Insect Pest Management and Ecological Research.* Cambridge University Press, U.K.

Walter, G. H. & Benfield, M. D. (1994) Temporal host plant use in three polyphagous Heliothinae with special reference to *Helicoverpa punctigera* (Wallengren) (Noctuidae: Lepidoptera). *Australian Journal of Ecology,* **19,** 458 – 465.

Waterson, D. & Urquhart, C. A. (1995) *Forest Protection, Research Division Series Number E6: Leaf Beetles (Coleoptera: Chrysomelidae).* State Forests of New South Wales, Australia.

Whitlock, M. C. & McCauley, D. E. (1999) Indirect measures of gene flow and migration: *F* ST is not equal to 1/(4 *Nm* + 1). *Heredity,* **82,** 117 – 125.

Wirth, T., Le Guellec, R., Van Cassel, M. & Veuille, M. (1998) Molecular and reproductive characterization of sibling species in the European earwig (*Forficula auricularia). Evolution,* **52,** 260 – 265.

Wylie, F. R. & Peters, B. C. (1993) Insect pest problems of eucalypt plantations in Australia. 1. *Queensland. Australian Forestry,* **56,** 358 – 362.

Yeh, W. B., Yang, C. T. & Kang, S. C. (1997) Identification of two sibling species, *Ephemera formosana* and *E. sauteri* (Ephemeroptera: Ephemeridae), based on mitochondrial DNA sequence analysis. *Zhonghua Kunchong,* **17,** 257 – 268.

Chapter 4

Converse Bergmann cline in a *Eucalyptus* herbivore, *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae): phenotypic plasticity or local adaptation?

This chapter has been published as:

Schutze, M.K. and Clarke, A.R. Converse Bergmann cline in a *Eucalyptus* herbivore, *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae): phenotypic plasticity or local adaptation? *Global Ecology and Biogeography*. **17 (3),** 424-431.

Statement of Joint Authorship

Schutze, M.K.

Designed and developed experimental protocol. Carried out field and laboratory work, and analysed data. Wrote manuscript and acted as corresponding author

Clarke, A.R.

A.R. Clarke was the principal supervisor of study design and experimental protocols, and assisted in the interpretation of data and the construction of the manuscript.

N.b. While a single measurement of body size (pronotum width) is reported in this chapter, fifteen body size measurements were taken as part of the overall thesis. The remaining fourteen measurements were not included in the final publication due to referee insistence. Supplementary results (including all measurements taken) are presented in Appendix 1 to this thesis.

Converse Bergmann cline in a *Eucalyptus* herbivore, *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae): phenotypic plasticity or local adaptation?

Abstract

Aim To measure latitude-related body size variation in field collected *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae) individuals and to conduct common-garden experiments to determine whether such variation is due to phenotypic plasticity or local adaptation.

Location Four collection sites from the east coast of Australia were selected for contemporary field collections: Canberra (latitude 35º19'S); Bangalow (latitude 28º43'S); Beerburrum (latitude 26º58'S); and Lowmead (latitude 24º29'S). Museum specimens collected over the past 100 years and covering the same geographic area as contemporary field collections came from one state, one national, and one private collection.

Methods Body size (pronotum width) was measured for 118 field collected beetles and 302 specimens from collections. We then reared larvae from the latitudinal extremes (Canberra and Lowmead) to determine whether the size cline was the result of phenotypic plasticity or evolved differences (= local adaptation) between sites.

Results Beetles decrease in size with increasing latitude, representing a converse Bergmann cline. A decrease in developmental temperature produced larger adults for both Lowmead (low latitude) and Canberra (high latitude) individuals, and those from Lowmead were larger than those from Canberra when reared under identical conditions.

Main conclusions The converse Bergmann cline in *P. atomaria* is likely the result local adaptation to season length.

Keywords Leaf beetle, latitude, temperature, body size, converse Bergmann's Rule, season length

Introduction

Body size variation in animals is perhaps the single most important quantitative character measure as it strongly influences most physiological and fitness traits (Blanckenhorn & Demont, 2004). Given this importance, large-scale systematic variation of body size over latitudinal gradients has been of interest to biologists for over 150 years (Bergmann, 1847; Atkinson, 1994; Blackburn *et al.*, 1999; Blanckenhorn & Demont, 2004). Reports of such trends in nature are so pervasive in the literature (*e.g.,* see Blanckenhorn & Demont 2004 for a review of arthropod examples) that they have resulted in the construction of numerous rules which attempt to provide mechanistic explanations for the observed phenomenon of body size variation with latitude.

The environmental variable most often associated with changing latitude is temperature. Consequently, temperature has been at the core of most attempts to relate variation in body size to geographical gradients, with Bergmann's Rule the most often invoked (Bergmann, 1847; Blackburn *et al.*, 1999). Put simply, Bergmann's Rule states that individuals are larger at higher latitudes. Bergmann's original hypothesis was based on the observation of mammals and explained in terms of heat-conservation; the larger the animal in a colder climate, the lower its surface area-to-volume ratio, and hence the greater its capacity to retain heat (thus conferring an advantage). This explanation has been largely dismissed for endotherms and is even less likely for ectotherms, whose body temperatures fluctuate rapidly and are highly dependant on ambient conditions (heat conservation, therefore, is not usually possible) (McNab, 1971; Atkinson & Sibly, 1997). Furthermore, the reverse scenario, *converse* Bergmann's Rule – individuals are smaller at higher latitudes (Park, 1949) – has been documented for many species and was equally represented as conventional Bergmann clines in a recent study of 48 arthropod species (Blanckenhorn & Demont, 2004).

For ectotherms, temperature directly influences body size through its effects on metabolic rates and development time (Sibly & Atkinson, 1994; Van der Have & De Jong, 1996): lower developmental temperatures typically result in larger individuals (temperature-size rule; Carleton, 1960; Vannote & Sweeny, 1980; Lonsdale & Levinton, 1985; Atkinson, 1994; Partridge *et al.*, 1994; Van der Have & De Jong, 1996; Atkinson & Sibly, 1997; Ramsden & Elek, 1998; Chown & Gaston, 1999;

Reeve *et al.*, 2000; but see Walters & Hassall, 2006 for a reverse trend in the grasshopper, *Chorthippus brunneus*). Consequently, if a Bergmann cline is the result of phenotypic plasticity in a species which follows the temperature-size rule, we may expect larger individuals at higher latitudes (colder temperatures), thus conforming to a conventional Bergmann's cline. Importantly, however, this does not imply a Bergmann cline in an ectotherm species is the inherent result of phenotypic plasticity, as adaptive explanations – such as starvation resistance as documented in the ant lion *Myrmeleon immaculatus* (Arnett & Gotelli, 2003) – may be equally valid.

To determine whether a Bergmann cline or its converse is the product of adaptive mechanisms or phenotypic plasticity, common-garden experiments are required. For instance, should an ectotherm species adhere to the temperature-size rule during developmental trials and also conform to a converse Bergmann cline in the wild, we may justifiably conclude genetic differences between populations are driving latitudinal body size variation (*i.e.,* an adaptive mechanism) (Masaki, 1978; Mousseau & Roff, 1989; Blanckenhorn & Fairbairn, 1995; Blanckenhorn & Demont, 2004). The next step is to determine the likely adaptive mechanism driving such a cline. In many cases converse Bergmann clines in an ectotherm species are proposed to be mediated by the interaction of temperature effects on growth, season length, and average development time for the organism (Blanckenhorn & Demont, 2004), with higher latitude seasons providing less time (and resources) for individuals to mature, thereby producing smaller adults (Carleton, 1960; Roff, 1980; Fischer & Fiedler, 2002; Blanckenhorn & Demont, 2004).

Paropsis atomaria Olivier (Coleoptera: Chrysomelidae) is a widely distributed Australian endemic leaf beetle. Adults and larvae feed on the foliage of at least 20 species of *Eucalyptus* L'Her (Myrtaceae) (CABInternational, 2005) and the beetle is an emergent pest of eucalypt plantations in Queensland (Qld) and New South Wales (N.S.W.). *Paropsis atomaria* consists of at least two partially isolated populations along the east coast of Australia, from the temperate south (latitude ~35º) to the sub-tropical north (latitudes 28º – 24º) (Schutze *et al.*, 2006). Beetles from the southern areas of the distribution (Canberra, Australian Capital Territory (A.C.T.)) are active from October until March / April (Carne, 1966), whereas northern populations living in south east Queensland experience a longer field season and adults are active from

as early as September through to at least mid-April (Nahrung, 2006). The large geographical range, together with genetic differentiation between populations along that range, renders *P. atomaria* ideal for investigating a Bergmann cline and its underlying mechanism.

In this study, we first describe a converse Bergmann cline in *P. atomaria* body size across latitude using both recent and historical collections. To determine whether the cline results from phenotypic plasticity in response to temperature or is the product of local adaptation, we conducted common-garden experiments with wild caught beetles from the two extremes of the latitudinal gradient. We conclude the converse Bergmann cline seen for *P. atomaria* is likely the product of local adaptation to season length.

Materials and methods

Temperature data

To determine the degree of correlation between latitude and temperature, long-term climatic data was sourced from the Australian Bureau of Meteorology. We calculated the average daily temperature (ºC) between the months of October and April (*P. atomaria* field season) based on the closest data record site for each collection locality: Lowmead: Gladstone Radar (23°51'36"S, 151°13'36"E; averages based on data from 1957 – 2004); Beerburrum: Caloundra signal station (26°48'00"S, 153°09'00"E; averages from 1899 – 1992); Bangalow: Lismore central street (28°48'36"S, 153°17'24"E; averages from 1884 – 2003); and Canberra: Canberra airport (35°17'60"S, 149°11'60"E; averages from 1939 – 2004).

Body size of field collections

Material

Collection sites were selected based on the following criteria: 1) they occurred across a significant part of the species range, ensuring tropical and temperate locations were included; and 2) sufficient numbers of individuals were present for analysis. Consequently, the following four sites were chosen: tropical/sub-tropical Lowmead (central Qld, 24º29'22"S, 151°42'14"E), Beerburrum (south-east Qld, 26º58'02"S, 153°03'06"E), Bangalow (north-east N.S.W., 28º43'11"S, 153°31'07"E) and temperate Canberra (A.C.T., 35º18'51"S, 149°09'16"E) (Fig. 1) (*n.b.* for brevity, site

latitude information hereafter only given to nearest degree except for specific collection localities). *Paropsis atomaria* was collected from trees within forestry plantations at every site except Canberra (collected within the Jerrabomberra wetlands).

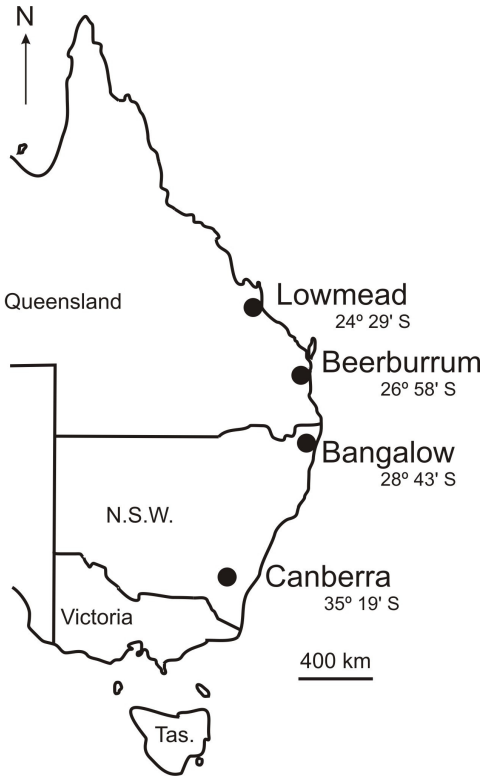


Figure 1. Geographical locations where *Paropsis atomaria* individuals were collected for this study.

Adult beetles were identified based on gross morphology (Waterson & Urquhart, 1995) and hand collected into 70% ethanol. Due to the gregarious and largely sedentary nature of *P. atomaria* (Carne, 1966), every effort was taken to sample from multiple sites within each sampling location in order to reduce the possibility of collecting directly related individuals. Beetles from Beerburrum, Bangalow, and Canberra were collected during March and April of 2003, whilst beetles from Lowmead were collected in February of 2005. Our analysis also included research collection specimens from the Agricultural Scientific Collections Unit (Orange Agricultural Institute), the Australian National Insect Collection (A.N.I.C.), and one private collection.

Character selection

Width of pronotum was selected as the relative measure of adult body size from an original survey of 15 body parts, as it was straightforward to measure, present in all specimens, and not subject to distortion (as is the case for total body length or width, which is inaccurate due to distortion in the resting elytra). Measurements were made by a single observer (MKS) for each individual from the four collection sites using a calibrated eye-piece micrometer on a stereomicroscope to the nearest 0.1 mm.

Statistical analysis

All statistical procedures were run in SPSS v. 14.0 for WINDOWS.

We analysed the relationship between temperature and latitude using Pearson correlation analysis.

For *de novo* collections, we treated latitude as a categorical variable and used a two-way ANOVA to determine the effects of sex, latitude, and their interaction on body size (pronotum width): size ~ sex + latitude + sex*latitude. For historical collection material, we treated latitude as a continuous variable (collection sites varied considerably) and used a Pearson correlation analysis to determine the direction and strength of the relationship between latitude and body size (pronotum width).

Common-garden experiments

Study insects

We collected beetles from the two latitudinal extremes of the current study: Canberra, A.C.T. (35º18'51"S, 149°09'16"E) and Lowmead, central Qld (24º29'22"S, 151°42'14"E) during December 2005 and January 2006.

Approximately 50-100 beetles from each site were maintained on *E. tereticornis* foliage in outdoor cultures in Brisbane Queensland for the duration of the trial. Eggs from stock cultures were allowed to hatch and larvae permitted to consume the egg chorion prior to rearing in controlled temperature cabinets at four temperatures: 16 ºC, 20 ºC, 24 ºC, and 27 ºC. Larvae were supplied daily with fresh *E. pilularis* leaves taken from potted or plantation trees. On any one day all leaves supplied to larvae came from one source, with individual shoots randomised before being placed in rearing containers so as to minimise any potential for diet to bias treatments.

Twenty neonate individuals were placed in each petri dish with foliage and moistened filter paper. Third and fourth instar larvae were transferred to larger containers for the remainder of development. Pre-pupal larvae were removed from rearing containers and placed in petri dishes until adult eclosion. The number of 20-larvae replicates ranged between 10 – 13 for each location (Canberra and Lowmead) and temperature. Not all replicates survived through to adult eclosion, especially Lowmead individuals reared at 27 ºC. Total development time was the number of days from egg eclosion until 50% of the surviving cohort emerged as adults. After 2 – 3 days adults were placed into 70% alcohol for preservation, from which pronotum width was measured (as for field material).

Statistical analysis

The following statistical models were conducted for common garden experiments: a two-way ANOVA testing development time (days) ~ rearing temperature + location + rearing temperature*location, with Tukey *post hoc* tests for temperature for each location, followed by pairwise ANOVA between locations for each temperature trial; and a three-way ANOVA for body size (pronotum width) ~ sex + rearing temperature + latitude + interactions, with Tukey *post hoc* tests for temperature for each location, followed by pairwise ANOVA for each temperature trial between each location for both sexes.

Results

Temperature – latitude correlation

The average daily temperature for each of the four collection localities during the October to April *P. atomaria* field season was as follows: Lowmead (latitude 24ºS): 25.70 ºC; Beerburrum (latitude 27ºS): 23.09 ºC; Bangalow (latitude 29ºS): 22.77 ºC; and Canberra (latitude 35ºS): 17.48 ºC. Pearson correlation analysis of temperature against latitude revealed a strong significant negative correlation (r = -0.992, P = 0.008) (Fig. 2).

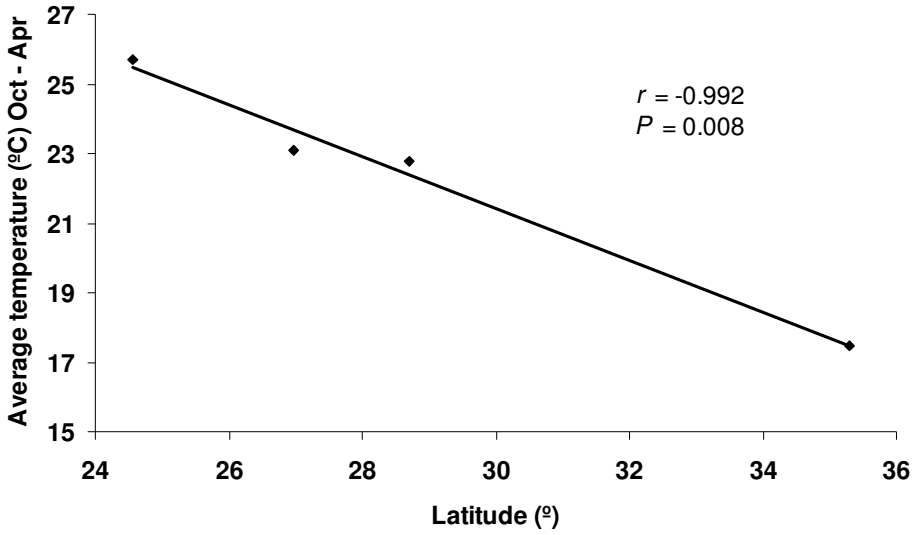


Figure 2. Average daily temperatures for *Paropsis atomaria* field season plotted against latitude for each of the four field collection sites: Lowmead, Beerburrum, Bangalow and Canberra (left-right); Pearson correlation co-efficient $r = -0.992$, $P < 0.05$. See text for calculation of averages.

Body size of field collections

For *de novo* material, sex and latitude significantly affected body size, but their interaction did not (Table 1). Average male pronotum width across all locations (N = 63) was significantly less than that of females (N = 55) (♂ = 5.5 ± 0.5 mm, ♀ = 6.0 ± 0.4 mm; ANOVA d.f. = 1, M.S. = 7.132, $F = 36.823$, $P < 0.001$). For females, Canberra (latitude 35ºS) individuals were significantly smaller than those collected from Bangalow (latitude 29ºS) and Beerburrum (latitude 27ºS), which in turn were significantly smaller than Lowmead (latitude 24ºS) females. For males, Canberra, Bangalow, and Beerburrum beetles were significantly smaller than Lowmead beetles (Fig. 3).

Table 1. Two-way ANOVA of the effect of sex and location (and their interaction) on *Paropsis atomaria* pronotum width for *de novo* collected field material.

Effect	d.f.	M.S.	*F*	*P* value
Sex	1	8.990	97.532	< 0.001
Location	3	3.927	42.602	< 0.001
Sex*Location	3	0.027	0.297	0.83
Error	110	0.092		

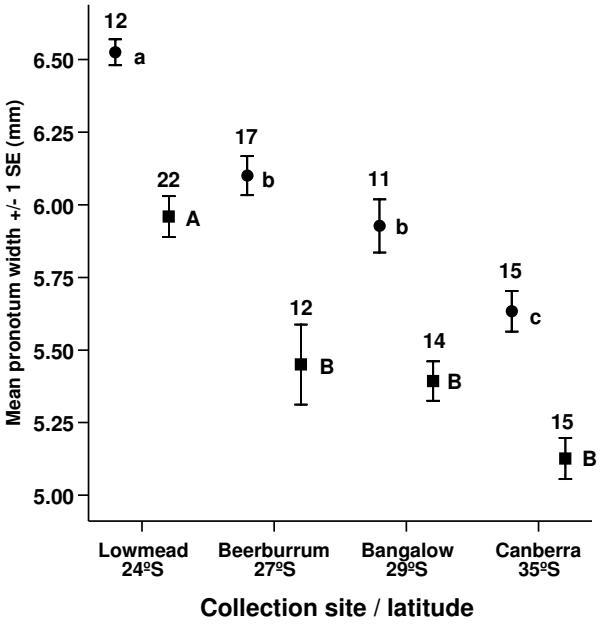


Figure 3. Plot of pronotum width against latitude for *de novo* field collected *Paropsis atomaria* (circles = females; squares = males). Latitude rounded to nearest degree. Numbers above plots = number of individuals measured. Different letters denote statistically significant difference ($P < 0.05$) in pronotum width between locations for each sex (females lower case; males upper case). Points slightly offset for clarity.

One-hundred and forty-nine males and 153 females from historical collection material were measured for pronotum width (latitudes ranged from 19°11'60"S to 37°38'60"S). Pronotum widths for males was again significantly less than that of females (♂ = 5.6 ± 0.4 mm, ♀ = 6.1 ± 0.3 mm; ANOVA d.f. = 1, M.S. = 20.262, F = 173.015, $P < 0.001$). Pearson's correlation analysis revealed a significant negative relationship between pronotum width and latitude for males (r = -0.357, $P < 0.001$), but a weaker, non-significant negative correlation for females (r = -0.110, $P = 0.185$) (Figure 4).

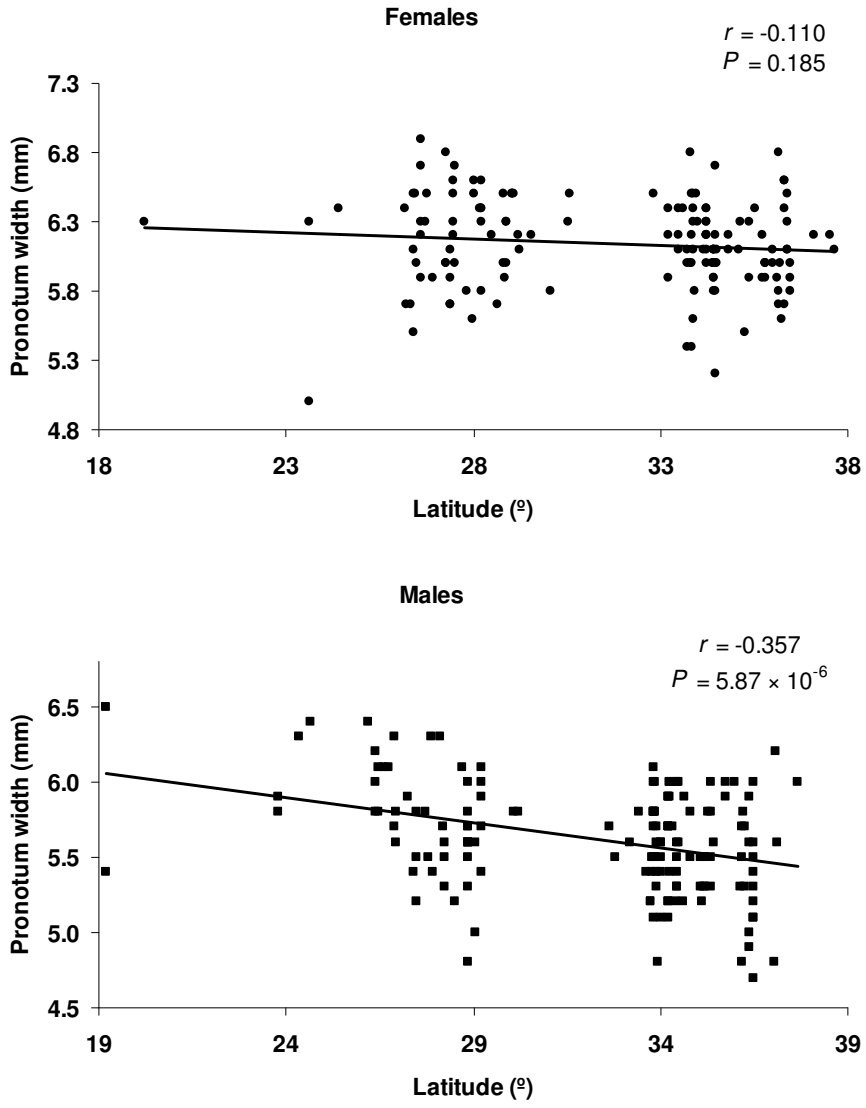


Figure 4. Relationship between pronotum width and latitude for historically collected (*i.e.,* collected prior to this study) *Paropsis atomaria* females (circles) and males (squares) demonstrating converse Bergmann cline. Gap between clusters due to lack of specimens collected from those latitudes.

Common garden experiments

Development time

All individuals for each trial were analysed together regardless of sex (impossible to sex larvae or rear individually due to gregarious feeding behaviour). There was a significant effect of both location and temperature on development time of larvae, but no interaction between the two (Table 2). *Post hoc* tests revealed a significant difference in development time between all developmental temperatures for Canberra

individuals, and similarly so for Lowmead individuals except for between 24 ºC and 27 ºC, for which mean development time was not significantly different between temperature treatments (Fig. 5). Developmental times between populations for each temperature were the same except for the extremes (16 ºC and 27 ºC), in which Canberra individuals developed faster than Lowmead individuals (significant for the 16 ºC trial, $P = 0.028$; and close to statistical significance for the 27 ºC trial, $P = 0.059$) (Fig. 5).

Table 2. Two-way ANOVA of the effect of location and temperature (and the interaction) on total development time in days (egg – adult) for larvae reared at 4 temperatures (16 ºC, 20 ºC, 24 ºC, and 27 ºC).

Effect	d.f	M.S.	*F*	*P* value
Location	1	10.405	5.41	0.023
Temperature	3	4516.934	2348.699	< 0.001
Location*Temperature	3	3.544	1.843	0.149
Error	59	1.923		

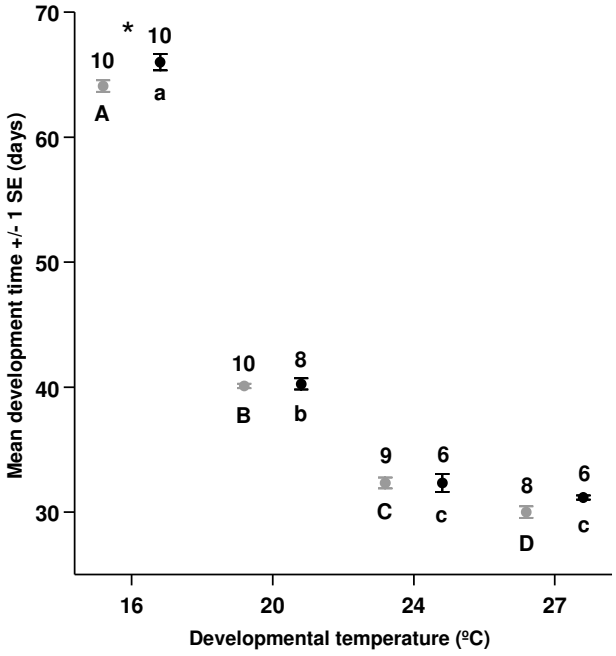


Figure 5. Mean development time (days) for each population of *Paropsis atomaria* (Canberra 35ºS = grey and Lowmead 24ºS = black) reared at four temperatures (16 ºC, 20 ºC, 24 ºC, and 27 ºC). Numbers above plots = number of replicates. Different letters denote significant difference (Tukey *post hoc* comparisons, $P < 0.05$) in total development time between each temperature (Canberra upper case and Lowmead lower case). Asterisk denotes significant difference (pairwise ANOVA, $P < 0.05$) in development time between populations reared at the same temperature. Points slightly offset for clarity.

Body size

Sex, location, and temperature all significantly affected adult body size (none of the interactions were significant) (Table 3). Females were, again, significantly larger than males; northern beetles (Lowmead; 24ºS) were significantly larger than southern beetles (Canberra; 35ºS) when reared at the same temperature for all comparisons except between Lowmead and Canberra males reared at 24 ºC; and *post hoc* tests revealed higher temperatures produced significantly smaller adult females, and smaller (but not significant) males (Fig. 6).

Table 3. Three-way ANOVA results for the effect of sex, location, and temperature (and interactions) on adult body size (pronotum width, mm) for larvae reared at 4 temperatures (16 ºC, 20 ºC, 24 ºC, and 27 ºC).

Effect	d.f.	M.S.	*F*	*P* value
Sex	1	9.714	196.185	< 0.001
Location	1	3.061	61.646	< 0.001
Temp	3	0.284	5.719	0.001
Sex*Location	1	0.092	1.848	0.176
Sex*Temperature	3	0.043	0.858	0.464
Location*Temperature	3	0.016	0.326	0.807
Sex*Location*Temperature	3	0.006	0.120	0.948
Error	212	0.050		

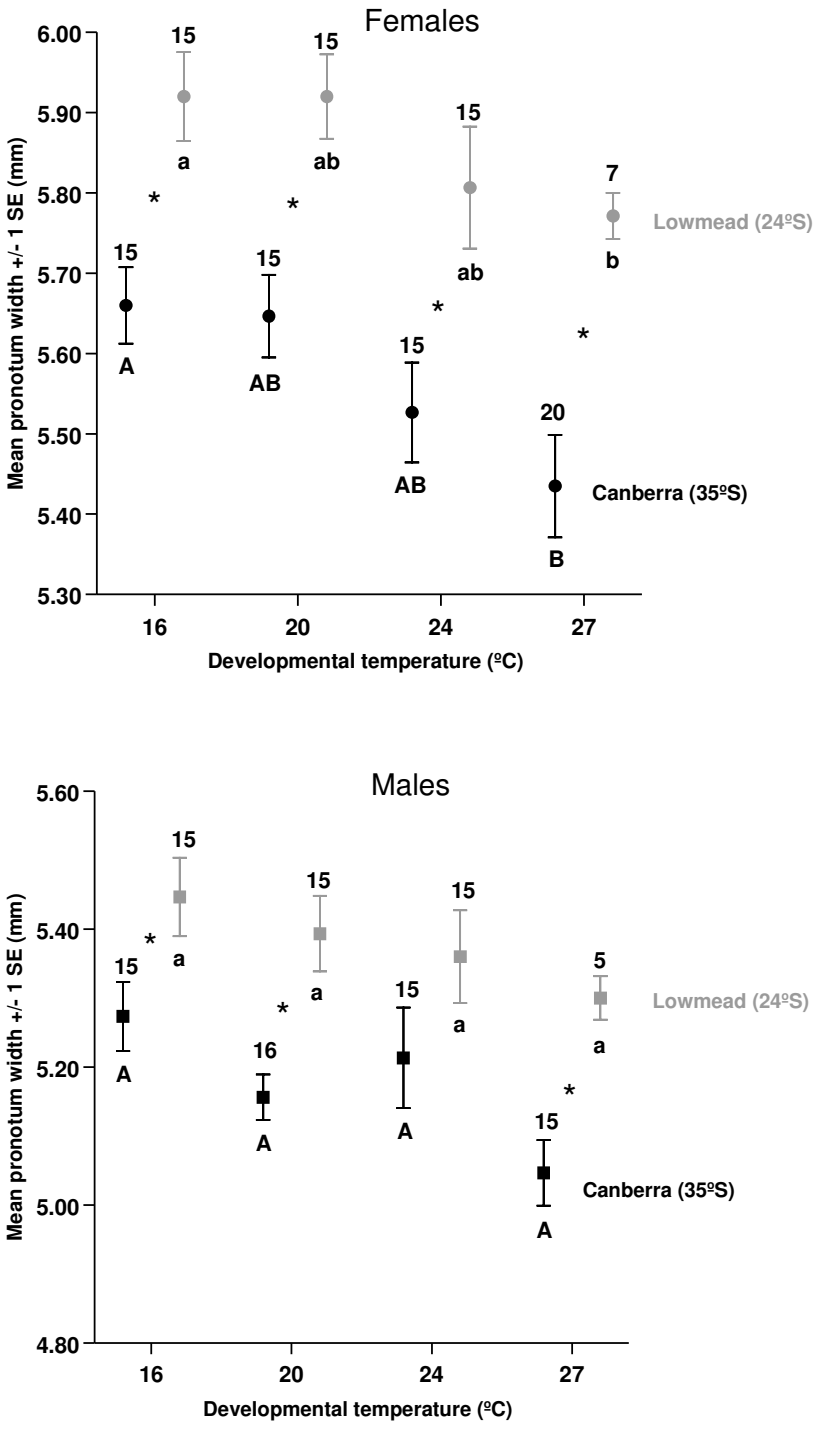


Figure 6. Effects of larval rearing temperatures (16 ºC, 20 ºC, 24 ºC, and 27 ºC) on adult body size (pronotum width, mm) for females (circles) and males (squares) of *Paropsis atomaria* from two population origins (Canberra indicated in black and Lowmead indicated in grey). Numbers above plots = number of individuals measured. Different letters denote significant differences (Tukey *post hoc* comparisons, $P < 0.05$) in average pronotum width between temperature trials for each population (Canberra upper case and Lowmead lower case). Asterisks denote significant difference (pairwise ANOVA, $P < 0.05$) in pronotum width between populations reared at the same temperature. Points slightly offset for clarity.

Discussion

Paropsis atomaria conforms to both a converse Bergmann cline and the temperature-size rule. Wild-caught adults demonstrate a clear trend of decreasing size with increasing latitude (Figs 3 & 4), and common-garden experiments showed that rearing larvae at higher temperatures generally resulted in smaller adults (Fig. 6). Furthermore, northern beetles were consistently larger than southern beetles, regardless of rearing temperature (Fig. 6). Therefore, we conclude the observed converse Bergman cline is due to genetic differences between populations, representing a case of local adaptation rather than phenotypic plasticity.

Adaptive explanations for Bergmann clines in ectotherms include starvation resistance (Arnett & Gotelli, 2003), adaptive co-variation among life-history traits (Angilletta *et al*., 2004), adaptive phenotypic plasticity (Partridge *et al*., 1994), and voltinism mediated by season length (Roff, 1980; Blankenhorn & Demont, 2004). The starvation resistance hypothesis, as seen for the ant-lion *Myrmeleon immaculatus*, is an example whereby particular geographic regions within a latitudinal gradient experience unpredictable seasonal conditions resulting in highly variable food availability for some populations (Arnett & Gotelli, 2003). Consequently, it becomes an adaptive advantage for populations in these unpredictable environments (usually at higher latitudes) to have an increased body size as a means to resist starvation during periods of food unavailability. Whilst valid for the above example, we do not believe this mechanism applies to *P. atomaria*, as we observe a *converse* Bergmann cline, counter to what may be expected under a starvation resistance hypothesis should resource predictability relate similarly to such latitudinal variation. Additionally, there is no reason to believe any particular region lacks available resources compared to another, as *Eucalyptus* is the dominant genus inhabiting Australian forests and constitutes at least 92 % of native forests and woodlands (Morrow, 1976), with *P. atomaria* recorded from more than 20 species (CABInternational, 2005).

Adaptive co-variation among life-history traits may produce a conventional Bergmann cline in ectotherm species with populations experiencing large differences in age at maturation. This is the case for Sceloporine lizards, in which larger individuals living in colder climates (higher latitudes) may mature a full year later than smaller body-sized animals exposed to warmer conditions (Angilletta *et al*.,

2004). Whilst *P. atomaria* is similar to lizards in that it is an ectotherm that adheres to the temperature size rule, *P. atomaria* individuals reach maturity within a single season (significantly less than a year). Therefore, season length across a latitudinal gradient may play a more important role in determining rate to maturation and consequent adult size in a rapidly developing insect such as *P. atomaria*. Indeed, season length is considered important in determining the direction of the cline (Bergmann or converse Bergmann) for arthropods in particular (Roff, 1980; Mousseau, 1997; Blanckenhorn & Demont, 2004). Populations of *Teleogryllus* cricket in Japan, for example, demonstrate a converse Bergmann cline in which northern (lower latitude) individuals mature earlier (and are correspondingly smaller) than their southern counterparts (Masaki, 1972) and this is regarded an adaptive response to season length.

Several factors indicate season length is an important factor producing a converse Bergmann cline in *P. atomaria*. Our results demonstrate development time and size at maturation is genetically controlled, with development time for southern (high latitude) populations generally shorter across the four developmental temperatures studied (Fig. 5), with an associated decrease in adult body size compared to northern, low latitude beetles (Fig. 6). We propose that reduced development time is an adaptive response to shortened season length in southern temperate regions compared to sub-tropical regions in the north, where increased season length permits a longer growth period resulting in increased average adult body sizes (= increased potential fecundity). Such local adaptation is considered possible due to restricted gene flow between temperate and sub-tropical populations of *P. atomaria*, with high pairwise F_{ST} comparisons between the southern population (Canberra) and all northern collection localities (Bangalow, Beerburrum, and Lowmead) (Schutze *et al*., 2006).

Furthermore, other factors, especially host plant quality, may influence final adult body size in *P. atomaria* within and between populations across the latitudinal gradient. Indeed, *P. atomaria* develops at variable rates depending on which host it is reared, resulting in correspondingly variable adult body sizes (Carne, 1966; Schutze in prep.). We believe host plant response contributes to the weaker correlation of body size for historical collection material with latitude compared to our analysis of *de novo* material. Measurements for historical material were taken from specimens

collected over 100 years, which had consequently developed under a wide range of biotic and abiotic environmental conditions. This individual-to-individual variation in turn will have masked, but not hidden, the converse cline effect. We recommend further trials comparing host-plant response between populations be conducted to determine if locally adapted populations have also acquired variable developmental responses to different species of *Eucalyptus*.

Acknowledgements

We thank Stephen Monteith, Helen Nahrung, Alexsis Wilson, Martin Henery and Angela Duffy for assistance with field collections. We also thank Simon Lawson of QDPI&F (Forestry), Richard Lunney of Integrated Tree Crops (ITC) and NSW State Forests for access to plantation sites. We also thank the curators of the Orange Agricultural Institute, the Australian National Insect Collection and Gunter Maywald for the generous loaning of material used in this study.

References

Angilletta, M.J.Jr., Niewiarowski, P.H., Dunham, A.E., Leaché, A.D. & Porter, W.P. (2004) Bergmann's clines in ectotherms: illustrating a life-history perspective with Sceloporine lizards. *The American Naturalist*, **164**, E168-E183.

Arnett, A.E. & Gotelli, N.J. (2003) Bergmann's rule in larval ant lions: testing the starvation resistance hypothesis. *Ecological Entomology*, **28**, 645-650.

Atkinson, D. (1994) Temperature and organism size - a biological law for ectotherms. *Advances in Ecological Research*, **25**, 1-58.

Atkinson, D. & Sibly, R.M. (1997) Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology and Evolution*, **12**, 235-239.

Bergmann, C. (1847) Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Gottinger studien*, **3**, 595-708.

Blackburn, T.M., Gaston, K.J. & Loder, N. (1999) Geographic gradients in body size: a clarification of Bergmann's rule. *Diversity & Distributions*, **5**, 165-174.

Blanckenhorn, W.U. & Demont, M. (2004) Bergmann and converse Bergmann latitudinal clines in arthropods: Two ends of a continuum? *Integrative and Comparative Biology*, **44**, 413-424.

Blanckenhorn, W.U. & Fairbairn, D.J. (1995) Life history adaptation along a latitudinal cline in the water strider Aquarius remigis (Heteroptera: Gerridae). *Journal of Evolutionary Biology*, **8**, 21-41.

CABInternational (2005) Forestry Compendium. CAB International, Wallingford UK.

Carleton, R. (1960) The application of Bergmann's and Allen's rules to the poikilotherms. *Journal of Morphology*, **106**, 85-108.

Carne, P.B. (1966) Ecological characteristics of the eucalypt defoliating chrysomelid *Paropsis atomaria* Ol. *Australian Journal of Zoology*, **14**, 647-672.

Chown, S.L. & Gaston, K.J. (1999) Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews of the Cambridge Philosophical Society*, **74**, 87-120.

Fischer, K. & Fiedler, K. (2002) Reaction norms for age and size at maturity in response to temperature: a test of the compound interest hypothesis. *Evolutionary Ecology*, **16**, 333-349.

Lonsdale, D.J. & Levinton, J.S. (1985) Latitudinal differentiation in copepod growth: An adaptation to temperature. *Ecology*, **66**, 1397-1407.

Masaki, S. (1972) Climatic adaptation and photoperiodic response in the band-legged ground cricket. *Evolution*, **26**, 587-600.

Masaki, S. (1978) Climatic adaptation and species status in the lawn ground cricket. *Oecologia*, **35**, 343-356.

McNab, B.K. (1971) On the ecological significance of Bergmann's Rule. *Ecology*, **52,** 845-854.

Morrow, P.A. (1976) The significance of phytophagous insects in the *Eucalyptus* forests of Australia. *The role of Arthopods in Forest Ecosystems* (ed. by W.J. Mattson), pp. 19-29. Springer-Verlag, New York.

Mousseau, T.A. & Roff, D.A. (1989) Adaptation to seasonality in a cricket: patterns of phenotypic and genotypic variation in body size and diapause length along a cline in season length. *Evolution*, **43**, 1483-1496.

Mousseau, T.A. (1997) Ectotherms follow the converse to Bergmann's Rule. *Evolution*, **51**, 630-632.

Nahrung, H.F. (2006) Paropsine beetles (Coleoptera: Chrysomelidae) in south-eastern Queensland hardwood plantations: identifying potential pest species. *Australian Forestry*, **69**, 270-274.

Park, O. (1949) Application of the converse Bergmann principle to the carabid beetle, *Dicaelus purpuratus*. *Physiological Zoology*, **22**, 359-372.

Partridge, L., Barrie, B., Fowler, K. & French, V. (1994) Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution*, **48**, 1269-1276.

Ramsden, N. & Elek, J.A. (1998) Life cycle and development rates of the leaf beetle *Chrysophtharta agricola* (Chapuis) (Coleoptera: Chrysomelidae) on *Eucalyptus nitens* at two temperature regimens. *Australian Journal of Entomology*, **37**, 238-242.

Reeve, M.W., Fowler, K. & Partridge, L. (2000) Increased body size confers greater fitness at lower experimental temperature in male *Drosophila melanogaster*. *Journal of Evolutionary Biology*, **13**, 836-844.

Roff, D.A. (1980) Optimizing development time in a seasonal environment: The ups and downs of clinal variation. *Oecologia*, **45**, 202-208.

Schutze, M.K., Mather, P.B. & Clarke, A.R. (2006) Species status and population structure of the Australian Eucalyptus pest *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae). *Agricultural and Forest Entomology*, **8**, 323-332.

Sibly, R.M. & Atkinson, D. (1994) How rearing temperature affects optimal adult size in ectotherms. *Functional Ecology*, **8**, 486-493.

Van der Have, T.M. & De Jong, G. (1996) Adult size in ectotherms: temperature effects on growth and differentiation. *Journal of Theoretical Biology*, **183**, 329-340.

Vannote, R.L. & Sweeny, B.W. (1980) Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. *The American Naturalist*, **115**, 667-695.

Walters, R.J. & Hassall, M. (2006) The temperature-size rule in ectotherms: may a general explanation exist after all? *The American Naturalist*, **167**, 510-523.

Waterson, D. & Urquhart, C.A. (1995) "Forest Protection, Research Division Series Number E6: Leaf Beetles (Coleoptera: Chrysomelidae)." State Forests of New South Wales, Beecroft.

Chapter 5

Larval development of two geographically isolated populations of *Paropsis atomaria* on two species of *Eucalyptus*

This chapter is in prep for submission as:

Schutze, M.K. and Clarke, A.R. Larval development of two geographically isolated populations of *Paropsis atomaria* on two species of *Eucalyptus*. *Australian Journal of Entomology*.

Statement of Joint Authorship

Schutze, M.K.

Designed and developed experimental protocol. Carried out field and laboratory work, and analysed data. Wrote manuscript and acted as corresponding author

Clarke, A.R.

A.R. Clarke was the principal supervisor of study design and experimental protocols, and assisted in the interpretation of data and the construction of the manuscript.

Larval development of two geographically isolated populations of *Paropsis atomaria* on two species of *Eucalyptus*

Abstract

Paropsis atomaria Olivier (Coleoptera: Chrysomelidae) is an endemic, *Eucalyptus* L'Her. (Myrtaceae) feeding beetle with a distribution that extends along nearly the entire east coast of Australia. Beetle populations from across the range display low gene flow between northern and southern populations, together with variation in adult body size. This study investigated potential for differential host plant utilisation by geographically isolated populations of *P. atomaria* by measuring southern (Canberra, Australian Capital Territory) and northern (Lowmead, central Queensland) larval developmental response to rearing on two host species: *Eucalyptus cloeziana* (that have a distribution sympatric with the northern beetle population), and *E. pilularis* (sympatric with the southern population). Developmental characteristics studied included the following: larval survival, larval development time, and pupal weight. *Eucalyptus cloeziana* (northern host) was superior compared with *E. pilularis*, showing increased survival rates for both northern and southern *P. atomaria* individuals, together with reduced developmental times. Southern *P. atomaria* pupal weights were generally lower than northern pupae regardless of the host plant larvae were reared on. Northern *P. atomaria* individuals took significantly longer to mature than did southern beetles and were significantly heavier when reared on *E. cloeziana*. No such difference existed for populations reared on *E. pilularis*. Pupal weight was significantly reduced for northern *P. atomaria* individuals reared on *E. pilularis* (southern host). Our results demonstrate similar host plant utilisation for northern and southern populations of *P. atomaria*, with both populations performing better on *E. cloeziana*. The only difference between populations was pupal mass attained, which may be due to other factors such as underlying genetic differences between populations resulting from adaptation to other factors.

Keywords: Chrysomelidae, Coleoptera, Myrtaceae, host specialisation, larval performance, leaf beetle, local adaptation

Introduction

Phytophagous insect species that possess a broad host range and that occur over large geographical areas may consist of multiple localised populations with specialist host affiliations (Jaenike, 1990). Each population, if sufficiently isolated from other populations and subjected to local selective pressures is predicted, over time, to develop adaptations particular to local environmental conditions (Fujiyama et al., 2005). If a tight association exists between populations and their respective host plants, this may lead to the evolution of host races and potential for new species (Ballabeni et al., 2003). Further to this, adaptation to one suite of host plants may coincide with a decrease in the suitability of other host plants not found within the range of a localised population, as explained by trade-off theory (Dethier, 1954; Mackenzie, 1996). There are, however, many examples where such a straightforward trade-off does not exist, as some herbivore populations exhibit increased fitness on novel compared with natal hosts (Ballabeni et al., 2003; Joshi and Thompson, 1995). Additionally, differential host plant use by herbivore populations may not necessarily result from adaptation to a particular host species, but may reflect pleiotropic effects of genes involved in host plant utilisation which are influenced by a wide range of non-host plant related selective pressures (Fujiyama et al., 2005).

For an individual to become specialised on a host plant – or a suite of hosts – requires host-related behaviours that increase an individual's realised fecundity and enhance survival and fitness of offspring (Jaenike, 1990). Detection of host specialisation can be a complex biological process, that is further complicated conceptually, by continued refinement and debate over definitions of host races and intermittent stages of host specialisation (see Dres and Mallet (2002) for a review). Generally, however, current theory requires populations of a species meet a number of criteria before they can be referred to as host races. These requirements include: populations are genetically differentiated, sympatric, use different hosts, and there is appreciable (but not panmictic) gene flow among them (Dres and Mallet, 2002). Additionally, while not considered mandatory to host race identification, improved fitness on natal hosts over alternative hosts is considered supporting evidence for existence of host races (Dres and Mallet, 2002). Measures of fitness often focus on those related to adult behaviour (oviposition and host food preference) and larval development (growth, development rate, survival, and potential fecundity) (Gratton

and Welter, 1998; Harris et al., 2001; Moon and Stiling, 2006; Steinbauer et al., 1998; Verdon et al., 2007), with increased fitness on one host relative to another host being indicative of some degree of host specialisation.

Paropsine leaf beetles (Coleoptera: Chrysomelidae) are common in Australia, with both adult and larval beetles feeding externally on the leaves of *Eucalyptus* L'Her (Myrtaceae) hosts (Simmul & de Little 1999). Several species are recognised pests of *Eucalyptus* plantations, where feeding causes production losses (Baker et al., 2002; Clarke et al., 1997; Nahrung and Allen, 2003; Schutze et al., 2006). One such species, *Paropsis atomaria* Olivier, is an emergent pest of plantations in New South Wales and Queensland and is recorded from at least 20 species of *Eucalyptus* (CABInternational, 2005). As noted for other paropsine species (Baker et al., 2002; Patterson et al., 1996), different *Eucalyptus* species vary in their suitability as food plants for *P. atomaria*, with different host species inducing variable developmental responses relating to larval survival, larval development time, and pupal weight (Carne, 1966a, b).

The geographical distribution of *P. atomaria* is extensive and ranges from the temperate south (South Australia, Victoria, and the Australian Capital Territory (A.C.T.)) to the tropics of Queensland, with molecular evidence demonstrating reduced gene flow between temperate and tropical populations (Schutze *et al.*, 2006). Furthermore, the distribution of Australia's eucalypt flora is highly fragmented, with many species possessing a limited geographical range (Brooker and Kleinig, 1983, 1994), and this potentially exposes local populations of *P. atomaria* to a restricted suite of *Eucalyptus* species. Whilst Carne (1966a) had studied host utilisation by *P. atomaria* of different *Eucalyptus* species within the Canberra region, no study has examined potential differential host utilisation between *P. atomaria* individuals collected from other regions across its wide distribution. Consequently, there remains a lack of information for this species regarding the potential of either differential host utilisation through pleiotropic effect on host-related genes, or the presence of host races adapted to sympatric eucalypt species.

Population structuring in *P. atomaria* is evident across its range, with geographically isolated populations having revealed differential haplotype composition (Schutze *et al*., 2006). The same study, however, found no evidence for strict haplotype / host-plant association, therefore indicating an absence of host races. While this conclusion

remains sound based on genetic evidence, the COI gene used by Schutze *et al.* (2006) is a potentially unlinked neutral marker and may have failed to reveal local host adaptation should it exist. It therefore remains possible that even though genetic data revealed no evidence of host races, local adaptation to host plants may yet have occurred in *P. atomaria* populations.

Furthermore, adult body size of *P. atomaria* over a latitudinal gradient had previously been found to conform to a converse Bergman cline (*i.e.,* smaller beetles at higher latitudes), and this cline was found to be under genetic control and the likely result of local adaptation to season length rather than phenotypic plasticity (Schutze & Clarke, 2008). This finding supports the analysis of Schutze *et al.* (2006), in that genetic variation between northern and southern populations of *P. atomaria* exists, but it goes further by demonstrating how this variation is not simply neutral, but does have the potential to affect physiological traits within the species. Such variation may extend to differential host plant use as outlined above.

We have therefore chosen to continue our earlier studies of *P. atomaria* to examine the influence of different eucalypt hosts on larval development of two populations (northern and southern) of *P. atomaria*. We aim to test for potential differential larval development between populations and to examine the possibility of either (i) local adaptation to host plant or (ii) pleiotropic effects altering developmental characteristics as influenced by adaptation to non-host related selective pressures. If physiological response to host – as observed through larval growth characteristics – remains unchanged between beetle populations, then further support would be lent to an absence of host races (and consequently no local adaptation to host species or host-associated pleiotropic effect). However, differential larval development on host species between populations would suggest either the presence of incipient host races or a pleiotropic effect on host plant utilisation.

Materials and methods

To test differential host-plant use between populations of *P. atomaria*, we reared two populations of beetles identified by Schutze *et al.* (2006) as having low levels of gene flow between them (northern population, Lowmead, central Queensland; and southern population, Canberra, A.C.T.), on two species of host eucalypt chosen for their distributional characteristics (*E. cloeziana*, sympatric with northern *P. atomaria*

population; and *E. pilularis*, sympatric with the southern *P. atomaria* population). Measures of larval fitness taken included: survival to pupation, total development time, and pupal weight.

Field collection of adult beetles

We collected adult *P. atomaria* beetles from two source populations: a mixed *Eucalyptus* species forest at Jerrabomberra wetlands in Canberra (35º18'51"S, 149º09'16"E), Australian Capital Territory (A.C.T.) (southern beetles), and from a forestry plantation site (*E. grandis X E. camaldulensis* hybrids) at Lowmead (24º29'22"S, 151º42'14"E), central Queensland (northern beetles). These locations were selected as they contained abundant numbers of beetles and it was from each of these sites that previous studies revealed disparate population genetic structure (Schutze et al., 2006) and adult body size (Schutze & Clarke, 2008). A minimum of 50 adults were collected from each locality and maintained at ambient temperature on *E. tereticornis* foliage in outdoor cultures in Brisbane, south-east Queensland (27º27'S, 152º58'E) for the duration of the trial.

Host plants

Eucalyptus cloeziana and *E. pilularis* were the study host plants chosen to measure larval development. Each host plant possesses a different natural distribution (Fig 1): *E. pilularis* is a southern eucalypt species sympatric with the southern (Canberra) population of *P. atomaria*, whilst *E. cloeziana* has a restricted northern distribution sympatric with the northern (Lowmead) population of *P. atomaria*.

Eucalyptus cloeziana and *E. pilularis* were obtained from the Queensland Department of Primary Industries & Fisheries and were established as saplings maintained in outdoor pots. Supplemental foliage was required occasionally for both species and was collected from a forestry plantation located at Beerburrum, south-east Queensland (26º58'02"S, 153º03'06"E). Supplemental foliage was fed to all replicates equally to avoid bias.

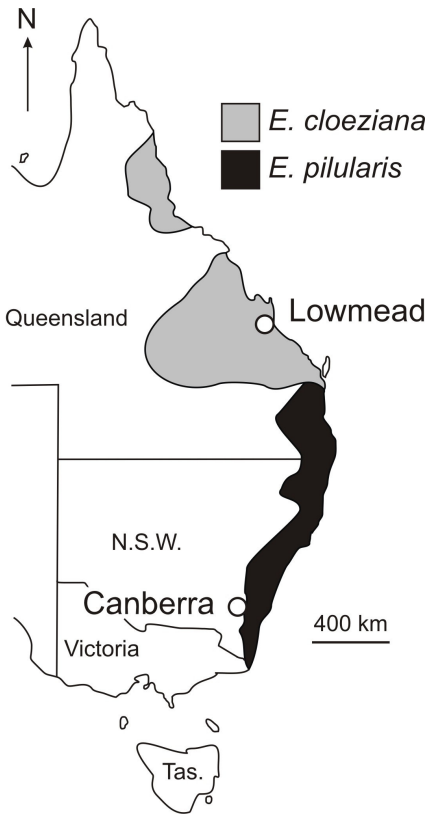


Figure 1. Map showing location of two source populations of *Paropsis atomaria* used in the current study: Lowmead and Canberra; and distributions of *Eucalyptus cloeziana* and *E. pilularis* (data sourced from Australia's Virtual Herbarium: http://www.anbg.gov.au/avh/).

Experiments

Three aspects of larval development were measured for each population on each host plant: survival to pupation, development time (days), and average pupal weight (grams).

Egg batches were removed daily from field cages and maintained at 24 ºC prior to emergence. Neonate larvae were permitted to consume their egg chorion, after which they were segregated into groups of 20 individuals and placed in petri dishes with a supply of newly emerged flush foliage (*E. cloeziana* or *E. pilularis*). Larvae of *P. atomaria* are obligate group feeders in their early instars (Carne 1966a) and must establish on young foliage (Larsson & Ohmart 1988), which dictated the experimental design. Larvae from the same egg batch were separated to control for potential maternal effects. Ten 20-larvae replicates for both Lowmead and Canberra were established for *E. cloeziana* and *E. pilularis* trials. All trials were maintained in constant temperature cabinets at 24 ºC with a 16D: 8L regimen. Foliage was replaced and petri dishes were cleaned of frass daily, taking care not to disturb feeding larvae.

Survival was recorded as the number of individuals surviving to pupation for each trial. To test the possibility that host plant produced a differential survival rate between males and females for each source population, a chi-squared test on total numbers reaching pupation was conducted for both sexes on each host. As no host plant effect was apparent (see Results), males and females were grouped with proportion surviving arcsin square root transformed prior to two-way ANOVA to determine effect of host plant, population, and interaction between the two on survival.

Development time was total number of days from egg eclosion to 50% adult emergence. Results were analysed using two-way ANOVA to determine effects of source population, host plant, and their interaction on development time (days).

Once all individuals had pupated, pupae were sexed based on characters detailed in Reid and Ohmart (1989) and weighed (grams). Males and females are significantly different in weight (data not shown) and were analysed separately with two-way ANOVA to determine the effect on pupal weight by source population, host plant, and their interaction. Where significant interaction effects of host and location were detected by the two-way ANOVA, a sliced ANOVA was used to split the analysis to examine the effects of each factor at individual levels of the other.

Results

Survival to pupation

There was no difference in the ratio of males and females surviving to pupation between host plants for either population (Canberra $\chi^2 = 0.165$, d.f. = 1, $P = 0.685$; Lowmead $\chi^2 = 0.019$, d.f. = 1, $P = 0.890$), hence males and females were combined for each ANOVA.

Two-way ANOVA revealed a significant host plant effect on survival to pupation ($F_{1, 36} = 13.826$, $P = 0.001$), but no effect of source population ($F_{1, 36} = 2.254$, $P = 0.142$) or the interaction between host plant and source population ($F_{1, 36} = 1.764$, $P = 0.193$). Survival to pupation for Canberra larvae was significantly higher on *E. cloeziana* (59 %) compared with *E. pilularis* (21.5 %) ($F_{1, 18} = 26.67$, $P < 0.001$), and whilst Lowmead survival was similarly reduced on *E. pilularis,* it was not

statistically significant (survival to pupation on *E. cloeziana* = 38.5 %; *E. pilularis* = 25 %; $F_{1, 18} = 1.88.$, $P = 0.188$).

Development time

Two-way ANOVA revealed a significant host plant effect on development time ($F_{1, 30} = 16.693$, $P < 0.001$), but development time was not affected by origin of population ($F_{1, 30} = 1.832$, $P = 0.186$) or the interaction between host plant and population origin ($F_{1, 30} = 1.832$, $P = 0.186$). Canberra larvae reared on *E. cloeziana* developed significantly faster than those reared on *E. pilularis* ($F_{1, 17} = 17.725$, $P = 0.001$), whereas there was no significant difference in development time between host plants for Lowmead individuals ($F_{1, 13} = 3.146$, $P = 0.100$) (Fig. 2).

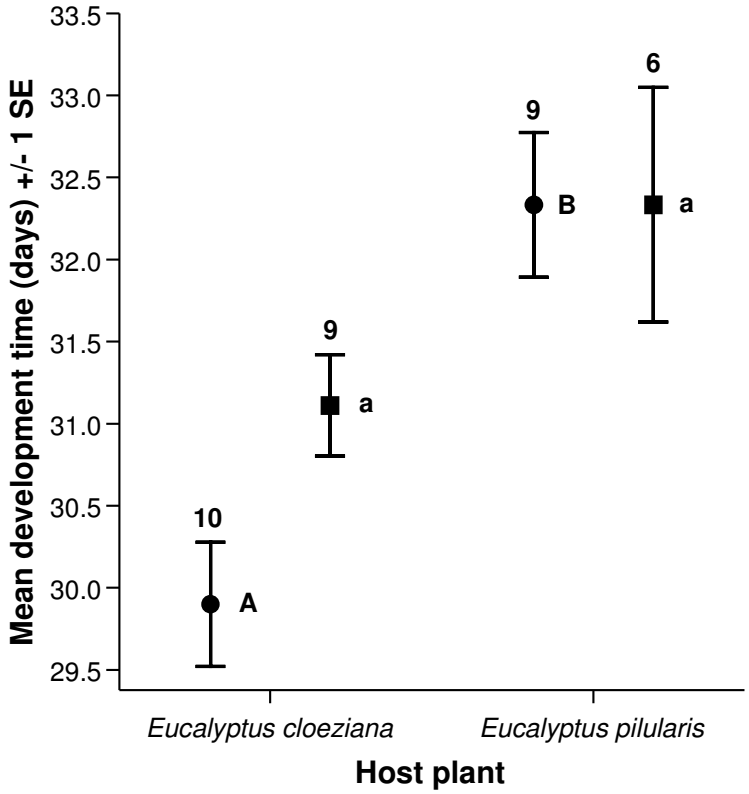


Figure 2. Mean ± 1 S.E. development time (days) of *Paropsis atomaria* from egg eclosion to adult emergence for two populations (Canberra: circle and Lowmead: square) reared on two host plants: *Eucalyptus cloeziana* and *E. pilularis.* Different letters denote significant difference ($P < 0.05$) for between host-plant comparisons calculated within each beetle population. Numbers above plots equal number of replicates.

Pupal weight

There was a significant effect of location, host plant, and their interaction on pupal weight for both males and females (Table 1) and hence both sexes were analysed separately in subsequent analyses.

Lowmead males and females reared on *E. cloeziana* were significantly larger than Lowmead males and females reared on *E. pilularis* (♀ $F_{1, 65} = 8.515$, $P = 0.005$; ♂ $F_{1, 58} = 19.075$, $P < 0.001$), whereas Canberra male and female pupal weights did not differ between host plants (♀ $F_{1, 92} = 0.012$, $P = 0.912$; ♂ $F_{1, 65} = 0.110$, $P = 0.741$) (Fig. 3).

As there was a significant interaction effect of host and location on pupal weight for both males and females (see Table 1), sliced ANOVA revealed a significant difference in pupal weight between Canberra and Lowmead individuals when reared on *E. cloeziana* (♀ $F_{1, 157} = 27.07$, $P < 0.001$; ♂ $F_{1, 123} = 22.30$, $P < 0.001$), but not so for those reared on *E. pilularis* (Fig. 3).

Table 1. Two-way ANOVA of the effects of location, host plant (and interaction) on pupal weights (g) attained by female and male *Paropsis atomaria* from two source populations (Lowmead and Canberra) reared on *Eucalyptus cloeziana* and *E. pilularis*.

	Effect	d.f.	MS	*F*	*P* value
♀	Location	1	0.005	15.096	< 0.001
	Host plant	1	0.002	4.869	0.029
	Location*Host plant	1	0.001	4.221	0.042
	Error	157	< 0.001		
♂	Location	1	0.001	6.819	0.010
	Host plant	1	0.001	5.621	0.019
	Location*Host plant	1	0.001	8.395	0.004
	Error	123	< 0.001		

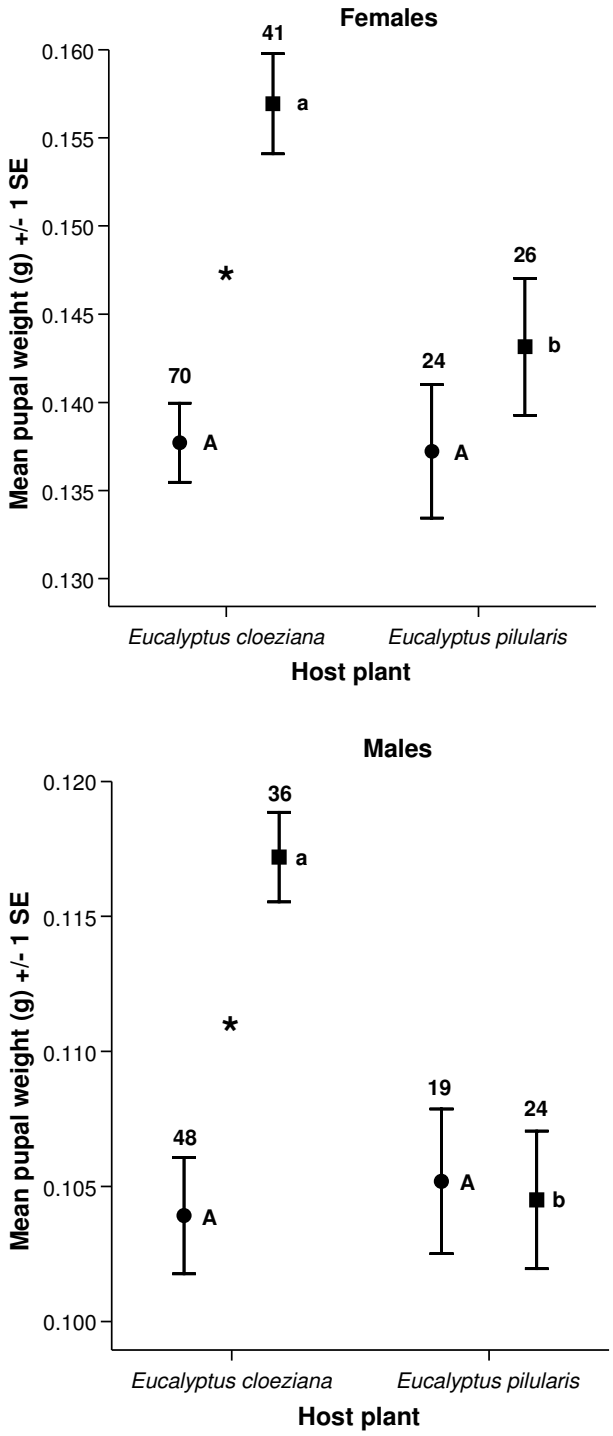


Figure 3. Mean ± 1 S.E. pupal weights (grams) of *Paropsis atomaria* females and males, for two populations (Canberra: circles, and Lowmead: squares) reared on two host plants: *Eucalyptus cloeziana* and *E. pilularis*. Asterisk denotes significant difference ($P < 0.05$) in pupal weight between beetle populations reared on the same host plant. Different letters denote significant difference ($P < 0.05$) in pupal weights within a beetle population between host plants. Numbers above plots represent number of individuals measured.

Discussion

At least two genetically differentiated populations of *P. atomaria* are present along the east coast of Australia (Schutze et al., 2006), with individuals from northern populations (northern NSW to central Qld) inherently larger in body size compared with those from the south (Canberra, ACT) (Schutze & Clarke, 2008). The current study addressed whether there is also differential host plant utilisation between individuals from northern (Lowmead) and southern (Canberra) populations of *P. atomaria*. Further, considering that *P. atomaria* is a largely sedentary species (Carne, 1966a), combined with the fragmented distributions of Australia's eucalypt flora (Brooker and Kleinig, 1983, 1994), we tested the hypothesis that respective northern and southern populations may have adapted to local, sympatric eucalypt species.

Paropsis atomaria is a polyphagous species that feeds on at least 20 recognised hosts within the genus *Eucalyptus* (CABInternational, 2005). *Eucalyptus* is the dominant plant genus in Australia and constitutes 92 % of native forests and woodlands (Morrow, 1976), with most environments containing mixed-species stands belonging to multiple eucalypt subgenera (Noble, 1989). Our trial included only two host plant species that represent a small proportion of the many potential hosts to which wild populations of beetles are exposed. Therefore, it may be argued *a priori*, that beetles from each population would be unlikely to experience strong selective pressures to become specially adapted to the two hosts we used as many other potential host species are present in the field, and in some cases at greater densities. This scenario contrasts with those systems with tight associations between herbivore populations and locally available host plant species. For example, different populations of the chrysomelid *Oreina elongata* Suffrian are naturally exposed to specific hosts and, as a consequence, each population has evolved specialisation relative to its available host (Ballabeni et al., 2003). As populations of *P. atomaria* do not have such strict associations with local host species, it is less likely to evolve host races – a hypothesis supported by previous genetic studies (Schutze et al., 2006).

Nevertheless, we have demonstrated similar host plant utilisation between the two study populations. While the northern host, *E. cloeziana*, produced higher survival rates and shorter development time for larvae from both populations, especially those from Canberra, a significant difference in pupal weight was found between populations. Whilst there was no difference in average pupal weight of southern

individuals reared on either *E. cloeziana* or *E. pilularis*, there was a significant reduction in pupal weight for northern Lowmead individuals reared on the allopatric host, *E. pilularis* (relative to its sympatric host, *E. cloeziana*). As increased pupal weight confers increased potential fecundity (Carne, 1966a), the significant reduction in pupal weight for Lowmead individuals reared on *E. pilularis* may therefore imply reduced fitness of *P. atomaria* on this host, and consequently a diminished suitability of *E. pilularis* compared with *E. cloeziana*.

Alternatively, influences other than direct host plant adaptation may produce the differential host use observed. Differential host use can result from pleiotropic effects, such as adaptation to other environmental influences that affect genes associated with host use. Furthermore, host specialisation on eucalypt species not included in our study may also occur. As previously mentioned, adult body size in *P. atomaria* varies over a latitudinal gradient with northern adults inherently larger than southern adults (Schutze & Clarke, in press). Consequently, pleiotropic effects influencing host plant use may flow on from local adaptation to environmental factors affecting adult body size (such as season length). Due to local adaptation to season length, Canberra individuals may be genetically constrained to reach a maximum body size which can not be exceeded even when presented with a superior host species, whereas inherently larger northern beetles are capable of greater body sizes given such higher quality resources. Examination of developmental response across a wider range of *Eucalyptus* species may reveal tighter host specialisation occurring on other species; however we believe this unlikely due to the large number of *Eucalyptus* species used by *P. atomaria* resulting in a low likelihood of host specialistion (as outlined above).

While we have demonstrated some differential host plant utilisation by geographically isolated populations, we see little evidence for host adapted local populations. Most of the traits measured demonstrated consistent improved performance on *E. cloeziana* over *E. pilularis*, a result consistent with a fixed, species-wide preference for *E. cloeziana* not influenced by local adaptation. We recommend extending trials to include aspects of adult behaviour, especially oviposition and adult feeding preferences. Preliminary unpublished observations by the authors indicate, for example, that whilst *E. cloeziana* appears the better host for larval development compared with *E. pilularis*, it was the least favoured host by

females for oviposition by either population. An inverse relationship between oviposition preference and larval development has been shown previously in *P. atomaria*, albeit for beetles sourced from a single population (Carne, 1966a). Inclusion of further data will contribute to elucidating the mechanisms that explain disparity between populations and can aid in determining whether *P. atomaria* consists of populations adapted to specific host eucalypt species.

Acknowledgements

We thank those who assisted with collection of field material, namely Martin Henery, Katarina Mikac, and Angela Duffy. We thank ITC Plantations for access to eucalypt plantations, Queensland DPI&F for access to eucalypt plantations, saplings, and facilities, and Alexsis Wilson and Helen Nahrung for assistance with lab-work and intellectual input on this project. We acknowledge QUT for financial support to M.K.S. through the QUTPRA.

References

Baker, S. C., Elek, J. A., and Candy, S. G. (2002). Comparison of feeding efficiency, development time and survival of Tasmanian eucalyptus leaf beetle larvae *Chrysophtharta bimaculata* (Olivier) (Coleoptera: Chrysomelidae) on two hosts. *Australian Journal of Entomology* **41**, 174-181.

Ballabeni, P., Gotthard, K., Kayumba, A., and Rahier, M. (2003). Local adaptation and ecological genetics of host-plant specialization in a leaf beetle. *Oikos* **101**, 70-78.

Brooker, M. I. H., and Kleinig, D. (1983). "Field guide to Eucalypts: South-eastern Australia," Inkata Press, Sydney.

Brooker, M. I. H., and Kleinig, D. (1994). "Field Guide to Eucalypts: Northern Australia," Inkata Press, Sydney.

CABInternational (2005). Forestry Compendium. CAB International, Wallingford UK.

Carne, P. B. (1966a). Ecological characteristics of the eucalypt defoliating chrysomelid *Paropsis atomaria* Ol. *Australian Journal of Zoology* **14**, 647-672.

Carne, P. B. (1966b). Growth and food consumption during the larval stages of *Paropsis atomaria* (Coleoptera: Chrysomelidae). *Entomologia Experimentalis et Applicata* **9**, 105-112.

Clarke, A. R., Zalucki, M. P., Madden, J. L., Patel, V. S., and Paterson, S. C. (1997). Local dispersion of the *Eucalyptus* leaf-beetle *Chrysophtharta bimaculata* (Coleoptera: Chrysomelidae), and implications for forest protection. *Journal of Applied Ecology* **34**, 807-816.

Dethier, V. G. (1954). Evolution of feeding preferences in phytophagous insects. *Evolution* **8**, 33-54.

Dres, M., and Mallet, J. (2002). Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society London Series B* **57**, 471-492.

Fujiyama, N., Matsumoto, K., Kobayashi, N., Ohta, Y., and Katakura, H. (2005). Differentiation in the ability to utilize *Pterostyrax hispida* (Ebenales: Styracaceae) among four local populations of the phytophagous ladybird

beetle *Henosepilachna yasutomii* (Coleoptera: Coccinellidae). *Population Ecology* **47**, 91-98.

Gratton, C., and Welter, S. C. (1998). Oviposition prefence and larval performance of *Liriomyza helianthi* (Diptera: Agromyzidae) on normal and novel host plants. *Environmental Entomology* **27**, 926-935.

Harris, M. O., Sandanayaka, M., and Griffin, W. (2001). Oviposition preferences of the Hessian fly and their consequences for the survival and reproductive potential of offspring. *Ecological Entomology* **26**, 473-486.

Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics.* **21**, 243-273.

Joshi, A., and Thompson, J. N. (1995). Trade-offs and the evolution of host specialization. *Evolutionary Ecology* **9**, 82-92.

Larsson, S., and Ohmart, C. P. (1988) Leaf age and larval performance of the leaf beetle *Paropsis atomaria*. *Ecological Entomology* **13**, 19-24.

Mackenzie, A. (1996). A trade-off for host plant utilization in the Black Bean Aphid, *Aphis fabae*. *Evolution* **50**, 155-162.

Moon, D. C., and Stiling, P. (2006). Trade-off in oviposition strategy: choosing poor quality host plants reduces mortality from natural enemies for a salt marsh planthopper. *Ecological Entomology* **31**, 236-241.

Morrow, P. A. (1976). The significance of phytophagous insects in the *Eucalyptus* forests of Australia. *In* "The role of Arthropods in Forest Ecosystems" (W. J. Mattson, ed.), pp. 19-29. Springer-Verlag, New York.

Nahrung, H. F., and Allen, G. R. (2003). Geographic variation, population structure and gene flow between populations of *Chrysophtharta agricola* (Coleoptera: Chrysomelidae), a pest of Australian eucalypt plantations. *Bulletin of Entomological Research* **93**, 137-144.

Noble, I. R. (1989). Ecological traits of the *Eucalyptus* L'Herit. subgenera *Monocalyptus* and *Symphyomyrtus*. *Australian Journal of Botany* **37**, 207-224.

Patterson, K. C., Clarke, A. R., Raymond, C. A., and Zalucki, M. P. (1996). Performance of first instar *Chrysophtharta bimaculata* larvae (Coleoptera: Chrysomelidae) on nine families of *Eucalyptus regnans* (Myrtaceae). *Chemoecology* **7**, 94-106.

Reid, C. A. M., and Ohmart, C. P. (1989). Determination of the sex of pupae of *Paropsis atomaria* Olivier, and the related paropsina (Coleoptera: Chrysomelidae). *Journal of the Australian Entomological Society* **28**, 29-30.

Schutze, M. K., Mather, P. B., and Clarke, A. R. (2006). Species status and population structure of the Australian Eucalyptus pest *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae). *Agricultural and Forest Entomology* **8**, 323-332.

Schutze, M.K., and Clarke, A.R. (2008). Converse Bergmann cline in a *Eucalyptus* herbivore, *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae): phenotypic plasticity or local adaptation? *Global Ecology and Biogeography.* DOI: 10.1111/j.1466-8238.2007.00374.x

Simmul, T. L., and de Little, D. W. (1999). Biology of the Paropsini (Chrysomelidae: Chrysomelinae). In Cox, M. L. (ed.), *Advances in Chrysomelidae Biology 1*, Backhuys Publishers, Leiden, The Netherlands, pp. 463-477.

Steinbauer, M. J., Clarke, A. R., and Madden, J. L. (1998). Oviposition preference of a *Eucalyptus* herbivore and the importance of leaf age on interspecific host choice. *Ecological Entomology* **23**, 201-206.

Verdon, A., Margraf, N., Davison, A. C., Rahier, M., and Naisbit, R. E. (2007). Conserved oviposition preferences in alpine leaf beetle populations despite host shifts and isolation. *Ecological Entomology* **32**, 62-69.

Chapter 6

Thermal requirements, field mortality and population phenology modelling of *Paropsis atomaria* Olivier, an emergent pest in subtropical hardwood plantations

This chapter has been published as:

Nahrung, H.F., Schutze, M.K., Clarke, A.R., Duffy, M.P., Dunlop, E.A. and Lawson, S.A. (2008) Thermal requirements, field mortality and population phenology modelling of *Paropsis atomaria* Olivier, an emergent pest in subtropical hardwood plantations. *Forest Ecology and Management*, **255 (8)**, 3515-3523.

Statement of Joint Authorship

Nahrung, H.F.

Contributed towards experimental design, field data collection, and data interpretation. Wrote parts of manuscript, co-ordinated manuscript construction and acted as corresponding author.

Schutze, M.K.

Designed and developed experimental protocols (developmental temperature data). Carried out field and laboratory work, and analysed data. Contributed to interpretation of data and writing of manuscript.

Clarke, A.R.

A.R. Clarke was the principal supervisor of study design and experimental protocols, and assisted in the interpretation of data and the construction of the manuscript.

Duffy, M.P.

Designed and undertook field trials and contributed to interpretation of results. Contributed to manuscript content.

Dunlop, E.A.

Implemented field and laboratory data into the construction of DYMEX model.

Lawson, S.A.

Contributed to experimental design, interpretation, and manuscript construction.

Abstract

Paropsis atomaria is a recently emerged pest of eucalypt plantations in subtropical Australia. Its broad host range of at least 20 eucalypt species and wide geographical distribution provides it the potential to become a serious forestry pest both within Australia and, if accidentally introduced, overseas. Although populations of *P. atomaria* are genetically similar throughout its range, population dynamics differ between regions. Here we determine temperature-dependent developmental requirements using beetles sourced from temperate and subtropical zones by calculating lower temperature thresholds, temperature-induced mortality, and day-degree requirements. We combine these data with field mortality estimates of immature life stages to produce a cohort-based model, ParopSys, using DYMEX™ that accurately predicts the timing, duration, and relative abundance of life-stages in the field and number of generations in a spring-autumn (September to May) field season. Voltinism was identified as a seasonally plastic trait dependent upon environmental conditions, with two generations observed and predicted in the Australian Capital Territory, and up to four in Queensland. Lower temperature thresholds for development ranged between 4 and 9 °C, and overall development rates did not differ according to beetle origin. Total immature development time (egg – adult) was approximately 769.2 ± s.e. 127.8 DD above a lower temperature threshold of 6.4 ± s.e. 2.6 °C. ParopSys provides a basic tool enabling forest managers to use the number of generations and seasonal fluctuations in abundance of damaging lifestages to estimate the pest risk of *P. atomaria* prior to plantation establishment, and predict the occurrence and duration of damaging lifestages in the field. Additionally, by using local climatic data the pest potential of *P. atomaria* can be estimated to predict the risk of it establishing if accidentally introduced overseas. Improvements to ParopSys' capability and complexity can be made as more biological data become available.

Keywords: eucalypt, DYMEX™, voltinism, seasonal plasticity

1. Introduction

Commercial hardwood production forests are a recent initiative in subtropical Australia, with large-scale eucalypt planting recently exceeding 90 000 ha (Parsons et al., 2006). The concomitant emergence of insect pests associated with plantations, and the growth and economic losses they cause, are among the most serious problems faced by plantation managers (Ohmart, 1990). For example, paropsine chrysomelid beetles cause significant defoliation that can affect the growth rate, height, volume, and possibly pulpwood quality of trees (Candy et al., 1992; Elek, 1997; Elliott *et al*., 1998), and are major pests in the commercial eucalypt-growing regions of Australia (de Little,1989; Simmul & deLittle, 1999), South Africa (Tribe, 2000) and New Zealand (Withers, 2001).

Paropsis atomaria Olivier (Coleoptera: Chrysomelidae) is one such paropsine pest of eucalypts. This species has four larval instars and long-lived adults that all feed on the new growth of trees, removing apical leaves and resulting in a characteristic broom-topped appearance to trees (Cumpston, 1939; Carne, 1966a). *Paropsis atomaria* is the most abundant paropsine beetle in plantations of *Eucalyptus cloeziana* (F. Muell.) (Nahrung, 2006), and other eucalypt species (Lawson, pers. obs.), and as such poses a risk to hardwood productivity in New South Wales (NSW) and Queensland (Qld) (Stone, 1993; Lawson & King, 2002; Schutze et al., 2006). Although initially not considered a major problem in commercial eucalypt plantations (see Wylie and Peters, 1993; Elliott et al., 1998; Strauss, 2001), *P. atomaria* is now a documented pest of *E. grandis* (Hill ex Maiden), *E. cloeziana* and *E. pilularis* Smith in Qld and NSW, and of *E. camaldulensis* Dehnh.*, E. dunnii* Maiden and *E. pilularis* Smith in NSW (Simmul & de Little 1999), and is associated with *Corymbia citriodora* subsp. *variegata* (F. Muell.) A.R. Bean & M.W. McDonald in Queensland (Nahrung, 2006), and several eucalypt species in Victoria (Collett, 2001) and South Australia (Phillips, 1996). Its pest potential is further evidenced by its specific inclusion as a containment hazard in the United States (Eisler, 1999; Kliejunas et al., 2003) and as a regulated pest in New Zealand for eucalypt-associated imports from Australia (MAF, 2003).

Paropsis atomaria represents a single genetic species (Schutze et al., 2006) throughout its geographical distribution from South Australia and Victoria to northern Qld, and it has a broad host range of around 20 eucalypt species

(CABInternational, 2005). Published research on *P. atomaria* originates predominantly from the Australian Capital Territory (ACT), and although we can now reliably use these data in relation to subtropical populations because populations are genetically similar (Schutze et al., 2006), climatic variation between regions may result in significant differences in population dynamics. For example, in the ACT, *P. atomaria* is bivoltine (Carne, 1966a), but in south-east Qld (SEQ) it can undergo up to four generations each year (Nahrung, 2006; Duffy, 2007).

Understanding the life history and population dynamics of pests is paramount to achieving their long-term management (Cox, 1994; Nylin, 2001), while seasonal predictability of the appearance and duration of susceptible life-stages is essential for effective application of control measures. Further, the requirements for accurate forest health reporting (Stone & Coops, 2001), certification of forests for sustainability (Stone & Coops, 2001; Govender, 2002), and the contentiousness of pesticide use (Jenkin & Tomkins, 2006), mean that effective targeted management strategies are important. Here we present underpinning research and a population phenology model, ParopSys, to help deliver such targeted management for *P. atomaria*.

Laboratory trials were used to determine thermal requirements of immature stages of *P. atomaria* and these results were integrated with estimations of field mortality through the DYMEX^{TM} modelling programme (Maywald et al. 2004), which has been used to produce predictive models for other hardwood forestry pests, including gumleaf skeletoniser (Farr 2002) and autumn gum moth (Steinbauer et al. 2004). Lower temperature thresholds, development rates, and mortality were calculated using beetles originating from temperate and subtropical regions of Australia. Phenological sampling (not used in model construction) was conducted to assess the model's ability to predict voltinism, lifestage peaks and durations.

2. Materials and Methods

2.1. Thermal requirements and thresholds for immature P. atomaria *lifestages*

Although Carne (1966a) reported development rates of an ACT population of *P. atomaria* at 5 – 6 constant temperatures (7.2 - 29.4 °C), we conducted our own development trials for this study. We considered that inherent inaccuracies in reading from the development time curves presented and subjectively determining the linear portion of Carne's results may cumulatively render DD estimates unreliable. Further, the question of local adaptation and isolation-by-distance (see Schutze et al., 2006) and differences in voltinism (Nahrung, 2006) between temperate and subtropical populations may also mean that results from the ACT do not apply to subtropical populations. We therefore conducted a new series of constant temperature development trials using *P. atomaria* collected from the ACT and Queensland. These experiments also formed part of a larger study (see Schutze & Clarke, in press) examining the species status, causes of intraspecific body size variation, and host plant utilisation of *P. atomaria* throughout its geographical range.

Paropsis atomaria were collected from two field sites (ACT (Canberra) 35 º18'51"S, 149 º09'16"E and Qld (Lowmead) 24º29'22"S, 151 º42'14"E) in December 2004 and January 2005, and 50-100 beetles from each site were maintained in separate outdoor colonies on *E. tereticornis* Smith foliage.

Egg batches were collected daily from rearing colonies, placed in Petri dishes (one egg batch per dish), and maintained at one of four trial temperatures: 16 ºC, 20 ºC, 24 ºC and 27 ºC. Between nine and thirteen replicate egg batches were used for each temperature/population origin treatment, with egg development time recorded as the number of days from egg batch laying to larval eclosion.

For larval development trials, larvae hatched from egg batches in each colony were divided between temperature treatments to control for possible maternal effects. Twenty neonate larvae (which had been allowed to feed on their egg chorion) were placed in each replicate container (Petri dish: 10 – 13 replicates per population), together with foliage and moistened filter paper. Temperatures used were the same as for the egg development trials. Larvae were supplied daily with fresh *E. pilularis* leaves taken from potted or plantation trees. To control for possible diet effects, on any one day all leaves supplied to larvae came from one source, with individual shoots randomised before being placed in rearing containers.

Replicates were checked daily and instar changes noted: instar duration was calculated based on when 50% of surviving individuals had moulted into the next stage. Once greater than 50% of larvae in any one replicate reached Liii, all larvae were transferred to larger containers for the remainder of larval development. When individuals reached the pre-pupal stage (characterised by cessation of activity and longitudinal compression, see Cumpston, 1939; Carne, 1966a), they were removed from rearing containers and placed in clean Petri dishes until adult eclosion. Between 10 and 13 replicates were conducted for each population (ACT and Qld) and treatment temperature, but not all replicates survived through to adult eclosion, especially Qld individuals reared at 27 ºC (due to increased mortality during development).

Six immature developmental stages were used in DD and T_0 calculations (egg, Li, Lii, Liii, Liv, pre-pupa+pupa), and development time for each stage was considered as the number of days until 50% of the surviving cohort reached the subsequent stage. Pre-pupal and pupal stages were combined at the outset because they are non-feeding, difficult to sample in the field, and ecologically inactive. Data were analysed using mean development rate (the reciprocal of development time) for each developmental stage for each treatment temperature. A linear regression model was fitted to the development rate for each of the six immature life stages described above, yielding for each an equation in the form $y = a + bx$, where y is the rate of development (1/days), x is temperature, a is the intercept and b is the slope. An Analysis of Covariance (ANCOVA) was conducted for each developmental stage to determine whether development rate differed between population origin. Total immature development time did not differ between ACT and Qld populations except at 16 ºC (Schutze & Clarke, in press); nor was there any difference in development rate for each immature stage separately (see Results) so mean development data for each site were pooled for DD and T_0 calculations. Because early instars (Li and Lii) are difficult to differentiate in the field (Duffy, 2007), they were combined into an additional developmental stage to enable model development and validation, and permit application of field-based mortality estimates. The lower temperature thresholds (T_0) for development were estimated by solving the regression equation for development rate = 0 (x-intercept, ie the temperature below which no development occurs), and the number of DD required for each life stage was

estimated by 1/b for each immature life stage (as in Nahrung et al. 2004). Standard errors for T_0 and DD estimates were calculated using the methods of Campbell et al. (1974).

2.2. Mortality of immature life stages

2.2.1. Laboratory estimates Using data from the development rate experiments outlined above, mortality was compared between beetle origin (ACT and Qld) and treatment temperature, following arcsine-square-root transformation of mortality rates, using a two-way ANOVA, with post-hoc differences between temperature treatments identified using Fishers LSD test. Stage-specific mortality as a function of temperature was also determined, and compared using a two-way ANOVA (temperature*development stage). Overall laboratory egg-Li mortality data were estimated using the average hatch rate of unparasitised field-collected egg batches reported by Duffy (2007) and Duffy et al. (in press).

2.2.2. Field estimates To compare laboratory mortality estimates and mortality in the presence of natural enemies (see Nahrung et al., in press), field surveys counting the number of eggs, early instar larvae (Li+Lii), Liii, and Liv were conducted at two-weekly intervals between September 2004 and April 2005 at two *E. cloeziana* plantation sites as follows: *Site I* 26°04′30.72″S 152°44′8.88″E Mean average daily temperature was 22.48 ± 0.19 °C, maximum mean 28.75 °C and minimum mean 13.75 °C; and *Site II* 26°11′20.4″S 152°29′40.2″E Mean average daily temperature was 22.66 ± 0.20 °C, maximum mean 29.5 °C and minimum mean 13.75 °C.

Eight sections across each plantation were representatively selected on each census date (different sections and trees each time), and three branches from each of six trees within each section were visually searched for *P. atomaria* lifestages. The number of egg batches and larvae of each instar on these 144 branches was counted and used to determine the average number of every lifestage present per branch throughout the field season. The population difference between egg and final instar larvae was calculated to estimate overall field mortality rates of immature stages. Mortality between each developmental stage was estimated using the total of all life-stages recorded during the season at each site. Proportional mortality was calculated using the difference between the number of individuals in successional stages, and between egg and final instar larvae for overall immature mortality. An average egg

batch size of 76 eggs per batch (Duffy, 2007) was used to estimate subsequent mortality rates.

2.3. Population modelling

2.3.1. Model description and overview

The model, ParopSys, describes the life cycle processes and population dynamics of *P. atomaria* in relation to climate. ParopSys was created using DYMEX™ V2 (Maywald et al., 2004). The life cycle processes for ParopSys were modelled on a daily time step for 236 days, representing the phase of the *P. atomaria* life cycle when the beetles are active on foliage (i.e. flying, feeding and mating) during the months of September to May (Duffy, 2007). The first time step (Day 1) begins on the 21st of September (when beetles were first observed in the field) and concludes on the 15th May (after which no adults were observed in the field, Duffy, 2007). Immigration and emigration are not explicitly considered, as they are assumed to be equal with no net effect on the numbers of eggs.

ParopSys identifies eight discrete life stages in the *P. atomaria* life cycle: egg, early-instar larvae (1st and 2nd instar), 3rd instar larvae, 4th instar larvae, pupae (pre-pupae and pupae), pre-reproductive adults, overwintering adults, and sexually mature adults (reproductive) (Figure 1). A series of functions describe the lifecycle processes, including development and mortality rates for each life stage, as well as the transfer of individuals from one life stage to the next, and adult fecundity and rates of reproduction. Field and laboratory data presented within this paper were used wherever possible to derive life cycle functions. Where this data set was insufficient, data published in Carne (1966a) or unpublished laboratory estimates were used. Relationships of *P. atomaria* to environmental factors other than temperature were not explicitly considered.

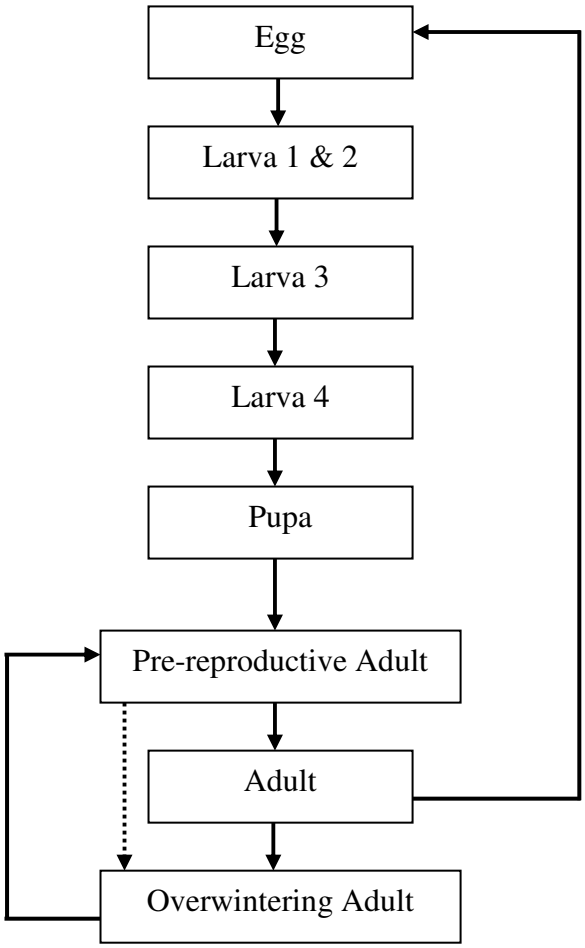


Figure 1: Schematic diagram of the life cycle of *Paropsis atomaria* used in the DYMEX™ model.

2.3.2. Egg and larval development

The thermal thresholds and functions for rates of egg and larval development used in ParopSys are presented in Table 1. T_0 were entered as the threshold temperature, above which a linear relationship between temperature and development time occurred. High temperature-induced reduction in development rate was not considered in this model.

2.3.3. Mortality

Table 2 shows the average estimated mortality of each immature *P. atomaria* life stage experienced under field conditions. However, Table 2 does not quantify field mortality of pupae and adult beetles. Mortality experienced in laboratory trials was

used instead, with a correction for increased mortality that would be experienced in the field by these stages. This was derived by calculating the average mean difference between field and laboratory mortality data for the egg through to 4th larval instar stages, and multiplying the pupal and adult lab mortality rates by this average value. In the model, pupae experience a 0.08 mortality rate on exit, while adult mortality and longevity was modelled using a constant mortality parameter of 0.006 per day (mortality associated with field predation and other biotic and abiotic factors) combined with mortality due to age. Beetles were assumed to live for 85.6 days based on mean longevity of adult beetles (average of Carne, 1966 and Nahrung, unpubl. data). Rates for each possible cause of mortality in the field were not quantified explicitly. Therefore, in ParopSys, *P. atomaria* cohorts experience a combined total mortality on exit from each life stage.

2.3.4. Stage transfer

Stage transfer of egg and larval stages in ParopSys was determined by analysing the relationship between accumulated degree days and the proportion of individuals developing into each life stage at each temperature. The resulting pattern of transfer is predominantly linear (Maywald, et al. 2004; our results not shown) and hence ParopSys uses a linear-above-threshold transfer function to determine transfer rate (i.e. the daily proportion of individuals in a cohort moving into a new stage). This function results in a spread of individuals transferring between stages whereby transfer commences at the lower heat threshold (accumulated degree days) and is completed at the upper heat threshold. Prepupal and pupal stages were considered together for the purposes of development rate estimation and therefore also in the model. As with the larval stages, development and transfer from the fourth larval instar used a linear above threshold function (Table 1).

2.3.5. Pre-reproductive adult development and fecundity

Development rates for pre-reproductive adult beetles (pre-oviposition period) were derived from Carne (1966a). Newly-emerged adults undergo a period of maturation before being capable of oviposition, modelled as a linear above threshold function in the model (development rate = 0.0018, T_0 = 2.7 °C). Potential reproductive capacity of 640 eggs per female (Carne, 1966a) was used to describe fecundity. Because DYMEX^{TM} does not specifically model sex ratios, a mean fecundity of 320 eggs per beetle was applied in the model based on the observed 1:1 operational sex ratio of

this beetle (Duffy, 2007). A pulse function was used to describe the temporal distribution of egg-laying, with batches of 32.5 eggs per adult being laid every seven days (Carne, 1966a).

2.3.6. Overwintering adults

Carne (1966a) reported that *P. atomaria* overwintering in ACT populations is triggered by daylength and terminated in response to temperature, but he did not provide specific data that we could use in our model. Furthermore, the translation to SEQ populations may not be accurate. Arbitrary days of the year were therefore used in ParopSys to initiate (27th April) and terminate (21st September) overwintering. These dates coincide with changes in beetle activity observed in the field (Duffy, 2007). Adults are the only lifestage that overwinter, and the model assumes that all pre-reproductive individuals present will overwinter between the 27th of April and the 21st September, with half (Nahrung, unpubl. data) surviving to reproduce. In the model lifecycle, surviving overwintered adults resume activity as pre-reproductive adults and thus undergo a pre-oviposition period before eggs are produced (Carne, 1966a) (Figure 1).

2.3.7. Meteorological Data

Meteorological data used in ParopSys were obtained from the Silo Data drill website (http://www.nrw.qld.gov.au/silo/datadrill/). This is spatially interpolated data and may not equate exactly with localised conditions. A circadian temperature model was used to drive temperature related functions in ParopSys. This enables hourly calculations of the average temperature to be derived, which are based on the interpolation of the daily maximum and minimum temperatures using a composite sine and exponential function.

2.3.8. Model initialization

For all sites, the model was run for the period from the 21st September 2005 to the 15th May 2006. It was assumed that only adults survive over winter, so the lifecycle module was initialised with 0.5 pre-reproductive adults per day for ten days from the start date. Pre-reproductive adults were used to initialise the model instead of reproductive adults because overwintering adults need to feed for a period of time before reproduction (Carne, 1966; see above).

2.4. Field validation

2.4.1. South East Queensland

To provide data to validate ParopSys, a third plantation, Site III, 26°05′97.2″S 152°43′7.54″E, was sampled during the 2005/2006 season. Mean average daily temperature was 23.00 ± 0.19°C, with a maximum mean of 29.75°C and minimum mean of 14.25°C. Samples as in 2.2.2 were taken every two weeks between October 2005 and April 2006 to provide phenological data that were then used to check the accuracy of the model in predicting the onset, duration and peaks of each developmental stage in the field, and to predict the number of beetle generations.

2.4.2. Temperate vs. subtropical conditions

To test the model's validity over a range of environmental conditions that occur in the extremely wide natural distribution of *P. atomaria* and for the populations where developmental data were compared in this study, the model was run over the same time period (September 2005 to May 2006) using climatic data for Canberra (temperate south eastern Australia) and Lowmead (subtropical central Qld).

3. Results

3.1. Thermal requirements and thresholds for immature P. atomaria *lifestages*

Development rates did not differ according to source of beetle origin for any developmental stage (ANCOVA, $F_{1,5} = 0 – 0.62$, $P = 0.47 – 0.99$), suggesting that the differences in voltinism reported between them (compare Carne, 1966, Nahrung, 2006) is probably a seasonally plastic trait dependent upon field conditions. Data from ACT and Qld populations were therefore combined to produce developmental thresholds (T_0) and DD requirements for ParopSys (Table 1).

Table 1. Developmental thresholds (T_0), thermal requirements (DD) and proportion of development time for immature lifestages of *Paropsis atomaria.*

Immature stage	**Regression equation***	**R^2 P-value**	**T_0 ± s.e. (°C)**	**DD ± s.e.**	**Proportion of immature life cycle****
Egg	$y = 0.008x - 0.0713$	0.98 <0.001	8.9 ± 0.7	125.0 ± 7.0	0.20
Li	$y = 0.0115x - 0.0607$	0.887 <0.001	5.3 ± 2.4	87.0 ± 12.7	
Lii	$y = 0.0156x - 0.0862$	0.884 <0.001	5.6 ± 2.4	64.1 ± 9.5	
Li+Lii	$y = 0.0066x - 0.0358$	0.887 <0.001	5.4 ± 2.9	166.7 ± 26.8	0.21
Liii	$y = 0.0145x - 0.0876$	0.927 <0.001	6.0 ± 1.8	69.0 ± 7.9	0.09
Liv	$y = 0.0037x - 0.0147$	0.703 0.01	4.0 ± 6.9	270.3 ± 72.2	0.11
pp+p	$y = 0.0051x - 0.041$	0.964 <0.001	8.0 ± 1.1	196.1 ± 15.5	0.40

*Temperature range 16 – 27 °C. Linear regression model $y = a + bx$ where y is the rate of development (1/days), x is temperature, a is the intercept and b is the slope.
** at an average spring/summer temperature of 23 °C

Total immature development time (egg – adult) was approximately 769.2 ± s.e. 127.8 DD above T_0 6.4 ± s.e. 2.6 °C: about 49 days at the average field temperature of 23 °C. As a proportion of total development time under average field conditions, the longest stage durations were for fourth instar larvae and pre-pupae+pupae (Table 1), while the shortest was Lii and Liii.

Overall Li-adult mortality in the laboratory was higher from Qld-sourced *P. atomaria* (2-way ANOVA, $F_{1,72} = 4.4$, $P = 0.04$) with ACT larvae exhibiting greater survival at 20 °C and 27 °C than those from Qld; survival at 16 °C and 24 °C did not differ according to origin. Overall Li-adult mortality (Figure 2) increased with temperature (2-way ANOVA, $F_{3,72} = 13.9$, $P < 0.001$), best described ($R^2 = 0.98$) by the polynomial function $y = -0.028x^2 + 0.28x + 0.17$. The interaction between beetle origin and temperature on overall immature mortality was almost significant (2-way ANOVA, $F_{3,72} = 2.8$, $P = 0.05$). Nevertheless, to obtain stage-specific mortality as a function of temperature for ParopSys, we combined data from ACT and Qld populations.

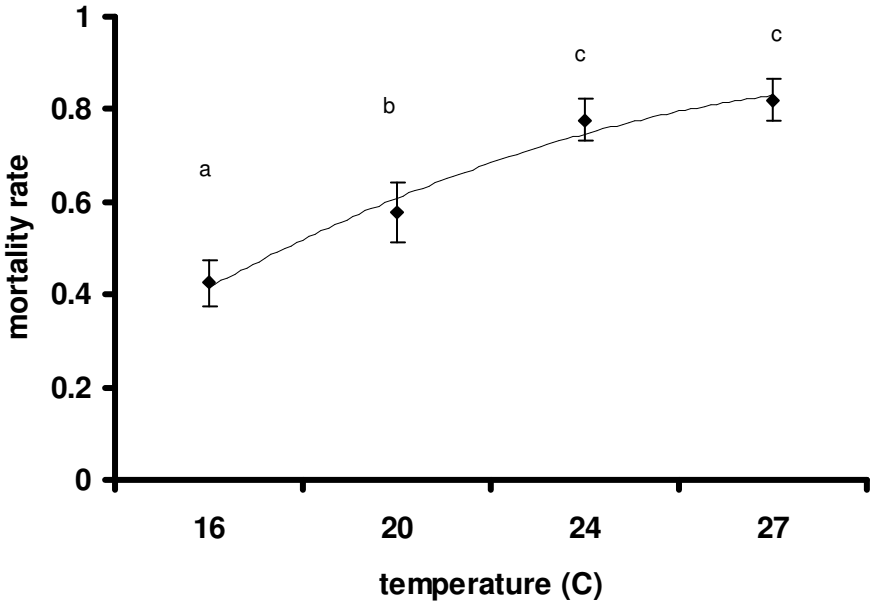


Figure 2: Mean ± s.e. mortality rate between Li and adult *Paropsis atomaria* at four constant temperatures in the laboratory. Different letters denote means that differ significantly.

Stage-specific transfer mortality differed according to developmental stage and temperature (2-way ANOVA, stage: $F_{3,361} = 63.1$, $P < 0.001$; temperature: $F_{4,361} = 14.2$, $P < 0.001$; Figure 3), but with a significant interaction between factors (stage*temperature: $F_{12,361} = 5.03$, $P < 0.001$). Mortality at temperatures 24 °C and above did not differ significantly (Fishers LSD post-hoc test).

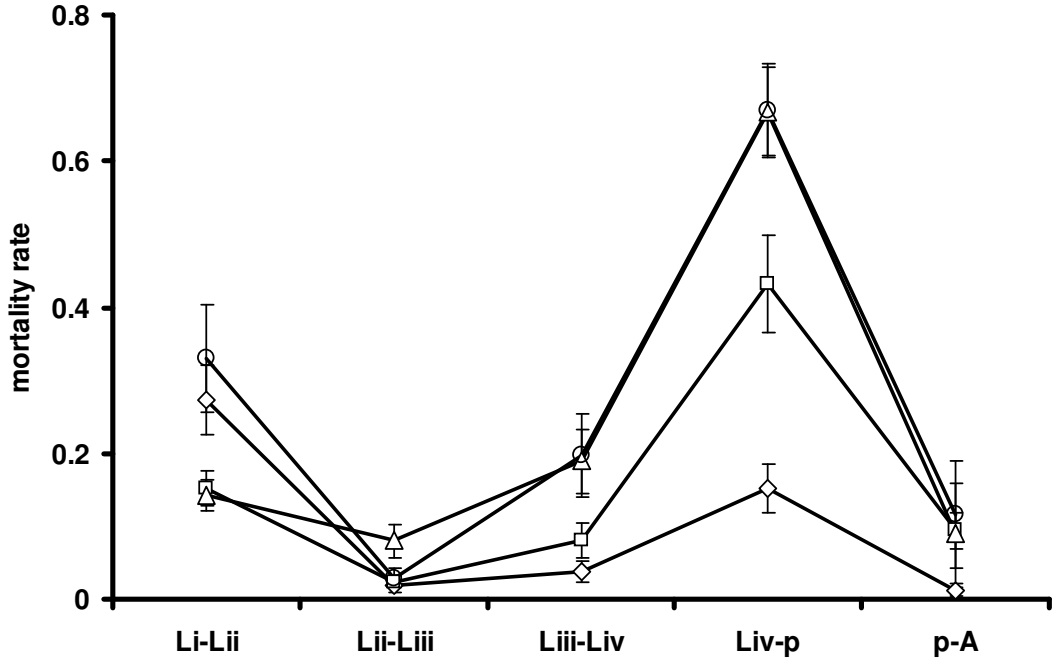


Figure 3: Stage-specific average ± s.e. mortality of immature *Paropsis atomaria* at four temperatures: 16 °C (diamonds), 20 °C (squares), 24 °C (triangles) and 27 °C (circles).

3.2. Field mortality of immature life stages

Less than 8% of eggs survived to become fourth instar larvae (Table 2). The highest mortality occurred between egg and early instar larvae (first and second instars) at Site I, and between early instar larvae and third instar larvae at Sites II and III. These data do not reflect loss from larval parasitoids which generally emerge from fourth instar larvae or pre-pupae, and nor is loss from Liv onwards determined.

Table 2: Estimated mortality (proportion of eggs and larvae lost) at each immature life stage of *Paropsis atomaria* in the field at two sites (Li = first instar, Lii = second instar, Liii = third instar, Liv = fourth instar).

Life Stage	Site I	Site II	Average ± se
Egg to Li+Lii	0.81	0.68	0.75 ± 0.1
Li+Lii to Liii	0.60	0.74	0.67 ± 0.1
Liii to Liv	0.38	0.04	0.21 ± 0.2
all larvae (Li to Liv)	0.75	0.75	0.75 ± 0
Egg - Liv	**0.95**	**0.92**	**0.94 ± 0.02**

3.3. Population modelling and field validation

3.3.1. South East Queensland

To validate the model's predictive capability, we compared field-derived phenological data obtained from Site III for September 2005 to May 2006 and predictions made by ParopSys (Figure 4). The model shows a very good fit with the field data in terms of number of generations, timing of generational peaks, and the relative sizes and shapes of each peak. Three *P. atomaria* generations were observed during the active beetle season at Site III and the model was accurate in predicting the same number of generations. Timing of each peak in the model differed somewhat from the field data, but was mostly within the margin associated with fortnightly collection of field data (Figure 4). Relative sizes of each population peak were very well predicted by the model for all life stages, with the greatest variation occurring in the timing and size of the first generation for both eggs and total larvae. The size and timing of the final, highest population peak was very close to that of the observed field data for all life stages (Figure 4).

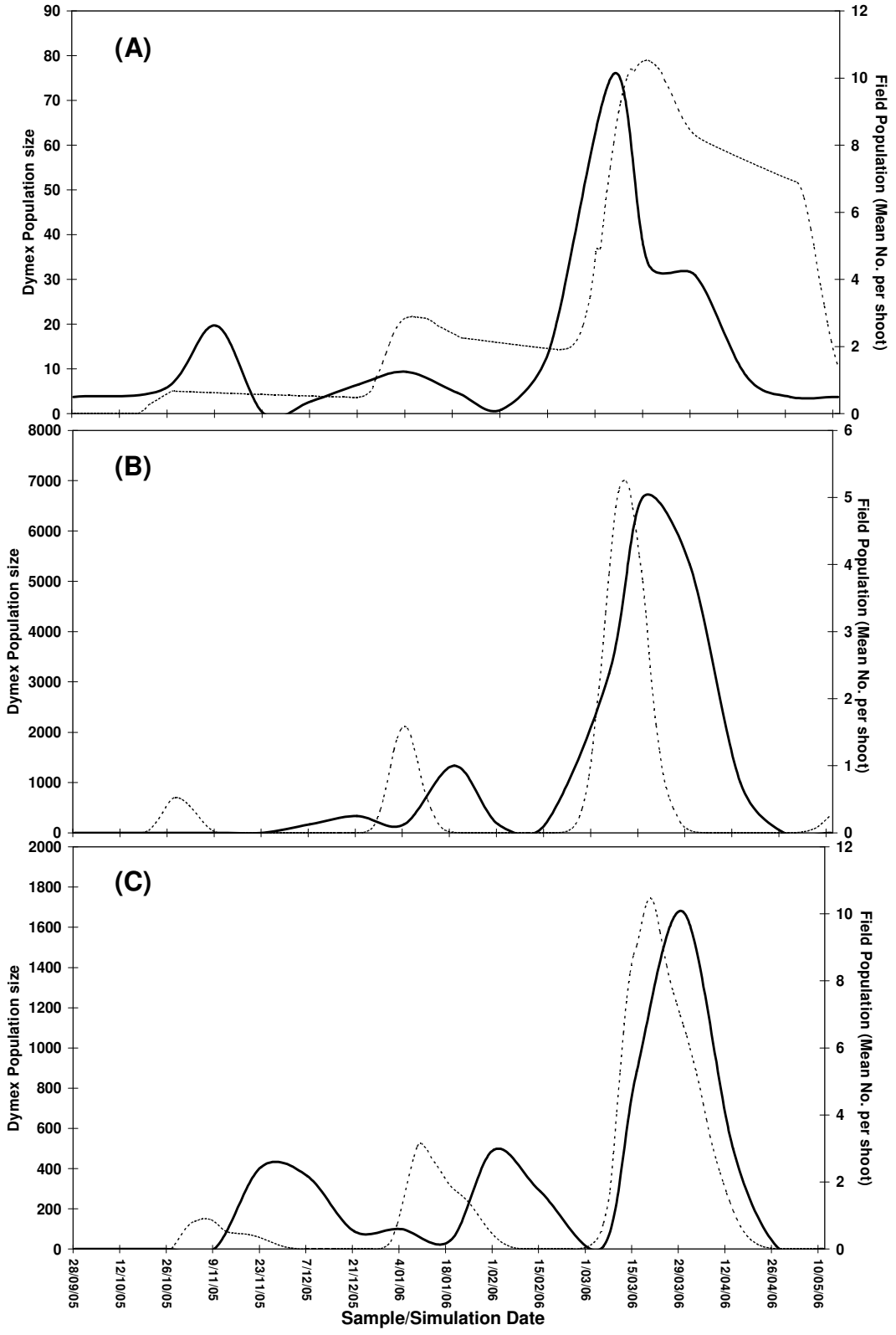


Figure 4: Field validation of ParopSys model between 28 Sept 2005 and 10 May 2006 for Site III, South East Queensland - (A) Adults (B) Eggs (C) Total larvae. Solid lines represent field phenological data collected fortnightly (right y-axis); dotted lines represent DYMEX™ model data using Data Drill meteorological data for Site III (left y-axis). DYMEX™ population numbers are dependent on numbers initialised and so relate relatively to field data (numbers per shoot).

3.3.2. Temperate vs subtropical conditions

Results of the simulations comparing temperate and subtropical populations are shown in Figure 5. ParopSys correctly predicted bivoltinism in Canberra (see Carne, 1966a) and that *P. atomaria* would have had four generations during the season at Lowmead. The model initialized both locations with the same number of pre-reproductive adults but peak adult populations were around ten-fold higher at Lowmead than at Canberra. The model predicts one more generation per year at Lowmead compared to Site III in SEQ.

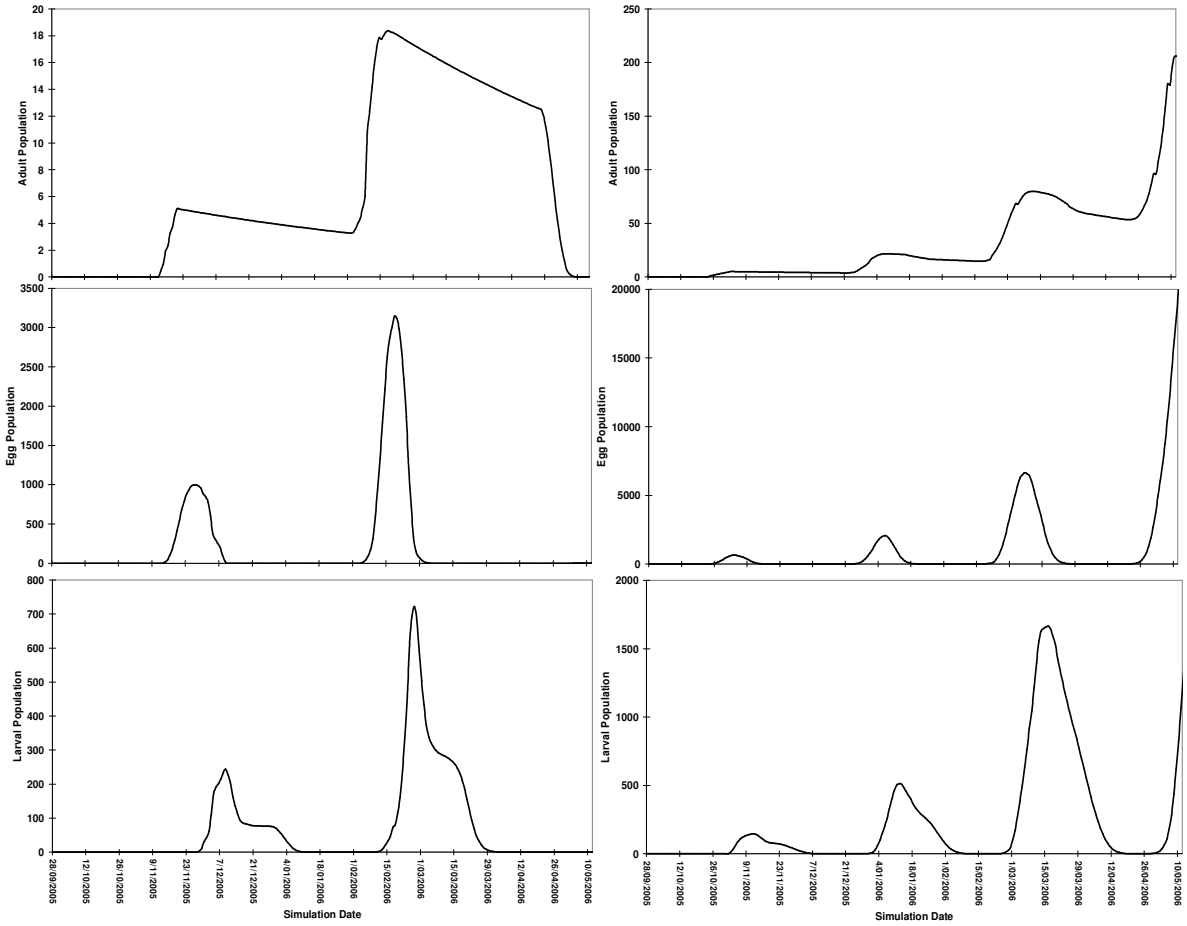


Figure 5: DYMEX^{TM} model predictions for adult, egg and total larval populations of *Paropsis atomaria* for ACT (Canberra) and Queensland (Lowmead) between 28 Sept 2005 and 10 May 2006. DYMEX^{TM} population numbers are dependent on numbers initialised and so relate relatively to field data (numbers per shoot).

4. Discussion

4.1. Development and mortality

Our temperature-development results coincide with those originally calculated by Carne (1966a) for ACT beetles, and they are also consistent across populations sourced from within relative extremes of *P. atomaria*'s geographical distribution. Such low variation in development rates between populations over spatial and temporal scales supports the finding that *P. atomaria* is genetically similar throughout its range (Schutze et al., 2006). Therefore, the difference in the number of *P. atomaria* generations observed between regions suggests that voltinism is a seasonally plastic trait influenced by environmental conditions such as temperature. This is supported by the high degree of accuracy provided by our model in which temperature was the only environmental variable included. We do, however, consider other factors – especially photoperiod and host plant quality – contribute towards determining voltinism in *P. atomaria* (see Carne, 1966a).

Photoperiod is an important factor that can indirectly influence voltinism through its role as a trigger in diapause initiation and termination. Photoperiod is considered the most influential and seasonally reliable diapause cue in insects, while temperature is considered the second most important environmental regulator (Tauber & Tauber, 1976, Tauber et al., 1986). In the paropsine *Chrysophtharta agricola* (Chapuis) (Coleoptera: Chrysomelidae), for example, whilst temperature was secondarily responsible for inducing diapause under controlled conditions, photoperiod was the dominant environmental factor (Nahrung & Allen, 2004b). Further, the interplay between photoperiod and temperature may be critical for the induction of diapause, as demonstrated for the flea beetle, *Argopistes coccinelliformis* Csiki (Coleoptera: Chrysomelidae) (Inoue, 2001). Carne (1966a) reported that newly-emerged *P. atomaria* adults are responsive to photoperiodic cues for reproductive diapause: in the ACT, adults emerging from pupation in February attain reproductive maturity, whereas those that emerge after the first week of March enter diapause without reproductive development. Further work is required to elucidate diapause cues under subtropical conditions: a limitation of ParopSys is our use of an arbitrary date, rather than a specific environmental cue, for simulating the initiation of reproductive diapause. Indeed, altering the start date by one week to 14 September gave a better fit with field data for the timing of peaks (output not shown), suggesting that we

didn't accurately identify the start of the season. Nevertheless, ParopSys generates field-corroborated voltinism accurately for subtropical and temperate regions.

Host plant quality and availability is also likely to contribute to the number of generations produced in a season, whereby if host plant quality is poor, or suitable hosts are not present, oviposition may be delayed until suitable larval food sources appear (see Carne, 1966a). Host plant influence is an important factor for paropsine population dynamics (Ohmart 1991) as for *Chrysophtharta bimaculata* (Olivier), an important paropsine pest of Tasmanian eucalypt plantations (Steinbauer et al., 1998). In this case, host plant quality (amount of flush foliage present) plays a more important role in stimulating oviposition than does host species (Steinbauer et al., 1998). Further, *P. atomaria* populations located merely 20 km apart in SEQ exhibited variable voltinism within the same field season (Nahrung, 2006; Duffy, 2007) – a result unexplainable using temperature or photoperiod data alone. Delayed oviposition by females due to poor early-season host plant quality (*i.e.* less flush foliage) was proposed as the driving cause influencing variable voltinism in that case (Duffy 2007). The potential importance of flush foliage in driving *P. atomaria* populations is not unexpected considering the importance of host plant quality on successful *P. atomaria* larval establishment, with first instars suffering extremely high levels of mortality on older, tougher leaves (Ohmart et al., 1987, Larsson & Ohmart, 1988).

Early instars suffer the greatest mortality in the field despite their gregarious behaviour potentially increasing defence (Sillen-Tulberg 1988) and feeding establishment (Nahrung et al. 2001). Early paropsine instars experience high mortality under laboratory conditions in *C. bimaculata* (Baker et al., 2002) and *C. agricola* (Nahrung et al., 2001), and our overall egg-Liv field mortality estimates for *P. atomaria* were similar to these temperate species (deLittle et al., 1990; Nahrung & Allen, 2004a, respectively). Our laboratory trials revealed relatively low survival rates for fourth instar larvae, exacerbated by higher rearing temperatures, especially above 24 °C. Increased heat stress under experimental conditions may have caused high mortality at this life stage in the laboratory.

4.2. Population modelling

ParopSys is a simple DYMEX™ model based on temperature-dependent development thresholds, field- and laboratory-derived mortality data and general ecological knowledge of the beetle's behaviour. Despite this simplicity, validation against field data showed that ParopSys was accurate in predicting the number, timing, and relative size of *P. atomaria* generations. This varied somewhat with beetle stage: timing of adult population peaks most closely matched that of the field data, with larval peaks showing the greatest discrepancies with timing of egg peak heights intermediate between these (Figure 4). The model also suggested that an early field egg peak may have been missed because field sampling did not commence until 25 October 2005. For all stages, relative peak heights for each generation closely matched that of the field data, with the largest populations occurring from early March (adults) to late March – early April (larvae). Height of this final peak for all stages was approximately three-fold higher than for the earlier two peaks. This agrees with field observations of severe beetle damage at this time.

The model was also robust across climatic zones by correctly predicting bivoltinism at Canberra (ACT), and one extra generation at Lowmead (central Qld) compared to SEQ. Since 2004 forestry plantation companies in this area have reported severe *P. atomaria* defoliation of young *Eucalyptus* taxa in mid- to late-May, coinciding with peak larval and adult populations predicted by ParopSys (Lawson, unpubl. data), although phenological field data for Lowmead are not currently available. The model also predicts that *P. atomaria* is likely to be a more serious pest in the subtropics, with much larger populations compared with temperate areas.

4.3. Future improvements

At its current state of development, ParopSys does not incorporate sophisticated environmental and life cycle parameters that could improve its predictive ability. The interaction between leaf dynamics, herbivory, and rainfall (see e.g. Stone & Bacon, 1995), as well as tree growth rates could be incorporated to provide estimates of stand productivity and losses. Although the good fit of the model with field data suggests that beetle immigration and emigration is absent or equal within seasons, any such movement could potentially be linked to plantation proximity to native vegetation acting as a beetle and natural enemy source or sink (see Strauss, 2001).

Future improvements to ParopSys may also incorporate egg parasitism (Duffy et al., in press; Nahrung et al., in press; Nahrung & Duffy, 2008); basking behaviour of beetle stages that may affect development rates (e.g. Maddox, 1995); differential performance on different host species, and specific factors that determine diapause induction and termination (particularly in relation to daylength). The relationships between beetle size with geographic origin, host plant species/quality, and fecundity also deserves further investigation (see Carne, 1966a; Schutze & Clarke 2008).

Future versions of ParopSys will incorporate 'event scenarios' where the efficacy of number and timing of control measures, such as insecticides, can be evaluated as desktop studies. The model can also be linked with tree growth models to produce cost-benefit analyses of management strategies for *P. atomaria* when impact data become available. Linking the model with GIS software may also enhance its attractiveness to plantation managers. DYMEX^{TM} versions 2 and 3 incorporate a Climex-type mapping function (Maywald et al., 2004) that can be used in regional and global risk modelling for plantations and a climate change function that can be used to predict how the risk of serious damage by *P. atomaria* may change with currently available global warming scenarios.

5. Conclusion

The robustness of ParopSys for predicting numbers of generations, timing of population peaks (in particular the late season population peak, where the most severe and long-term impact on tree growth rates occurs), and size of the final population peak suggest that ParopSys may be helpful to plantation managers in developing risk models for current and future plantations. The model may also assist in predicting year to year fluctuations in *P. atomaria* damage and thus assist managers in planning forest health surveys, monitoring, and management responses.

Acknowledgments

Our sincere thanks to Nikki Sims & Andy Hulthen (both QUT) for laboratory assistance; Jacinta Hodnett, Janet McDonald, Daniel Hancox & Rebeccah Aigner (all Department of Primary Industries and Fisheries) for field assistance. Sincere thanks also to Gunter Maywald (CSIRO Entomology) for assistance with DYMEX^{TM}. MKS was in receipt of a QUT Postgraduate Research Award, and parts of this work were carried out under Australian Research Council Linkage Projects Program

(LP0454856) in conjunction with Forestry Plantations Queensland (formerly DPI-Forestry). We gratefully acknowledge all organisations for their support.

References

Baker, S.C., Elek, J.A., Candy, S.G., 2002. A comparison of feeding efficiency, development time and sutvival of Tasmanian eucalyptus leaf beetle larvae *Chrysophtharta bimaculata* (Olivier) (Coleoptera: Chrysomelidae) on two hosts. Australian Journal of Entomology 41, 174-181.

CABInternational, 2005. Forestry Compendium. Wallingford UK: CAB International.

Campbell, A., Frazer, B.D., Gilbert, N., Gutierrez, A.P., Mackauer, M., 1974. Temperature requirements of some aphids and their parasites. Journal of Applied Ecology 11, 431-438.

Candy, S.G., Elliott, H.J., Bashford, R., Greener, A., 1992. Modelling the impact of defoliation by the leaf beetle, *Chrysophtharta bimaculata* (Coleoptera: Chrysomelidae), on height growth of *Eucalyptus regnans*. Forest Ecology and Management 54, 69-87.

Carne, P.B., 1966a. Ecological characteristics of the eucalypt-defoliating chrysomelid *Paropsis atomaria* Ol. Australian Journal of Zoology 14, 647-672.

Carne, P.B., 1966b. Growth and food consumption during the larval stages of *Paropsis atomaria* (Coleoptera: Chrysomelidae). Ent. exp. & appl. 9, 105-112.

Collett, N., 2001. Insect pests of young eucalypt plantations. Agriculture Notes 6, December 2001. Department of Natural Resources and Environment, Heidelberg.

Cox, M.L., 1994. Diapause in the Chrysomelidae. In Jolivet, PH, Cox ML and Petitpierre, E. (Eds), Novel aspects of the biology of Chrysomelidae. Kluwer Academic Publishers, Dordrecht, pp. 469-502.

Cumpston M., 1939. Observations of the bionomics and morphology of seven species of the tribe Paropsini (Chrysomelidae). Proceedings of the Linnean Society of New South Wales 64, 353-366.

de Little, D.W., Elliott, H.J., Madden, J.L., Bashford, R., 1990. Stage-specific mortality in two field populations of immature *Chrysophtharta bimaculata* (Oliver) (Coleoptera: Chrysomelidae). Journal of the Australian Entomological Society 29, 51-55.

deLittle, D.W., 1989. Paropsine chrysomelid attack on plantations of *Eucalyptus nitens* in Tasmania. New Zealand Journal of Forestry Science 19, 223-227.

Duffy, M.P., Nahrung, H.F., Lawson, S.A., Clarke, A.R., in press. Direct and indirect effects of egg parasitism by *Neopolycystus* Girault sp. (Hymenoptera: Pteromalidae) on *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae). Australian Journal of Entomology.

Duffy, M.P., 2007. Population phenology and natural enemies of *Paropsis atomaria* in South-East Queensland. MSc thesis, School of Natural Resource Sciences, Queensland University of Technology. 100pp.

Eisler, R., 1999. Containment Hazard Reviews Report No. 35 Cumulative index to chemicals and to common and scientific names of species listed in containment hazard reviews 1 through 34. Biological Science Report USGS/BRD/BSR--1999-0002. Pauxtent Wildlife Research Center, Laurel, MD. 80 pp.

Elek, J.A., 1997. Assessing the impact of leaf beetles in eucalypt plantations and exploring options for their management. Tasforests 9, 139-154.

Elliott, H.J., Ohmart, C.P., Wylie, F.R., 1998. Insect Pests of Australian Forests: ecology and management. Melbourne, Inkata Press.

Elliott, H.J., Bashford, R., Greener, A., Candy, S.G., 1992. Integrated pest management of the Tasmanian *Eucalyptus* leaf beetle, *Chrysophtharta bimaculata* (Olivier) (Coleoptera: Chrysomelidae). Forest Ecology and Management 53, 29-38.

Elliott, H.J., Bashford, R., Greener, A., Candy, S. G., 1992. Integrated pest management of the Tasmanian *Eucalyptus* beetle, *Chrysophtharta bimaculata* (Olivier) (Coleoptera: Chrysomelidae). Forest Ecology and Management 53, 29-38.

Farr, J. D., 2002. Biology of the gumleaf skelentoniser, *Uraba lugens* Walker (Lepidoptera: Noctuidae), in the southern jarrah forest of Western Australia. Australian Journal of Entomology 41, 60-69.

Govender, P., 2002. Management of insect pests: have the goalposts changed with certification? Southern African Forestry Journal 195, 39-45.

Hopkins, R. J., Ekbom, B., 1999. The pollen beetle, *Meligethes aeneus*, changes to egg production rate to match host quality. Oecologia 120, 274-278.

Inoue, T., 2001. Life history of the flea beetle, *Argopistes coccinelliformis* Csiki (Coleoptera: Chrysomelidae) VII. Effects of photoperiod and temperature on induction of reproductive diapause in newly emerged adults. Applied Entomology and Zoology 36, 53-58.

Jenkin, B.M., Tomkins, B., 2006. Chemical pesticide usage by Australian plantation industries. FWPRDC Project PN06.4016. Forest and Wood Products Research and Development Corporation, Melbourne.

Kliejunas, J.T., Burdsall, H.H.J., deNitto, G.A., Eglitis, A., Haugen, D.A., Haverty, M.I., Micales, J.A., Tkacz, B.M., Powell, M.R., 2003. Pest risk assessment of the importation into the United States of unprocessed logs and chips of eighteen Eucalypt species from Australia. General Technical Report FPL-GTR-137. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 206 pp.

Larsson, J., Ohmart, C.P., 1988. Leaf age and performance of the leaf beetle *Paropsis atomaria*. Ecological Entomology 13, 19-24.

Lawson, S.A., King, J., 2002. Leaf beetles (Paropsines). Queensland Forest Research Institute, Agency for Food and Fibre Sciences, DPI. Brisbane: DPI Note FOR0027.

Maddox, C.D.A., 1995. Aspects of the biology of *Paropsisterna tigrina* (Chapuis) (Coleoptera: Chrysomelidae) the major pest species of *Melaleuca alernifolia* (Cheel) (Myrtaceae), Masters thesis, Department of Entomology, The University of Queensland.

M.A.F., 2003. Import Health Standard: Sawn wood from all countries. New Zealand Ministry for Agriculture and Forestry, Forest Biosecurity**:** 21 pp.

Maywald, G.F., Sutherst, R.W., Kriticos, D.J., 2004. DYMEX Professional: Modelling Natural Systems, Version 2.0, Hearne Scientific Publishing, Melbourne.

Nahrung, H.F., 2006. Paropsine beetles (Coleoptera: Chrysomelidae) in South-East Queensland hardwood plantations: identifying potential pest species. Australian Forestry 69, 270-274.

Nahrung, H.F., Allen, G.R., 2004a. Population dynamics of the chrysomelid leaf beetle *Chrysophtharta agricola* (Chapuis), a pest of *Eucalyptus nitens* plantations in Tasmania. TasForests 15, 67-84.

Nahrung, H.F., Allen, G.R., 2004b. Overwintering ecology of *Chrysophtharta agricola*: mechanisms of reproductive diapause induction and termination. Australian Journal of Zoology 52, 505-520.

Nahrung**,** H**.**F**.**, Allen**,** G**.** R**.,** Patel**,** V**.** S., 2004. Day-degree development and phenology modelling of the immature stages of *Chrysophtharta agricola*

(Chapuis) (Coleoptera: Chrysomelidae), a pest of eucalypt plantations. Australian Journal of Entomology 43, 177-183.

Nahrung, H.F., Duffy, M.P., 2008. Exploring the fourth trophic level: do hyperparasitoids influence biocontrol of a forestry pest in a native system? The New Zealand Entomologist 31, 6-12.

Nahrung, H.F., Duffy, M.P., Lawson, S.A., Clarke, A.R., in press. Natural enemies of *Paropsis atomaria* (Coleoptera: Chrysomelidae) in South-East Queensland eucalypt plantations. Australian Journal of Entomology.

Nahrung, H.F., Dunstan, P.K., Allen, G.R., 2001. Larval gregariousness and neonate establishment of the eucalypt-feeding beetle *Chrysophtharta agricola* (Coleoptera: Chrysomelidae: Paropsini). Oikos 94, 358-364.

Nylin, S., 2001. Life history perspectives on pest insects: What's the use? Austral Ecology 26, 507-517.

Ohmart, C. P., 1990. Insect pests in intensively-managed eucalypt plantations in Australia: some thoughts on this challenge to a new era in forest management. Australian Forestry 53, 7-12.

Ohmart, C.P., 1991. Role of food quality in the population dynamics of chrysomelid beetles feeding on *Eucalyptus*. Forest Ecology and Management 39, 35-46.

Ohmart, C.P., Thomas, J.R., Stewart, L.G., 1987. Nitrogen, leaf toughness and the population dynamics of *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae) – a hypothesis. Journal of the Australian Entomological Society 26, 203-207.

Parsons ,M., Gavran, M., Davidson, J., 2006. Australia's plantations 2006 National Report. Department of Agriculture, Fisheries and Forestry. 24pp.

Philips, C., 1996. Insects, diseases and deficiencies associated with eucalypts in South Australia. Primary Industries South Australia, Mt Gambier.

Schutze, M.K., Clarke, A.R. (2008). Converse Bergmann cline in a *Eucalyptus* herbivore, *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae): phenotypic plasticity or local adaptation? Global Ecology and Biogeography. DOI: 10.1111/j.1466-8238.2007.00374.x

Schutze, M.K., Mather, P.B., Clarke, A.R., 2006. Species status and population structure of the Australian Eucalyptus pest *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae). Agricultural and Forest Entomology 8, 323-332.

Sillen-Tullberg, B., 1988. Evolution of gregariousness in aposematic butterfly larvae: a phylogenetic analysis. Evolution 42, 293-305.

Simmul, T.L., deLittle, D.W., 1999. Biology of the Paropsini (Chrysomelidae: Chrysomelinae). In Advances in Chrysomelidae Biology. Cox, M. L. (Ed.) Leiden, Backhuys Publishers, pp 463-477.

Steinbauer, M. J., Clarke, A. R., Madden, J. L., 1998. Oviposition preference of a *Eucalyptus* herbivore and the importance of leaf age on interspecific host choice. Ecological Entomology 23, 201-206.

Steinbauer, M. J., Kriticos, D. J., Lukacs, Z., Clarke, A. R., 2004. Modelling a forest lepidopteran: phonological plasticity determines voltinism which influences population dynamics. Forest Ecology and Management 198, 117-131.

Stone, C., 1993. Insect problems of eucalypt plantations in Australia. Australian Forestry 56, 363-369.

Stone, C., Coops, N., 2004. Assessment and monitoring of damage from insects in Australian eucalypt forests and commercial plantations. Australian Journal of Entomology 43, 283.

Stone, C., Bacon, P. E., 1995. Leaf dynamics and insect herbivory in a *Eucalyptus camaldulensis* forest under moisture stress. Australian Journal of Ecology 20, 473-481.

Stone, C., Matsuki, M., Carnegie, A., 2003. Pest and disease assessment in young eucalypt plantations: field manual for using the Crown Damage Index, editor Parsons, M. (Ed.) Canberra, National Forest Inventory, Bureau of Rural Sciences.

Strauss, S.Y., 2001. Benefits and risks of biotic exchange between *Eucalyptus* plantations and native Australian forests. Austral Ecology 26, 447-457.

Tauber, M.J., Tauber, C.A., 1976. Insect seasonality, diapause maintenance, termination and post-diapause development. Annual Review of Entomology 21, 81-107.

Tauber, M. J., Tauber, C.A., Masaki, S., 1986. Seasonal adaptations of insects. Oxford University Press, New York. 411pp.

Tribe, G.D., 2000. Ecology, distribution and natural enemies of the *Eucalyptus*-defoliating beetle *Trachymela tincticollis* (Blackburn) (Chrysomelidae:

Chrysomelini: Paropsina) in southwestern Australia, with reference to its biological control in South Africa. African Entomology 8, 23-45.

Withers, T.M., 2001. Colonisation of eucalypts in New Zealand by Australian insects. Austral Ecology 26, 467-476.

Wylie, F.R., Peters, B.C., 1993. Insect pest problems of eucalypt plantations in Australia. 1. Queensland. Australian Forestry 56, 358-362.

Chapter 7

General Discussion

Thesis summary

In this thesis, different aspects of genetic and phenotypic variation within *Paropsis atomaria* were investigated as an approach to assess if this economically important taxon represented a single genetic species or a complex of undetected cryptic species. Comparisons of work by previous authors had revealed evidence for differences in life history characteristics among individuals sourced from geographically distant populations, including different optimum development temperatures and seasonal phenologies. Also, wide host range suggested that *P. atomaria* was unusual among the Chrysomelidae, a group that predominantly have narrow host ranges. Consequently, as *P. atomaria* is an emergent pest of forestry, it was important to determine if populations from different parts of the natural geographical range, or that use different host plants in the same location, were conspecific. Determining this would therefore permit the use of all historical data accumulated for *P. atomaria*, regardless of from where the data was collected.

Detailed empirical studies were used to investigate underlying population structure of the species, morphological variation between populations, developmental responses with respect to host plant utilisation, and population phenology across the range, to determine if cryptic species were present, and if not, what explained intraspecific variation in *P. atomaria*. First, molecular techniques were used to indicate that *P. atomaria*, as currently defined, is essentially a single gene pool with partial geographical isolation among populations from the northern extent of the range compared with the southern extreme (Chapter 3). Second, while morphometric analysis further supported the single taxon hypothesis (see Appendix 1), variation in body size was present among individuals, with body size correlated with latitude in accordance with a converse Bergmann cline. Some of this variation did have a genetic component (Chapter 4), reinforcing the recognition of some intraspecific genetic differentiation between southern and northern populations. Third, variation in the insect's biotic environment (specifically host plant) also influenced body size differentially between populations (Chapter 5), but that populations did not differ in

their overall performance when reared on two eucalypt species. Finally, experimental data and computer modelling prediction reveal voltinism to be a plastic trait largely mediated by temperature (Chapter 6). Consequently, each line of investigation, whether genetic, morphological, physiological, or developmental, shows no evidence of a cryptic species complex within *P. atomaria*. Furthermore, preliminary mating trials (see Appendix 2) further demonstrated a lack of evidence for different species within the taxon, as individuals from the extent of the range recognised each other as mates under artificial conditions, and hence the results are uninterpretable (see Walter, 2003). Phenotypic variation in body size, developmental characteristics, and life histories is therefore a complex interaction between genotype, and both abiotic (climatic) and biotic (host plant) factors. Specifically, temperature imposed a direct effect on individual body size as explained by the Temperature-Size rule, whereas latitudinal variation in body size is most likely an adaptive response to season length. Moreover, the nature of phenotypic plasticity differed among individuals from different localities, with host plant-related pupal mass variation for northern individuals affected more so than for southern populations, however seasonal plasticity in voltinism was unchanged across the distribution. Therefore, phenotypic differences in *P. atomaria* are intraspecific, and not evidence of a cryptic species complex.

These data show that restriction of gene flow operates in concert with phenotypic differentiation among populations. The temptation can be, therefore, to invoke local adaptation as the chief explanatory mechanism for observed variation across *P. atomaria*'s wide geographical distribution. The data presented here reveal, however, how multiple intimate interactions between the organism and its environment produce a more complex scenario, and that alternative explanations to local adaptation exist. As a consequence, the evidence both for and against local adaptation explaining the observed patterns will be developed below, taking into consideration important factors that could also contribute to the variation reported here.

Local adaptation

Local adaptation can be defined as the pattern of resident genotypes within a population possessing, on average, higher relative fitness within their local habitat compared with genotypes originating from other habitats (Kawecki and Ebert, 2004).

As the habitat within which an individual resides can directly influence the relative fitness of its specific genotype (Falconer, 1952; Via and Lande, 1985), and the process by which genotypes vary between environments is largely, but not exclusively, the result of selective processes operating on different local populations (Mayr, 1942), local genotypes are predicted to perform less well when removed from their natal habitat and placed in a foreign environment. Importantly, however, local adaptation by populations to their immediate environment is not a simple, inevitable process (Walter, 2003). While directional selection or random genetic drift may affect organisms, those individuals are also invariably under strong stabilising selection for other traits, such as the necessity to maintain a specific fertilisation system within a given environment (Paterson, 1993). Thus, local adaptation, if it occurs, should therefore be viewed as a process brought about in response to strong localised selective pressures imposed on a geographically constrained population. Issues pertinent to local adaptation in phytophagous insects, such as *P. atomaria*, are discussed below.

Selective pressures relevant to phytophagous insects

Phytophagous insects are, by definition, ectothermic animals that feed on plants. Consequently, two important factors are immediately apparent to determining variation and potential local adaptation in phytophagous insects, and these are: climate (abiotic) and the host plant (biotic). The increased abundance of phytophagous insects compared with their non-plant feeding relatives is testament to the importance of the variety of plant life in promoting such diversity among plant-feeding insect clades (Jaenike, 1990), while the importance of temperature and relative humidity in affecting insect fitness is also widely acknowledged (Chown and Gaston, 1999).

Host plant specialisation is widespread across phytophagous insect groups, with generalist species often consisting of either locally specialised populations (Fox and Morrow, 1981) or potentially cryptic species complexes (Clarke and Walter, 1995). Further, the relative dietary breadth of a species (from monophagous to polyphagous) is considered, by some, to be important in affecting the relative rate of gene flow among populations – specialist phytophagous insects are hypothesised to show more restricted gene flow compared with generalist species, and hence be more prone to speciation (Futuyma and Moreno, 1988; however see contrary evidence proposed by

Peterson and Denno, 1998). Considering the wide host range, the potential for host specialisation in *P. atomaria* would be predicted by the argument above.

Climatic variables including season length, temperature, humidity, and photoperiod can all impose marked effects on insect behaviour, physiology, and related ecological characteristics (Chown and Gaston, 1999; Chown and Klok, 2003; Inoue, 2001; Sibly and Atkinson, 1994). As a consequence, seasonal climatic fluctuations (both long term predictability and short term local unpredictability) have the potential to impose pressure therefore on regional populations of non-migrating insects to complete development and become reproductively active within a given season, thereby resulting in local adaptation to proximate climatic factors (Blanckenhorn and Fairbairn, 1995). The extensive range of *P. atomaria* over a wide latitudinal gradient therefore implies that local adaptation to seasonal pressures is likely to cause adaptive differentiation.

As previously emphasised, strong directional selective forces are required for significant ecological change to take place within populations. Without biotic and abiotic environmental influences resulting in effective differential fitness between genotypes, there is no impetus for local adaptation to occur, with local adaptation more likely in cases of strong selection against genotypes adapted to alternative environments (Kawecki and Ebert, 2004).

Is there evidence of local adaptation in P. atomaria*?*

Gene flow – One of the principle considerations for determining the potential for local adaptation is the degree of gene flow among populations (Ballabeni et al., 2003), with restricted gene flow considered necessary for local adaptation to occur, as any potential for fixation of locally advantageous traits will generally be swamped by high levels of migration between populations (Kawecki and Ebert, 2004; Peterson and Denno, 1998). Factors affecting gene flow can include: geographic distance between populations, dispersal capability, ecological specialisation, phenological isolation, habitat patchiness, habitat persistence, and the frequency and nature of extinction / re-colonisation events (Hastings, 1983; Peterson and Denno, 1998). For *P. atomaria*, each of these influences may contribute differentially to affecting gene flow between populations, and consequently are of varying significance.

The effect of geographical distance on gene flow is tied intimately with the dispersal capabilities of a species. For those taxa with great dispersal potential over wide geographical distances, the rate of gene flow among populations is large; however sedentary species are likely to exhibit heightened genetic differentiation at a much smaller spatial scales (Peterson and Denno, 1998). *Paropsis atomaria* population structure revealed higher levels of gene flow among northern populations compared with gene flow between northern and southern populations (Chapter 3). This may be due to isolation by distance, as dispersal by *P. atomaria* is considered relatively poor (Carne, 1966). Furthermore, the accuracy of our computer-generated phenology model, ParopSys, provides additional support for poor dispersal within *P. atomaria* and consequently low gene flow between populations (Chapter 6). If, for example, migration rates between populations during a given season were high, we would expect greater stochasticity in the predictions relative to field validation results. Instead, predicted and observed phenologies are tightly correlated, indicating a single cohort of individuals remains at the same location for the duration of the season.

There is little evidence of ecological specialisation affecting gene flow in *P. atomaria*, as shown by a lack of significant population genetic association with host plant species, together with similar host utilisation characteristics for northern and southern populations. Therefore, the effects of geographical distance and dispersal capability are considered the principle determinants of gene flow between populations of *P. atomaria*. Thus, limited gene flow between geographically distant populations does provide the potential for local adaptation to occur.

Genetic maintenance of phenotypic variation – Local adaptation is often considered to be an alternative explanation to phenotypic plasticity (Kawecki and Ebert, 2004). Whilst local adaptation is the fixation within a population of specific genetically determined traits in response to consistent environmental selective pressures, phenotypic plasticity enables individuals to express a range of differential phenotypic traits in direct response to specific environmental conditions (Nylin and Gotthard, 1998; Via et al., 1995). Further, phenotypic plasticity may be favoured in species that reside within heterogeneous environments that experience significant temporal variation (Kawecki and Ebert, 2004; Via and Lande, 1985). As a consequence, local adaptation may be more likely for species in situations with little temporal variation, but greater spatial diversity across habitats.

For *P. atomaria*, trials investigating effect of temperature on body size clearly demonstrated a genetic component to body size that correlated with latitudinal variation in the form of a converse Bergmann cline (Chapter 4). This result is supportive of local adaptation over phenotypic plasticity. Considering the environments within which *P. atomaria* lives, local adaptation to seasonal constraints is considered possible as there is comparatively little temporal variation in a given habitat between years, but greater spatial variation between habitats, particularly with respect to latitude-associated season length.

Affinity for sympatric host plants – Given reduced gene flow and partial isolation among *P. atomaria* populations, local adaptation within a population may theoretically result in increased fitness of individuals to their local food source, as local specialisation to proximate biotic resources, such as host plants, is often considered likely for generalist herbivores (Fox and Morrow, 1981). Furthermore, given strong directional selective pressures, populations may become rapidly adapted to their proximate environment (Carroll et al., 2001; Lee, 2002). The Soapberry Bug, *Jadera haematoloma*, for example, is hypothesised to consist of two recently diverged (~100 generations) races in Florida, U.S.A., one constituting individuals adapted to their original hosts (Balloon Vine, *Cardiospermum corindum*), and another geographically isolated population that adapted rapidly to the introduced southeast Asian Goldenrain Tree, *Koelreuteria elegans* (Carroll and Boyd, 1992; Carroll et al., 2001). Such adaptation was explained as strong directional selection for beak length in order to access seeds within fruit of varying radii; long beaked individuals were found on thicker native fruits, with short beaked individuals on small introduced fruits. Furthermore, there was evidence for evolved trade-offs, with each population performing less well on non-natal hosts during reciprocal rearing experiments on the two host plants (Carroll et al., 2001). Trade-offs, while often considered important during local adaptation are, however, not always apparent – especially in studies of host plant specialisation as phytophagous insects are often shown to perform better on novel hosts compared with natal species (Joshi and Thompson, 1995).

For *P. atomaria*, there is no evidence for local host plant specialisation. No significant association was detected between haplotype diversity and host plant of origin (Chapter 3), and while northern populations reach greater adult body sizes,

with reduced development time, and having higher survival rate on the sympatric host species (*Eucalyptus cloeziana*), this host is also better for the southern population of *P. atomaria* (compared to *E. pilularis*) (see Chapter 5). Indeed there is little reason to consider *P. atomaria* to have become locally specialised on any host plant species, as the influence of host plant species and their respective geographical distributions are unlikely to be an influential selective pressure for *P. atomaria.* While the geographic distributions of different eucalypt host species utilised by *P. atomaria* are fragmented – a condition favouring local host plant specialisation in phytophagous insect species (Fox and Morrow, 1981) – host quality seems less likely to depend on which species is utilised by the insect, but, similarly to other Australian paropsines, more so the quality of foliage present as defined by age, toughness, and nitrogen concentration (Ohmart et al., 1987; Steinbauer et al., 1998). Indeed, *P. atomaria* individuals seem capable of feeding on many eucalypt species due to a capacity for metabolising defensive terpenoids (major secondary compounds contained within *Eucalyptus* foliage) (Ohmart and Larsson, 1989) and as such are capable of feeding on most eucalypt species. Nevertheless, northern beetles are significantly larger than southern individuals when reared on the superior quality host (*E. cloeziana*); a difference not apparent when individuals from both populations are reared on the poorer host, *E. pilularis*. This may be due to the poorer nutritional quality of the *E. pilularis* preventing northern beetles from maximising their pupal weight given a better host (such as *E. cloeziana*). Southern beetles may, however, be incapable of growing larger even when presented with a better quality host, as revealed by their failure to attain a higher pupal mass when reared on the superior host, *E. cloeziana* – a circumstance potentially due to genetic constraints preventing individuals from growing larger and possibly due to local adaptation to season length (Chapter 5). In summary, there seems little evidence to suggest local adaptation to sympatric host plant species in *P. atomaria*.

Summation – For this thesis, I have demonstrated that there is no evidence to indicate *P. atomaria* constitutes a cryptic species complex, and that variation in a simple observable character like body size appears to be under the influence of both genetic and environmental factors. Furthermore, underlying causes of intraspecific variation have potentially very different origins. Adult body size in *P. atomaria* is likely a by-product of both genetic determinants and developmental processes that occur during

larval development. Smaller adult body size, for instance, results from poor nutrition or high stress during development, or reduced time (*i.e.,* short season) to reach maturity. *Paropsis atomaria* body size changes as a consequence of *direct temperature effect*, with a negative correlation between increasing rearing temperature and adult body size (*plastic effect*) (Chapter 4, page 74, Fig. 6). However, as larger *P. atomaria* occur at lower latitudes (Chapter 4, page 69, Fig. 3), body size also has a genetic component that I postulate to be explained by *local adaptation* to season length. Furthermore, body size in *P. atomaria* is influenced additionally by host plant quality (Chapter 5, page 91, Fig. 3), which imposes a differential effect on host plant utilisation among *P. atomaria* populations (the northern population responds differently compared with the southern population) and this represents a combination between environmental and genetic effects on development and ultimately body size in this beetle with respect to host plant. Finally, seasonal plasticity was demonstrated within *P. atomaria*, and that it is largely a function of direct temperature and seasonal effect imposing limits to the number of generations possible within a field season. Thus, in this widely dispersed beetle, with limited gene flow between disjunct populations, variation in morphological and physiological traits are a combination of local adaptation, phenotypic plasticity, and phenological plasticity; not just local adaptation alone. This study emphasises the need to look more critically at within species variation and not to automatically assume local adaptation as an explanation for all variation observed between geographically and genetically disjunct populations.

Implications for pest management

The work described here has a number of potentially important considerations for future management of *P. atomaria* populations, as some ecological characteristics are shared across populations, while other characteristics are different among them. Such observations are relevant to considerations pertaining to insecticide resistance, population modelling, and host susceptibility. Most importantly, however, the knowledge that *P. atomaria* constitutes a single species with largely shared biological attributes across the distribution means historical research into the taxon may be applied to populations across its geographical range.

The ability of local populations to disperse, together with associated probability of local adaptation, has practical applications for pest management. Resistance to

control measures (*e.g.,* insecticide resistance), is often considered a form of adaptation by populations to local selective pressures (*i.e.,* chemical pesticides), and by understanding relative rates of dispersal and gene flow, we can better predict the potential for resistance to evolve. By examining the population genetic structure of the destructive Tasmanian forestry pest, *Chrysophtharta bimaculata*, for example, it was revealed that due to high levels of gene flow among populations across the island, localised resistance was unlikely if low levels of control agents were applied (Congdon et al., 1997). Similarly for *P. atomaria*, a greater understanding of gene flow in the species may provide insight into the potential for resistance to build up (*i.e.,* high levels of gene flow may reduce the probability of localised resistance). Such assumptions must, however, be approached with due caution, as resistance to insecticides may occur through relatively minor changes involving single allelic substitutions, and hence be easily lost due to disadvantageous pleiotropic effect (Walter, 2003).

The consistency of developmental characteristics as evidenced by comparisons between northern and southern populations shows that populations have not evolved different phenologies. The application of this knowledge, combined with the understanding phenology to be largely under the influence of ambient temperature therefore permits accurate modelling of the life cycle for *P. atomaria* across the season (see Chapter 6). The input of further information into the model, such as pesticide application events, may therefore be undertaken with confidence, as any predictions can be applied across the species' range.

Finally, evidence of differential host plant utilisation by different populations across the geographical distribution of *P. atomaria* is noteworthy. As northern beetles are genetically predisposed to reach a greater body size given a better quality host plant, this may have implications for the plantation of specific eucalypt host species in northern regions of the distribution. The continued planting of better hosts in lower latitudes may allow northern individuals to reach larger sizes, which may increase their potential fecundity (see Carne, 1966). Such increased fecundity and subsequent population number consequently elevates the damage potential of *P. atomaria*, and can affect population density throughout the season. This prediction could be tested using the current phenology computer model, ParopSys. The knowledge that some host species are poorer hosts and will reduce potential for large bodied northern

populations to reach their maximum size may therefore emerge as an effective future management option for the eucalypt hardwood industry.

References

Ballabeni, P., Gotthard, K., Kayumba, A., and Rahier, M. (2003). Local adaptation and ecological genetics of host-plant specialization in a leaf beetle. *Oikos* **101**, 70-78.

Blanckenhorn, W. U., and Fairbairn, D. J. (1995). Life history adaptation along a latitudinal cline in the water strider *Aquarius remigis* (Heteroptera: Gerridae). *Journal of Evolutionary Biology* **8**, 21-41.

Carne, P. B. (1966). Ecological characteristics of the eucalypt defoliating chrysomelid *Paropsis atomaria* Ol. *Australian Journal of Zoology* **14**, 647-672.

Carroll, S. P., and Boyd, C. (1992). Host race radiation in the Soapberry Bug: Natural history with the history. *Evolution* **46**, 1053-1069.

Carroll, S. P., Dingle, H., Famula, T. R., and Fox, C. W. (2001). Genetic architecture of adaptive differentiation in evolving host races of the soapberry bug, *Jadera haematoloma*. *Genetica* **112-113**, 257-272.

Chown, S. L., and Gaston, K. J. (1999). Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews of the Cambridge Philosophical Society* **74**, 87-120.

Chown, S. L., and Klok, C. J. (2003). Altitudinal body size clines: latitudinal effects associated with changing seasonality. *Ecography* **26**, 445-455.

Clarke, A. R., and Walter, G. H. (1995). "Strains" and the classical biological control of insect pests. *Canadian Journal of Zoology* **73**, 1777-1790.

Congdon, B. C., Lange, C. L., and Clarke, A. R. (1997). Geographical variation and gene flow in the eucalyptus defoliating beetle *Chrysophtharta bimaculata* (Coleoptera: Chrysomelidae). *Journal of Applied Ecology* **34**, 1287-1292.

Falconer, D. S. (1952). The problem of environment and selection. *The American Naturalist* **86**, 293-298.

Fox, L. R., and Morrow, P. A. (1981). Specialization: Species property or local phenomenon? *Science* **211**, 887-893.

Futuyma, D. J., and Moreno, G. (1988). The evolution of ecological specialization. *Annual Review of Ecology and Systematics.* **19**, 207-233.

Hastings, A. (1983). Can spatial variation alone lead to selection for dispersal? *Theoretical Population Biology* **24**, 244-251.

Inoue, T. (2001). Life history of the flea beetle, *Argopistes coccinelliformis* Csiki (Coleoptera: Chrysomelidae) VII. Effects of photoperiod and temperature on induction of reproductive diapause in newly emerged adults. *Applied Entomology and Zoology* **36**, 53-58.

Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics.* **21**, 243-273.

Joshi, A., and Thompson, J. N. (1995). Trade-offs and the evolution of host specialization. *Evolutionary Ecology* **9**, 82-92.

Kawecki, T. J., and Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters* **7**, 1225-1241.

Lee, C. E. (2002). Evolutionary genetics of invasive species. *TREE* **17**, 386-391.

Mayr, E. (1942). "Systematics and the Origin of Species," Colombia University Press, New York.

Nylin, S., and Gotthard, K. (1998). Platicity in life-history traits. *Annual Review of Entomology* **43**, 63-83.

Ohmart, C. P., and Larsson, S. (1989). Evidence for absorption of eucalypt essential oils by *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae). *Journal of the Australian Entomological Society* **28**, 201-205.

Ohmart, C. P., Thomas, J. R., and Stewart, L. G. (1987). Nitrogen, leaf toughness and the population dynamics of *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae) - A hypothesis. *Journal of the Australian Entomological Society* **26**, 203-207.

Paterson, H. E. H. (1993). "Evolution and the recognition concept of species. Collected writings," The Johns Hopkins University Press, Baltimore.

Peterson, M. A., and Denno, R. F. (1998). The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. *The American Naturalist* **152**, 428-446.

Sibly, R. M., and Atkinson, D. (1994). How rearing temperature affects optimal adult size in ectotherms. *Functional Ecology* **8**, 486-493.

Steinbauer, M. J., Clarke, A. R., and Madden, J. L. (1998). Oviposition preference of a *Eucalyptus* herbivore and the importance of leaf age on interspecific host choice. *Ecological Entomology* **23**, 201-206.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S. M., Schlichting, C. D., and Van Tienderen, P. H. (1995). Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology and Evolution* **10**, 212-217.

Via, S., and Lande, R. (1985). Genotype-Environment Interaction and the Evolution of Phenotypic Plasticity. *Evolution* **39**, 505-522.

Walter, G. H. (2003). "Insect Pest Management and Ecological Research," Cambridge University Press, Cambridge, UK.

Appendix 1

Morphometric study of *Paropsis atomaria* (supplement to Chapter 4)

Introduction

Morphometric variability between populations is a traditional technique used to identify cryptic species complexes, and while the advent of molecular approaches has revolutionised cryptic complex studies, morphometric techniques continue to be applied (Hoberg et al, 1999; Bain et al., 2003). However, while molecular approaches have become affordable, morphometric analysis remains a key tool in cryptic species identification due to its ease of application and minimal requirement for specialised tools and training. Consequently, a morphometric analysis was undertaken as an additional approach towards identifying whether *Paropsis atomaria* constituted a cryptic species complex. Results of this analysis demonstrate no clear evidence for a cryptic species complex; however latitudinal body-size trends revealed a converse Bergmann cline which is detailed in Chapter 4 of this thesis.

Materials and methods

Fifteen body measurements (Fig 1, Table 1) were selected for examination across *P. atomaria* individuals collected from the following four locations in Australia: Lowmead, central Queensland; Beerburrum, south-east Queensland; Bangalow, north-east NSW; and Canberra, ACT. See Chapter 4 for measurement methodology. Also included for comparison were samples of *P. deboeri*, a Tasmanian species closely allied with *P. atomaria* (see Chapter 2).

Discriminant function analysis in SPSS was used to examine the fifteen body measurements and to make a qualitative assessment of morphometric similiarity among populations.

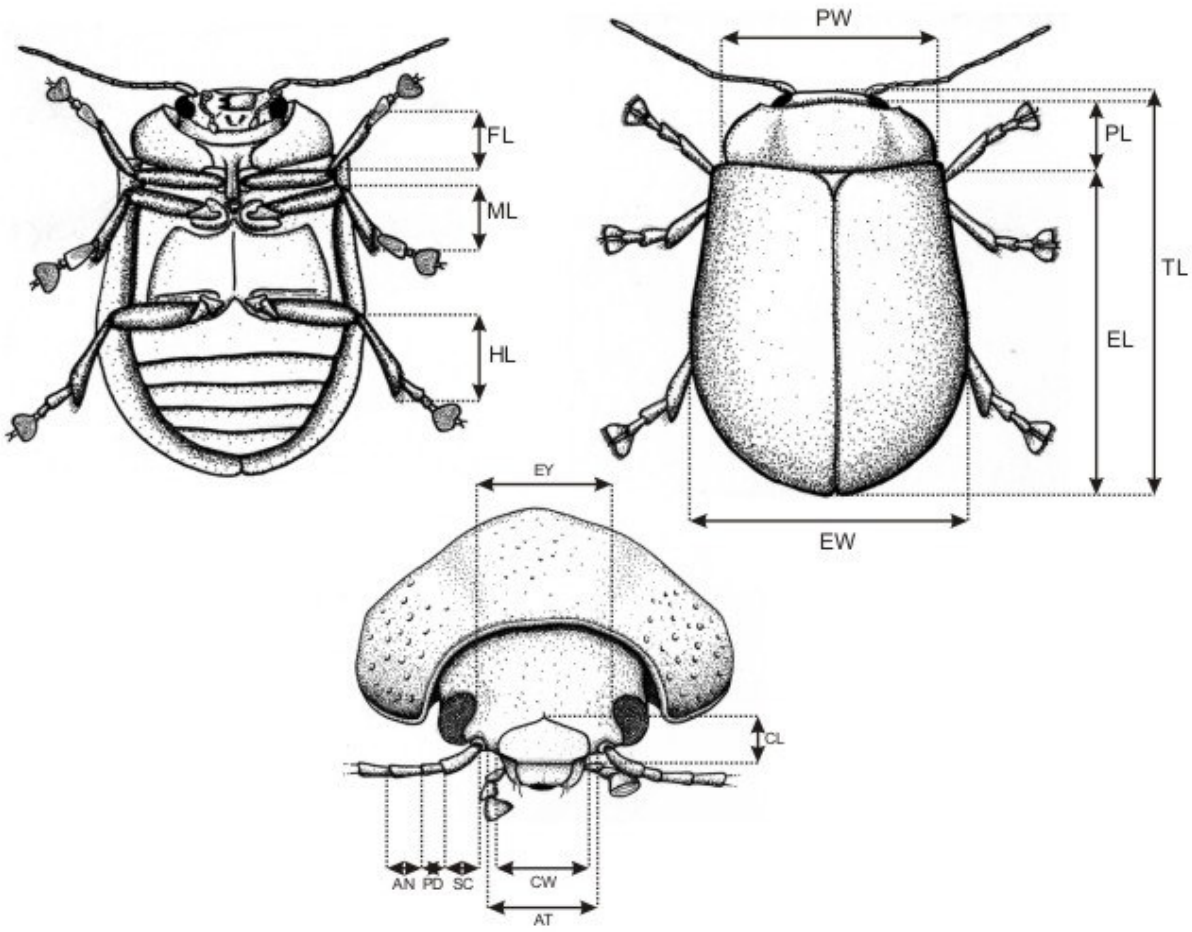


Figure 1. All measurements take for *Paropsis atomaria* individuals collected from the field and reared in common-garden trials.

Table 1: Fifteen measurements selected for morphometric study of *Paropsis atomaria* individuals collected from four sites.

Abbreviation	Character description
AN	Length of first antennal segment
AT	Distance between base of antennae
CL	Length of clypeus
CW	Width of clypeus
EL	Length of elytra
EW	Width of both elytra (maximum width of body)
EY	Distance between eyes
FL	Length of fore tibia
HL	Length of hind tibia
ML	Length of mid tibia
PD	Length of pedicel
PL	Length of pronotum
PW	Width of pronotum
TL	Total length from tip of head to tip of elytra/abdomen

Results and Discussion

Table 2 shows the measurements for each of the body measurements of *P. atomaria* samples from each location (mm, means and standard deviations). Note that males and females are analysed separately due to significant differences in size between the sexes.

Discriminant function analysis (Fig 2) reveales no clear evidence for any population of *P. atomaria* emerging as different from any other. While each population occupies a slightly different region in space, there is significant overlap between them. This is in contrast to the close relative, *P. deboeri*, which is clearly different from all populations of *P. atomaria* (Fig 2). Note, however, that only multiple samples of female *P. deboeri* were examined, with only a single male specimen available. This lack of *P. deboeri* specimens for the male analysis may explain the increased disparity between populations seen for the male results, as a similar pattern emerges for females when *P. deboeri* samples are removed from the female analysis (results not shown).

Due to the overlap between populations in the discriminant analysis, there is insufficient evidence for cryptic species based on a morphometric approach, however as outlined above, the latitudinal gradient trend is elaborated upon in Chapter 4.

Sample	Sex	*N*	SC mean±SD	PD mean±SD	AN mean±SD	EY mean±SD	AT mean±SD	CW mean±SD	CL mean±SD	PW mean±SD	PL mean±SD	EW mean±SD	EL mean±SD	TL mean±SD	FL mean±SD	ML mean±SD	HL mean±SD
BBR	M	12	0.64±0.06	0.27±0.03	0.43±0.05	2.01±0.14	1.62±0.11	1.35±0.09	0.64±0.06	5.45±0.48	2.40±0.21	7.70±0.70	7.07±0.58	10.15±0.89	1.98±0.19	2.15±0.19	2.38±±0.18
	F	17	0.71±0.04	0.29±0.02	0.48±0.04	2.25±0.11	1.81±0.09	1.52±0.07	0.71±0.07	6.10±0.28	2.63±0.17	8.61±0.46	9.12±0.67	12.08±0.78	2.26±0.11	2.44±0.18	2.67±0.13
BAN	M	14	0.62±0.05	0.28±0.02	0.46±0.04	2.04±0.09	1.64±0.06	1.35±0.05	0.64±0.03	5.39±0.25	2.34±0.13	7.51±0.31	7.16±0.29	10.34±0.47	2.04±0.11	2.21±0.14	2.43±0.14
	F	11	0.72±0.04	0.29±0.02	0.49±0.05	2.25±0.10	1.80±0.08	1.50±0.05	0.68±0.04	5.93±0.30	2.56±0.11	8.39±0.28	8.95±0.50	12.51±0.71	2.25±0.15	2.48±0.17	2.66±0.17
LOW	M	22	0.68±0.05	0.28±0.02	0.48±0.02	2.24±0.11	1.78±0.09	1.46±0.07	0.66±0.15	5.96±0.33	2.57±0.14	8.31±0.42	7.81±0.73	10.94±0.73	2.15±0.13	2.28±0.16	2.51±0.17
	F	12	0.74±0.03	0.30±0.01	0.51±0.01	2.42±0.08	1.93±0.06	1.60±0.04	0.73±0.05	6.53±0.15	2.80±0.14	8.96±0.21	9.75±0.46	13.00±0.50	2.36±0.12	2.54±0.11	2.76±0.12
CAN	M	15	0.64±0.05	0.28±0.02	0.44±0.04	2.00±0.11	1.58±0.09	1.31±0.07	0.61±0.04	5.13±0.28	2.21±0.12	7.06±0.58	7.27±0.64	9.99±0.97	1.93±0.15	2.12±0.16	2.33±0.16
	F	15	0.69±0.03	0.29±0.03	0.48±0.03	2.21±0.08	1.78±0.06	1.49±0.05	0.66±0.04	5.63±0.27	2.45±0.11	7.90±0.44	8.44±0.45	11.59±0.55	2.09±0.10	2.32±0.15	2.51±0.15

Table 2. Measurements for each of the fifteen selected measurements (mm ± standard deviation) for the morphometric analysis of *Paropsis atomaria* collected from the following four sites: Beerburrum, Qld (BBR); Bangalow, N.S.W. (BAN); Lowmead, Qld (LOW); and Canberra, A.C.T. (CAN).

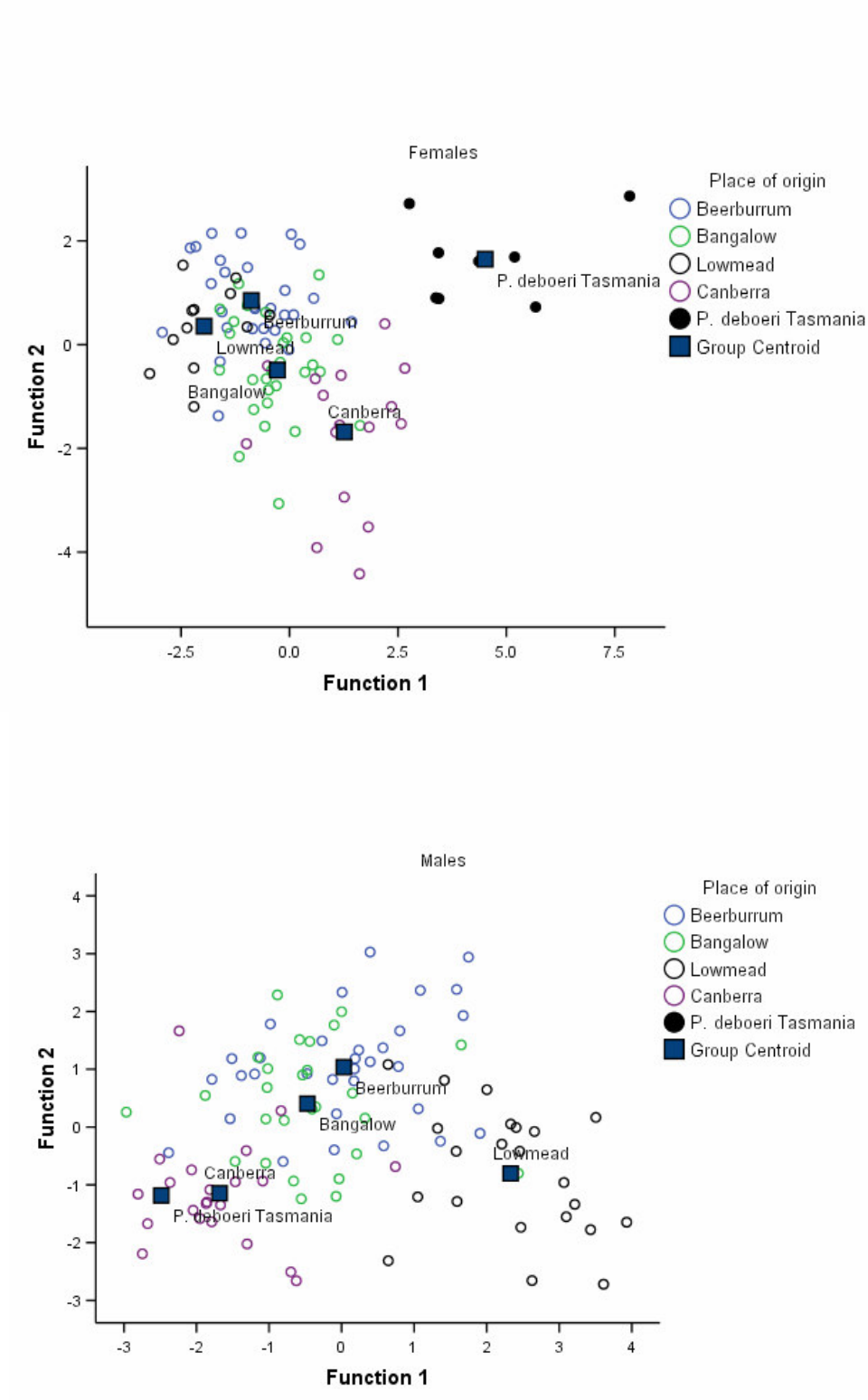


Figure 2. Canonical discriminant results for morphometric data based on fifteen measurements taken from female (top) and male (bottom). *Paropsis atomaria* samples sourced from four collection localities (Beerburrum, Qld; Bangalow, N.S.W.; Lowmead, Qld; and Canberra, A.C.T.) and also for individuals of *P. deboeri* (close relative of *P. atomaria*) from Tasmania. Note that only a single male specimen was available for *P. deboeri* analysis.

References

Bain R.H., Lathrop A., Murphy R.W., Orlov N.L., and Cuc H.T. (2003). Cryptic Species of a Cascade Frog from Southeast Asia: Taxonomic Revisions and Descriptions of Six New Species. *American Museum Novitates* **3417(1):** 1-60.

Hoberg E.P., Monsen K.J., Kutz S., and Blouin M.S. (1999). Structure, Biodiversity, and Historical Biogeography of Nematode Faunas in Holarctic Ruminants: Morphological and Molecular Diagnoses for *Teladorsagia boreoarcticus* n. sp. (Nematoda: Ostertagiinae), a Dimorphic Cryptic Species in Muskoxen (*Ovibos moschatus*). *The Journal of Parasitology* **85(5):** 910-934.

Appendix 2

Cross mating trial between Canberra (A.C.T.) and Lowmead (Qld) individuals

Introduction

Whether individuals accept each other as mates can contribute towards assessing the species status of a study taxon. Consequently, mating trials investigating the potential for individuals to mate can then be regarded as a key component to cryptic species identification. The problem arises, however, in the interpretation of results. Very few experimental outcomes are capable of unequivocally determining species status given the recognition concept of species (Walter, 2003). Due to the confounding effects of laboratory conditions, only in a case of a) controls mating and b) crosses not mating, can relative confidence be assigned to the existence of multiple biological species. If, however, controls *and* crosses freely accept each other as mates under laboratory conditions, a case for or against cryptic species can not be resolved, regardless of the viability of resultant offspring (Walter, 2003). Consequently, as cross-mating trials between geographically disjunct populations of *Paropsis atomaria* revealed both control and experimental crosses mated freely, there is no evidence of a cryptic species.

Materials and methods

Individuals collected from two localities (Canberra, A.C.T. and Lowmead, Qld) were paired in plastic containers to observe mating behaviour. The following pairings were made: Canberra male X Canberra female; Canberra male X Lowmead female; Lowmead male X Lowmead female; and Lowmead male X Canberra female.

Pairs were observed for 300 minutes at a time, recording behaviour every five minutes. Behaviour was recorded as either: no mating behaviour; mounting; or copulation. Copulation was recorded when the male genitalia was clearly inserted into the female, whereas mounting was recorded as when the male was mounted but with no copulation. Only total duration of copulation is presented (Table 1).

Results and Discussion

Table 1 reveals that for each cross, whether control or experimental, there was acceptance between males and females as potential mates with at least some pairs engaging in extended periods of copulation. Consequently, as both control and experimental crosses were successful, results can not be interpreted to either support or refute a case of a cryptic species complex within *P. atomaria*. While experimental crosses do mate under laboratory conditions, field conditions may pose a very different reality as other elements of the currently largely unknown mate recognition system may act to segregate populations and result in assortative mating within their natural environment. Evidence for a cryptic species complex would exist should a lack of mate recognition between populations occur in the wild; however, laboratory trials as presented fail to do so.

Table 1. Total duration of copulation (minutes) between paired *Paropsis atomaria* individuals from one of two source populations (Can = Canberra, A.C.T.; Low = Lowmead, Qld). Numbers with '+' denote those pairs that were still copulating at the termination of the experiment (observations made over 300 minutes). Total number of replicates for each trial (including those that did not mate) given in parentheses.

Can ♀ X Can ♂ (n = 17)	Can ♀ X Low ♂ (n = 17)	Low ♀ X Low ♂ (n = 21)	Low ♀ X Can ♂ (n = 8)
85	140+	200	165+
85	145	105	140+
145	300+	265+	135
175	95	130	185
10	185	165	285+
80+		130	25
180		190	
45		200	
35		185	
		250	
		180	

References

Walter, G. H. (2003). "Insect Pest Management and Ecological Research," Cambridge University Press, Cambridge, UK.