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Date:

2012

Citation:

Hill, M. P. (2012). A predictive framework to assess response of invasive invertebrates to climate change: pest mite species of Australian grains. PhD thesis, Science - Zoology, The University of Melbourne.

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**A predictive framework to assess response of invasive
invertebrates to climate change: pest mite species of
Australian grains**

Submitted by
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Submitted in total fulfillment of the requirements of the degree of
Doctor of Philosophy

September 2012

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Produced on archival quality paper

ABSTRACT

Climate change is set to place enormous pressure on both biodiversity and agricultural production. Important vectors of agricultural damage, such as pest invertebrates, are likely to respond to climate change in different ways. Differing pest invertebrate responses in grain crops will translate to shifts in outbreak frequency and persistence of pests, changes to pest species assemblages, and alter biocontrol by natural enemies. Successful management will thus require predictions of how climate change will affect individual species in terms of distributions and abundance. Climate change predictions for species are often based on models that characterize distributions through species-environment relationships. However, there are important factors relating to the ecology and evolutionary biology of species that are not incorporated and will mediate climate change response. This thesis aims to establish a transferrable framework, employing multiple, complimentary lines of enquiry, to build on distribution models and understand how climate change may affect different crop pests.

I focus on two important mite species groups, the blue oat mites (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*). These mite species are invasive, and so understanding how they have adapted since being introduced into Australia will help predict response to climate change in the future. The first part of this thesis applies environmental niche models to distribution data of the three cryptic *Penthaleus* species, to make preliminary assessments of response to climate change. These models found that the distributions of each species are governed by different climate variables, and that species assemblages are likely to shift under climate change. The remainder of the thesis builds on such models by applying a more integrated approach to assess climate change response of *H. destructor*. This species provides an ideal candidate to develop this framework as the biology and ecology is well understood, and its introduction and spread in Australia has been well documented.

I first build environmental niche models for *H. destructor*, and test key climatic variables that limit distributions. The invasive distribution of *H. destructor* is not predictable from its native distribution alone, and different climate variables limit the present distribution in Australia than did historically. By revealing differences in important variables, hypotheses of traits that may have shifted during invasion can be developed. For *H. destructor* traits related to temperature appear to have shifted over time. A laboratory study was then used to characterize thermal tolerance traits in both native and invasive ranges of *H. destructor*. The results suggest a shift in temperature response within Australian populations. To understand if these changes in thermal tolerance traits are correlated with population differences, DNA markers were developed and analysed across populations in both native and invasive ranges. These markers revealed high levels of gene flow between Australian populations, indicating high levels of dispersal across the continent. This may aid in the spread of any adaptive shifts in important traits, like thermal tolerance. These findings point to *H. destructor* persisting as an important pest species under climate change.

Understanding the impacts of climate change on different pest invertebrate species is required for developing targeted management strategies. By combining different tools to investigate the species-environment relationships for *H. destructor*, it was possible to make a more informed prediction of climate change response. Importantly, the methods presented in this thesis allow for species-specific hypotheses to be generated and tested. The strength of the framework is that it uses simple tools in concert, so is transferrable to a broad range of pest invertebrates.

DECLARATION

This is to certify that:

- i. the thesis comprises only my original work towards the PhD except where indicated in the Preface,
- ii. due acknowledgement has been made in the text to all other material used,
- iii. the thesis is less than 100,000 words in lengths, exclusive of tables, maps, bibliographies and appendices

Matthew Peter Hill

19th September 2012

PREFACE

The following chapters of this thesis have either been published or have been submitted for consideration under the following titles. The nature and contribution of each author for each chapter is also described below.

Chapter 2. Hill, M. P., Hoffmann, A. A., McColl, S. & Umina, P. A. (2011) Distribution of cryptic blue oat mite species (*Penthaleus* spp.) in Australia: current and future climate conditions. *Agricultural and Forest Entomology*, **14**, 127–137.

Hill, M.P. (PhD Candidate).

Developed methods, conducted analyses and wrote manuscript.

Hoffmann, A.A.

Supervised development of work and edited manuscript.

McColl, S.

Conducted Western Australian field collections

Umina, P.A.

Supervised development of the work and edited manuscript.

Chapter 3. Hill, M. P., Hoffmann, A. A., Macfadyen, S., Umina, P. A. & Elith, J. (2012) Understanding niche shifts: using current and historical data to model the invasive redlegged earth mite, *Halotydeus destructor*. *Diversity & Distributions*, **18**, 191–203.

Hill, M.P. (PhD Candidate)

Developed methods, conducted analyses and wrote manuscript.

Hoffmann, A.A.

Supervised development of work and edited manuscript.

Macfadyen, S.

Supervised development of work and edited manuscript.

Umina, P.A.

Supervised development of work and edited manuscript.

Elith, J.

Developed methods, contributed to writing and edited manuscript.

Chapter 4. Hill M. P. & Hoffmann, A. A. (**in review**) Increased resistance and plasticity in thermal tolerance traits support niche-shift predictions for an invasive mite, *Halotydeus destructor*. *Global Ecology and Biogeography*.

Hill, M.P. (PhD Candidate)

Developed methods, collected samples, conducted experiments, conducted analyses and wrote manuscript.

Hoffmann, A.A.

Supervised development of work, assisted with analysis and edited manuscript.

ACKNOWLEDGEMENTS

First and foremost I wish to thank Ary Hoffmann for being an exceptional supervisor and guiding me through my PhD. I have learned an incredible amount by being his student, grown immensely as a scientist, and will be forever grateful for the advice and mentoring that he provided. I also sincerely thank Paul Umina and Sarina Macfadyen for being excellent supervisors on this project. I am extremely lucky to have benefited from the time and dedication they have given, the discussions and guidance, and different points of view that have helped me formalize my thoughts.

Jane Elith gave a considerable amount of time to help me learn new modelling methods and I am very grateful to now possess these skills. Adam Miller lead me through the laboratory work needed to develop the microsatellite library and guided me on the analysis required. Special thanks to Linda Thomson, who has always been available for advice and supportive discussions and also provided me with opportunities to teach and work as a researcher within the laboratory, both of which I am immensely grateful. Special thanks also to Michael Nash who has always been willing to lend his support and talk about science and beyond over the period of my candidature, as well as assisting on a memorable field trip. Nancy Endersby, Andrew Weeks and Anthony van Rooyen all provided time, advice and assistance for the genetics laboratory work. Elise Furlan was a great help on the field-collecting trip to Tasmania. Emily Thomson was indispensable in helping me manage the funds and paperwork involved with this project.

The Loftus-Hills memorial award from the Zoology Department allowed for me to go to South Africa, and I am very grateful to Steven Chown for hosting me in his laboratory there. Thanks to Suzaan Kritzinger-Klopper, Charlene Scheepers and Natalie Theron for assistance with South African fieldwork, and Erika Nortje for providing valuable laboratory assistance.

I am forever grateful to my good friends in the laboratory – Pip, Clare, Kat, and Vale, who at different times provided reassurance, friendship and motivation. I also want to thank Re for being so understanding and supportive of me during the write-up time of this thesis.

Finally I wish to thank my loving parents, Peter and Carolyn, for being supportive of me through the entirety of my education, providing support and assistance whenever I have required it. I would never have got to this stage without them!

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CHAPTER 1: GENERAL INTRODUCTION

Anthropogenic climate change is one of the greatest modern challenges to biodiversity, ecosystem function, agricultural production and human sustainability. Sea level rise, more frequent extreme weather events, longer hot spells and general increases in temperature and aridity will place enormous pressure on natural systems, urban settlements and primary production. The effects of such changes on food production are set to be devastating with the IPCC forecasting a 10-20% decline in overall global crop yields by 2050 (IPCC, 2007). For Australia, recent evidence is compelling: there have been no other warmer periods than post-1950 in the last 1000 years (Gergis *et al.*, 2012), and the most recent decade (2000-09) was the hottest on record (Braganza & Church, 2011). These are linked to anthropogenic causes (Gergis *et al.*, 2012). With extreme temperatures already experienced throughout much of Australia's grain growing and pasture regions, climate change is forecasted to decrease the yield of crops such as wheat by around 25% in the south eastern parts of Australia (Anwar *et al.*, 2007). Where climate defines suitable area for dry-land cropping, these areas are expected to contract under different climate change scenarios (Nidumolu *et al.*, 2012). To offset predicted lower crop yield, a number of management changes are required. These include changes in farming practices and location of certain crops (Anwar *et al.*, 2007; Olmstead & Rhode, 2011), breeding more resistant varieties (Turner *et al.*, 2011), and management of pest species such as invertebrates (Hoffmann *et al.*, 2008).

Many species of invertebrates are invasive pests and not only cause huge losses to biodiversity by outcompeting native species, but represent some of the most recognized vectors of agricultural damage (Ziska *et al.*, 2010). Climate change will result in a range of potential impacts on pest invertebrates (reviewed in Harrington & Woiwood, 1995), including changes in population dynamics such as growth rate and overwintering success, as well as an increase in the number of generations per year and changing interactions with other species (Van der Putten *et al.*, 2010; Sutherst *et al.*, 2011). Greater risks are likely to come from changes in geographic distributions of pests and invasions by new pests (Harrington & Woiwood, 1995). For example, the

distribution shifts and greater outbreak potential of coffee borer beetle, *Hypothenemus hampei*, in Africa (Jaramillo *et al.*, 2009), mountain pine beetle, *Dendroctonus ponderosae*, in North America (see de la Giroday *et al.*, 2012) and pine processionary moth, *Thaumetopoea pityocampai*, in Europe (Battisti *et al.*, 2005) are all linked to climate change.

Within Australia, pest invertebrate species cause extensive damage to pasture and broadacre crops such as oilseeds, cereals and pulses. While drivers such as changes in pesticide use and increased irrigation are likely to be associated with shifts in species distributions, climate change is also likely to be driving shifts in the geographic distribution of some pest invertebrate species (Hoffmann *et al.*, 2008). With changes in distribution come changes to phenology and persistence that ultimately lead to pest outbreaks and spread of vector-borne plant pathogens. Some species, such as armyworm, may be responding negatively to climate change (Hoffmann *et al.*, 2008). Others such as the slug, *Milax gagates* that perform better in arid conditions, may benefit from drier conditions under climate change (Nash, 2008), and so too the transmission of plant viruses, such as Yellow dwarf by aphid vectors (Parry *et al.*, 2012). Clearly, pest species will respond to climate change differently and it is thus important to investigate individual species responses through a framework that is broadly applicable.

To meet the challenges that climate change will impose on food production, we need to be able to accurately predict how pest species will respond. To date, research on how invertebrate pests will respond to climate change is relatively rare (Mika *et al.*, 2008; Ziter *et al.*, 2012). Such research could help in the development of management recommendations to be used by growers in order to aid crop protection in the future (Steffen *et al.*, 2011; Turner *et al.*, 2011). For this to be achieved, a good understanding of how species have adapted in the past, how they respond to current variability in weather, and when and how this is likely to lead to pest outbreaks across agricultural landscapes is required. An essential component of this is to predict species distributions across the landscape.

1.1 Predicting distributions of insects

1.1.1 Predicting pest species distributions

The link between insects and climate was researched widely even before the field of ecology was formally recognized. When Uvarov (1931) reviewed this subject, he cited over 1000 papers, many of which dated back to the 19th century or earlier. At this time the field of “climatic analysis of insect distribution” was first formulated. Meteorological data was available to ecologists and the understanding of how large-scale climatic variables influenced distributions was becoming apparent (e.g. Grinnell, 1917). While there existed a few studies that studied the effect of climatic variables on pest insects (e.g. green bug, *Toxoptera graminum* (Ruggles & Wadley, 1927) and Mediterranean fruit fly, *Ceratitidis capitata* (Gjullin, 1931)) it was William C. Cook who outlined methods of predicting the distributions of pest invertebrate species (Cook, 1931). Cook (1929) first described how weather station data could be used to determine climatic zonations for pest insects. He explained how these zones could be correlated with the frequency of limiting climatic conditions and thus provide maps describing where outbreaks of species such as the pale western cutworm, *Porosagrotis orthogonia* (Cook, 1924), and the true armyworm, *Cirphis unipuncta* (Cook, 1929), were more likely to occur. Cook went on to describe a framework for predicting insect distributions that tied weather data to insect distributions and known physiological parameters (Cook, 1931). While the effects of twentieth century climate change were not apparent at this time, and the field of invasion biology was just emerging, this provided a robust framework for predicting the distributions of pest invertebrates in relation to climate.

There have been many other advances in the field of pest ecology since the time of Cook (e.g. Andrewartha & Birch, 1982), but predicting the distribution of a pest species still remains an essential component of understanding the effects of climate change. The goal of this thesis is to use modern tools to reinterpret the ideas of Cook (1931) into a predictive framework for pest invertebrate distributions. Central to this framework, that includes biological invasions and the response to recent climate change, is the concept of the *niche*.

1.1.2 Niche theory and concepts

The niche is an ecological concept that ties biotic and environmental elements together (Keller & Golley, 2000) to define the distribution or functional role of a species. To accurately describe the niche for a given species would involve measuring every environmental condition, biotic interaction and resource that an organism requires (Porter & Kearney, 2009). In reality, this is an impossible task, however the niche as a concept, still serves as a useful tool for understanding species requirements across space and time (Soberón & Nakamura, 2009; Wiens *et al.*, 2009). In terms of predicting the distribution of species, there are different and equally important interpretations of the niche that need to be defined when investigating processes and traits that determine niche boundaries (Colwell & Rangel, 2009; Porter & Kearney, 2009; Wiens *et al.*, 2009).

Niche definitions

The first formal definition of the niche was provided by Joseph Grinnell, who described the niche in terms of areas of distributions of species and the different variables that govern the range of species (Grinnell, 1914; 1917). That is to say, that the spatial extent of the range and geographical expression of a species' niche are approximately the same (Tingley *et al.*, 2009). This provides a somewhat restricted definition of the niche, employing broad ecological variables that do not interact with each other (Soberón & Nakamura, 2009) to set the range limits of species (Wiens, 2011). This simplicity allows for an operational and straightforward niche concept (Soberón & Nakamura, 2009), and when employed can provide strong explanations of species range boundaries (Tingley *et al.*, 2009). Such an interpretation of the niche lends itself to being particularly useful to understand biogeographical patterns (Wiens, 2011).

While the Grinnellian niche encapsulates broad environmental processes, Charles Elton defined the niche in terms of biotic interactions and resource limitations that shape the distribution of a species (Elton, 1927; Soberón, 2007). This interpretation of the niche presents the species as playing a functional role within a community (Wiens *et al.*, 2009) and thus presents a much finer-scale concept than that of Grinnell. The

Eltonian niche employs axes of resource utilization and provided the foundation for later elaborations by Hutchinson (1957) and MacArthur (1972), to become widely used in ecological studies (Wiens *et al.*, 2009). This interpretation of the niche allows for understanding of biophysical requirements of the species to be measured and associated with landscape features to define niche boundaries.

George Evelyn Hutchinson presented a niche-distribution duality that provides perhaps the most important distinction of niche concepts. Hutchinson (1957) described the niche as taking both the form of the *fundamental* niche - the direct physiological requirements of a species, and the *realised* niche – the proportion of the fundamental niche actually exhibited by the species at a point in time, due to limits set by both biotic and abiotic interactions (Wiens *et al.*, 2009). This Hutchinsonian definition of the niche allows for both the Grinnellian and Eltonian niche interpretations to be employed in a suite of species-environment relationships within physical (environmental) and geographical (biotope) space (Colwell & Rangel, 2009; Wiens *et al.*, 2009).

Recent advances in species distribution models (see section 1.1.3) have seen the advent of the *potential* niche. This describes limits set by the fundamental niche that may permit population persistence and growth, but the species has not dispersed to fulfill yet (Soberón, 2007; Soberón & Nakamura, 2009). This concept is particularly important for invasive species that are not in equilibrium with the environment.

Niche conservatism

Niche shifts describe transgression between species-environment relationships across ranges (e.g. Fitzpatrick *et al.*, 2007; Broennimann *et al.*, 2007), or over time (e.g. Kharouba *et al.*, 2009). Conversely, a species that occupies geographical regions corresponding to regions of niche space set by the fundamental niche is said to have displayed niche conservatism (Colwell & Rangel, 2009). For studies of climate change, niche conservatism refers to species that track climatic change to preserve species-environment relationships: species must undergo elevational and latitudinal range shifts to stay within their favourable climate zones (Colwell & Rangel, 2009). This process can cause problems for species not able to disperse as fast or as far as the

changing climate dictates. One way we can study the process of niche shifts and niche conservatism in response to climate change is to construct different types of species distribution models. These models can be used to inform which traits may limit the niche, and the level of niche evolution displayed in species invasion and response to climate change.

1.1.3 Species distribution models

Species distribution models are increasingly popular for describing the niche of a species and detecting niche shifts. There are a number of modelling tools available, which have varying advantages and explicabilities, and utilize different interpretations of the species niche. Commonly used in conservation biology research (Pearson *et al.*, 2007; Habel *et al.*, 2011), species distribution models may also be applied to invertebrate pest species, especially when questions need to be asked of species invasion or climate change. Table 1.1 gives examples of recent research showing the application of species distribution models for a variety of pest insects. Presently, the majority of pest insect distribution models determine potential areas for species invasions, though more are starting to address responses to climate change.

Table 1.1. Examples of species distribution models for pest invertebrate species. I = invasion risks , C = response to climate change.

Common name	Species	Models	Type	Use	Locality	Reference
Fig fly	<i>Zaprionus indianus</i>	MAXENT / GARP / Mahalanobis distances	Correlative	I	Global	Alves da Mata <i>et al.</i> , 2010
Western corn root-worm	<i>Diabrotica virgifera virgifera</i>	ENFA / Mahalanobis distances	Correlative	I/C	Northern Hemisphere	Aragón & Lobo, 2012
Mediterranean fruit fly / Natal fruit fly	<i>Ceratitis capitata</i> / <i>Ceratitis rosa</i>	GARP / PCA	Correlative	I	Africa / Europe / Global	De Meyer <i>et al.</i> , 2008
European grapevine moth	<i>Lobesia botrana</i>	Physiology /demographic	Mechanistic	I	California / USA	Gutierrez <i>et al.</i> , 2012
Light brown apple moth	<i>Epiphyas postvittana</i>	Temperature/demographic	Mechanistic	I	California	Gutierrez <i>et al.</i> , 2010
Light brown apple moth	<i>Epiphyas postvittana</i>	CLIMEX / MAXENT	Semi-mechanistic / Correlative	I	Global	Lozier & Mills, 2011
Bird cherry-oat aphid	<i>Rhopalosiphum padi</i>	CLIMEX	Semi-mechanistic	I	Global	Macfayden & Kriticos, 2012
Pea leafminer	<i>Liriomyza huidobrensis</i>	CLIMEX	Semi-mechanistic	I/C	North America	Mika & Newman, 2010
Swede midge	<i>Contarinia nasturtii</i>	CLIMEX	Semi-mechanistic	I/C	North America	Mika <i>et al.</i> , 2008
Brown marmorated stink bug	<i>Halyomorpha halys</i>	MAXENT	Correlative	I	North America / Global	Zhu <i>et al.</i> , 2012

Much of the recent advance in species distribution modelling has been made possible by the increasing availability of global weather station data and computational power to process these. This has allowed for the formation of geospatial databases that offer high-resolution layers of averaged monthly climate data (e.g. WorldClim (Hijmans *et al.*, 2005), ANUCLIM (v6.1, Fenner School of Environment and Society, Australian National University)). These data can be transformed into biologically relevant trends and patterns of rainfall, temperature, humidity and solar radiation (e.g. BIOCLIM variables (Nix & Busby, 1986)), and used to determine limiting factors to species distributions (Elith & Leathwick, 2009). Instead of models being restricted to a few sites, it is possible to project models across entire countries, continents or globally to understand macroecological processes of invasion and climate change (Peterson, 2003; Araújo *et al.*, 2005; Elith & Leathwick, 2009). There is also a range of future climate models (Global Circulation Models - GCMs) that are based on different scenarios of severity of climate change. These allow for future forecasts of climate change to be incorporated into species distribution models. Choice of climate change scenario can alter model outputs, so this needs to be taken into consideration when building models, to determine which scenarios are likely to be relevant to the models (Beaumont *et al.*, 2008; Mika & Newman, 2010). As no one model may be considered the “best” (Beaumont *et al.*, 2008), an ensemble forecast of a range of GCMs can outperform single GCMs and provide greater confidence in model outputs (Fordham *et al.*, 2011).

Correlative models

Correlative species distribution models are based on the realized Grinnellian niche and are typically referred to as environmental niche models (ENMs) (Jiménez-Valverde *et al.*, 2011; Wiens, 2011). By correlating known distribution points with environmental layers as predictor variables or covariates, it is possible to predict species range boundaries. There are a variety of correlative methods available to predict the distribution of species, ranging from regression methods such as GLM (Generalised Linear Models) and GAM (General Additive Models), bioclimatic methods such as BIOCLIM and ENFA (Environmental Niche Factor Analysis) and through to more recently developed machine-learning methods, MAXENT (Maximum Entropy) and BRT (Boosted Regression Trees).

Because biotic, abiotic and dispersal constrain the niche, ENMs can only provide partial information on the full range of environmental conditions that the species may survive (i.e. not estimating the fundamental niche, or even potential niche) (Jiménez-Valverde *et al.*, 2011). Environmental Niche Models rely heavily on well-sampled and unbiased data to construct models that encapsulate as broad a range of species-environment relationships as possible. For many pest invertebrates, often the only data available is for where a species has been found or abundance data, thus the correlative models in Table 1 are all based on presence-only data. The correlative ENM, MAXENT, discriminates something close to the realized or potential niche of a species from the landscape the species is found in, by identifying limiting variables and estimating the habitat suitability of geographic areas (see Elith *et al.* (2011) for a more detailed explanation). To investigate niche change, ENM models, like those built with MAXENT, can be projected into new geographic space (e.g. Fitzpatrick *et al.*, 2007; Broennimann *et al.*, 2007; Rödder & Lötters, 2009; Medley, 2010). However, some degree of caution must be taken when projecting ENMs as the relationship of variables projected may result in under-prediction of areas that will contain non-analogue climates in space or time.

Semi-mechanistic

One of the most common modelling tools used for pest insect distributions is CLIMEX. This tool uses a semi-mechanistic approach to examine the relationship between climate, species distributions and patterns of growth (Macfadyen & Kriticos, 2012). Models can be fitted using a combination of empirically measured parameters and point distribution records. When abundance patterns are not well known, they can be inferred from development rate experiments or observations and these become methods of validation (Kocmánková *et al.*, 2011; Macfadyen & Kriticos, 2012). The CLIMEX model works through a series of weekly growth and stress indices that are combined to produce an Ecoclimatic Index (EI). The EI describes regions that are unsuitable for the species to persist, through to those that provide a “perfect” environment for the species. This is interpreted within a scale of 0 -100 (Olfert *et al.*, 2011), with over 30 considered to convey ideal conditions (Sutherst *et al.*, 2004), and below 10 an unfavorable environment, though rating interpretations will be species-specific to some extent. The strength of CLIMEX lies in its ability to project models

to new environments without relationships between variables confounding projections. This makes CLIMEX suited to predicting new geographical regions for invasive invertebrate species, and also responses to climate change. However, the stress indices are normally derived from the realized distribution, and due to the nature of the climate data used, CLIMEX models do not capture microclimate effects. In this way, CLIMEX is closer to a correlative approach, an important consideration when employing this model type.

Mechanistic models

Mechanistic models are often referred to as process- (Morin & Thuiller, 2009) or trait-based (Kearney *et al.*, 2008) models and use explicit biological processes built on species-specific observations (Morin & Thuiller, 2009). Mechanistic models aim to translate morphological and physiological traits of a species with key environmental variables and the terrain (Kearney *et al.*, 2008; Kearney & Porter, 2009) and thus require little to no information about the distribution of a species to construct the model (Kearney *et al.*, 2008).

While mechanistic models are not always used for species distributions, they can be coupled with spatial variables, like those used as predictor variables in correlative models, to determine the probability of a given location, or grid cell, to meet the organism's resource requirements (Kearney & Porter, 2004). Information regarding physiological response to climatic variables can be compiled into a framework to understand activity, reproduction and survival thresholds. Mechanistic models that may be most useful for the prediction of pest invertebrate distributions include life history and phenology models (e.g. Gutierrez *et al.*, 2008; Gutierrez *et al.*, 2010; Gutierrez *et al.*, 2012) or thermodynamic niche models (e.g. Kearney *et al.*, 2008; Kearney & Porter, 2009). Like correlative ENMs, they can be interpreted through the Hutchinsonian niche duality, but instead characterize axes of the fundamental Eltonian niche. Mechanistic models can also include information about the microclimate – the environmental conditions an individual of a species will experience and utilize (Kearney *et al.*, 2009). Species interactions can also be incorporated (Gutierrez *et al.*, 2008) to predict abundance of species given a location

and presence of food resources or a predator, which then form axes of the realized Eltonian niche.

Species that are in the process of an adaptive shift (e.g. the cane toad, *Bufo marinus*) may not be best described through use of correlative approaches (Kearney *et al.*, 2008). This is important in terms of understanding the effects of climate change on pest invertebrates, as research should aim to include phenotypic and genotypic flexibility (Bale *et al.*, 2002). Mechanistic models can also incorporate levels of variation or plasticity in distribution limiting traits (Kearney & Porter, 2009; Kearney *et al.*, 2009; Kolbe *et al.*, 2010). This allows for hypotheses of adaptive shifts to be estimated under selective conditions such as climatic change.

1.1.4 Niche-limiting traits

When applied appropriately, the different types of species distribution models are able to generate hypotheses about the niche of an organism, and then direct further research towards understanding traits that limit the niche. Adaptation in limiting traits may lead to niche shifts and mediate a species response to climate change (Chown *et al.*, 2010; Hoffmann & Sgrò, 2011). For the mosquito *Aedes aegyptii*, Kearney *et al.* (2009) identified egg desiccation resistance as limiting the inland distribution of the species and incorporated this into a model predicting how adaptation in this trait could facilitate range expansion under climate change. Thus, one of the great challenges is to determine which traits may be limiting current distributions (Wiens, 2011) and then to measure these to incorporate them into predictions. Adaptation or persistence in changing environments may also be mediated through phenotypic plasticity: rapid phenotypic adjustment to environmental variation (see Chown & Terblanche, 2006). For invasive species, studies that measure traits in both native and invasive ranges and across a range of environmental gradients would be extremely beneficial, though are rare (Alexander & Edwards, 2010). The key traits that are relevant to niche shifts during invasion and under climate change are likely to be:

1. Thermal tolerance: Maximum and minimum temperature limits for activity and survival. As temperature plays a large part in determining the niche of a

species (Bale *et al.*, 2002), understanding thermal tolerance traits can help to determine species-environment relationships (Terblanche *et al.*, 2006). Thermal tolerance traits are used widely to investigate ecological and evolutionary processes for terrestrial arthropods (Hoffmann *et al.*, 2005; Terblanche *et al.*, 2006; Mitchell & Hoffmann, 2010; Alford *et al.*, 2012). Species often exhibit differences in thermal tolerance limits across environmental gradients, including elevation and latitude (Gaston & Chown, 1999; Hoffmann *et al.*, 2005), with variation across latitude, including phenotypic plasticity, more evident for lower than upper limits of terrestrial arthropods (Hoffmann *et al.*, 2005; Terblanche *et al.*, 2006; Alford *et al.*, 2012; Hoffmann *et al.*, 2012).

2. Desiccation resistance: Water loss potential. For terrestrial arthropods, their small size and high surface to volume ratio means they are susceptible to desiccation (Johnson *et al.*, 2011). Variation in desiccation resistance has been linked to distributional patterns (Kellermann *et al.*, 2009). For example, tropical species *Drosophila* have low desiccation resistance (and low heritability of this resistance), compared to widely distributed *Drosophila* species (Kellermann *et al.*, 2009; Hoffmann & Sgrò, 2011).
3. Photoperiodism: Physiological reaction to day or night length. While day length will not be affected by climate change, photoperiodism interacts with temperature and varies across latitude, to initiate lipid storage and diapause with the onset of winter (van Asch & Visser, 2007; Lehmann *et al.*, 2012; Urbanski *et al.*, 2012). Adaptation in photoperiodic response has allowed the mosquito, *Aedes albopictus*, to undergo range expansion in North America (Urbanski *et al.*, 2012). Adaptations in photoperiodic traits may also facilitate earlier emergence (to coincide with milder winter temperatures) and increase herbivore damage under climate change (van Asch & Visser, 2007).

Whereas mechanistic models can directly incorporate variation in niche-limiting traits, correlative models can determine which environmental covariates are most important to the niche and which may limit the distribution of species across a geographic range. These can lead to hypotheses about which traits are likely to govern the distribution of the species and should be investigated empirically (e.g. Banta *et al.*, 2012). This provides an opportunity to use correlative models in tandem with

experiments to measure these traits, rather than building full mechanistic models, which can be time consuming. Alternatively, a few niche-limiting traits for pest invertebrate species could be used with distribution information in CLIMEX-like models that are able to draw from multiple knowledge domains (Macfadyen & Kriticos, 2012).

1.1.5 Genetic variation

Within a species, populations and individuals will display some level of genetic variation. Populations of a species are thus likely to respond differently from one another, however species distribution models assume that all individuals within a species will exhibit the same response to environmental variables across their entire range. By measuring genetic variation, estimates of genetic diversity and an indication of demographic population processes, such as gene flow, can be made (Balloux & Lugon-Moulin, 2002). Genetic variation also allows for measurements of spread of adaptations such as pesticide resistance across the landscape (Endersby *et al.*, 2006).

Neutral variation

Measures of neutral genetic variation, such as allele exchange between populations, can be used to determine levels of population differentiation (Balloux & Lugon-Moulin, 2002). Populations that differ genetically may respond differently to climate change, thus while neutral genetic markers may not provide an answer as to what genes are involved, a well-constructed survey of genetic variation based on neutral markers can provide an understanding of genetic divergence and gene flow (Chen & Dorn, 2010). Further, by understanding population structure and relatedness within an invasive species, it may be possible to determine the pathway and time of invasion, and how established populations now vary across the landscape (Estoup & Guillemaud, 2010).

Studies that combine neutral genetic markers and species distribution models are currently rare, though there are some examples from conservation biology (e.g. Arteaga *et al.*, 2011; Habel *et al.*, 2011). A review by Chan *et al.* (2011) discusses

how best these methods could be integrated within a phylogeographical framework. While future correlative and mechanistic models will likely incorporate a range of phenotypes from genetic analyses into dynamic models, present examples are mainly side-by-side analysis of population genetics and ENMs (e.g. Lozier & Mills, 2009). Combining ENMs with population genetic analyses can help to identify endangered genetic lineages and prioritize conservation research (Habel *et al.*, 2011). Other examples include using an ENM to create an environmental resistance covariate and determine its relatedness to genetic structure and effective population size (Wang, 2012), and using an ENM to calculate predicted nucleotide diversity loss under climate change (Dubey *et al.*, 2012). Methods that combine ENMs with population genetics should lead to an increased understanding of how rapid adaptive shifts, especially in niche-limiting traits, could occur across populations.

Adaptive variation

Adaptive genetic variation refers to genes that have effects on fitness (Holderegger *et al.*, 2006) and can be used to determine the adaptive potential of populations. Quantitative genetic experiments such as quantitative trait locus (QTL) mapping can identify changes related to adaptive shifts in different functional traits. For instance, QTL could measure traits involved with shifts in niche-limiting traits such as thermal response, or even identify candidate genes that are associated with *invasiveness* (Prentis *et al.*, 2008). However, even though rapid evolutionary change has been observed for a range of pest invertebrate and invasive species, putative adaptive traits are rarely considered at the genetic level. Changes in niche-limiting traits can be observed at the level of phenotypes. Examples include the rapid adaptation in critical photoperiod for the mosquito *Aedes albopictus* in its invasive range (Urbanski *et al.*, 2012) and shortened juvenile development time and increased invasiveness in the cinnabar moth, *Tyria jacobaeae* (McEvoy *et al.*, 2012). These types of data can be used in mechanistic models, such as in models developed for the mosquito *Aedes aegypti* (Kearney *et al.*, 2009) that considered the impact of adaptive shifts in egg desiccation resistance.

In terms of adaptation to climate change, clinal studies provide an important source of information on the types of traits and nature of genetic polymorphisms associated

with climatic adaptation. For instance Hoffmann and Weeks (2007) reviewed clinal studies in *Drosophila melanogaster* from the east coast of Australia and reviewed how these studies have made it possible to link candidate genes to adaptive shifts (Hoffmann & Weeks, 2007). By having a clear understanding of candidate loci involved with adaptive shifts, it should eventually be possible to link such information to accurate predictions of evolutionary potential in pest invertebrate populations responding to climate change.

1.2 Review of Earth Mite research

In grain crops in Australia, some of the most recognized pests are mite species. Earth mites such as the redlegged earth mite, *Halotydeus destructor*, and blue oat mites, *Penthaleus* spp., and emerging pest mite species, *Balaustium* spp. mites and *Bryobia* spp. mites, have all increased in outbreak incidence between 1994 and 2008 (Arthur *et al.*, 2011; Hoffmann *et al.*, 2008). Both *Penthaleus* spp. and *Halotydeus destructor* have received extensive surveying efforts (particularly Wallace & Mahon, 1971; Weeks & Hoffmann, 1999; Robinson & Hoffmann, 2001; Arthur *et al.*, 2011) and provide ideal candidates to construct ENMs and determine niche-limiting climatic variables. As *H. destructor* has received much more research attention (see Ridsdill-Smith, 1997), it is used here to apply the framework of ENMs, niche-limiting traits and genetic variation to predict responses to climate change. While this thesis is focused on pest mites of grain crops, the broad framework developed here is applicable to other areas of agriculture and to conservation biology.

1.2.1 *Halotydeus destructor*

The redlegged earth mite, *Halotydeus destructor* Tucker (Acari: Penthaleidae), is a polyphagous mite native to the Western and Northern Cape provinces of South Africa. The species is invasive and also found in Mediterranean-type climates in Australia and New Zealand (Ridsdill-Smith, 1997; Wallace & Mahon, 1971), but is a more serious economic pest in Australia (Ridsdill-Smith & Pavri, 2000). *Halotydeus destructor* emerges during cooler-moist months around April-May. The mites occur mainly in regions with a cool wet winter between May and October (Ridsdill-Smith & Annells, 1997) and undergo three generations, before entering into obligate summer

diapause around October- November (Ridsdill-Smith *et al.*, 2005). Due to its pest status, *H. destructor* has received considerable monitoring and research attention mainly focusing on pesticide efficacy, competitive interactions and potential biological control (e.g. Umina & Hoffmann, 1999; Weeks & Hoffmann, 2000; Halliday & Paull, 2004; Ridsdill-Smith *et al.*, 2008). An adult mite is about 1 mm long and may occur in densities of around 10,000 mites per square metre (Ridsdill-Smith, 1997). The mites are particularly damaging at the establishment phase, where outbreaks can result in entire crop re-sowing (Robinson & Hoffmann, 2001), and reduce the productivity of established pasture (James & O'Malley, 1991). The earliest method of control for *H. destructor* was to watch for the emergence of young mites and then harrow the earth to “crush” the young mites before they reached maturity (Tucker, 1925). Today, control is largely achieved through pesticide application, and this has led to problems with evolved resistance in some populations (Umina, 2007).

Lifecycle

Halotydeus destructor reproduces sexually (Weeks *et al.* 1995) and, in pasture, populations contain more females (70 – 84%) than males (Ridsdill-Smith, 1997). The three generations each take about six to eight weeks on pasture (Ridsdill-Smith & Annells, 1997) and in ideal constant temperature conditions (18°C day/ 11°C night) they take five weeks (Ridsdill-Smith & Gaull, 1995). It takes around 27-31 days for an egg to develop through to adult under natural conditions and the adult will then live for 26-56 days (Ridsdill-Smith, 1997). Winter eggs were found to develop fastest when kept at 27.5°C (22% per day) and post-diapause eggs at 22.5°C (8.7% per day) (James & O'Malley, 1991). Thus, winter eggs are likely adapted to exploit warmer winter temperatures and the increasing temperatures in spring (end of growing season) (James & O'Malley, 1991). These characteristics of the winter eggs of *H. destructor* suggest that milder winters brought about by climate change will benefit the development of the mite and lead to increasing issues with this pest.

Over the summer months, *H. destructor* enters into obligate diapause to survive the hot dry summers. This important part of the life cycle of *H. destructor* has been well studied as it may be crucial to control measures (Ridsdill-Smith *et al.*, 2005). Diapause egg production is at the end of the active season (Umina & Hoffmann,

2003) and the females die leaving the diapause eggs in their cadavers (Wallace, 1970a, Ridsdill-Smith & Annells, 1997). The long developmental period of diapause eggs safeguards against premature hatching during occasional heavy summer rainfalls (Norris, 1950). Diapause is then broken by high temperatures, requiring an equivalent of a month with soil temperatures over 50°C (Wallace, 1970b; Ridsdill-Smith & Annells, 1997).

Distribution changes

H. destructor was first recorded in the Western Cape province of South Africa in 1908 by Jack, where it was causing widespread damage to vegetable crops (Baker, 1995). In 1917 the species was reported in Bunbury, Western Australia and this was attributed to being transported through ships' ballast (Newman, 1925; Johnson, 1930; Baker, 1995). Thus it is very likely that there were subsequent introductions of *H. destructor* individuals from the same or similar South African populations giving scope for a large number of individuals to establish in Western Australia. Initially it was proposed that *H. destructor* was transported through the movement of seedlings and sheep fleeces (Baker, 1995), however it is now thought that the dispersal of diapause eggs by wind and on farm machinery is responsible for the spread of this species (Umina *et al.*, 2012). After being reported in Western Australia in 1917, *H. destructor* spread rapidly across Australia to be recorded in Victoria by 1921 (Newman, 1923) and had a recognized pest-status by 1934 (Swan, 1934); it has since remained a seasonal pest species.

In Australia *H. destructor* is unlikely to be limited by host plant availability, as it is commonly associated with a wide range of plants, grain crops and pasture species, including clover (*Trifolium* spp.), Paterson's curse (*Echium plantagineum*), *Plantago* spp., capeweed (*Arctotheca calendula*), bristly ox-tongue (*Picris echioides*) and *Oxalis* spp. (Ridsdill-Smith, 1997). *Halotydeus destructor* can also feed on algae and mosses (MacLennan *et al.*, 1998), which can provide important food resources for young mites. With many of these host plant species already established in Australia, *H. destructor* was able to spread rapidly across the entire southern grain-belt of Australia (southern Western Australia, South Australia, Victoria, New South Wales and Tasmania).

An extensive survey of *H. destructor* in Australia was carried out in the 1960s to accurately describe the distribution of this species (Wallace & Mahon, 1971). This survey covered areas far beyond the known distribution of *H. destructor* and included the Northern Territory, northern Western Australia, south-eastern Queensland and inland New South Wales. Given that ample time for full dispersal had elapsed since colonization, the range limits of *H. destructor* were thought to be dictated by climate, in particular moisture. Distribution limits coincided closely with the 205 mm rainfall isohyet across the growing season (May – October) (Wallace & Mahon, 1971). Wallace and Mahon determined that summer rain above 225 mm (December – March) was detrimental to mite survival, as was mean monthly maximum temperature of the hottest month (usually January) above 33°C (Wallace & Mahon, 1971; Ridsdill-Smith *et al.*, 2005). These climatic patterns were also incorporated into a simple model of the mite distribution, used to determine the onset of diapause (Ridsdill-Smith *et al.*, 2005).

Thirty years after Wallace and Mahon (1971), further sampling efforts in eastern Australia show that *H. destructor* has undergone range expansion and exists further north (for example, 120 kms further north in north eastern New South Wales) and inland than earlier reported (Weeks & Hoffmann, 1999; Robinson & Hoffmann, 2001). More recently, distribution data from pest outbreaks, control failures and field observations indicate that *H. destructor* occurs in hotter and drier areas, well beyond the long-term average 205 mm rainfall isohyet (Robinson & Hoffmann, 2001; Arthur *et al.*, 2011). This suggests that *H. destructor* has undergone a recent range expansion, perhaps due to adaptation in a niche-limiting trait. Ridsdill-Smith and Annells (1997) concluded that *H. destructor* abundance is regulated by two main traits: rate of oviposition, and mortality of active mites. There has been plenty of opportunity for these traits to have evolved in response to new environmental pressures, and further evolution could mediate responses to future climate change. However, the range expansion may also be due to other factors, including shift in climate or through changes in agricultural practices, such as increased irrigation. Due to the extensive sampling of *H. destructor*, data on this species are suitable for constructing ENMs to detect any shifts between native and invasive ranges and subsequent range expansion in Australia.

Thermal tolerance

The niche of *H. destructor* is likely to be limited by a range of environmental factors, including temperature, moisture and light. The winter active life history of the adult mites means they are well adapted to cool and wet environments and have a highly thermal resistant diapause stage to buffer against summer extreme temperatures. It is important to determine key niche-limiting traits of *H. destructor*, the role they have played in successful invasion, and how these will influence the species' response to climate change. Early studies (Solomon, 1937a; Solomon, 1937b) considered responses of *H. destructor* to temperature, moisture and light. These early studies tended to involve observations rather than quantitative experiments, but they form useful anecdotes of how *H. destructor* responds to environmental parameters, and can aid the design of experiments to characterize traits.

Temperature has been used as a guide to determine *H. destructor* emergence in the field. In Western Australia it is thought that mites will first appear on pasture the first week the maximum temperature falls below 21.5 °C (Ridsdill-Smith & Annells, 1997). Upper limits for *H. destructor* activity were first investigated by Solomon (1937b). Between 18 and 26°C was the range for optimal activity. From 30-32°C temperature started to play a much more dominant role on mite survival than humidity, and at 38°C all mites died (Solomon, 1937b). The lower limits for *H. destructor* activity have not been investigated explicitly, however low temperatures reduce metabolic activity and mites are able to survive at 1.5°C for two weeks with no changes to feeding damage, or up to 3 weeks with reduced fecundity (Liu & Ridsdill-Smith, 2000a). *Halotydeus destructor* populations are adversely affected by cold weather extremes (Liu & Ridsdill-Smith, 2000a) as low abundance generally corresponds with the coldest temperatures of winter (Ridsdill-Smith & Annells, 1997).

Desiccation resistance

Due to their small size and thin permeable cuticle, *H. destructor* is highly susceptible to water loss. Desiccation is avoided through buffering by host plants that provide a

more suitable surface-air boundary microclimate. *Halotydeus destructor* spends most of its time on leaves where it is less affected by air humidity (Solomon, 1937b), and in low temperatures mites survive longer with food plants available, however the presence of food plants does not greatly delay the effect of lethal high temperatures ($> 34^{\circ}\text{C}$) (Solomon, 1937b). The mites spend considerable time close to the ground and on the soil, and this plays a part in reducing desiccation due to wind and leads to increased mite production (Thackray *et al.*, 1997).

Photoperiodic response

The effects of light and day length play roles in the behaviour and diapause of *H. destructor*. In strong sunlight the mites prefer to feed on the underside of leaves (also where eggs are laid – particularly lower leaves) (Solomon, 1937a), though early in the morning and in late afternoon when temperature is low and humidity high, mites will feed on top (Solomon, 1937a; Liu & Ridsdill-Smith, 2000b). Day length provides some cues to initiate diapause, though experimental data is required to determine which life stage is most sensitive to photoperiodic changes (Ridsdill-Smith *et al.*, 2005). In a model to predict summer diapause for *H. destructor*, up to 80% of the observed variability in production of diapause eggs was attributed to day length, and duration of the long-term growing season accounted for another 10%. While day length can be detected directly by the mites, the long term growing season would be detected indirectly, perhaps due to quality (or greenness) of host plants (Wallace, 1970a; Ridsdill-Smith *et al.*, 2005). This work led to the formulation of the TIMERITE model (<http://timerite.wool.com/>, accessed September 2012), to optimize the time for pesticide application for *H. destructor*, before the mites start producing resistant diapause eggs (Ridsdill-Smith *et al.*, 2005).

Genetic variation

There have been two studies on genetic variability of *H. destructor* to date. Firstly, Weeks *et al.* (1995) examined levels of genetic differences based on three polymorphic markers across Victorian populations. Secondly, Qin (1997) examined five polymorphic markers across Australia and South Africa. According to these studies, populations of *H. destructor* exist in Hardy-Weinberg equilibrium and this is

maintained across different generations (Weeks *et al.*, 1995). *Halotydeus destructor* is not divided into races or strains (common alleles same in all populations) (Weeks *et al.*, 1995) and the geographic origin of Australian *H. destructor* is likely to be Cape Town, based on possessing the lowest genetic distance from Australian populations (Qin, 1997). South African populations are more divergent than Australian populations although are all morphologically similar (Qin, 1997). There is some evidence for regional structuring of populations in South Africa. Populations in the Northern Cape province have a different allele to populations in the south near Cape Town (where the same allele occurs as found in Australian populations). Qin (1997) found no evidence of subdivision of populations of *H. destructor* in Australia. Weeks *et al.* (1995) found allele frequencies differed between Victorian sites, although *F* statistics indicated little differentiation over all loci. Thus, there was no association between genetic distance and geographic distance in Victorian populations of *H. destructor*, perhaps reflecting a large population size. A sample from Western Australia did not differ in allele frequencies from the Victorian sites, suggesting that a large population from Western Australia colonized Victoria (consistent with historical movements of *H. destructor*). Weeks *et al.* (1995) also found no correlation between allele frequencies and four environmental variables. This may indicate that there is little divergence (especially environmentally driven) between populations of *H. destructor*, but a more comprehensive study is required.

Both of the previous genetic studies are based on limited loci and include low numbers of populations (Weeks *et al.*, 1995) or individuals (Qin 1997). Population genetic studies for *H. destructor* need to be expanded, to allow for more accurate estimates of gene flow. This information can then be used to compare patterns of neutral variation reflecting population processes to trait divergence reflecting adaptive processes. Such information is also important to determine the extent of spread of resistance genes (Umina, 2007; Umina *et al.*, 2012).

1.2.2 *Penthaleus* spp.

The blue oat mites, *Penthaleus* spp. (Acari: Penthaleidae), are important pests whose future management will benefit from a prediction of likely responses to climate

change. These mites are widespread pests across the circumpolar regions in both hemispheres of the world including Australia (Umina & Hoffmann, 1999; Umina & Hoffmann, 2004). The origin of *Penthaleus* spp. is unknown, but they were first reported in Australia in 1921 (Froggatt, 1921), and by 1934 were recognized as an agricultural pest (Swan, 1934). They feed on pastures and a variety of crops by penetrating the epidermal cells of plants and removing the cellular contents. This can lead to decreases in crop yields and available feed for livestock (Umina & Hoffmann, 2004). Recent advances in the taxonomy of *Penthaleus* spp. have revealed that this is a cryptic species complex (Halliday, 2005a; Qin & Halliday, 1996a; Weeks & Hoffmann, 1999), with three formally described species: *Penthaleus major* (Dugés), *Penthaleus falcatus* (Qin & Halliday) and *Penthaleus tectus* (Halliday), whereas prior research assumed only one, *P. major*.

Like *H. destructor*, *Penthaleus* spp. have a winter-active life cycle and complete two to three generations per year with an obligate diapause over summer (Umina & Hoffmann, 2003; Weeks & Hoffmann, 1999). However, *Penthaleus* spp. all produce diapause eggs earlier than *H. destructor*, with *P. major* producing diapause eggs almost immediately after emergence, *P. falcatus* producing them in early winter and *P. tectus* towards the end of the growing season (Umina & Hoffmann, 2003). This means that targeted control, like TIMERITE, cannot be applied generally to these mites (Umina & Hoffmann, 2003), and differences in such traits may result in differences in response to climate change. The distributions of *Penthaleus* spp. in Australia are all different from one another, though they overlap with each other and the distribution of *H. destructor*. However *Penthaleus* spp. occur in northern New South Wales, where *H. destructor* is not found (Robinson & Hoffmann, 2001; Umina & Hoffmann, 2003). *Penthaleus* spp. also display different tolerance levels to pesticides and this has led to increased control failures and economic losses, as chemical control is one of the only viable options for management (Umina & Hoffmann, 1999).

All *Penthaleus* species reproduce via obligate parthenogenesis (Weeks *et al.*, 1995; Weeks & Hoffmann, 1999). Within Australia, these three species have different plant hosts (Umina & Hoffmann, 2004). The type of host plant and other environmental conditions can also alter the competitive ability of the different *Penthaleus* species

(Umina & Hoffmann, 2005). For example, competitive advantage can change between *H. destructor*, *P. major* and *P. falcatus* in pasture, whereas *P. tectus* appears to be a stronger competitor on wheat and oats (Umina & Hoffmann, 2005). Such differences are important to consider when developing targeted control strategies (Halliday, 2005a). CLIMEX models applied to *Penthaleus* spp. were unable to determine differences between species in response to climate (Robinson & Hoffmann, 2001). However, there is little known physiological information for *Penthaleus* spp., so the well-surveyed distribution data means that an ENM approach may give a better understanding of climatic limits to distributions. However, as identification of *Penthaleus* spp. is only certain in Australia, this restricts available distribution data to studies identified under the current taxonomic revision in Australia (Halliday, 2005a; Qin & Halliday, 1996a), rather than the global distributions of these species.

1.3 Aims of thesis

Currently, there is no predictive framework for understanding pest invertebrate species and the impacts of climate change. This thesis aims to use tools from different disciplines as part of a coherent framework to predict the response of species of earth mites to climate change: namely ENMs, thermal physiology experiments and a population genetics assessment. This framework draws parallel to that of Cook (1931), but expands on this with clearly defined niche concepts and the advent of new tools such as ENMs and population genetics. Figure 1.1 outlines the framework.

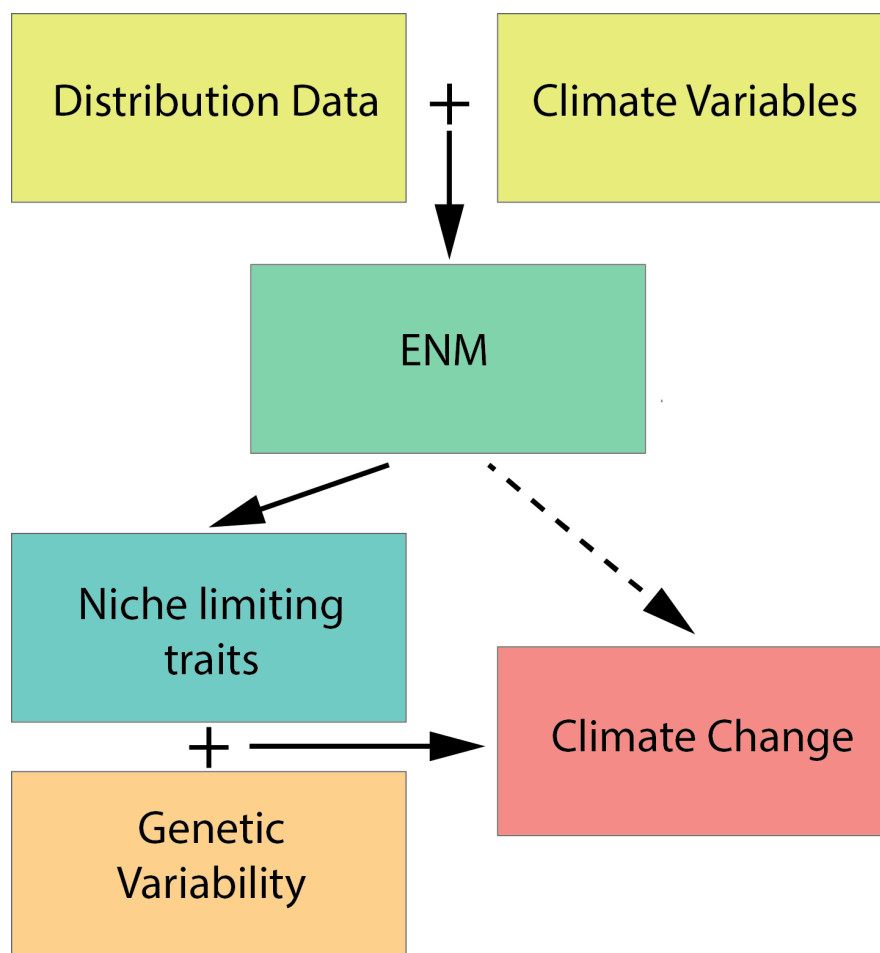


Figure 1.1. Schematic of framework explored in this thesis. Widely sampled distribution data and relevant climate layers are first required to build Environmental Niche Models (ENMs). These ENMs can be projected directly into future climate change (dashed line) scenarios to give an idea of how suitable climate space may shift. By investigating niche-limiting traits and population genetics in concert with ENMs, it is possible to get a more accurate prediction of how climate change will affect the species being investigated.

Many pest species have recorded distribution data and these form the foundation of this framework. These distribution data coupled with high-resolution climate layers can be used to determine the realized Grinnellian niche. By then determining which traits are likely to be limiting the niche of these species, it is possible to examine how these may have shifted for species that have undergone invasion and range expansions, and the extent that phenotypic plasticity may mediate this. Population genetics can be used to determine how divergent populations are and how much gene flow there is between them, to determine potential spread of adaptive shifts and different responses to climate change.

In this thesis I apply this framework using two of Australia's most recognized pest mite groups: the redlegged earth mite, *Halotydeus destructor*, and blue oat mites, *Penthaleus* species. They vary in their biology (Umina & Hoffmann, 2005; Arthur *et al.*, 2011) and pesticide tolerance (Umina & Hoffmann, 1999; Arthur *et al.*, 2008) but all cause extensive damage to a range of crops (Umina & Hoffmann, 2004; Arthur *et al.*, 2011). Climate change may already be driving distribution shifts in these species (Hoffmann *et al.*, 2008), which makes it even more important to understand the response of these species to climate in order to implement better management strategies in the future.

1.4 THESIS OUTLINE

Chapter two of this thesis aims to determine species-environment relationships for each of the *Penthaleus* species. All known distribution information is compiled and then Environmental Niche Models are constructed to test for important niche differences between this cryptic species complex. These models are then projected into future climate change scenarios to examine how suitable climate for each of the three species will change into the future. While this is not a complete predictive assessment it forms the basis for hypotheses of response to climate change.

Chapter three uses Environmental Niche Models to examine niche shift in *Halotydeus destructor* across its native range in South Africa and its invasive range in Australia. This chapter aims to determine if the environmental space occupied by *H. destructor* is different in South Africa and Australia, and if the recent range expansion

in Australia corresponds with changes in species-environment relationships. This chapter also explores some recently developed tools to investigate limiting factors and differences in environmental space between geographic regions.

Chapter four takes the ENMs of chapter three and uses them to select populations in South Africa and Australia that appear to occupy different niches. This chapter aims to test hypotheses that *H. destructor* occupies different niches in South Africa and Australia, by investigating thermal tolerance traits as potential mechanisms. Thermal tolerance traits characterized for Australian populations of *H. destructor* allow for comments to be made on potential evolutionary responses to climate change.

Chapter five looks at the population genetics of *Halotydeus destructor* and extends earlier work beyond allozyme markers. This chapter first aims to test the hypothesis that Australian populations are originally from near Cape Town in South Africa. The chapter also aims to determine if eastern Australian populations are genetically divergent from Western Australia, and if there is any other substructuring to Australian populations. The data from this chapter allow for estimates of gene flow and population connectedness.

Chapter six provides a general discussion on the findings of this thesis and presents future directions for research of *H. destructor*, *Penthaleus* spp. and prediction of pest invertebrate species responses to climate change.

CHAPTER 2: Distribution of cryptic blue oat mite species (*Penthaleus* spp.) in Australia: current and future climate conditions

2.1 Abstract

Invertebrate pests, such as blue oat mites (*Penthaleus* spp.), cause significant economic damage to broad-acre crops in Australia. Climate is a major driver of invertebrate species distributions and climate change is expected to shift pest assemblages and pest prevalence across Australia. At this stage little is known of how individual species will respond to climate change.

I mapped the current distribution for each of the three pest *Penthaleus* spp. in Australia and built environmental niche models for each species using the correlative modelling program, MAXENT. Predictor variables useful for describing the climate space of each species were determined and the models were projected into a range of future climate change scenarios to assess how climate change may alter species-specific distribution patterns in Australia.

The distributions of the three cryptic *Penthaleus* spp. are best described with different sets of climatic variables. Suitable climate space for all species decreases under the climate change scenarios investigated here. The models also indicate that the assemblage of *Penthaleus* spp. is likely to change across Australia, particularly in Western Australia, South Australia and Victoria. These results show the distributions of the three *Penthaleus* spp. are correlated with different climatic variables, and that regional control of mite pests is likely to change in the future. A further understanding of ecological and physiological processes that may influence distribution and pest status of mites is required.

2.2 Introduction

Globally, invertebrate pests cause significant economic losses to broad-acre agriculture. Blue oat mites, *Penthaleus* spp. (Acari: Penthaleidae), are widespread pests of a variety of crops and found across the circumpolar regions in both hemispheres of the world, including Australia (Umina & Hoffmann, 1999; Umina *et al.*, 2004). *Penthaleus* spp. were first reported in Australia in 1921 (Froggat, 1921), and by 1934 were recognised as an important agricultural pest (Swan, 1934). They feed on pastures and a variety of crops by penetrating the epidermal cells of plants and removing the cellular contents. This can lead to decreases in crop yields and available feed for livestock (Umina *et al.*, 2004). The *Penthaleus* complex currently consists of three recognised pest species in southern Australia: *Penthaleus major* (Dugés), *Penthaleus falcatus* (Qin & Halliday) and *Penthaleus tectus* (Halliday) (Robinson & Hoffmann, 2001). The latter two were only recently discovered (Weeks *et al.*, 1995; Qin & Halliday, 1996a; Weeks & Hoffmann, 1999), with studies prior to this considering the *Penthaleus* complex as a single species, referred to as *P. major*. The three species are morphologically cryptic and reproduce via obligate parthenogenesis (Weeks *et al.*, 1995; Weeks & Hoffmann, 1999). Despite the morphological similarities, there are clear differences in the biology and ecology of each species. These include differences in host plants (Umina & Hoffmann, 2004), timing of diapause egg production (Umina & Hoffmann, 2003) and tolerance levels to chemicals (Umina & Hoffmann, 1999; Weeks & Hoffmann, 1999). Such differences are important to consider when developing targeted control strategies (Halliday, 2005a).

Although often occurring sympatrically, *Penthaleus* spp. differ in their distributions within Australia (Robinson & Hoffmann, 2001). Wallace and Mahon (1971) first mapped the distribution of *Penthaleus* spp. and concluded that the inland distribution was determined by a 190 mm minimum rainfall isohyet during the active season (May-October); however this finding preceded the separation of the three species. The distributions of the species in eastern Australia have subsequently been mapped (Weeks & Hoffmann, 1999; Robinson & Hoffmann, 2001). Both *P. major* and *P. falcatus* were found to occupy approximately the same area, although the former was far more abundant. *Penthaleus tectus* was found only in two disjunct regions; one in

north-western Victoria and the other in north-eastern New South Wales. Weeks and Hoffmann (1999) also found that the distribution of *P. major* and *P. falcatus* extends further inland than previously recorded for *Penthaleus* spp. by Wallace and Mahon (1971). Expansion inland may indicate a range expansion, a shift in climate or an adaptive shift in mite physiology. The distribution for *Penthaleus* spp. in Western Australia has not been mapped, although *P. major* has been collected in the State (Qin & Halliday, 1996b).

Climate records for southern agricultural regions of Australia already show rising temperatures and reduced precipitation, and future climate projections suggest warmer and drier conditions (Whetton, 2011). Climate change operating over a global scale greatly influences life history traits (Jaramillo *et al.*, 2009), pest phenology (Harrington *et al.*, 2007; Parmesan, 2007) and species distributions (i.e. range shifts) (Karban & Strauss, 2004; Hoffmann *et al.*, 2008) through changing temperature and precipitation regimes. Although distributions are ultimately driven by a range of abiotic and biotic factors, climate (particularly temperature) is one of the major drivers shaping distribution patterns in most invertebrate species (Bale *et al.*, 2002). Climate has been shown to broadly influence distributions of *Penthaleus* spp. (Robinson & Hoffmann, 2001), as well as other pest mites within Australia, such as *Aceria tosichella* (Carew *et al.*, 2008), *Balaustium medicagoense* (Arthur *et al.*, 2011) and *Halotydeus destructor* (Robinson & Hoffmann, 2001). Although a full understanding of factors such as physiological mechanisms and land-use is required to predict seasonality and fine-scale distributions, predictions of broad climatic patterns that influence species distributions can be made by combining well sampled distributions with long-term averaged climate data (Graham & Hijmans, 2006; Arthur *et al.*, 2011).

Species distribution modelling in the form of environmental niche models (ENM) allows for distribution patterns to be correlated with broad predictor variables such as climate (Phillips *et al.*, 2004; Phillips *et al.*, 2006; Elith *et al.*, 2006). Climate space is closely related to suitable habitat, though fine-scale and biotic interactions will ultimately define local distributions for pest species of agriculture. Such modelling can identify species-specific climate space that can help elucidate ecological processes (Beaumont *et al.*, 2009). Environmental Niche Models can be projected into

future climate change scenarios to examine how available climate space may shift across a landscape (e.g. Pearson & Dawson, 2003; Thuiller *et al.*, 2004; Elith & Leathwick, 2009; Yates *et al.*, 2010), ultimately identifying areas that will be suitable for population growth. In terms of management, this process can assist in targeting surveys of pests and in preparing growers for emerging problems. Robinson and Hoffmann (2001) used a process-based model, CLIMEX, to elucidate key responses of *Penthaleus* spp. to climate. This modelling method provided some insight into different climate variables that may be associated with *P. major* and *P. falcatus*, but not for *P. tectus*, and the method was also unable to separate the distribution for *P. falcatus* from the more widespread *P. major*.

In this paper I provide comprehensive distribution maps for all three pest *Penthaleus* spp. in Australia that extend maps provided by Weeks and Hoffmann (1999) and Robinson and Hoffmann (2001). This information is used to develop correlative models describing suitable climate space, and to identify variables that are correlated with broad distribution patterns. I then project models of mite distributions into future climate space to assess the likely effects of climate change on the broader distribution and pest status of *Penthaleus* species.

2.3 Materials and Methods

2.3.1 Distribution mapping of *Penthaleus* species

Due to previous confusion over the taxonomy and distribution of *Penthaleus* spp. (Umina *et al.*, 2004), I only considered data from within Australia in this study. To compile accurate distributions of *Penthaleus* spp. across Australia, I combined historical data with a comprehensive field survey in Western Australia where information was lacking. In July 2006, a total of 202 sites were sampled across the south-western agricultural region of Western Australia, an area thought to cover the distribution of earth mites based on findings by Wallace and Mahon (1971). The exact location of each site was recorded using a Navman global positioning system (Model iCN 320). Collection of samples took place during the active season of the mites from roadside vegetation and adjacent paddocks every 20 - 50 km. Samples were collected by vacuum using a Stihl blower-vac (Model SH 55) with a fine gauze sieve (200 μ m)

attached. They were placed directly into 70% ethanol and later sorted to species by observing dorsal setae morphology under 40× magnification. Mites were obtained from a variety of plant types at each site to maximise the likelihood of collecting all species present.

Additional *Penthaleus* spp. distribution points were obtained from sampling that targeted other pest mite species (see Arthur *et al.*, 2011). These samples were collected from 2005-2007 and included Victoria, South Australia and New South Wales. A few of these sites yielded *Penthaleus* spp. and from 901 samples I obtained 68 points (*P. major* = 57; *P. falcatus* = 4; *P. tectus* = 7). Distribution data was also included from pest outbreak surveys conducted from 2007-2009 (see Arthur *et al.*, 2011). These were samples received from agricultural industry personnel following mite outbreaks or chemical control failures, and contributed 34 distribution points (*P. major* = 26; *P. falcatus* = 3; *P. tectus* = 5). Participants were asked to include the location of the property where the mites were collected and this was used to determine GPS coordinates through Google Earth (Google Inc., 2009, accessed October 2009). All mites were identified under 40× magnification.

Other distribution data for the three *Penthaleus* spp. was taken from Robinson and Hoffmann (2001), which describes distributions for the *Penthaleus* spp. across eastern Australia by combining survey data and distribution points taken from Weeks and Hoffmann (1999). Maps from Robinson and Hoffmann (2001) were overlaid in ArcMap 9.2 (ESRI 2005) and point data was plotted to extract GPS coordinates.

2.3.2 Climate variables

To use as predictor variables, BIOCLIM variables were obtained from WorldClim (<http://www.worldclim.org> accessed December 2010; Hijmans *et al.*, 2005), which offers high-resolution layers of averaged monthly climate data (1950-2000) from globally distributed weather stations. The primary 19 BIOCLIM variables are derived from averaged monthly temperature and precipitation data (Nix & Busby, 1986) and describe means, trends and seasonal variations of temperature and precipitation, which are more likely to represent physiological limits for species (Graham &

Hijmans, 2006). These variables have been used widely for climate modelling over different temporal periods and for a range of different species' distributions (e.g. Giovanelli *et al.*, 2008; Lozier & Mills, 2009; Murienné *et al.*, 2009; Wang *et al.*, 2010), including pest mites (Arthur *et al.*, 2011). Grid cells were at a resolution of 30 arc seconds, approximately 0.83 km² at the equator.

2.3.3 Distribution modelling

The program MAXENT (version 3.3.2i; Phillips *et al.*, 2004; 2006) was used for modelling the *Penthaleus* spp. distributions. MAXENT is a presence-only based method that correlates known distributions with predictor variables such as environmental variables, and gives model output in terms of habitat suitability. MAXENT has been applied to a variety of ecological modelling applications (e.g. Ficetola *et al.*, 2007; Giovanelli *et al.*, 2008; Lozier & Mills, 2009) and has also been used to project models into future climate space (e.g. Penman *et al.*, 2010; Yates *et al.*, 2010).

While some studies use all 19 BIOCLIM variables as a "complete" dataset (e.g. Giovanelli *et al.*, 2008; Evans *et al.*, 2009), I selected a more informative subset (e.g. Rödder *et al.*, 2009). To do this, each *Penthaleus* species was considered independently. To test for spatial correlation, values of each BIOCLIM layer for each species locality were extracted and Pearson's coefficient tests were performed between each pair of variables using R (R Development Core Team, 2009). Any pair where $r \geq 0.80$ were considered correlated (see Lozier & Mills, 2009). After correlated variables were identified, preliminary models were constructed and the MAXENT jack-knife test was used to examine the importance of each variable and its relationship to each species (see Ficetola *et al.*, 2007). For pairs of variables that were correlated, the variable that added the least unique information and least to model performance was omitted. The models were run again on the reduced dataset in a step-wise fashion. To ensure that reducing the number of predictor variables was not compromising model performance, AUC (Area Under the Curve for the ROC [Receiver-Operator Characteristic]) values were examined for each run of the model (see Elith *et al.*, 2006; Ficetola *et al.*, 2007; Lozier & Mills, 2009). The output for

each run was examined to ensure that the model was not over-predicting habitat suitability. This process was repeated until the model was built on a subset of the most informative predictor variables, without compromising AUC. An advantage of this method is that a reduced number of predictor variables can help avoid multicollinearity issues (Heikkinen *et al.*, 2006; Ficetola *et al.*, 2007). For each model with a final predictor variable set, MAXENT was run with 25% of the training dataset randomly chosen as a test dataset. Each final run of the model was run with 10-fold cross-validation. The remainder of the MAXENT modelling parameters were left at program default (see Phillips & Dudík, 2008).

2.3.4 Future climate scenarios

The A1FI (fossil-intensive) SRES (Special Reports on Emission Scenarios) (IPCC, 2007) future climate change scenario was used in this study. The SRES reflect different societal responses and emission rates whilst accounting for population growth (IPCC, 2007). This particular scenario incorporates a “fossil intensive” projection of emissions incorporated with alternative directions of technological change. BIOCLIM variables were constructed for an ensemble of 23 General Circulation Models (GCM) from the Intergovernmental Panel on Climate Change 4th Assessment Report, for three time periods, 2030, 2050 and 2070 (IPCC, 2007). These were built on a national 9-second Digital Elevation Model (DEM) v3 (GeoScience Australia) aggregated to 36 seconds (~1km), and the change grids and downscaling methods supplied in ANUCLIM v6.1 (Fenner School of Environment and Society, Australian National University). An ensemble of multiple GCMs filters out individual model bias and allows for greater confidence to be placed on outcomes for future projections (see Beaumont *et al.*, 2008). I set a presence / absence threshold at 10% of habitat suitability values from the original models for each species.

2.4 Results

2.4.1 Current distributions of *Penthaleus* spp. in Australia

The survey data greatly increases the distribution knowledge of *Penthaleus* spp. across Australia. In total there are 537 distribution points (236 from this study; 301 from the literature) for *P. major* (Fig. 2.1a), 170 distribution points (16 from this study; 154 from the literature) for *P. falcatus* (Fig. 2.1b) and 66 distribution points (12 from this study; 54 from the literature) for *P. tectus* (Fig. 2.1c). The distribution of *P. major* is widespread across the southern regions of Australia, with the species prominent in Western Australia, South Australia, Victoria, New South Wales and Tasmania (Fig. 2.1a). New distribution points from this study fell within the expected range of previous work. *Penthaleus major* was the most common species collected and is the only *Penthaleus* species previously confirmed in Western Australia (Robinson & Hoffmann, 2001). In Western Australia, the distribution of *P. major* extended as far as Northampton, about 50 km north of Geraldton, and as far inland as Southern Cross, approximately 370 km east of Perth. *Penthaleus major* was found at 153 out of 202 sites sampled in Western Australia.

For the first time, *P. falcatus* and *P. tectus* were reported from Western Australia. *Penthaleus falcatus* was found at seven sites across the sampled area in Western Australia (Fig. 2.1b), three sites in Victoria and a single site in New South Wales. *Penthaleus falcatus* was generally collected from the weed *Hypochaeris glabra* and some grasses which occurred infrequently in paddocks and roadside vegetation. As found previously in eastern Australia, the distribution of *P. falcatus* does not extend as far north as *P. major* in Western Australia. *Penthaleus tectus* was found at seven sites in a restricted area in the south-east of Western Australia (Fig. 2.1c). This area is approximately 250 km by 150 km, and adds to the two disjunct populations previously found in eastern Australia. *Penthaleus tectus* was collected from wild oats and thick-bladed grasses in pasture paddocks, roadside vegetation and adjacent to cereal crops. As well as being reported for the first time in Western Australia, *P. tectus* was recorded from South Australia for the first time.



Figure 2.1. Current distributions of *Penthaleus* spp. within Australia. Closed circles indicate points new from this study, open circles indicate distribution points reported in Weeks & Hoffmann (1999) and Robinson & Hoffmann (2001). (a) Distribution for *Penthaleus major*, $n = 537$. (b) Distribution for *Penthaleus falcatus* $n = 170$. (c) Distribution for *Penthaleus tectus*, $n = 66$. Inset shows names of Australian States and the major regions where grain crops are grown (source: Australian Collaborative Land Use Mapping Program. Available at: <http://adl.brs.gov.au/mapserv/landuse/> Accessed: April 2010). NSW = New South Wales, Vic = Victoria, Tas = Tasmania.

All of the points added for Victoria and New South Wales from this study fell within the disjunct distributions described by Robinson and Hoffmann (2001) (Fig. 2.1c). The new data for *P. tectus* now indicates that the species has disjunct distributions in Western Australia, Victoria and New South Wales, with a single population recorded from South Australia. *Penthaleus tectus* has not been detected in Tasmania.

2.4.2 Climatic factors influencing species distributions

Using MAXENT I was able to determine three climatic variables for each of *P. major*, *P. falcatus* and *P. tectus* that describe the different distributions within Australia (Table 2.1). These variables displayed little to no spatial correlation with each other and the combination of variables for each given species was unique. For all three species, distributions were influenced by "Precipitation of Coldest Quarter (bio19)" and this variable contributed highly to the *P. major* and *P. tectus* models (51.6% and 75.7%, respectively) whilst only contributing 4.7% to the *P. falcatus* model. *Penthaleus major* was influenced by "Mean Temperature of Warmest Quarter (bio10)" and "Mean Temperature of Coldest Quarter (bio11)". The distribution of *P. falcatus* was influenced by "Annual Mean Temperature (bio1)" and "Precipitation of Driest Quarter (bio17)". The model for *P. tectus* incorporated "Temperature Seasonality (STDEV *100) (bio4)" and "Precipitation Seasonality (Coefficient of Variation) (bio15)". The AUC values for the final model for all three species were very close to 1, with 1 indicating optimal model performance (*P. major* = 0.900; *P. falcatus* = 0.919; *P. tectus* = 0.957).

Table 2.1. BIOCLIM variables identified and used for climate modelling for each *Penthaleus* species. Values represent the percentage contribution of each variable to species models after Pearson's and jack-knifing tests.

	BIOCLIM Variable	<i>P. major</i>	<i>P. falcatus</i>	<i>P. tectus</i>
bio1	Annual Mean Temperature		54.9	
bio4	Temperature seasonality			12.6
bio10	Mean Temperature of Warmest Quarter	31.5		
bio11	Mean Temperature of Coldest Quarter	16.9		
bio15	Precipitation Seasonality			11.7
bio17	Precipitation of Driest Quarter		40.5	
bio19	Precipitation of Coldest Quarter	51.6	4.7	75.7

The predicted range for each species is shown in Figures 2-4. The model for *P. major* (Fig. 2.2) shows that the species has suitable climate space to occupy much of the southern regions of Australia. Known distribution points all fall within the predicted suitable habitat. The model also shows an area in southern Queensland as being moderately suitable, although this area is yet to be surveyed extensively. King Island, north of Tasmania, appears to be highly suitable for *P. major*; this area has not been

sampled. In South Australia there is an area of suitable habitat extending north from Adelaide. The model for *P. falcatus* projects an expansive range in eastern Australia, extending further inland than *P. major* in New South Wales (Fig. 2.3). While not as widespread as *P. major*, highly suitable habitat for *P. falcatus* is also predicted to extend into southern Queensland. This model also predicts suitable climate space in Western Australia extending further along the southern coastline than known localities. As for *P. major*, South Australia holds suitable climate space for *P. falcatus*, extending north of Adelaide. The eastern half of Tasmania is also shown as having highly suitable climate space for *P. falcatus*. Predicted climate space for *P. tectus* is tightly associated with the known distribution points in Victoria, New South Wales and Western Australia (Fig. 2.4). Suitable climate space is projected for southern Queensland, though not as far north or inland as either *P. major* or *P. falcatus*. The model also predicts that a large area of South Australia has suitable climate space, though there is only one record of *P. tectus* in this state. The model does not predict suitable climate space for *P. tectus* in Tasmania.

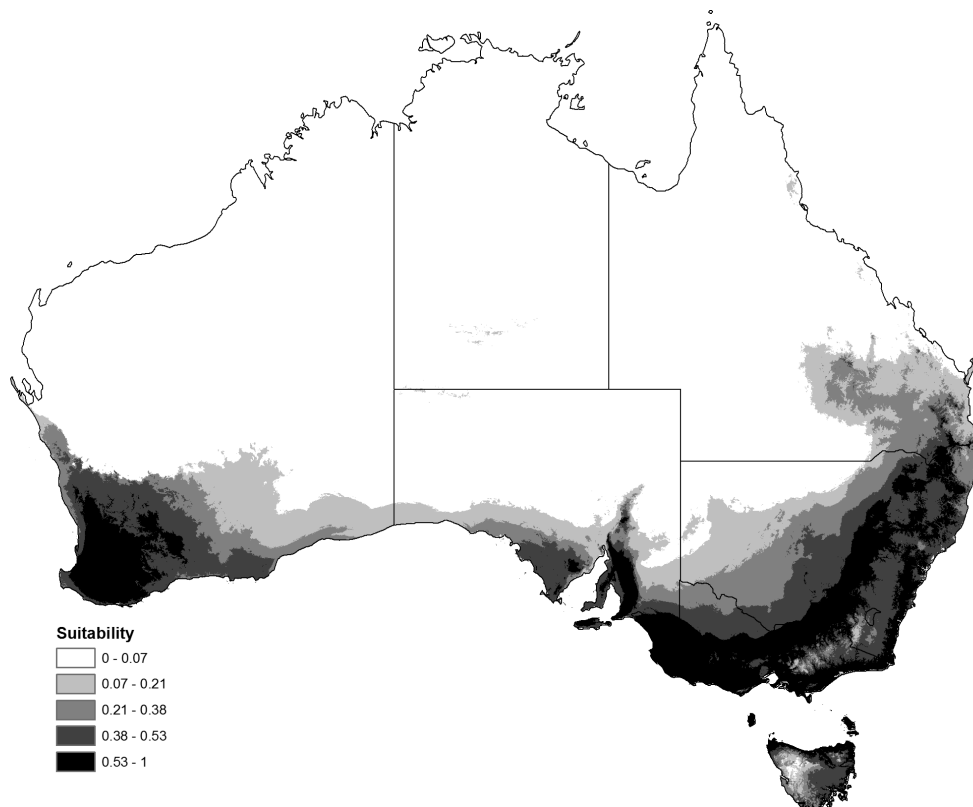


Figure 2.2. MAXENT model output of habitat suitability for *Penthaleus major* in Australia. Shading represents suitability of area in terms of climate space. Model built on present climate (1950-2000 averaged) data and three predictor variables (Table 1). AUC value for model = 0.900.

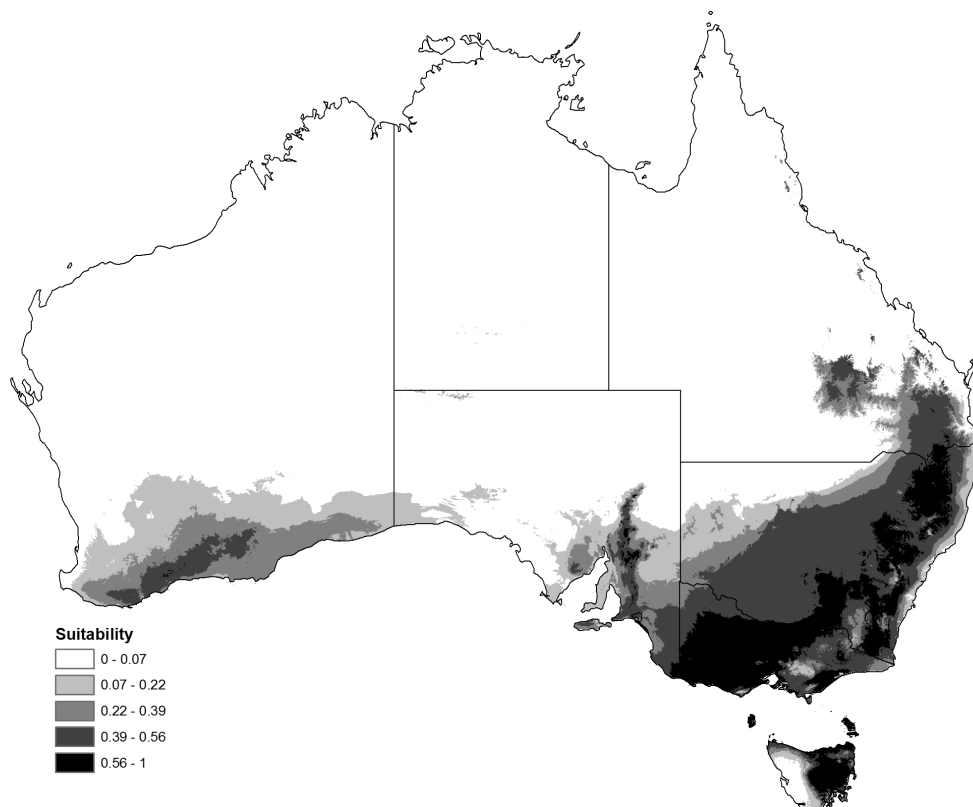


Figure 2.3. MAXENT model output of habitat suitability for *Penthaleus falcatus* in Australia. Shading represents suitability of area in terms of climate space. Model built on present climate (1950-2000 averaged) data and three predictor variables (Table 1). AUC value for model = 0.919.

2.4.3 Future distributions of species under climate change

Under present climate change predictions the climate space for all *Penthaleus* species tends to decrease over time and become fragmented at the southern borders. For *P. major*, the climate space within inland New South Wales decreases by 2030 and continues for each time period thereafter (Fig. 2.5a-c). The northern border is projected to retreat from southern Queensland over time. In Victoria, the climate space for *P. major* remains similar although it retreats somewhat south in western Victoria. In South Australia, the climate space of *P. major* becomes fragmented over time, becoming disjunct from the Victorian climate space by 2070. In Western Australia, the suitable climate space for *P. major* becomes smaller by retreating towards the south-western corner, separating from the other two species (Fig. 2.5a-c).

The climate space for *P. major* in Tasmania is projected to remain the same for the time period investigated.

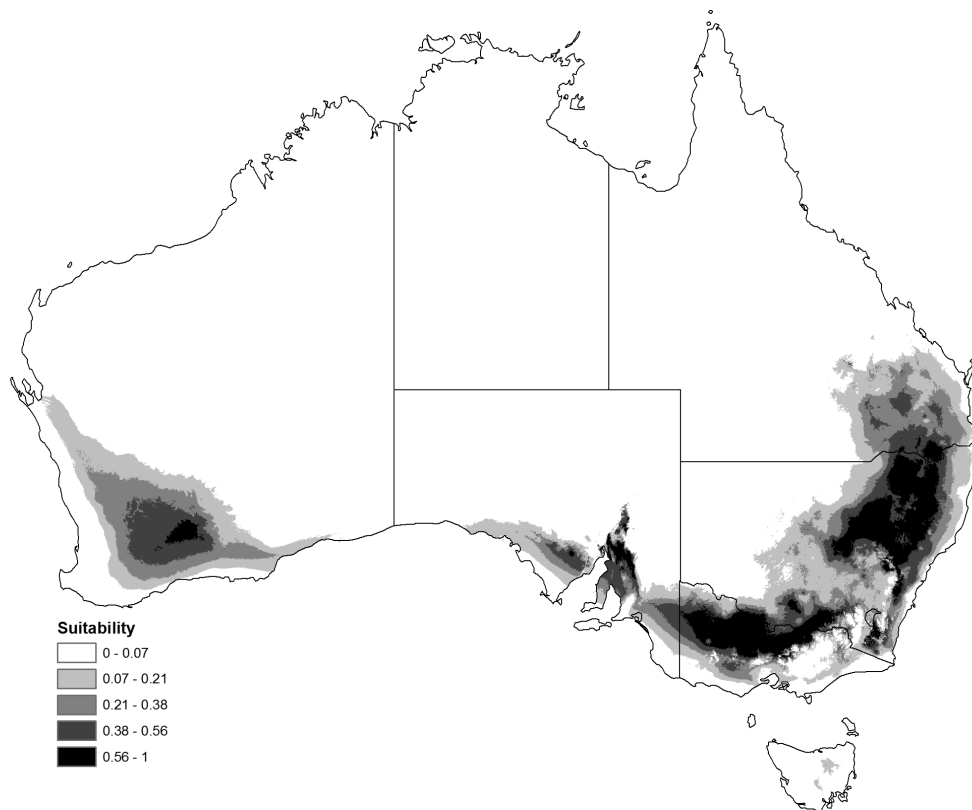


Figure 2.4. MAXENT model output of habitat suitability for *Penthaleus tectus* in Australia. Shading represents suitability of area in terms of climate space. Model built on present climate (1950-2000 averaged) data and three predictor variables (Table 1). AUC value for model = 0.957.

While remaining further inland than *P. major*, the Victorian-New South Wales climate space for *P. falcatus* is projected to retreat both southward and towards the eastern coast of Australia (Fig. 2.5d-f). In South Australia, the *P. falcatus* climate space is significantly reduced to small fragments within a narrow band by 2070. The climate space in Western Australia is also predicted to retreat substantially into two small fragments in the south (Fig. 2.5d-f). Within Tasmania, the climate space changes very little for *P. falcatus* over the next 60 years.

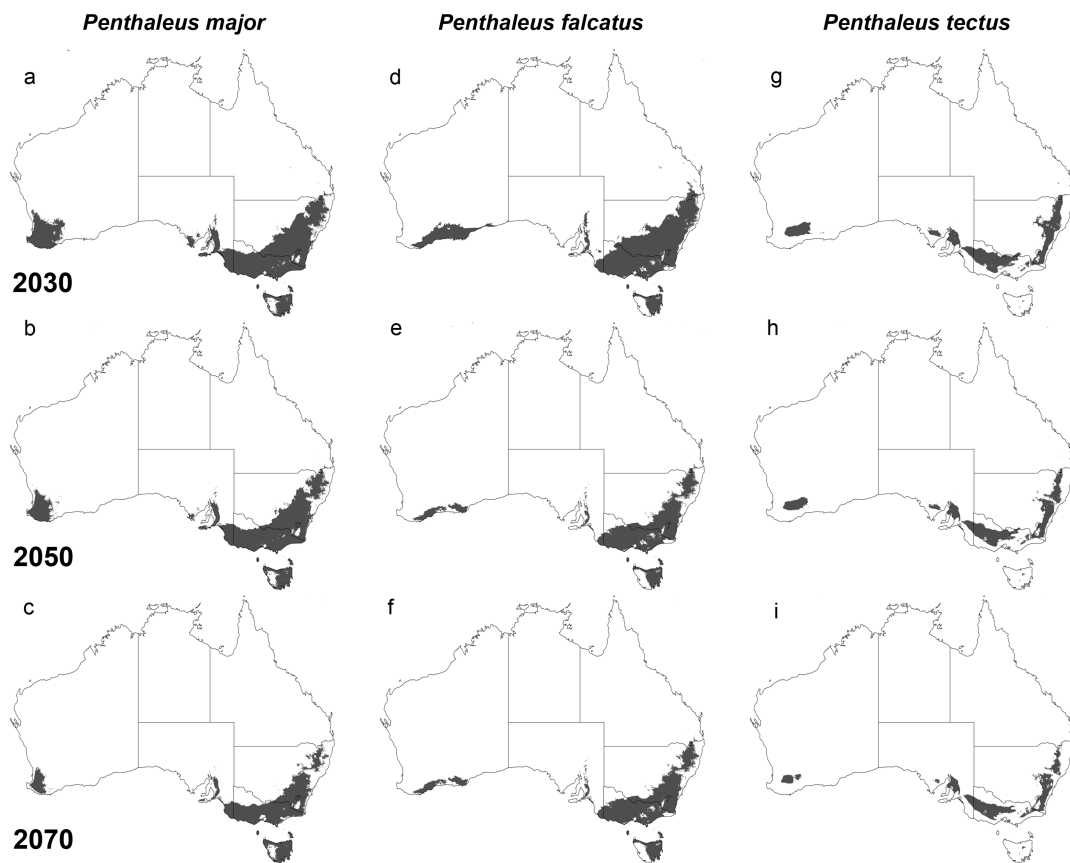


Figure 2.5. Climate change projections for *Penthaleus* spp. in Australia for 2030, 2050 and 2070 under A1FI SRES. Each projection uses the same set of variables as the present-day distribution models. a – c: *Penthaleus major* projections; d – f: *Penthaleus falcatus* projections; g – i: *Penthaleus tectus* projections. Shading represents suitability of area in terms of climate space.

The future climate space for *P. tectus* is projected to broadly encompass the present area, although over time become smaller and further fragmented (Fig. 2.5g-i). This means that in areas such as South Australia and western Victoria, the climate space of *P. tectus* will occupy a different area to that of *P. major* and *P. falcatus*. Similar to the other species, the future climate space of *P. tectus* in New South Wales is expected to retreat southwards and towards the east coast. The position of suitable climate space for *P. tectus* in Western Australia remains similar to the current one, but diminishes through time under each future climate projection (Fig. 2.5g-i). In Western Australia, the future climate space for *P. tectus* is further east than for *P. major* and further north of *P. falcatus*, resulting in non-overlapping climate space projections. There is only a very small amount of suitable climate space for *P. tectus* in Tasmania under future projections.

2.5 Discussion

I present here the current and most accurate Australian distributions for the three cryptic *Penthaleus* species, including Western Australian distributions for the first time. Wallace and Mahon (1971) mapped the distribution of *Penthaleus* spp. in Western Australia, however this was prior to the identification of the three separate species. The distribution of *P. major* mapped in this study broadly agrees with Wallace and Mahon (1971). However, these findings contrast with the situation in eastern Australia, where *P. major* seems to have expanded its range (Robinson & Hoffmann, 2001; Umina *et al.*, 2004). Weeks and Hoffmann (1999) and Robinson and Hoffmann (2001) collected *P. major* further inland than this species had previously been found (c.f. Wallace & Mahon, 1971). This could be a reflection of changing climatic conditions or differing farming practices in eastern Australia, which has created additional suitable habitats for this species.

This study identified *P. falcatus* and *P. tectus* in Western Australia for the first time. Given the widespread and overlapping distributions of *P. major* and *P. falcatus* in eastern Australia, Umina *et al.* (2004) speculated the latter would exist in Western Australia. Although *P. falcatus* was only collected at 7 out of the 202 sites sampled in Western Australia, these sites were relatively widespread across the sampled area. This suggests the low numbers of *P. falcatus* collected in Western Australia may not be due to unsuitable climatic conditions. Instead, *P. falcatus* may be restricted by some other factor, such as host plant availability. *Penthaleus falcatus* is less polyphagous than both *P. major* and *P. tectus*, being largely restricted to some broad-leaved weed species and brassica crops (Weeks & Hoffmann, 1999; Umina & Hoffmann, 2004). These plants were relatively rare across the sampled area (S. McColl, unpublished data), particularly compared with grasses and cereal crops, which are known hosts of *P. major* and *P. tectus*. The presence of *P. falcatus* in Western Australia is important considering the high inherent tolerance of this species to many currently registered pesticides (Umina & Hoffmann, 1999; Robinson & Hoffmann, 2001). In eastern Australia this high tolerance has been correlated with control failures in the field, where *P. falcatus* have persisted after multiple pesticide applications at up to twice the recommended field rate (Umina & Hoffmann, 1999; Robinson & Hoffmann, 2001). Robinson and Hoffmann (2001) showed that control

failures concerning the *Penthaleus* species were disproportionately skewed towards *P. falcatus*, despite this species being relatively rare.

Penthaleus tectus is now known from four disjunct regions in separate States in Australia. This species was found at seven sites within a restricted area northeast of Perth in Western Australia and recorded at one locality in South Australia. The MAXENT model highlights an area north of Adelaide that provides further suitable habitat in South Australia, which indicates future field surveys should target this area. The absence of *P. tectus* in Tasmania may reflect a lack of sampling, although models for the current distribution failed to identify suitable climate space for this species and future projections also predict only a very restricted area of suitable habitat in Tasmania.

The MAXENT models describe separate geographical distributions (or climate space) for the three *Penthaleus* species and link these to different climatic variables, something that was not achieved in an earlier attempt using the semi-mechanistic modelling method, CLIMEX (Robinson & Hoffmann, 2001). Due to a lack of any experimentally determined variables for *Penthaleus* spp., CLIMEX models were unable to discriminate individual species biology and relate these to known distributions, in particular for *P. tectus*. The current and comprehensive distribution for *Penthaleus* spp. enabled us to use MAXENT to generate a suite of potential variables that are associated with known localities for each species, describe suitable climate space and predict how this may change in the future. This is particularly insightful for the morphologically cryptic *Penthaleus* species, which, while often occurring sympatrically, show clear differences in climatic responses (Robinson & Hoffmann, 2001).

The prominence of “Precipitation of Coldest Quarter” in models for both *P. major* and *P. tectus*, and to a lesser extent *P. falcatus*, is supported by the winter-active biology of the mites and strengthens the relevance of the correlative approach used here. It is interesting that *P. falcatus* was strongly influenced by “Precipitation of the Driest Quarter”, as during this time the species is in diapause. This may imply *P. falcatus* has a less-resistant diapause stage than the other species. Because the area where *P. falcatus* is presently found is expected to become drier over the summer

period, a less-resistant diapause stage could help explain why climate space for this species decreases markedly in areas such as Western Australia and South Australia under these projections. In addition, the current distributions for *P. falcatus* and *P. tectus* in Western Australia are largely outside the habitat predicted suitable in these models. This could be due to differences in physiological responses between populations in eastern and Western Australia. Genetic clonal types of *P. major* have been shown to differ in their relative fitness on both spatial and temporal scales (Weeks & Hoffmann, 1998). Additionally, clones of both *P. major* and *P. falcatus* can differ in their response to pesticides, which may reflect selection on a local and geographic level (Umina & Hoffmann, 1999). Although the clonal types of each *Penthaleus* spp. present in eastern and Western Australia have not been directly compared, any differences that exist could affect the observed distribution of the species as a whole. This is particularly apparent for *P. falcatus* and *P. tectus*, as these species appear quite rare in Western Australia.

Correlative models assume all distribution points of a species represent populations that are in equilibrium with climate (Heikkinen *et al.*, 2006). By sampling widely across environmental gradients where a species occurs, correlative models are able to capture at least some variation in predictor variable response across populations (Phillips *et al.*, 2006; Beaumont *et al.*, 2009), simply by incorporating more combinations of environmental conditions (Thuiller *et al.*, 2004; Heikkinen *et al.*, 2006). Sampling widely will also incorporate genetic diversity at some level; however the potential evolutionary ability of populations to adapt to changing conditions would require experimental determination (Hoffmann & Sgrò, 2011). If *Penthaleus* spp. populations do adapt to future climate, these models still provide a prediction of the core future climate space, although the distribution may extend beyond this.

Penthaleus spp. are found throughout the circumpolar regions of the world (Umina *et al.*, 2004), although these records are sparse and species may have been misidentified. Thus, these models cannot be validated with international datasets. Nevertheless, having models constructed in Australia for correctly identified *Penthaleus* spp. will provide useful validation datasets for future models of these species. Seasonal fluctuations will allow *Penthaleus* spp. to expand (or retract) beyond the present ranges projected by these models, although sampling over a range of years, as in this

study, accounts for much of this variation. In addition to climate, the distributions of *Penthaleus* spp. are likely to depend on a range of variables including environmental components (soil type), biotic interactions (competitive exclusion, host plant availability) and farming practices (irrigation and land use) (Hoffmann *et al.*, 2008). Any effect of climate change on these variables is likely to affect *Penthaleus* spp. distributions. While variables such as land use and vegetation information operate at a much finer scale than the climate predictors used in this study, these models may be further enhanced by incorporating such information (Ficetola *et al.*, 2007; Kharouba *et al.*, 2009).

The models show assemblages of *Penthaleus* spp. are expected to change substantially under future climate projections. Although the effects of climate change are not entirely predictable, these models give insight into the overall patterns of future distributions of the three *Penthaleus* species. The models predict localities where ideal conditions are likely to exist in the future for each species, reflecting a broad magnitude of climate impact rather than fine-scale simulation (Pearson & Dawson, 2003). Conditions such as microclimate and host-plant availability will provide some refuge for species under a changing climate. Assuming the availability of suitable host plants, outbreaks of *Penthaleus* spp. would be expected to be more frequent within predicted future climate space than outside it. The climate space available for *Penthaleus* spp. over the next 60 years is predicted to retract across most regions of Australia. To further understand how climatic variables influence present and future distributions of *Penthaleus* spp., physiological parameters (such as moisture and temperature profiles) could be obtained for each species and used in mechanistic models (Kearney *et al.*, 2010a).

Penthaleus spp. distributions are defined largely by climate (Wallace & Mahon, 1971; Robinson & Hoffmann, 2001) and these models reveal current and future climate space where the species are expected to persist as pests. By using correlative models I was able to determine broad climatic processes that influence cryptic *Penthaleus* spp. distributions and how projected climate change may influence them. The results will provide an important basis for future planning of *Penthaleus* spp. management strategies. Future modelling to investigate pest status of earth mites under climate change should focus on specific mechanistic models to incorporate species biological

traits and adaptive potential. This will help identify the potential of each species to shift physiology in response to a changing climate. Areas which displayed congruence between GCMs should be considered carefully, especially those for 2030. In terms of biotic interactions it is also important to understand how climate may affect other pest earth mite species, such as *H. destructor*, which directly competes for resources with all three *Penthaleus* species (Weeks & Hoffmann, 2000, Umina & Hoffmann, 2005).

CHAPTER 3: Understanding niche shifts: using current and historical data to model the invasive redlegged earth mite, *Halotydeus destructor*.

3.1 Abstract

Niche conservatism is key to understanding species responses to environmental stress such as climate change, or arriving in new geographical space such as biological invasion. *Halotydeus destructor* is an important agricultural pest in Australia and has been the focus of extensive surveys that suggest this species has undergone a niche shift to expand its invasive range inland to hotter and drier environments. I employ modern environmental niche modelling methods to examine niche conservatism in *H. destructor*, and highlight ecological differences between historical and current distributions.

I compile comprehensive distribution datasets for *H. destructor*, representing the native range in South Africa, its invasive range in Australia in the 1960s (40 years post-introduction), and its current range in Australia. Using MAXENT, I build environmental niche models and reciprocally project them between South Africa and Australia, and investigate range expansion with models constructed for historical and current datasets. I use several recently-developed model exploration tools to examine the climate similarity between native and invasive ranges, and subsequently examine climatic variables that limit distributions.

The invasive niche of *H. destructor* in Australia transgresses the native niche in South Africa and the species has expanded in Australia beyond what is predicted from the native distribution. These models support the notion that *H. destructor* has undergone a more recent range shift into hotter and drier inland areas of Australia since establishing a stable distribution in the 1960s.

My use of historical and current data highlights that invasion is an ongoing dynamic process, and demonstrates that once a species has reached an established range it may still expand at a later stage. I also show that model exploration tools help understand

factors influencing the range of invasive species. The models generate hypotheses about adaptive shifts in *H. destructor*.

3.2 Introduction

Invasive species lead to significant losses of biodiversity and are harmful to agricultural production, but may serve as valuable model organisms for investigating mechanisms underlying ecological and evolutionary processes over relatively short timescales (Sax *et al.*, 2007). Central to the understanding of invasive species is the concept of the niche (Alexander & Edwards, 2010). Through defining the niche in the sense of G. E. Hutchinson as a suite of species-environment relationships within physical (environmental) and geographical (biotope) space (Colwell & Rangel, 2009; Wiens *et al.*, 2009), it is possible to investigate ecological processes such as biological invasion across large geographic scales. The Hutchinsonian idea of the niche may take the form of the *fundamental* niche - the direct physiological requirements of a species, or the *realised* niche – the proportion of the fundamental niche actually exhibited by the species at a point in time, due to limits set by both biotic and abiotic interactions (Wiens *et al.*, 2009). When this interpretation of the niche is applied to invasive species, it is apparent that climatic similarity of both native and invasive ranges of a species is crucial to invasion success (Ficetola *et al.*, 2007; Thomas, 2010), and that the native range of a species is often able to predict its potential invasive range (Sutherst & Maywald, 2005; Venette & Cohen, 2006).

In an invasive range, a species encounters a geographically isolated set of environments that may or may not allow persistence due to limitations of the fundamental niche that evolved within its native range. An invasive species that occupies geographical regions corresponding to regions of niche space set by the fundamental niche is said to have displayed niche conservatism (Colwell & Rangel, 2009). Conversely, niche shifts describe transgression between species-environment relationships across ranges (e.g. Fitzpatrick *et al.*, 2007; Broennimann *et al.*, 2007), or over time (e.g. Kharouba *et al.*, 2009). Niche conservatism is key to understanding species response to environmental stress such as climate change, or arriving in new geographical space as in biological invasion (Wiens *et al.*, 2009). However, it is difficult to test for niche conservatism, as it is impossible to characterise the complete fundamental niche (Kearney *et al.*, 2008; Jiménez-Valverde *et al.*, 2011). In order to describe niche conservatism, SDM (species distribution models) are commonly employed tools (e.g. Broennimann *et al.*, 2007; Kharouba *et al.*, 2009; Beaumont *et*

al., 2009) that characterise something much closer to the realised niche (Jiménez-Valverde *et al.*, 2011). Therefore, when SDMs are used to measure niche conservatism, they inherently encompass a broad range of factors and include the possibility that “shifts” are not a result of change in the fundamental niche. Niche shifts may occur if the native range only holds a subset of the full range of the fundamental niche due to interspecific competition (the presence of predators and pathogens) or a geographical barrier, or a limited set of possible environments - the species in its invasive range might simply be expressing other parts of the fundamental niche (Broennimann *et al.*, 2007; Rödder & Lötters, 2009, Alexander & Edwards, 2010; Medley, 2010). Alternatively, the species may have adapted, resulting in a change in a species’ response to environmental variables over time (Broennimann *et al.*, 2007; Ficetola *et al.*, 2010). A niche shift may also arise through species dispersal and colonization, driving expansion geographically into new environmental habitats (Alexander & Edwards, 2010). These processes of range expansion may be facilitated by changes in climatic conditions, land use or through evolutionary adaptation.

Environmental niche modelling (ENM) methods such as MAXENT (Phillips *et al.*, 2004; 2006) can model presence-only data within a presence-background modelling framework, in order to produce biologically relevant models for distributions of species in their native range. These can be useful for exploring suitability of habitats in invaded ranges (e.g. Fitzpatrick *et al.*, 2007; Rödder & Lötters, 2009). Reciprocal Distribution Modelling (RDM) (Fitzpatrick *et al.*, 2007) is a method of testing for niche conservatism through constructing ENMs for both native and invasive ranges and reciprocally projecting them onto the alternate range (Fitzpatrick *et al.*, 2007; Medley, 2010). To be effective, a RDM must assume that distributions in both ranges are broad enough to characterise species-environment relationships (Fitzpatrick *et al.*, 2007). This is achieved through comprehensive sampling and establishing that the species has dispersed to all possible environments within the invasive range. Using RDMs, niche shifts have been described for various species such as the fire-ant, *Solenopsis invicta* (Fitzpatrick *et al.*, 2007), spotted knapweed, *Centaurea maculosa* (Broennimann *et al.*, 2007), the Mediterranean house gecko, *Hemidactylus turcicus* (Rödder & Lötters, 2009) and the mosquito, *Aedes albopictus* (Medley, 2010). However, such models need to be applied carefully because they often require

extrapolation into un-sampled environments (Elith & Leathwick, 2009). Model exploration tools extend RDM methods to encourage scrutiny, reduce uncertainty associated with correlative model projections, and allow for predictor variables to be examined spatially across distribution datasets. These tools can demonstrate areas of range shift and important variables associated with niche shifts (Elith *et al.*, 2010).

The redlegged earth mite, *Halotydeus destructor* Tucker (Acari: Pentheleidae), is a polyphagous mite native to the Western Cape of South Africa. *Halotydeus destructor* was first reported in Western Australia in 1917 and was recorded in Victoria by 1921 (Newman, 1923). In Australia it is unlikely to be limited by host plant availability, as it is commonly associated with a wide range of plants, grain crops and pasture species such as subterranean clover, *Trifolium subterraneum*, and the South African-introduced *Arctotheca calendula* (capeweed) (Ridsdill-Smith, 1997). It is now a major winter-active pest across southern Australia, completing three generations between May and October with an obligate summer diapause (Ridsdill-Smith *et al.*, 2005). The species has also been reported from New Zealand, though it is not presently a significant pest (Ridsdill-Smith, 1997).

An extensive survey of *H. destructor* in Australia was carried out in the 1960s to accurately describe the distribution of this species (Wallace & Mahon, 1971). This survey covered areas far beyond the known distribution of *H. destructor* and included the Northern Territory, northern Western Australia, south-eastern Queensland and inland New South Wales. Given that ample time for full dispersal had elapsed since colonization, and because host plants were widely distributed, the range limits of *H. destructor* established at that time were thought to be dictated by climate and particularly moisture; distribution limits coincided closely with the 205 mm rainfall isohyet (Wallace & Mahon, 1971). However, 30 years later, further sampling efforts in eastern Australia show that *H. destructor* existed further north and inland than earlier reported (Weeks & Hoffmann, 1999; Robinson & Hoffmann, 2001). More recently, distribution data from pest outbreaks, control failures and field observations indicate that *H. destructor* occurs in hotter and drier areas, well beyond the long-term average 205 mm rainfall isohyet (Arthur *et al.*, 2011). If the 1960s range of this species represented an equilibrium distribution, these data suggest a recent range

expansion, perhaps due to climatic changes (in particular rainfall), changes in agricultural practice and/or an adaptive shift in physiology.

The apparent niche shift and range expansion in *H. destructor* provide an opportunity to investigate niche conservation during invasion through new modelling tools. In this paper, I build robust models for *H. destructor* by combining data on distribution shifts with correlative modelling techniques. These approaches can provide insights into climatic influences driving species range expansions and potential adaptive physiological shifts now and into the future. I present current distribution information for *H. destructor* in Australia and South Africa, and then investigate climatic variables associated with any niche-shift for *H. destructor* by building RDM methods and expanding on these with recently developed model exploration tools. I identify niche shift for *H. destructor* in Australia by combining historic and current distribution information with long-term averaged climate datasets. Finally, the models are used to form hypotheses and point to future experiments to test them.

3.3 Methods

3.3.1 Species data

Species locality data for *H. destructor* were obtained from a variety of sources. The majority of the distribution information was taken from published literature, extracted as an electronic image and then assigned geographical coordinates in ArcGIS (version 9.2; ESRI 2005). I used this technique to extract data for a historical dataset from sampling conducted in the 1960s (Wallace & Mahon, 1971), which yielded 583 points for Australia. I also constructed a current distribution dataset by extracting point data from Robinson & Hoffmann (2001), which included points from Qin (1997) and Weeks & Hoffmann (1999). This provided 271 locality points. To allow for error in this technique I created a buffer of 25 km around each location assigned to literature records, and randomly sampled a new point in each buffer. This was repeated 10 times for both datasets. All models (described below) were run with the originally assigned location and the ten randomly jittered locations. The resulting mapped predictions were compared using ENMtools (version 1.3, Warren *et al.*, 2008) niche overlap analysis (Schoener's *D* statistics). I then performed a one-tailed *t* test to

determine if the actual dataset was producing models different than those with random error incorporated.

Additional distribution points for *H. destructor* were obtained through surveys undertaken in 2006-2007 across southern Australia. This yielded 174 points from Western Australia, 19 from South Australia, 7 from New South Wales and 24 from Victoria. Samples were collected using a Stihl SH55 blower vacuum (Andreas Stihl AG & Co. KG, Waiblingen, Germany), mostly focussing on vegetation along roadsides. Exact localities were recorded with a using a Navman global positioning system (Model iCN 320; MiTAC International Corp., Taipei, Taiwan). Data from pest outbreak surveys were also included (see Arthur *et al.*, 2011). *Halotydeus destructor* specimens were identified from samples provided by agricultural workers following outbreaks or control failures from 2005 - 2007, contributing another 29 points.

The South African distribution of *H. destructor* is reported in Halliday & Paull (2004). Detailed locality data were obtained for 16 localities from specimens held in the Australian National Insect Collection (Canberra, Australian Capital Territory). I added to this an additional 15 records for *H. destructor* in South Africa from Eddie Ueckermann (pers. comm.).

3.3.2 Climate variables

I used two distinct sets of climate variables that are most appropriate for the two questions and to take advantage of access to a finer temporal resolution for a subset of the area of interest (Australia). For the RDM, bioclimatic variables were obtained from Worldclim (<http://www.worldclim.org> accessed December 2010; (Hijmans *et al.*, 2005)) to use as predictor variables. These variables are based on average monthly (1950-2000) temperature and precipitation data and describe means that define trends, seasonal variations of temperature and precipitation (Graham & Hijmans, 2006). Grid cells were at a resolution of 5 arc minutes, approximately 9.3 km x 9.3 km = 86 km² at the equator. A global aridity index from the CGIAR-CSI (Consortium for Spatial Information - Consultative Group for International Agriculture Research) database (<http://www.cgiar-csi.org> accessed December 2010)

was also included because I expect aridity to limit *H. destructor*. The aridity index was calculated as the mean annual precipitation divided by the mean annual potential evapo-transpiration (multiplied by 10000, for memory management), and therefore represents precipitation availability over atmospheric water demand.

To investigate range shift in Australia, I built bioclimatic variables with 10 km x 10 km grid cells for the two time periods (historical: 1921-1995 and current: 1975-2010) available in the ANUCLIM program (Version 6.1; The Fenner School of Environment and Society, Australian National University; Mike Hutchinson pers. comm.). Whilst these timeframes overlap, no distribution records in the historical dataset were collected after 1970 and no distribution records for the current dataset were collected before 1995, meaning the datasets could only fall within one of these timeframes. To make an aridity layer for the two time periods of interest in Australia, I followed the methods used by CGIAR-CSI (CGIAR, 2010). Due to data availability, a rainfall-adjusted radiation instead of a clear-sky measure was used and an estimate of the ratio of actual to possible hours of sunshine through the effect of clouds on radiation. The final aridity layers for Australia were highly correlated ($r = 0.97$) with the coarser CGIAR-CSI layer used in the RDM.

3.3.3 MAXENT modelling

There is a range of methods for invasive species modelling (see Venette *et al.* (2010)). I constructed *H. destructor* distribution models using MAXENT (Version 3.3.3i), a method specifically designed for presence-only data and shown to have good predictive performance across various applications (Elith *et al.*, 2010; Kearney *et al.* 2010; Medley, 2010). MAXENT has been described elsewhere (Phillips *et al.*, 2004; 2006; Elith *et al.*, 2011), so I only mention those settings and considerations important for this application. MAXENT uses information on the conditions in the region of interest as a basis for comparison with conditions at known presence sites. This means that the regions (or "background") need to be defined. I set the background for the native range to the borders of South Africa and for the remaining models the whole continent of Australia was used. The rationale here was to select regions that represented the areas potentially available to the species (i.e. to which it

might have dispersed if environments were suitable) that were also within the areas that could be considered surveyed given the methods and data available. All the climatic data were ‘unprojected’ and hence had varying cell areas. By default, MAXENT samples background points at the level of the grid cell and assumes equal cell area. To account for this I created my own background sample using the raster package in R (version 2.10.1; R Development Core Team 2009) and methods presented in Elith *et al.* (2011). For the Australian background I sampled 20,000 points that were weighted to reflect a random sample of area, not grid cells. I similarly sampled 10,000 points across South Africa. As considerable sampling for the species was performed along roadsides, I also tested for collection bias. Given no evidence of collection bias in environmental space (see Appendix 1.1), the only bias that I deal with is that resulting from unprojected data

Because climatic variables are often highly correlated and predictors need to be as proximal as possible, I reduced the initial variable set. I performed Pearson’s r correlation tests across all pairwise combinations of the 20 predictor layers for Australia and South Africa in R. Variable pairs were considered highly correlated if $r \geq 0.80$, and in such pairs only the variable which was more relevant to the winter-active life-history of *H. destructor* was retained. To further select variables, the jack-knife feature in MAXENT was used to assess performance of each variable in terms of AUC (area under the curve of receiver-operator characteristic) gain on both test and training data. The AUC score reflects the probability that a randomly chosen presence site will rank above a randomly chosen background site. An AUC score of 0.5 indicates randomness, whilst a ranking of 1.0 indicates perfect model performance (though with presence-background data, as used in MAXENT, the achievable maximum is < 1 (Phillips & Dudík, 2008)). As I am comparing models on a constant set of data, with constant background (i.e. no change in background extent), the use of AUC is reasonable here. My use of AUC was to assess which variables added least to predictive performance of the model, rather than assess overall model performance. Variables that added little to model performance were omitted from the analyses and the model was run again. Each iteration was compared to the initial model that employed all 20 variables, to ensure that models with fewer variables were not under- or over-predicting distributions. Final models were run with 10-fold cross-validation

and the AUC score examined to assess each model's predictive performance across the held out folds.

The complexity of MAXENT models can be controlled through choice of feature classes and regularization parameters (Elith *et al.*, 2011). For this paper, model names follow a “training-projection” convention, where S.Africa = South Africa; Aust.hist = historical Australian; Aust.curr = current Australian. For the S.Africa-S.Africa and Aust.curr-Aust.curr models I left MAXENT settings as default, apart from turning off threshold features. For the Aust.hist-Aust.hist model, I explored how complexity may influence model output, because I was interested in exploring whether my perception of suitable habitat was influenced by model tuning. I turned off threshold features and increased the regularization multiplier at various increments.

3.3.4 Reciprocal Distribution Modelling

To explore niche conservatism across ranges, I built S.Africa-Aust.curr and Aust.curr-S.Africa models. I tried various regularization increases for the S.Africa-S.Africa model to subsequently apply to the S.Africa-Aust.curr model, in an attempt to even out variable response curves. The aim here was to relax model fitting as much as possible in the projected range - to try and match model projections with the current distribution in Australia. I calculated two presence/absence thresholds for the projected ranges by calculating the Least Training Presence (LTP) (Pearson *et al.*, 2007) and a modified Least Training Presence – E (error that was set at 5%) in the training range (Donalisio & Peterson, 2011). The first of these provides a conservative estimate of habitat suitability, and the second allows for a percentage of localities to be geographical and ecological outliers, which for *H. destructor* are likely to be microclimate refuges. To investigate variable differences between ranges, I used multivariate environmental similarity surfaces (MESS) and the most dissimilar variable (MoD) of these MESS maps (Elith *et al.*, (2010), supplementary material), both within MAXENT. Some studies use Principle Component Analysis (PCA) to elucidate predictor variable contributions across ranges (Broennimann *et al.*, 2007; Beaumont *et al.* 2009; Medley, 2010), but these are not spatially explicit. The MESS feature allows for a pixel-by-pixel analysis of the relatedness of a given point to a

reference set of climate layers, whether spatial or temporal, to give a scale of similarity, including negative values (Elith *et al.*, 2010). Its value is driven by the variable for which that pixel is most dissimilar from the reference set, and reported as its distance (in percentiles) from the core of the distribution of values for that variable. The MoD maps report the variable with the smallest similarity at each point (Elith *et al.*, 2010).

3.3.5 Range expansion in Australia

To explore the possibility of recent range expansion for *H. destructor* in Australia, I constructed Aust.hist-Aust.curr and Aust.curr-Aust.hist models and then examined differences between them. First, I subtracted the logistic output of the Aust.hist-Aust.hist from the output of the Aust.curr-Aust.curr to identify which regions held more suitable climate space between models. Secondly, I used ENMtools to quantify niche overlap between Aust.hist and Aust.curr models with Aust.hist-Aust.curr and Aust.curr-Aust.hist projections. For this, mapped outputs from the ten "replicate" models generated during the 10-fold cross-validation were compared with each of the replicates of whichever other model was of interest, and the pairwise differences across these were examined using a Generalized Linear Model. Finally, to examine how variable importance may have changed for both historical and current models, I applied limiting factor analysis in MAXENT to each model (Elith *et al.*, 2010). At each pixel, the limiting factor is determined as the variable that, when changed to its average value at occupied sites, results in the largest positive model value change (Elith *et al.*, 2010).

Results

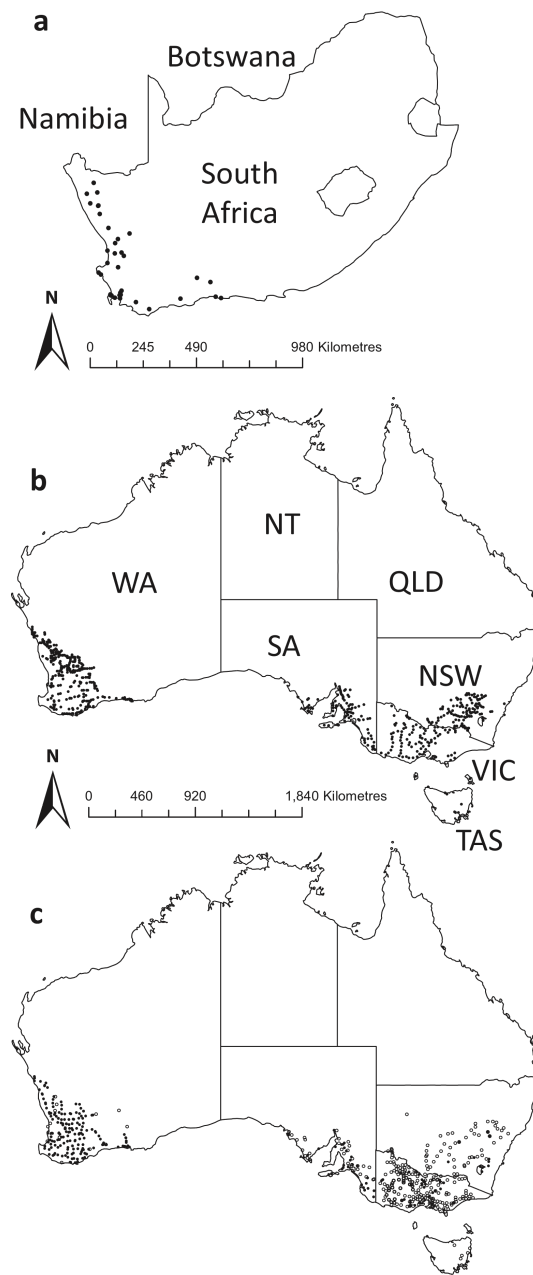


Figure 3.1. (a) Distribution of *Halotydeus destructor* in South Africa (•), sources are (Halliday & Paull, 2004) and Eddie Ueckermann, pers. comm. (b) Historical distribution of *Halotydeus destructor* from the 1960s, as described in Wallace & Mahon, (1971) and denoted as (•). State names: WA = Western Australia; SA = South Asutralia; NT = Northern Territory; QLD = Queensland; NSW = New South Wales; VIC = Victoria; ACT = Australian Capital Territory; TAS = Tasmania. (c) Current distribution of *Halotydeus destructor* in Australia. Distribution described in Robinson & Hoffmann (2001) (including data from Qin (1997) and Weeks & Hoffmann (1999)) and denoted by (○). Points added in this study are indicated by (•).

3.4.1 Distribution fits

The South African native distribution (Fig. 3.1a) is within the Western Cape and western Northern Cape Provinces of South Africa. The historical distribution for *H. destructor* in its introduced range in Australia (Fig. 3.1b) is taken from the comprehensive survey of Wallace & Mahon (1971). The current distribution records for *H. destructor* provide the most comprehensive information in Australia (Fig. 3.1c), however there is no recent data for Tasmania. All new data points added in this study fell within the distribution described by Robinson & Hoffmann (2001), showing an inland, northward expansion into New South Wales since the 1960s.

Table 3.1. Percentage contribution of predictor variables to each model. Percentage based on mean Area Under the Curve of Receiver-Operator Characteristic (AUC) test gain across 10 MAXENT cross-validation replicates. Arid = aridity index; bio4 = Temperature Seasonality (Coefficient of Variation); bio8 = Mean Temperature of Wettest Quarter; bio18 = Precipitation of Warmest Quarter; bio19 = Precipitation of Coldest Quarter.

	<i>n</i>	AUC	Variable contribution				
			arid	bio4	bio8	bio18	bio19
S.Africa	31	0.972	0	6.9	11.6	31.1	50.3
Aust.hist	583	0.941	3.9	3.9	45.7	6.2	41.6
Aust.curr	505	0.930	6.4	2.6	60.0	4.0	27.0

From 20 predictor variables, I ended up with a final suite of five variables (Table 3.1), with three consistently important across all three models. In the South African model, the aridity index was highly correlated with “Precipitation of Warmest Quarter” (bio18) ($r = 0.827$), and did not add to model performance. Instead, model performance was increased by incorporating “Temperature Seasonality (Coefficient of Variation)” (bio4). To maintain consistency (and as MAXENT can fit stable models even with highly correlated variables (Elith *et al.*, 2011)), I used both bio4 and aridity for all models. “Precipitation of Coldest Quarter” (bio19) was the most influential variable in all three models.

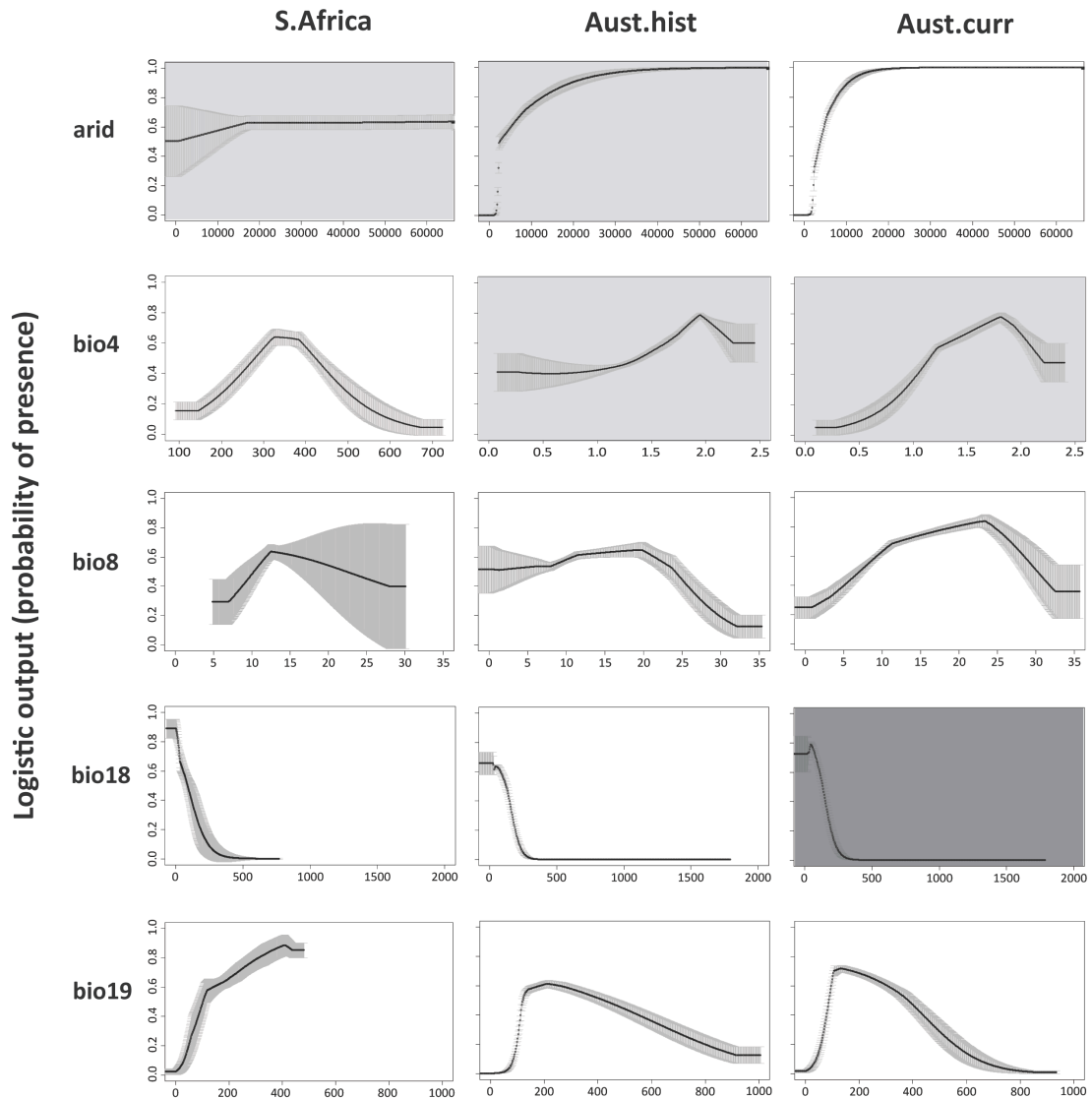


Figure 3.2. Variable response curves for four predictor variables across three models of *H. destructor* built in MAXENT. Variables that contributed less than 5% predictive power to the respective model are shaded grey. The black line on the graphs is the mean of 10 cross-validation replicates, the light grey is the standard deviation across these replicates. arid = aridity index (mean annual precipitation divided by the mean annual potential evapo-transpiration, multiplied by 10000)) ; bio4 = Temperature Seasonality (Coefficient of Variation); bio8 = Mean Temperature of Wettest Quarter (°C); bio18 = Precipitation of Warmest Quarter (mm); bio19 = Precipitation of Coldest Quarter (mm). The variable “bio4” is calculated differently in WORLDCLIM and ANUCLIM datasets so is plotted on different scales.

Figure 3.2 shows the response curves for the variables used in the models. As environmental space is multivariate, and these represent the response to one variable with others held constant, interpretation must be made cautiously. Nevertheless, these responses can provide testable ecological insights. The responses indicate that *H. destructor* is associated with winter temperatures (bio8) in the range 7 - 23°C (Fig. 3.2), within areas that are not too dry (as shown by higher aridity index ($\sim > 20,000$ = semi-arid and above) and winter rainfall around ~100-300 mm (bio19). The modest importance of the declining response to “Precipitation of the Warmest Quarter” (bio 18) reflects the unsuitability of tropical summer wet areas of Australia. Overall the shape of the response curves is similar between Aust.hist-Aust.hist and Aust.curr-Aust.curr models, though both models have different response curves to the S.Africa-S.Africa model. This reflects both the differing environmental conditions in the two areas, and the different relative importance of variables across these ranges/models (Table 3.1). Comparison of the historical and current responses suggests that *H. destructor* currently occurs in more arid areas than historically. This is reflected in the more gradual current decline in suitability at low values of the aridity index, the weakening of the strong historic decline at higher values of “Temperature of Wettest Quarter” (bio8) and higher arch of the curve for drier areas (<200 mm) in the current response for “Precipitation of Wettest Quarter” (bio19) (Fig. 3.2).

The three cross-validated models for historical, current and South African distributions all performed strongly in terms of high AUC scores (> 0.9) estimated on held-out cross-validation data within the training areas (Table 3.1). Testing of the extraction method from the literature confirmed model stability in the face of uncertainty. Variations in locations of up to 25 km did not significantly change model predictions (see Appendix 1.1).

3.4.2 Reciprocal Distribution Model

For the S.Africa-S.Africa model (Fig. 3.3a, left side), there is little prediction outside the convex hull surrounding the known distribution points, even when regularization was increased > 1.0 . The S.Africa-Aust.curr (Fig. 3.3a) model considerably under-projects to the invasive range, including eastern Victoria, though over-projects across the Great Australian Bight (the central area along the southern coast of Australia). The S.Africa-Aust.curr projection does encompass the Australian point of invasion, Fremantle in southwest Western Australia (Swan, 1934).

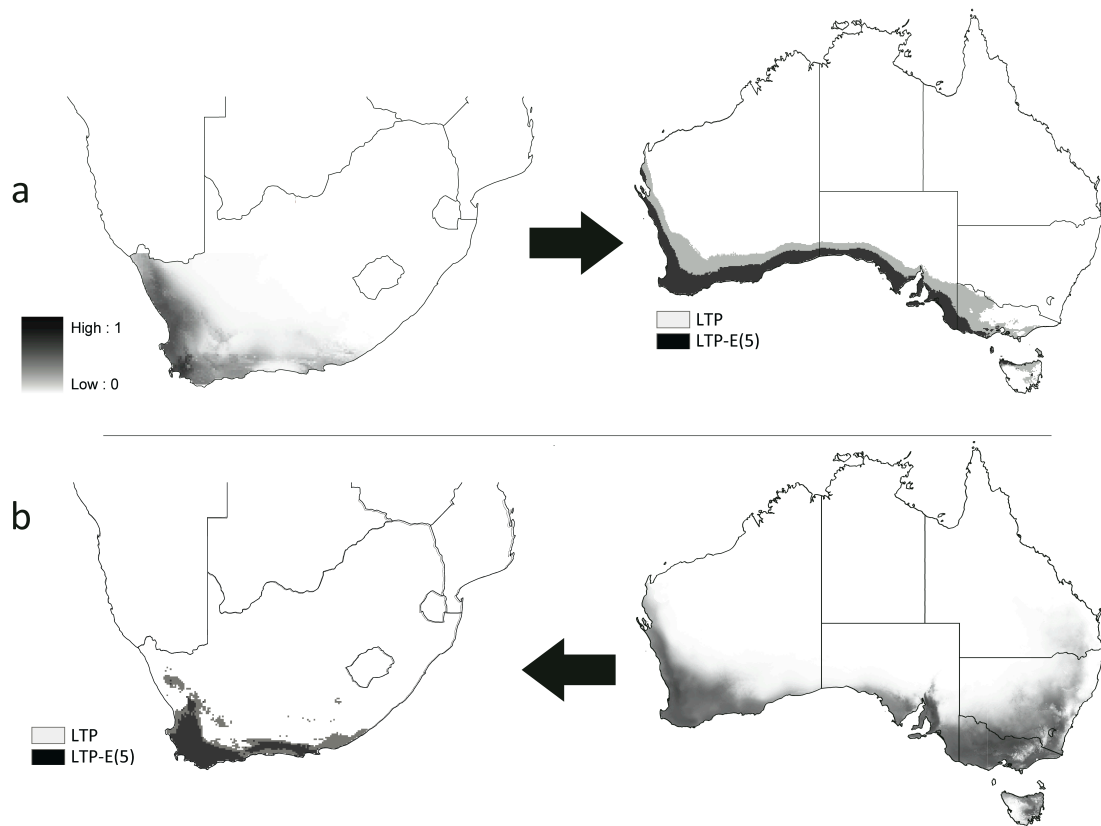


Figure 3.3. Reciprocal Distribution Models between South Africa current Australian distribution built on WORLDCLIM datasets. (a) S.Africa-Aust.curr: South Africa shows the MAXENT logistic output as a continuous suitability (0-1) layer, darker shading indicates higher climate space suitability. In Australia, the presence thresholds are least training presence (LTP) and least training presence omitting the lower 5% of records (LTP-E (5)). (b) Aust.curr-S.Africa: Australia shows the MAXENT logistic output as a continuous suitability (0-1) layer, darker shading indicates higher climate space suitability. In South Africa, the presence thresholds are least training presence (LTP) and least training presence omitting the lower 5% of records (LTP-E (5)).

Increasing the regularization of the S.Africa-Aust.curr model to 3.0 relaxed the model in the projected range, however I was still unable to find congruence with either the historical or current Australian distributions. The Aust.curr-S.Africa model also under-projects suitable climate space in the native range (Fig. 3.3b), successfully predicting the southern half of the native range, but not its northern half.

3.4.3 Range Expansion in Australia

Apart from the highland areas in the Victorian Alps, the Aust.hist-Aust.hist model (Fig. 3.4a) predicts continuous suitable climate space over south-eastern Australia, including the eastern half of Tasmania. The model also predicts Western Australia has suitable habitat across the entire southwest corner. The Aust.curr-Aust.curr model (Fig. 3.4b) is broadly congruent with the Aust.hist-Aust.hist model. The predictions are similar for Western Australia, though slightly broader in the southern parts of the range. In eastern Australia the distribution extends further inland and northwards, but also more suitable habitat towards the south-eastern corner of Victoria. Subtracting the Aust.hist-Aust.hist from the Aust.curr-Aust.curr models highlights the areas of range-expansion for *H. destructor* in Australia (Fig. 3.4c). This is mostly into the New South Wales Riverina area and the Victorian Mallee in the eastern half of the range, inland and north of the historical distribution. Increasing the regularisation parameter in MAXENT from 1 to >3 allowed us to smooth out the variable response curves. However, I was unable to “relax” the Aust.hist-Aust.hist model and push predictions into the range of the Aust.curr-Aust.curr model, suggesting that model complexity is not responsible for under-prediction. The comparison of niche overlap scores within and between models and model-projections is shown in Fig. 3.4d. Within the cross-validated replicates of both models (A & B), there is high overlap as expected (Fig 3.4d.). The replicates within the models overlap significantly more with each other than they do with models built on the reciprocal time series then projected to comparable data (D & E). The least overlap is between current models (fitted and predicted to current data) with historic models (C). The niche overlap values of the two latter model comparisons (D & E) suggest that based on the climate predictors used there may be some facilitation of range-expansion through climate, though it is not enough for reciprocal projections between time-series to be congruent.

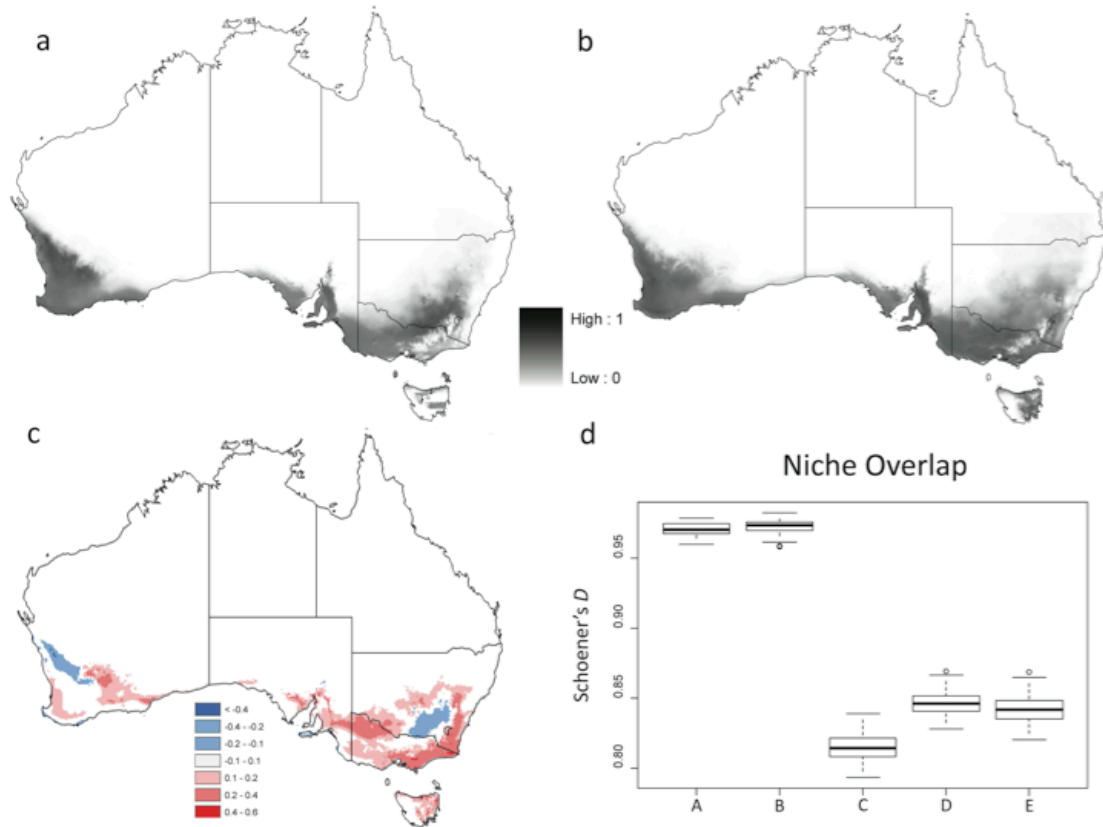


Figure 3.4. Range expansion in Australia. (a) MAXENT logistic output for *Halotydeus destructor* modelled on Aust.hist data, darker shading indicates more suitable climate space. (b) MAXENT logistic output for *Halotydeus destructor* modelled on Aust.curr data, darker shading indicates more suitable climate space. (c) Aust.hist logistic MAXENT output subtracted from Aust.curr MAXENT logistic output. Red areas indicate where more suitable climate space was predicted in the Aust.curr model than Aust.hist. Blue areas indicate where more suitable climate space was predicted in the Aust.hist model than the Aust.curr. (d) Distribution of niche overlap scores (Schoener's *D*) across replicates of the models and model projections; A = Aust.curr-Aust.curr/Aust.curr-Aust.curr; B = Aust.hist-Aust.hist/Aust.hist-Aust.hist; C = Aust.curr-Aust.curr/Aust.hist-Aust.hist; D = Aust.curr-Aust.curr/Aust.hist-Aust.curr; E = Aust.hist-Aust.hist/Aust.curr-Aust.hist. All paired replicate comparisons were significantly different from one another ($P < 0.001$ in all cases), except for the comparison of replicates between A & B ($P = 0.625$).

3.4.4 Model exploration

The MESS map for South Africa (Fig. 3.5b) shows the northern part of the native distribution is found in dissimilar climate space than encountered in the Australian background. Likewise, The MESS map for Australia (Fig. 3.5a) indicates several areas with predictor variables values outside the range encountered across the South African background. These areas identified as dissimilar climate space were not shown to be climatically suitable in the RDMs (Fig 3.3). The MoD maps (Figs. 3.5c & 3.5d) show variables that are most limiting and driving dissimilarity across the MESS maps. For South Africa, “Precipitation of the Warmest Quarter” (bio18) is most dissimilar across the native distribution area. In Australia, “Precipitation of Coldest Quarter” (bio19) is most dissimilar from the South African background across much of the distribution of *H. destructor*. The limiting factor maps (Figs. 3.5e & 3.5f) suggest - for each grid cell - which variable is limiting the species' distribution for the two datasets and models. The climate space that is largely unsuitable for *H. destructor* in the historical map (Fig. 3.5e) is limited by the variable “Precipitation of Coldest Quarter” (bio19). In the current map (Fig. 3.5f) aridity is restricting the northern expanse of the distribution. These results reflect observed changes in the response curves (Fig. 3.2). For both limiting factor maps, “Precipitation of the Warmest Quarter” (bio18) is restrictive down the east coast of Australia.

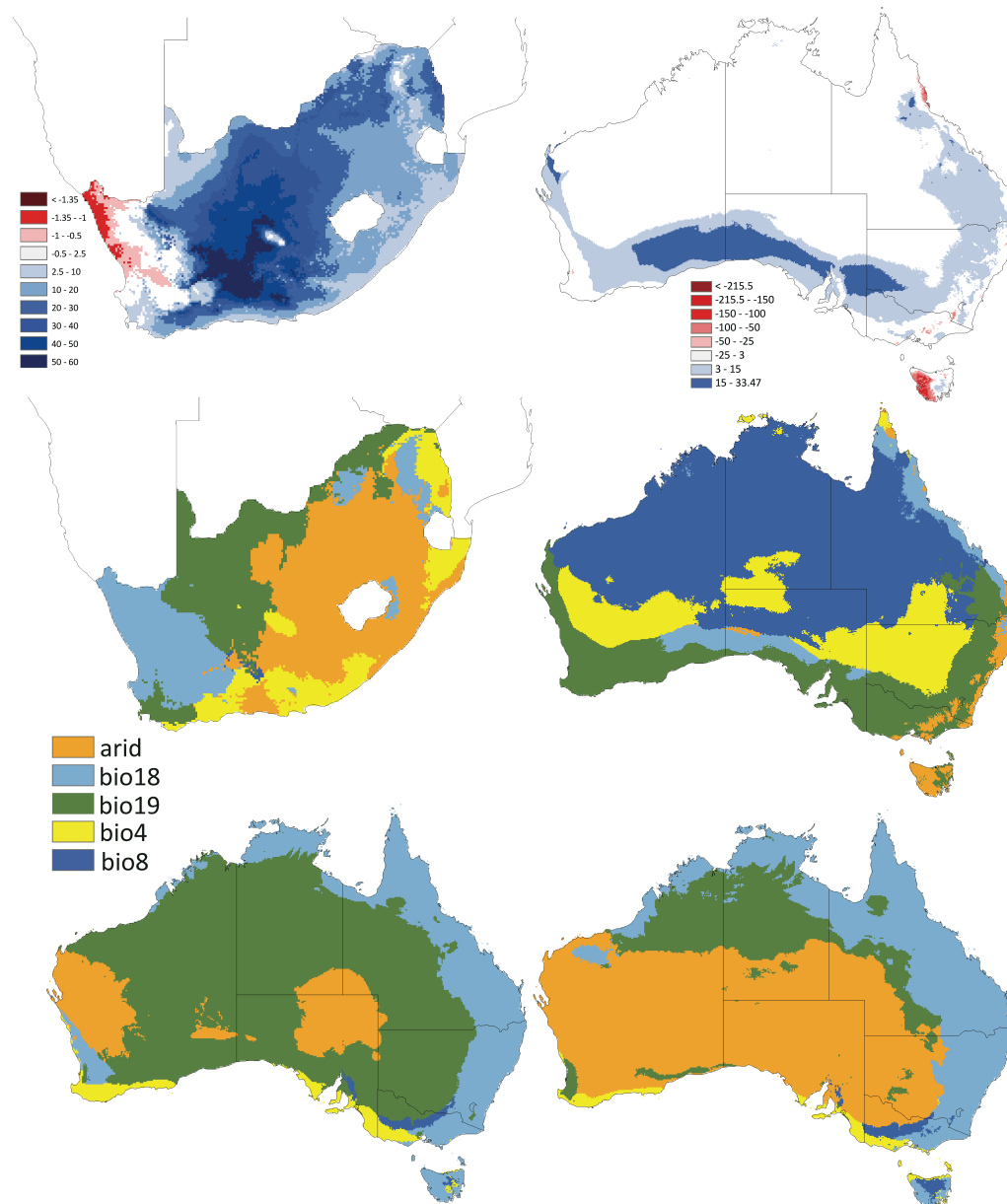


Figure 3.5. Model exploration maps. (a) Multivariate Environmental Similarity Surfaces (MESS) map for South Africa compared to Australia. Scale on MESS maps shows analogous areas (blue), negative values are shown in red and correlate to novel environments from the calibration layers (red) and areas around zero (white). (b) MESS map for Australia compared to South Africa. (c) Most Dissimilar variables (MoD) map for South Africa (from (a)). MoD maps show which variable influences MESS maps at each given pixel on map. (d) MoD map for Australia (from (b)). (e) Limiting factor map for Aust.hist MAXENT model. A limiting factor map shows the importance of each of the five predictor variables in terms of model performance (positive or negative relationship) at each pixel. (f) Limiting factor map for Aust.curr MAXENT model.

3.5 Discussion

Although niche conservatism between ranges is common for invasive species, environmental niche models (ENMs) (e.g. Broennimann *et al.*, 2007; Fitzpatrick *et al.*, 2007; Medley, 2010) points to several cases where niche shifts may have occurred (Alexander & Edwards, 2010). In the present study, models point to niche shift in *Halotydeus destructor*. The species occupies a broader range as an invasive species in Australia than predicted from its native South African range, as well as shifting its niche through range expansion in the last few decades. While the models imply potential shifts in the fundamental niche, a range of factors needs to be considered. Biological constraints such as predation and competition, or other ecological and abiotic factors, may influence the realized niche in the native range (Fitzpatrick *et al.*, 2007; Alexander & Edwards, 2010). Australia lacks significant predators of *H. destructor* (Halliday & Paull, 2004) and competition is restricted to *Penthaeus* mites and perhaps the lucerne flea, *Sminthurus viridis* (Weeks & Hoffmann, 2000; Umina & Hoffmann, 2005). It is unlikely that *H. destructor* is excluded from large areas via competition given that competitive success varies temporally and spatially (Umina & Hoffmann, 2005). Such fine-scale interactions are also unlikely to influence broad scale spatial patterns (Pearson & Dawson, 2003).

The Aust.curr-S.Africa model under-predicted the native range in South Africa. Two factors might contribute to this difference. Firstly, molecular work suggests that populations of *H. destructor* in Australia originated from near Cape Town (Qin, 1997), and the model may reflect the fact that southern populations of South African *H. destructor* near this origin express different physiological limits than northern populations. The Australian populations of *H. destructor* represent one gene pool (Weeks *et al.*, 1995), which may encompass only a subset of the South African gene pool; under-projection may therefore reflect founder effects (e.g. Medley, 2010). Secondly, under-prediction may be due to an artifact of correlative modelling. Values for the predictor variables in parts of the South African landscape suitable for *H. destructor* population persistence were outside the training (invasive distribution) values encountered in Australia. This, combined with the shapes of the modeled responses, resulted in the Aust.curr-S.Africa model not predicting an area above Cape Town as holding suitable climate space, even though native populations exist there.

While analyses such as PCA can determine if two or more distribution datasets cover similar environments (e.g. Broennimann *et al.*, 2007; Fitzpatrick *et al.*, 2007), un-encountered ranges of variables may result in reciprocal under-projection. I suspect that this second factor needs to be considered in other studies examining niche conservatism through ENMs.

The inward range expansion of *H. destructor* in Australia since the 1960s may reflect one or more drivers: (i) a change in farming practices, (ii) a shift in climate or (iii) adaptation. New farming practices in Australia since the 1960s have resulted in arable agriculture expanding into remote areas and areas under irrigation have increased in the eastern states. *Halotydeus destructor* is prone to desiccation (Solomon, 1937a; 1937b; Ridsdill-Smith, 1997), so water availability could directly or indirectly (through host plants) buffer against desiccation. Conversely, much of the area in Western Australia beyond the distribution is not used for extensive agriculture and contains few suitable host plants (Wallace & Mahon, 1971; M.P. Hill, pers. observ.), which may explain why the expansion had been more limited in this region. Conditions across most of the area where *H. destructor* is found have become warmer (mean annual temperature up to 1°C) and drier (mean annual rainfall down by 100 mm) between the historical and current datasets (see Appendix 1.2). However, winter rainfall has increased marginally across the areas where *H. destructor* has expanded (see Appendix 1.2), which may have facilitated a range expansion by providing higher moisture levels within the microclimate of *H. destructor*. The shift in variable importance between historical and current models suggests that winter rain is less limiting in the inland parts of the current distribution. Finally, *H. destructor* may have responded to climatic variables following evolutionary changes in physiological traits. The increase in the importance of the aridity variable moving from the Aust.hist-Aust.hist to Aust.curr-Aust.curr models could reflect an adaptive shift to allow persistence in hotter and drier conditions. Adaptive genetic change can occur rapidly in invasive populations (Alexander & Edwards, 2010). Australian populations of *H. destructor* have recently evolved resistance to several commonly used pesticides (Umina, 2007). The relative importance of these drivers could be elucidated through experimental tests (Rödger & Lötters, 2009; Medley 2010). For example, if adaptation is important, populations from inland/drier locations and populations from

wetter/cooler areas would be expected to differ physiologically when reared in a common environment.

An issue when describing niche conservatism through ENMs is that during invasion, species are not in a state of equilibrium with their climate and thus correlative methods may not reflect physiological limits (Hartley *et al.*, 2010). Whether species are in equilibrium depends on factors such as time since arrival and human aided dispersal (Thomas, 2010). *Halotydeus destructor* has been in Australia for almost 100 years (Ridsdill-Smith, 1997), providing ample time for the species to establish across suitable habitat. Further, *H. destructor* has a relatively short generation time and is able to complete three generations per year (Ridsdill-Smith, 1997). Movement is easily facilitated by wind dispersal and anthropogenic mediation along roadsides, where both *H. destructor* and host plants are often found. This suggests sufficient time and opportunity for the species to occupy all potentially available space in the invasive range.

Sporadic or biased data collection limits the power of inference from models (Fitzpatrick *et al.*, 2007; Hartley *et al.*, 2010) and may give false indication of niche conservatism. *Halotydeus destructor* is a major pest in Australia (Ridsdill-Smith, 1997; Umina & Hoffmann, 2005) and so has been the focus of extensive sampling across geographical space and environmental gradients. These data provide a range of environmental combinations for testing variables associated with distributions (Phillips *et al.*, 2006; Beaumont *et al.*, 2009). Despite good distribution data, correlative models are unable to encapsulate the fine-scale interactions of an organism with its microclimate (Buckley *et al.*, 2010; Hartley *et al.*, 2010; Kearney *et al.* 2010), but instead capture a range of ecological processes between the relatedness of distribution and spatial information (Kearney *et al.* 2010). Mechanistic models that examine limiting physiological processes of a species have been compared with correlative models to identify areas of model congruence and strengthen hypotheses of niche conservatism (Buckley *et al.*, 2010; Kearney *et al.*, 2010a). The models form a comparison for any future mechanistic modelling of *H. destructor*

Halotydeus destructor has potentially increased in outbreak frequency and prevalence within Australia over the last decade (Hoffmann *et al.*, 2008), making it important to

understand factors determining the distribution of this species. The models of *H. destructor* support the use of correlative models as valuable tools to investigate niche conservatism spatially and temporally, and for understanding niche shifts in biological invasions (Broennimann *et al.*, 2007; Fitzpatrick *et al.*, 2007; Medley, 2010). It was particularly useful to visualize non-analogue environmental space and identify the model-based limiting factors. While a complete understanding of niche conservatism in invasive species requires combining species distribution modelling, physiological and genetic approaches (Alexander & Edwards, 2010), the results herein identify niche shift in *H. destructor* and provide clear, testable hypotheses as to how range-shifts may occur.

CHAPTER 4: A predicted niche shift corresponds with increased thermal resistance in an invasive mite, *Halotydeus destructor*

4.1 Abstract

Predicted distributions of invasive species are often not congruent between their realized native and introduced ranges, but reasons for this are rarely investigated empirically. I tested for niche shift in an invasive species using a simple framework combining Environmental Niche Models (ENMs) and niche-limiting thermal tolerance traits.

The redlegged earth mite, *Halotydeus destructor*, a native to South Africa, is a major agricultural pest in Australia and has expanded its range to areas not predictable from its native range in the last 40 years. Revisiting recently constructed ENMs for *H. destructor*, I select populations in both native and invasive ranges that appear to occupy different niches. I characterize thermal tolerance traits and test for acclimation patterns of cold tolerance of these *H. destructor* populations to test for niche shifts.

Australian populations had an increased upper thermal threshold for movement and were able to recover from cold stress more rapidly than South African populations. Australian populations also differed in trait means from the likely source population in South Africa. Acclimation patterns were conserved across ranges for most populations, with 10°C acclimation lowering the onset of and recovery from cold tolerance and 15°C raising them when compared to field-acclimated populations.

These results support the prediction based on ENMs that *H. destructor* may have undergone a niche shift by adapting to environmental conditions in Australia. The increase in thermal resistance has implications for how this invasive species will respond to future climate change.

4.2 Introduction

The potential distribution and spread of invasive species can be predicted by characterizing a species' niche through measuring traits, or more commonly through different types of species distribution models (Kearney & Porter, 2009; Buckley *et al.*, 2010; Jiménez-Valverde *et al.*, 2011). One of the most common species distribution modelling methods for invasive species is the environmental niche model (ENM), which takes a known distribution of a species and correlates it with environmental variables, characterizing an approximate realized niche of a species (Elith & Leathwick, 2009; Jiménez-Valverde *et al.*, 2011).

These approaches suggest that niche conservatism is evident for many invasive species. In a recent survey of invasive plant species only 15% had undergone niche shifts (Petitpierre *et al.*, 2012). Nevertheless, predictions of invasive from native ranges, and the converse, based on ENMs or similar approaches may also prove inaccurate (Broennimann *et al.*, 2007; Medley, 2010; Hill *et al.*, 2012). When using ENMs to match distributions, it is assumed that the niche is conserved in space and time (Kolbe *et al.*, 2010; Medley, 2010), and also that the species is in equilibrium with the environment in which it occurs (Guisan & Thuiller, 2005; Gallien *et al.*, 2012). These assumptions may be violated for invasive species, especially when species have not reached their final distributional extent in invaded areas. However, when the distribution of an invasive species is well sampled in both its native and invasive ranges, reciprocally projecting ENMs between ranges may give an indication of niche shifts (Broennimann *et al.*, 2007; Medley, 2010; Hill *et al.*, 2012), or at least generate hypotheses about factors involved in potential niche shifts.

Niche shifts may be driven by a number of factors, including release from competitors, dispersal ability, climatic shift and adaptive shifts including increased plasticity within invasive populations (Sexton *et al.*, 2009). Adaptation, or “niche evolution” may involve a variety of traits within a given species, and enable a species to adapt to local conditions (Strayer *et al.*, 2006). One of the clearest examples of rapid adaptation in the invasive range is that of the cane toad (*Bufo marinus*) in Australia. The species has displayed extensive niche shift through morphological adaptation, locomotor speed and invasion velocity, facilitating a massive range-

expansion across northern Australia (Phillips *et al.*, 2006; Seebacher & Franklin, 2011). Rapid evolution in niche-limiting photoperiodism traits have also been shown for the invasive mosquito *Aedes albopictus* (Urbanski *et al.*, 2012). Niche-shifts through evolution can thus allow invasive species improved performance in new environments (Ebeling *et al.*, 2008), though often this may be due largely to phenotypic plasticity (e.g. Ebeling *et al.*, 2011) rather than the rapid evolution seen in toads and mosquitoes.

There is increasing evidence that thermal tolerance limits are linked to the distribution ranges of species (Hoffmann *et al.*, 2005; Terblanche *et al.*, 2006; Mitchell & Hoffmann, 2010; Alford *et al.*, 2012). By determining thermal limits for activity of populations or species, it is possible to gain some understanding of their likely distributions (Terblanche *et al.*, 2007), and their potential to invade new geographic regions and deal with climate change (Overgaard *et al.* 2011). Despite this, thermal tolerance traits have rarely been considered across both native and invasive ranges to examine niche conservatism. Terrestrial arthropod species often exhibit differences in thermal tolerance limits across environmental gradients, including elevation and latitude (Gaston & Chown, 1999; Hoffmann *et al.*, 2005), with plastic and inherent variation across latitude more evident for lower than upper thermal limits (Hoffmann *et al.*, 2005; Terblanche *et al.*, 2006; Alford *et al.*, 2012; Hoffmann *et al.*, 2012). It should be possible to explore the adaptive significance of this variation in invasive species by linking thermal traits to ENM predictions.

The invasive redlegged earth mite, *Halotydeus destructor* Tucker (Acari: Pentheleidae), is native to South Africa and an important agricultural pest in Australia (Ridsdill-Smith, 1997). Australian *H. destructor* populations are likely to originate from a population(s) near Cape Town (Qin, 1997) followed by a rapid spread across the southern grain-belt of Australia. *Halotydeus destructor* emerges during cooler-moist months around April-May and undergoes three generations, before entering summer diapause around October-November (Ridsdill-Smith, 1997; Ridsdill-Smith *et al.*, 2005). In the last 40 years *H. destructor* has undergone range expansion, moving inland to hotter and drier areas in eastern Australia. Due to its pest status, *H. destructor* has been extensively sampled and researched, mainly focusing on pesticide

efficacy and resistance (Umina *et al.*, 2012), competitive interactions (Umina & Hoffmann, 2005) and potential biological control (Halliday, 2005).

Environmental niche models were recently applied to *H. destructor* distributions in both its native and invasive ranges, and suggested a niche shift in this species (Hill *et al.*, 2012). *Halotydeus destructor* exists in environments in Australia different from those in the native range in South Africa, and vice versa. Further, the ENMs also identified that aridity now limits the inland distribution of *H. destructor*, as opposed to a historic limit associated with winter rain, which may reflect an adaptive shift facilitating range expansion (Hill *et al.*, 2012). Thus, changes in traits associated with aridity (particularly thermal and desiccation tolerance) may correlate with shifts in distributional limits. However, the range expansion could be due to other factors, including shifts in climate, changes in agricultural practices such as increased irrigation, or through changes to competitive, predatory or other biotic interactions in an area.

Halotydeus destructor therefore provides a unique opportunity to determine if apparent niche shifts in an invasive species can be linked to changes in thermal tolerance traits. I first revisit ENMs constructed by Hill *et al.* (2012) to select populations in both South Africa and Australia that appear to occupy different niches. Guided by these ENMs I test thermal tolerance traits in both native and invasive ranges, predicting differences in either basal thermal tolerance traits or plasticity that may have facilitated niche shifts. I also predict that South African populations display more variation in these thermal tolerance traits than do Australian populations, based on relatively higher genetic diversity in South African populations (Qin, 1997). Further, I predict that Australian populations will be most similar in thermal tolerance to the putative source population near Cape Town (Qin, 1997). The ENMs and thermal tolerance traits together therefore provide a framework to understand niche shifts in this invasive species.

4.3 Material and methods

4.3.1 Sampling and ENM construction

Halotydeus destructor has a wide range of host plant species that are common to both native and invasive ranges. I sampled roadside vegetation through agriculturally developed regions in both South Africa and Australia, targeting broad-leaved plants including clover (*Trifolium* spp.), Paterson's curse (*Echium plantagineum*), *Plantago* spp., capeweed (*Arctotheca calendula*), bristly ox-tongue (*Picris echioides*) and *Oxalis* species. Samples were taken using a Stihl SH55 blower vacuum (Andreas Stihl AG & Co. KG, Waiblingen, Germany), with a metal sieve and specimens placed directly into ethanol.

Methods for constructing ENMs for *H. destructor* are described in Hill *et al.* (2012). I added these new locality data, using GPS data recorded at each site, to revise the models (see Appendix 2.1). To determine if there were any significant changes from the models developed by Hill *et al.* (2012), I performed niche equivalency tests with Schoener's *D* in ENMtools (version 1.3; Warren *et al.*, 2010) between all replicates of the original models and all replicates of the reconstructed models.

4.3.2 Site selection

Using the ENMs, I selected sites in both South Africa and Australia that may reflect different niches that *H. destructor* occupies within both geographic regions. To examine these regions in terms of temperature differences, I sampled across ENM output and extracted monthly minimum and maximum values (see Appendix 2.2). I determined the northern inland limit of *Halotydeus destructor* by regularly sampling along roadsides, about 45 km North of Hay, New South Wales. I sampled four additional sites in New South Wales and Victoria and selected a further population at Conara, Tasmania. In South Africa I selected six populations from the southern end of the Western Cape province at Bredasdorp into the Northern Cape province at Steinkopf, based on population localities reported by Qin (1997). All samples were collected and experiments performed between June and October 2011. Experimental

samples were collected using the same technique as for point locality data, except that mites were kept alive in containers with moist paper towel and fresh *Trifolium* spp. leaves. This is a standard method of earth mite collection for ecological studies or pesticide efficacy (e.g. Umina & Hoffmann, 2005; Umina *et al.*, 2012). For each population, individuals were acclimated at either 10°C or 15°C (14h/dark:10h/light cycle) for two weeks, with fresh *Trifolium* spp. leaves supplied every few days (see below). These two temperatures reflect the lower limit to development (10°C) and close to the optimum development (18°C – but mites held at this temperature constantly for two weeks show mortality, so it was lowered to 15°C) from mass-rearing methods (see Ridsdill-Smith, 1997).

4.3.3 Experiments

All experiments were conducted using a thermoregulator controller (Australia: Grant GP-250/ South Africa: Grant PZ1) linked to a waterbath (Australia: Grant R2 / South Africa: Grant LTC) (Grant Instruments (Cambridge) Ltd, UK). I used a 50:50 mix of propylene glycol:water solution in these baths and the temperature controlled fluid was pumped through channels in aluminium blocks (see below), allowing for accurate changes to temperature. Thermal tolerance traits were measured through ramping assays employing a dynamically changing temperature, which are likely to be more ecologically relevant than static assays (Mitchell & Hoffmann, 2010; Overgaard *et al.*, 2011). As variation in size may impact on thermal tolerance traits, I selected young adult mites of similar size for each experiment. I did not attempt to determine sex of the mites, though avoided the larger adult mites, which are reproductive females (Ridsdill-Smith, 1997). In South Africa *Halotydeus destructor* occurs sympatrically with *Halotydeus anthropus* Qin & Halliday, and is not readily distinguishable in the field. All South African field samples were identified to species *post-hoc* examining coxal setae under 100X magnification (Qin & Halliday, 1996).

4.3.4 Heat tolerance

To assess how high temperatures affect the voluntary activity levels of *H. destructor*, I constructed an aluminium block (Hazell *et al.*, 2008), with an arena (7.5 mm depth,

25 mm diameter) connected to the waterbath. The entire top surface of the block was covered with a Perspex block (50 x 100 x 10 mm). A small fan was placed next to the arena to prevent condensation. I used Fluon (Blades Biological, UK) on the walls of the arena to keep individuals on the arena floor. However, some individuals overcame this barrier and were excluded from analysis. Experiments were recorded using a Dino-Lite AM411 digital microscope (AnMo Electronics Corporation, Taiwan) and DinoXcope software (AnMo Electronics Corporation, version 1.3.4). Video was analysed frame-by-frame using VLC media player (VideoLAN, version 1.1.10, available at: <http://www.videolan.org/>, accessed July 2011). Temperature of the block was recorded every 10s with an embedded Universal Temperature Probe (Model EI-1034; Electronic Innovations Corp., USA) connected to a LabJack data acquisition device (Model U3; LabJack Corp., USA).

The heat movement threshold (HMT) (Alford *et al.*, 2012) is defined as the temperature at which the organism is unable to maintain voluntary coordinated function such as walking (Hazell *et al.*, 2008). Heat coma temperature (HCT) is defined as when all movement of the organism, such as appendage twitching, ceases (Hazell *et al.*, 2008; Alford *et al.*, 2012). Each run consisted of an initial period of five minutes at 18°C (optimal rearing temperature - (Ridsdill-Smith, 1997)) and then a fast ramp-incline to 28°C (0.5°C/min). A ramp-incline (0.1°C/min) was then conducted from 28°C until complete heat coma (indistinguishable from mortality). I only considered individuals that could be scored for both traits (see Table 4.1 for sample sizes). The HMT and HCT experiments were carried out on the first day and the critical thermal minimum (CT_{MIN}) onset/recovery experiments on the third and (if necessary) fourth days post acclimation.

4.3.5 Cold tolerance

Thermal tolerance of cold temperature varies across environmental gradients for temperate arthropod species (e.g. (Hoffmann *et al.*, 2005; Terblanche *et al.*, 2006) and limit distributions of species such as *Drosophila* spp. (Kellermann *et al.*, 2012). I measured CT_{MIN} by constructing an aluminum block (100 x 100 x 10 mm) with 19 wells (5mm diameter). The wells were arranged in a circle and the middle well was used as a control, with a thermocouple (Type K) secured in place. Due to the

relatively quick ramping rate and the active nature of the mites, replicates consisted of around 10 individuals for ease of scoring. A piece of Perspex (100 x 100 x 3 mm) was placed on top of the arenas. Mites were viewed under 40X magnification and scored directly.

I tested field-collected, 10 °C -acclimated and 15 °C -acclimated individuals from each population with the CT_{MIN} ramping profile of Sinclair *et al.* (2006) which measures both an onset and recovery trait. The temperature was cooled from 18 °C down to 8 °C (0.5°C/min) and then taken down further to -5 °C (.25°C/min), to measure the onset of CT_{MIN}. I then held the temperature for 5 minutes before ramping up (0.25 °C/min) until complete reanimation of all individuals, recording this as the recovery trait from CT_{MIN} (Sinclair *et al.*, 2006). Only individuals that could be scored for both traits were considered for analysis. The number of individuals tested per population and country are shown in Table 4.1.

4.3.6 Statistical Analysis

All statistical analyses were performed in SPSS (version 20; 2011, IBM). To test for differences in HMT and HCT trait means between Australian and South African populations, I performed nested ANOVAs. Population was nested within country and included as a random effect. To test for differences in CT_{MIN} onset and recovery traits, I performed nested ANOVAs as for HMT/HCT, but added acclimation treatment as a fixed effect to the models and as an interaction term. I initially included an interaction term between acclimation and population (nested) but this term was incorporated into the error term to increase the denominator degrees of freedom in F tests, although the outcome of the analyses was the same regardless of the approach followed.

I tested if thermal tolerance trait means vary within Australia or within South Africa by constructing temperature covariates that may influence populations of *H. destructor*. The first consisted of the weather station data nearest to each sampling point (Australian Bureau of Meteorology [accessed February 2012], South African Weather Service [requested February 2012]). I obtained two weeks of temperature data prior to each field collection, summarized the daily upper and lower values into

temperature extremes, and built an isothermality index (daily min and max values divided by means for time period). To test for population differences I collapsed aggregated replicates to a single population mean and ran general linear models for each country respectively, incorporating these covariates.

Stellenbosch is approximately 50 km from Cape Town and is likely to represent a population close to the one that first colonized Australia. I tested if Australian populations were significantly different from this source population by performing one-way ANOVAs between Stellenbosch and Australian populations for each trait.

4.4 Results

4.4.1 Distribution modelling

I added 19 distribution points to the South African dataset and 91 to the Australian dataset, including 15 in Tasmania. This gave a total of 50 points for South Africa (originally 31) and 621 points for Australia (originally 530). The revised models following the methods outlined in Hill *et al.* (2012) are presented in Figure 4.1. I found no significant difference in niche overlap scores (Schoener's *D*) between all sets of replicates for the original and reconstructed models with the new distribution data. Consequently, the revised ENMs do not reveal any new information about the niches of *H. destructor*, but support the original ENMs of Hill *et al.* (2012).

4.4.2 Populations sampled

Figure 4.1 shows the reconstructed ENMs of Hill *et al.* (2012) and locations of populations sampled from geographic areas suggesting different environments from model predictions. In particular, the region of range expansion in Australia is characterized by having lower minimum temperatures across winter months than both the native South African distribution and the historical distribution in Australia (Appendix 2.2). I used six populations in South Africa and six populations in Australia to assess HMT and HCT (Table 4.1). For CT_{MIN} I only tested four populations in Australia and three in South Africa due to time limitations.

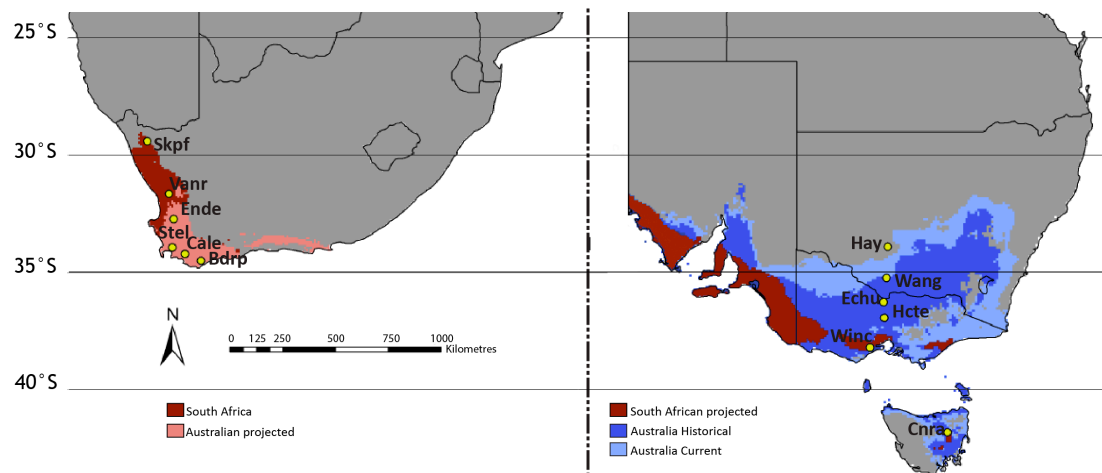


Figure 4.1. Population sampling of *Halotydeus destructor* in Australia (right) and South Africa (left) (yellow circles) on top of the revised ENMs of Hill *et al.* (2012). Refer to Table 1 for population names. Shading in South Africa represents model prediction for South Africa (dark red) and model projection from Australia (pink). Shading in Australia represents South African model projection (dark red), historical model prediction (blue) and present distribution prediction (light blue). All models were thresholded at least training presence – 5% error (LTE-5) to display core climatic suitability for each dataset.

4.4.3 Heat tolerance

For HMT and HCT, I tested 557 individuals of *H. destructor* across the twelve populations (Table 4.1). South African populations differed significantly for both heat tolerance traits, contributing to an overall population effect (Table 4.2; Fig. 4.2). There was no significant difference among populations of *H. destructor* in Australia for either HMT or for HCT (Table 4.2). Regression analysis indicated that isothermality of temperatures two weeks prior to collection was associated with Australian population means of HCT ($t = -3.184$, $df = 1$, $P = 0.033$), but not for South Africa ($t = 1.138$, $df = 1$, $P = 0.319$).

For both heat tolerance traits, means were higher in the invasive populations (Figure 4.2), and the effect of country was significant for HMT and marginally non-significant for HCT (Table 4.2). Heat movement threshold (Figure 4.2) was lower for South Africa (mean = 31.84°C) than Australia (mean = 33.58°C). Heat coma

temperature (Figure 2) was also lower in South Africa (mean = 34.96°C) compared with Australia (mean = 35.83°C).

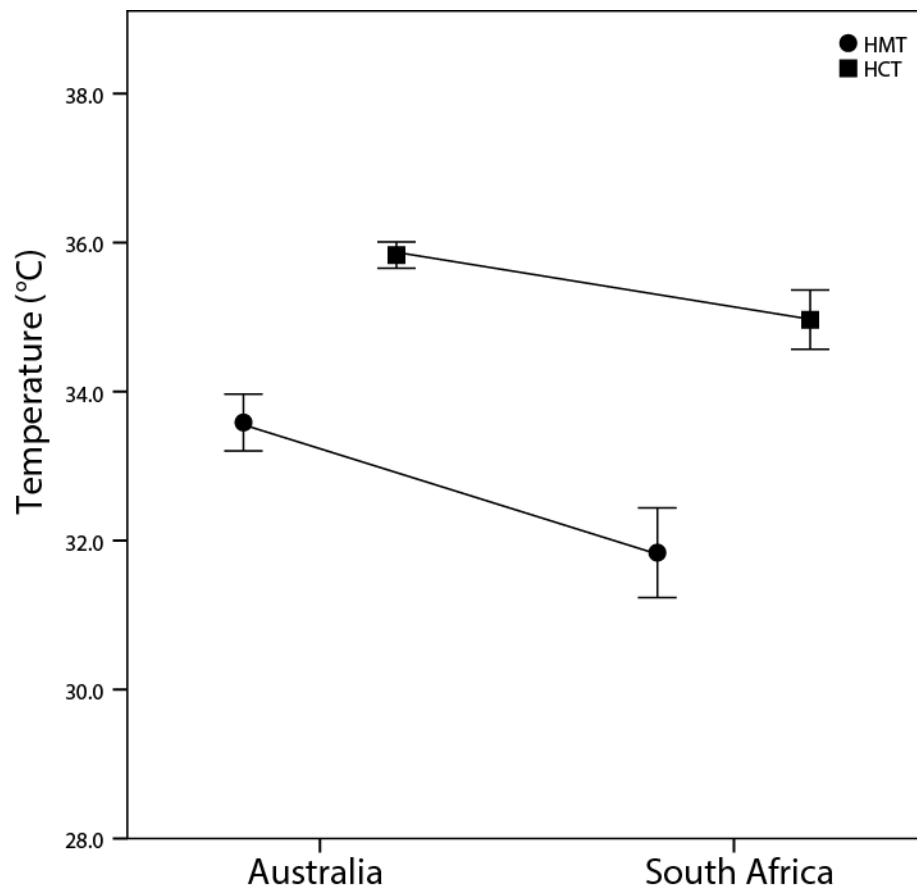


Figure 4.2. Heat Movement Threshold (HMT) (circles) and Heat Coma Temperature (HCT) (squares) for Australia and South Africa. Points represent mean of each country. Error bars signify one standard error for means.

4.4.4 Cold tolerance

I tested 675 *H. destructor* (Table 4.1) and Figure 4.3 shows the trait means for CT_{MIN} onset and recovery. For the CT_{MIN} experiments on field-collected populations, both CT_{MIN} traits were significantly different among populations from South Africa, in contrast to the Australian populations where a significant difference was detected for onset, but not recovery (Table 4.2). There was no interaction between country and acclimation treatment for either trait (Table 4.3) suggesting similar patterns regardless of acclimation. Onset was not significantly different between countries, but for the

CT_{MIN} recovery trait there was a significant country effect (Table 4.3) with Australian populations recovering from cold stress at a relatively lower temperature (means: South Africa = 7.84°C; Australia = 4.66°C). The acclimation treatment had a significant effect on CT_{MIN} recovery (Table 4.3). Generally, 10 °C acclimation decreased CT_{MIN} onset trait values when compared to 15 °C, and field acclimated mites tended to be intermediate (Fig. 4.3). However, the Hay population in Australia displayed a contrasting acclimation response to the rest of Australian populations for CT_{MIN} onset, and no acclimation response for recovery (Fig. 4.3). Within all Australian populations there was a significant interaction between acclimation and population for CT_{MIN} recovery, but this interaction was not significant for South Africa (Table 4.4). Because only three populations from South Africa were scored for cold tolerance, no attempt was made to test for correlations between cold tolerance and environmental factors.

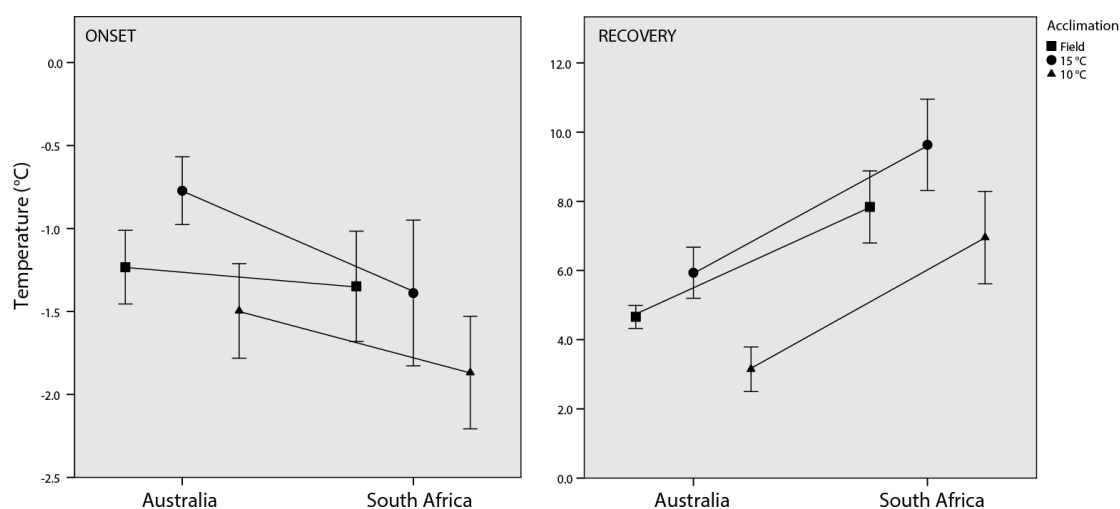


Figure 4.3. CT_{MIN} onset and recovery for Australia and South Africa. Points represent means of each treatment within country. Acclimation treatments are shown for field (squares), 15°C (circles), 10°C (triangles). Error bars represent one standard error.

4.4.5 Comparing Australian populations to source population

The one-way ANOVAs comparing Stellenbosch to Australian populations revealed significant differences in HCT (means: Stellenbosch = 34.96°C; Australia = 35.83°C) ($F_{6,7} = 4.469$, $P = 0.035$) and CT_{MIN} recovery (means: Stellenbosch = 7.17°C; Australia = 4.43°C) ($F_{5,60} = 6.901$, $P < 0.001$), a marginally non-significant difference for HMT (means: Stellenbosch = 32.47°C; Australia = 33.58°C) ($F_{6,7} = 3.552$, $P = 0.061$) and no significant difference for CT_{MIN} onset. Note that for CT_{MIN} recovery data were pooled across Australian populations that did not differ significantly, whereas the other comparisons were based on population means.

Table 4.1. Populations of *Halotydeus destructor* used in this study. Numbers represent individuals used in each experiment and treatment, heat movement threshold (HMT), heat coma temperature (HCT) and Critical thermal minimum (CT_{MIN}).

	Reference	Locality	Latitude	Longitude	Experiment / Treatment			
					HMT/HCT	CT _{MIN} Field	CT _{MIN} 10°C	CT _{MIN} 15°C
Australia	Hay	Hay	-33.9039	144.8844	39	36	31	35
	Wang	Wanganella	-35.2307	144.8198	52	37	35	20
	Hcte	Heathcote	-36.9448	144.7304	48	39	40	32
	Echu	Echuca	-36.2706	144.7037	46			
	Winc	Winchelsea	-38.2142	144.1177	44	34	37	22
	Cnra	Conara	-41.8299	147.4362	46			
South Africa	Skpf	Steinkopf	-29.3888	17.8030	47	29	36	15
	Vanr	Vanrhynsdorp	-31.6428	18.7191	44			
	Ende	Eendekuil	-32.7128	18.9226	52			
	Stel	Stellenbosch	-33.9286	18.8699	43	38	38	23
	Cale	Caledon	-34.2088	19.3991	48			
	Bdrp	Bredasdorp	-34.4966	20.0882	38	43	32	23

Table 4.2. ANOVAs for Heat movement threshold (HMT), Heat coma temperature (HCT), Critical thermal minimum (CT_{MIN}) onset and CT_{MIN} recovery of field collected populations of *H. destructor*.

Trait/Effect	df	Mean Square	F	Error	df (Error)	Significance
HMT						
country	1	18.324	6.009	3.05	10	0.034
population(country)	10	3.05	9.129	0.334	12	< 0.001
Australian populations	5	1.742	2.965	3.524	6	0.109
South African populations	5	4.357	53.948	0.081	6	< 0.001
HCT						
country	1	4.535	3.99	1.136	10	0.074
population(country)	10	1.136	10.378	0.11	12	< 0.001
Australian populations	5	0.377	2.72	0.833	6	0.128
South African populations	5	1.895	23.618	0.482	6	0.001
CT_{MIN} onset						
country	1	0.089	0.095	0.936	5.028	0.770
population(country)	5	0.949	6.121	0.155	21	0.001
Australian populations	3	0.787	5.299	0.149	12	0.015
South African populations	2	1.191	7.281	0.164	9	0.013
CT_{MIN} recovery						
country	1	67.608	10.481	6.451	5.042	0.023
Population(country)	5	6.532	3.975	1.643	21	0.011
Australian populations	3	1.756	0.926	1.896	12	0.458
South African populations	2	13.695	10.488	1.306	9	0.004

Table 4.3. ANOVAs for Critical thermal minimum (CT_{MIN}) onset and recovery of 10°C and 15°C acclimated populations of *H. destructor* between Australia and South Africa. (pop = population, treat = acclimation treatment).

Trait/Effect	df	Mean Square	F	Error	df (Error)	Significance
Onset						
country	1	3.086	2.125	1.452	5.013	0.205
treat	1	4.676	4.846	0.965	5.017	0.079
pop(country)	5	1.467	1.508	0.973	5	0.332
treat * country	1	0.19	0.197	0.964	5.019	0.675
pop(treat * country)	5	0.973	6.014	0.162	39	<0.001
Recovery						
country	1	181.794	10.449	17.399	5.015	0.023
treat	1	92.193	16.116	5.721	5.041	0.010
pop(country)	5	17.574	3.054	5.755	5	0.123
treat * country	1	0.001	0	5.715	5.047	0.989
pop(treat * country)	5	5.755	2.459	2.340	39	0.050

Table 4.4. ANOVAs for Critical thermal minimum (CT_{MIN}) onset and recovery of 10°C and 15°C acclimated populations of *H. destructor* within Australia and South Africa, respectively. (pop = Population, treat = acclimation treatment).

	Trait/Effect	df	Mean Square	F	Error	df (Error)	Significance
Onset							
Australia	treat	1	3.975	2.789	1.425	3.002	0.193
	pop	3	0.458	0.321	1.428	3	0.812
	treat * pop	3	1.428	7.597	0.188	23	0.001
South Africa	treat	1	1.241	4.29	0.289	2.004	0.174
	pop	2	2.981	10.289	0.29	2	0.089
	treat * pop	2	0.29	2.336	0.124	16	0.129
Recovery							
Australia	treat	1	55.167	7.462	7.393	3.007	0.072
	pop	3	5.762	0.778	7.404	3	0.579
	treat * pop	3	7.404	2.234	3.314	23	0.111
South Africa	treat	1	38.832	11.857	3.275	2.003	0.075
	pop	2	35.291	10.757	3.281	2	0.085
	treat * pop	2	3.281	3.487	0.941	16	0.055

4.5 Discussion

I predicted that Australian populations of *H. destructor* would differ in basal thermal tolerance traits and/or plasticity from South Africa, and that such differences may have contributed to a climatic niche shift. Australian populations of *H. destructor* had a higher trait mean for HMT, lower trait mean (quicker recovery) for CT_{MIN} recovery and some populations had a larger response to acclimation treatments (plasticity) for cold tolerance than did the South African populations. I also predicted that South African populations would vary more in thermal tolerance traits than the Australian populations, given the longer time frame for evolutionary divergence. I found significant differences in mean responses of populations from South Africa in contrast to the lack of overall difference among the Australia populations. The third prediction was that Australian populations would exhibit similar thermal tolerance responses to the likely source population of *H. destructor* near Cape Town, South Africa. This was not supported, as the Stellenbosch populations differed from Australian populations for HCT and for CT_{MIN} recovery.

These results for *H. destructor* are consistent with findings that invasive species can, through range expansion, occupy environments that are not predictable from the native range alone (Broennimann *et al.*, 2007). They also suggest that invasive species can adapt rapidly in the invasive range (e.g. Phillips *et al.*, 2006; Urbanski *et al.*, 2012), although further tests are required to determine if evolutionary changes have occurred in *H. destructor* as in these other species. In Australia, *H. destructor* has expanded into environments with colder minimum temperatures, and the lower recovery trait for CT_{MIN} may be reflective of an adaptive shift in cold tolerance. Environmental niche models described different environments for *H. destructor* distributions, and these appear to correlate with observed trait differences, such as cold tolerance. However, the ENMs also suggested that aridity limits the current Australian distribution, but desiccation resistance, which correlates with moisture availability as well as temperature, was not measured here. In an early study, temperatures above 30°C played a more dominant role than low humidity in determining survival for *H. destructor* over short time periods (Solomon, 1937). Future experiments could consider the performance of mites across different combinations of thermal and humidity conditions. Temperature and moisture

tolerances of other life stages, such as obligate diapause, also define niche limits. Diapause allows *H. destructor* to oversummer and withstand extreme temperatures, although this stage is sensitive to the amount of summer rain (Ridsdill-Smith, 1997; Ridsdill-Smith *et al.*, 2005). Thus adaptive shifts in diapause traits may allow for development in conditions that were not previously viable. Unfortunately, adaptive shift in diapause traits are difficult to measure due to the long development of post-diapause eggs and interaction of day length, temperature and moisture cues in initiating and breaking diapause (Ridsdill-Smith *et al.*, 2005).

A more complete understanding of the extent and form of adaptation in thermal tolerance traits will require cross-generational measurements of population/country differences as well as additional studies to assess the heritability of thermal responses (c.f. Chown *et al.*, 2009; Mitchell & Hoffmann, 2010). Because there were no significant interactions between acclimation and country, and because field temperature covariates used added little to explaining observed differences in thermal responses between countries, I suspect that evolutionary adaptation has occurred in basal traits, reflecting adaptive shifts for *H. destructor*, although this requires further testing. The active season of *H. destructor* in Australia coincides with frosty periods, most likely selecting for recovery from low temperature extremes. Cold tolerance traits such as CT_{MIN} have been shown to correlate with limits to the distributions of other temperate arthropods (Kellermann *et al.* 2012; Addo-Bediako *et al.* 2000). The expansion of *H. destructor* in Australia may reflect such limits. Hotter and drier days also occur more frequently in spring months in Australia towards the end of the active season and this may explain the small increase in HMT for Australian populations. This difference may facilitate population persistence in the area of Australian range expansion, where aridity correlates with distributional limits in contrast to predictions based on South African ENMs.

Acclimation responses were preserved across most populations. Plastic responses like acclimation offset temperature variation and buffer against extreme conditions (Seebacher & Franklin, 2011), and invasive species can display extended phenotypic plasticity over native counterparts (Slabber *et al.*, 2007), or differences in the form the plasticity takes (Chown *et al.*, 2007). Within this context, it is unclear why the inland Hay population from Australia has lost its acclimation response to cold conditions,

and additional populations from this region need to be tested across a wider range of temperatures.

Within Australian populations generally, low variability in thermal tolerances is inconsistent with ENM-generated hypotheses, as populations are found across environments that have different climatic extremes. However, these findings are in accordance with genetic work that points to reduced genetic variation being present in Australia (Weeks *et al.* 1995; Qin 1997). Qin (1997) measured allele frequency for five polymorphic loci across South African and Australian populations and found that sub-structuring in South African populations was high (F_{st} ranged 0.130 to 0.6435, mean 0.4340), whereas for Australian populations it was relatively low (F_{st} ranged from 0.027 to 0.217, average 0.089) (Qin, 1997). The relatively high rates of gene flow across the eastern Australian landscape (Weeks *et al.*, 1995) may also act to reduce any differences among populations, although more markers need to be considered in the genetic analyses.

The thermal tolerance results may provide some insight into how *H. destructor* might respond to climate change. *Halotydeus destructor* has expanded in Australia and seems to have adapted to colder extremes as well as hotter and drier conditions, which may be advantageous to a species faced with increasing climatic variability. Although *Halotydeus destructor* is likely to have currently reached distributional limits in Australia (Hill *et al.* 2012), it is well buffered against summer temperature extremes (Ridsdill-Smith *et al.*, 2005), and winter eggs of *H. destructor* are adapted to exploit warm winter temperatures (James & O'Malley, 1991). This makes it likely that this species will continue to be an important pest under forecasted more variable conditions with milder average winter temperatures.

The use of ENMs for modelling invasive species is often criticized for reasons including non-equilibrium between species and environment (Guisan & Thuiller, 2005; Gallien *et al.*, 2012). However, by using ENMs to generate hypotheses and select populations for testing thermal tolerance traits, I present a framework to investigate niche conservatism for invasive species. This framework led to the prediction that *H. destructor* has expanded into environments with colder extremes, and led to subsequent experiments on inland populations to test responses to thermal

extremes. By now expanding on these ENMs and thermal tolerance traits as well developing breeding designs to separate genetic and non-genetic factors, it should be possible to gain insight into evolutionary processes underlying *H. destructor* niche shifts and range expansion in Australia. Such studies will also help to predict how adaptive shifts may abet both biological invasions and responses to climate change.

CHAPTER 5: Population genetics of the invasive redlegged earth mite, *Halotydeus destructor*, using microsatellite markers

5.1 Abstract

Effective control strategies for the redlegged earth mite, *Halotydeus destructor*, will require an understanding of genetic differences within and between populations. Such information can give understanding of breeding systems, dispersal patterns and spatial connectivity of populations. Previous studies have detected limited population structure and moderate gene flow, however these have been limited to only a few allozyme markers and therefore a more comprehensive analysis is required. I extend this research by characterizing 14 microsatellite markers for *H. destructor* and assess allele frequencies using a sub-set of these across 22 locations to characterize genetic differences within and between South African and Australian populations. Genetic differentiation was low, suggesting relatively high gene flow and limited genetic structuring, although there was some differentiation between Western Australia and eastern Australia. The results are discussed in comparison to the earlier studies and in the context of future pest management of *H. destructor*.

5.2 Introduction

For successful management of pest invertebrate species it is important to understand the genetic structure of populations. This type of information, when based on neutral markers (i.e. those not influenced by selection), can provide insights into breeding systems, dispersal patterns and spatial connectivity of populations (e.g. Endersby *et al.*, 2006; Ciosi *et al.*, 2008; Yang *et al.*, 2012a; Zhang *et al.*, 2012). In addition, variation based on selected markers (i.e. those markers that contribute to adaptive variation and are under selection) can indicate the adaptive potential of populations and the extent to which populations might be adapted to local conditions.

Through using neutral polymorphic markers on invasive invertebrate pests, it is possible to understand processes such as invasion route (Estoup & Guillemaud, 2010), number of invasion events (Ciosi *et al.*, 2008; Yang *et al.*, 2012b), and demographic history and population connectivity (Zhang *et al.*, 2012; Yang *et al.* 2012a). Further, high amounts of gene flow can be indicative of high dispersal ability (Chen & Dorn, 2010) and genetic diversity can be used to measure the direction of gene flow.

In this chapter I develop neutral markers to examine genetic divergence between populations of the invasive redlegged earth mite, *Halotydeus destructor*. There have been two previous studies investigating genetic differentiation in *H. destructor* to date, both based on allozyme markers (Weeks *et al.*, 1995; Qin, 1997). Findings from these studies are discussed in detail in the introduction to this thesis, and briefly outlined below. Weeks *et al.* (1995) and Qin (1997) found limited population structure in *H. destructor* in Australia reflecting moderate gene flow, and no evidence for isolation by distance, which suggests that colonization across Australia involved long distance dispersal and/or transfer of large populations across space. However, both of these studies used a limited number of loci (three to five allozymes), whereas population genetic studies in recent times have tended to use larger numbers of microsatellite loci that tend to have a higher level of sensitivity to contemporary population processes than allozymes. An accurate evaluation of genetic divergence among populations and patterns of gene flow can help to assess the likely spread of pesticide resistance (Umina, 2007; Umina *et al.*, 2012) and thermal resistance alleles

(chapter four) in this species as well as indicating potential movement patterns following mite introductions.

In this chapter I extend the findings of Weeks *et al.* (1995) and Qin (1997) by developing a new marker library and conducting a comprehensive survey of *H. destructor* populations across Australia and South Africa. I test three hypotheses. The first is that Australian *H. destructor* represents a single population introduction from South Africa (near Cape Town). The second is that Australian populations will show less genetic diversity than South African populations: based on thermal tolerance data (chapter four) and allozyme differences (Qin, 1997). The final hypothesis is that populations in eastern Australia are differentiated from Western Australian populations, and do not comprise one large panmictic population.

5.3 Methods

5.3.1 Population sampling

Halotydeus destructor populations were sampled in Australia and South Africa between 2009 and 2011 (see chapters three and four). In South Africa I selected populations representing three groupings based on Qin (1997) and reflecting populations that appear to occupy different niches based on distribution models (chapter three). I also included a population from Stellenbosch in South Africa, which is close to Cape Town and represents the putative source of Australian populations. In Australia I determined three geographical regions that may reflect divergence between *H. destructor* populations: Western Australia, Eastern Australia (South Australia, Victoria and New South Wales) and Tasmania. I also included populations in inland eastern Australia that appear to have undergone recent range expansion (chapters three and four). Samples were collected by vacuum using a Stihl blower-vac (Model SH 55, Andreas Stihl AG & Co. KG, Waiblingen, Germany) with a fine gauze sieve (200 µm) attached. Samples were brought back to the laboratory, placed directly into ethanol, and then frozen at -80°C. In South Africa, *Halotydeus destructor* occurs sympatrically with *Halotydeus anthropus* Qin & Halliday, and is not readily distinguishable in the field. The key character used to differentiate *Halotydeus* spp. is *H. destructor* possessing two setae on each of coxae III and IV, *H. anthropus* only

possessing one (Qin & Halliday, 1996a). South African mites were identified to species under the microscope at 100X magnification.

5.3.2 Microsatellite marker development

For microsatellite marker development, I largely followed the methods of Miller *et al.* (2012a). A single DNA extraction was performed on approximately 400 individual mites (to yield at least 10 µg of DNA), using a Qiagen DNeasy kit following the manufacturers specifications. DNA was subsequently processed by the Australian Genome Research Facility (AGRF, Melbourne, Australia) where it was nebulized, ligated with 454 sequencing primers and tagged with a unique oligo sequence allowing sequences to be separated from pooled species DNA sequences using post-run bioinformatic tools. The DNA sample was analyzed using high throughput DNA sequencing on 1/16th of a 70 x 75 mm PicoTiterPlate on a Roche 454 Genome Sequencer FLX with the Titanium Sequencing kit XLR 70 (Margulies *et al.*, 2005). Using the software QDD (Megl  cz *et al.* 2010), 667 unique sequence contigs possessing microsatellite motifs were identified. Primer3 (Rozen & Skaletsky, 2000) was used to design optimal primer sets for each unique contig where possible, and I finalized a set of 40 contigs for analysis, all of which contained di-nucleotide repeats.

Individual mites were placed into Eppendorf tubes with two glass beads, 3 µl of Proteinase K and 100 µl of Chelex resin. Samples were then ground up using a mixer mill for 2 minutes before being incubated at 56°C for 1 hour and then incubated at 90°C for 8 minutes. DNA extractions were stored at -20 until and prior to PCR the samples were mixed by inversion, centrifuged at 13,000rpm for 2 min and DNA aliquot was taken from the bottom half of the supernatant above the chelex precipitate.

Loci were screened for polymorphism using template DNA from eight individuals, including four from Booligal, New South Wales and four from Toodyay, Western Australia. Loci were pooled into ten groups of four, labeled with unique fluorophores (FAM, NED, VIC, PET) and co-amplified by multiplex PCR using a Qiagen multiplex kit (Qiagen) and an Eppendorf Mastercycler S gradient PCR machine

following the protocol described by Blacket *et al.* (2012). PCRs were performed in 11 μ l reactions containing 5 μ l Qiagen multiplex mix, 0.125 μ M each forward tailed primer and 0.125 μ M corresponding fluorescently labeled primer, 0.25 μ M pigtailed reverse primer (Brownstein *et al.*, 1996), and 2 μ l template DNA. PCR cycling conditions consisted of an initial denaturation at 95°C for 15 min, followed by 40 cycles of 95°C for 20 s, 59°C for 90 s and 69°C for 60 s. A final extension step of 69°C for 30min preceded an indefinite hold period at 20°C.

Genotyping was performed using an Applied Biosystems 3730 capillary analyzer and product lengths were determined relative to a GS500LIZ_3730 size standard (Australian Genome Research Facility (AGRF, Melbourne, Australia)). Genotypes and product lengths were scored manually and assessed for polymorphisms using GeneMapper version 4.0 (Applied Biosystems). From a total of 39 loci, 14 were found to be polymorphic (Table 1) and the remaining 25 were either monomorphic or failed to amplify.

The subset of 14 polymorphic loci were pooled into three groups for multiplexing, based on observed locus specific allele size ranges, and further characterized using 30 individuals from both the Booligal and Toodyay populations. After successful amplification and genotyping of these populations, I extended the analysis to 19 further populations from Australia and five from South Africa. 30 mites were analysed per population.

5.3.3 Statistical analysis

Descriptive statistics were calculated for the microsatellite data using FSTAT version 2.9.3 (Goudet, 1995). I calculated allelic richness per population averaged over loci, Weir and Cockerham's measure of F_{IS} , a global estimate of F_{ST} (with 95% confidence limits) (Weir & Cockerham, 1984), population pair-wise measures of F_{ST} and their significance determined using permutations (1000), and pairs of loci tested for linkage disequilibrium using a log-likelihood ratio test. The software MICRO-CHECKER (Van Oosterhout *et al.*, 2004) was used to assess microsatellite loci for null alleles and scoring errors with the formulas outlined by Brookfield (1996).

Estimates of observed (H_O) and expected (H_E) heterozygosity were determined using the Excel Microsatellite Toolkit (Park, 2001) and deviations from Hardy-Weinberg equilibrium (HWE) were tested using Genepop version 3.4 (Raymond & Rousset, 1995). An analysis of molecular variation (AMOVA) was performed in GenAlEx (Peakall & Smouse, 2006) by using pairwise F_{ST} as the distance measure, with 10,000 permutations and missing data for loci set at 10%. The model for analysis partitioned variation among regions (South Africa, Western Australia, eastern Australia and Tasmania), among sample sites within regions, and within sample sites. A factorial correspondence analysis (FCA), implemented in GENETIX version 4.05 (Belkhir *et al.*, 2004), was used to summarize patterns of genetic differentiation between sample sites. The first two underlying factors that explain the majority of variation in multilocus genotypes across loci were plotted. Bayesian analyses were conducted to estimate the number of populations within the sample data using three software packages. The program STRUCTURE (Pritchard *et al.*, 2000) was used to identify the number of distinct clusters/populations, assign individuals to clusters and identify migrants and admixed individuals using genetic data only. To determine the number of populations (K) three independent simulations for $K = 1-10$ with 10,000 burn-in and 100,000 data iterations were run. Analysis was performed using the admixture model of population structure (i.e. each individual draws some fraction of their genome from each of the K populations) and allele frequencies were set as independent among populations. Results of all simulations were analysed with Structure Harvester (Earl & vonHoldt, 2012) to examine delta K using the Evanno method (Evanno *et al.*, 2005). Analysis of population genetic structure using a geographically constrained Bayesian model was performed using GENELAND (Guillot *et al.*, 2005). The inference algorithm was launched with a single step approach (Guillot, 2008) using the Dirichlet distribution as prior for allele frequencies and where K was allowed to vary from 1 to 22. Ten independent runs of 100,000 MCMC iterations were performed.

5.4 Results

Of the 14 polymorphic loci amplified, I chose to use eight markers based on conformation with HWE expectations across populations (Tables 5.1 & 5.3). A total of 648 individuals across 22 *H. destructor* populations were successfully genotyped for eight microsatellite loci (Figure 5.1; Table 5.2). These loci were characterized by moderate to high genetic variation (8 – 25 alleles per locus). A total of 128 alleles were detected, with a mean of 16 alleles per locus over all sites. Allelic richness over all loci ranged between 6.58 and 8.56 (Table 5.3). Expected heterozygosities were high and ranged from 0.68 to 0.77 (mean $H_E = 0.74$) (Table 5.3). Four of the five South African populations failed to amplify for the microsatellite loci and were removed from analysis (the exact reason for this lack of amplification was unclear). Marker independence was confirmed, as linkage disequilibrium analyses indicated no significant linkage between loci. Analysis with MICROCHECKER revealed minor sporadic patterns of potential null alleles across populations, however the only marker that appeared consistently influenced by null alleles was RL21 (4 of 20 collection sites). This marker is highly polymorphic (25 alleles) and apparent null alleles may be due to mutations at the priming sites. To avoid potential biases associated with this RL21, all analyses were conducted including and excluding this locus. Overall the results were not affected by the inclusion of this marker and results given below are based on all marker-based analysis.

Table 5.1. Primer sequences of 14 microsatellite loci isolated from *Halotydeus destructor*.

Locus	Primer Sequence (5' - 3')	Repeat motif
RL10	GCCTCCCTCGCGCCACGTTGGGCTGAATTTGAAAG GTTTCTTTCAAGCGTGACAAGCACATT	(CT)10
RL15	GCCTTGCCAGCCCGCGGCTAAGGACGAACTCTTTTG GTTTCTTACGAAGATCAAGTGAACGGG	(AG)10
RL21	CAGGACCAGGCTACCGTGTTTGTTCGTGTACTTGGC GTTTCTTGCGATGCCAAGAAAGAAGAG	(AG)11
RL23	CAGGACCAGGCTACCGTGACGCCAACCTGAAAGGAAAA GTTTCTTCGAAGCTGAAGTCGCTGAAT	(GA)11
RL30	CAGGACCAGGCTACCGTGCTCCAGATCACGAAGTTG GTTTCTTGAGAACTTTGGTGACGGCTC	(GA)13
RL32	CGGAGAGCCGAGAGGTGGAAAGCCTGAACAAGTTCGC GTTTCTTGACAGAGGGAAGGAAGAAAGT	(GA)14
RL37	CGGAGAGCCGAGAGGTGAAGGAAGAGCGAAACACGAA GTTTCTTAGGCCTTGAATTTGAAAG	(CT)9
RL40	CGGAGAGCCGAGAGGTGTTGAGAGCGAGAGGAAGAGG GTTTCTTGAGACATGTTTTCGGTCT	(GA)9
<i>Additional loci</i>		
RL01	GCCTCCCTCGCGCCACCTCTCACTGCCATTCATCA GTTTCTTAAAGAACATAGAACGAGAAGAGAGG	(CT)10
RL02	GCCTCCCTCGCGCCATCGAGCAAGATGCAAGATG GTTTCTTAAACAGAATGACTGTCAGCAAGG	(GA)10
RL05	GCCTCCCTCGCGCCACGAACTGGAGTGAAGTGTGC GTTTCTTCGATTAGTAGGGAGAGCGAAG	(CT)10
RL22	CAGGACCAGGCTACCGTGCCAAATAGAAGCCGACGAGA GTTTCTTTGATTGCTCACCATAGCAGC	(AG)11
RL28	CAGGACCAGGCTACCGTGTTGTGTCTTTCGCTCTCCCT GTTTCTTGATCCAGAATTGGAGAACCA	(AG)12
RL35	CGGAGAGCCGAGAGGTGGTCAATGGAGGAGTAAGCGG GTTTCTTACGCAATTCATGAAGGGAAG	(AG)9

Significant departures from Hardy–Weinberg Equilibrium (HWE) were observed ($P < 0.05$ across all loci) for 5 of the 22 sites (WAEI, WAON, MG01, NH01, TATW) (Table 5.3) and these remained significant after correction for multiple comparisons. Overall F_{ST} was low indicating limited genetic structuring ($F_{ST} = 0.048$, 95% confidence intervals = 0.041–0.056). However most pairwise comparisons of F_{ST} values were significant (although weak), suggesting some level of genetic differentiation (Table 4). This included populations collected from the same region, such as eastern Australia. After Bonferonni correction, 12 out of 231 (.05%)

comparisons remained non-significant. The highest F_{ST} values were recorded in comparisons with the Tasmanian population at Brighton (TATW) ($F_{ST} = 0.064 - 0.108$), whereas there were low values among some sites from Western Australia ($F_{ST} \sim 0.01$). These results point to limited but significant genetic differentiation. An AMOVA showed low differentiation between regions (South Africa, Western Australia, eastern Australia and Tasmania). Variation in microsatellite loci between these regions explained 4% ($P < 0.001$) of differentiation, whereas variation within populations explained 91% ($P < 0.001$). In eastern Australian populations, variation between populations explained 5% ($P < 0.001$) and within explained 88% ($P < 0.001$).

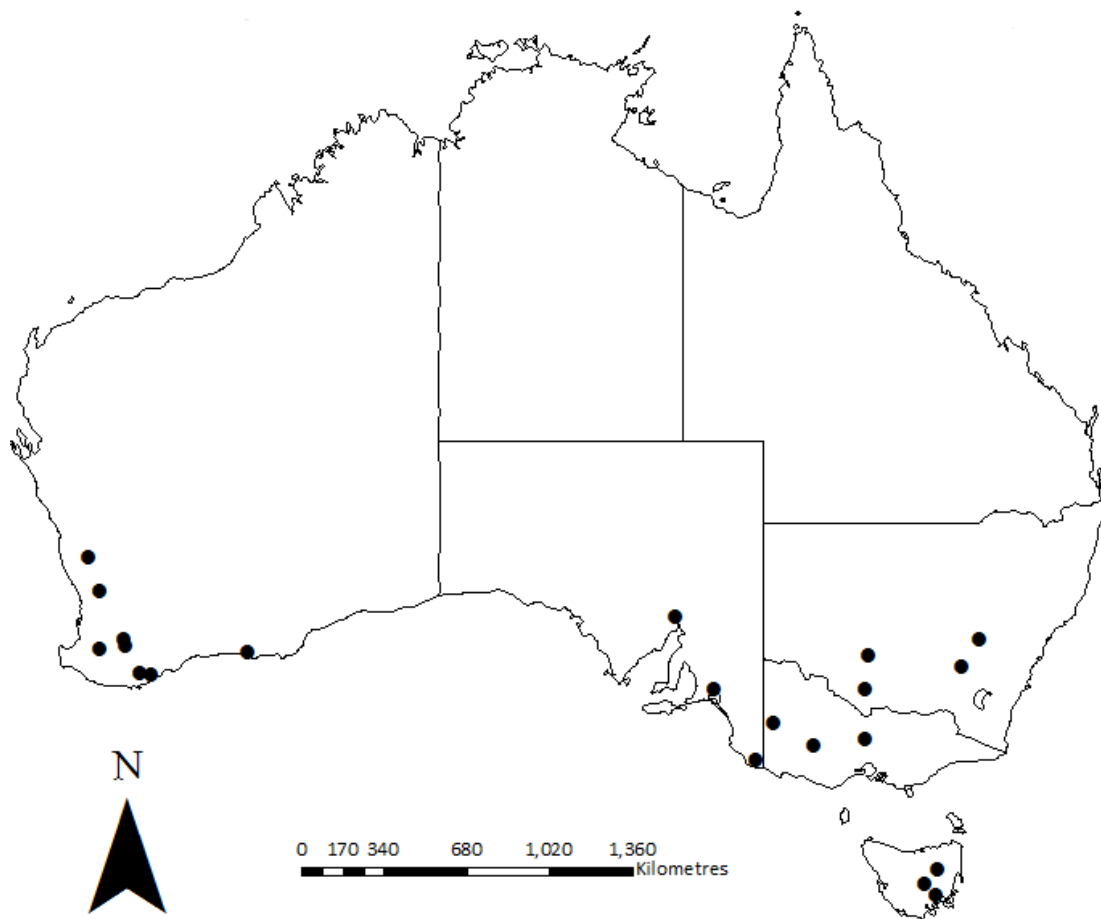


Figure 5.1. Sample locations for 21 Australian populations of *H. destructor* included in this study.

Table 5.2. Populations of *H. destructor* from each region and number of individuals included in genotyping.

Population	Locality	State	Latitude	Longitude	<i>n</i>
<i>Western Australia</i>					
WAOR	Wellstead	WA	-34.586	118.364	25
WARI	Kamballup	WA	-34.579	117.979	28
WAES	Esperence	WA	-33.782	121.957	29
WABL	Bleechmore	WA	-33.657	116.477	28
WABX	Baxter	WA	-33.537	117.441	30
WAEI	Wagin	WA	-33.318	117.350	30
WAFO	Toodyay	WA	-31.551	116.462	30
WAON	Watheroo	WA	-30.276	116.041	30
<i>Eastern Australia</i>					
TRWG	Wanganella	NSW	-35.148	144.811	29
NYG1	Young	NSW	-34.319	148.357	30
TRBG	Booligal	NSW	-33.904	144.884	30
NNC1	Canowindra	NSW	-33.436	148.780	30
MG01	Mount Gambier	SA	-37.792	140.713	30
AST7	Murray Bridge	SA	-35.150	139.181	30
SA44	Port Augusta	SA	-32.483	137.743	30
AR01	Ararat	Victoria	-37.245	142.912	29
TRHC	Heathcote	Victoria	-36.973	144.763	30
NH01	Nhill	Victoria	-36.399	141.396	30
<i>Tasmania</i>					
TATW	Brighton	Tasmania	-42.693	147.294	30
TATF	Bothwell	Tasmania	-42.404	147.104	30
TAFO	Ross	Tasmania	-41.830	147.436	30
<i>South Africa</i>					
SM19	Stellenbosch	Western Cape	-33.929	18.870	30

There was a significant association between genetic distance and geographic distance. Mantel tests showed a significant relationship between Slatkin's linearized F_{ST} and the natural log of geographic distance (Mantel $r = 0.35$, $P = 0.01$). Regression showed that this relationship was positive (Figure 5.2a; $R^2 = 0.12$), however much of this is likely attributed to the large distance between the South African population and Australia. When analysis was restricted to eastern Australian populations (to compare to Weeks *et al.* (1995)) this signal is no longer significant ($R^2 = 0.04$, Mantel test, $r = 0.21$, $P = 0.18$), and there is no obvious association between geographic and genetic distance (Figure 5.2b). These results suggest substantial gene flow within this region.

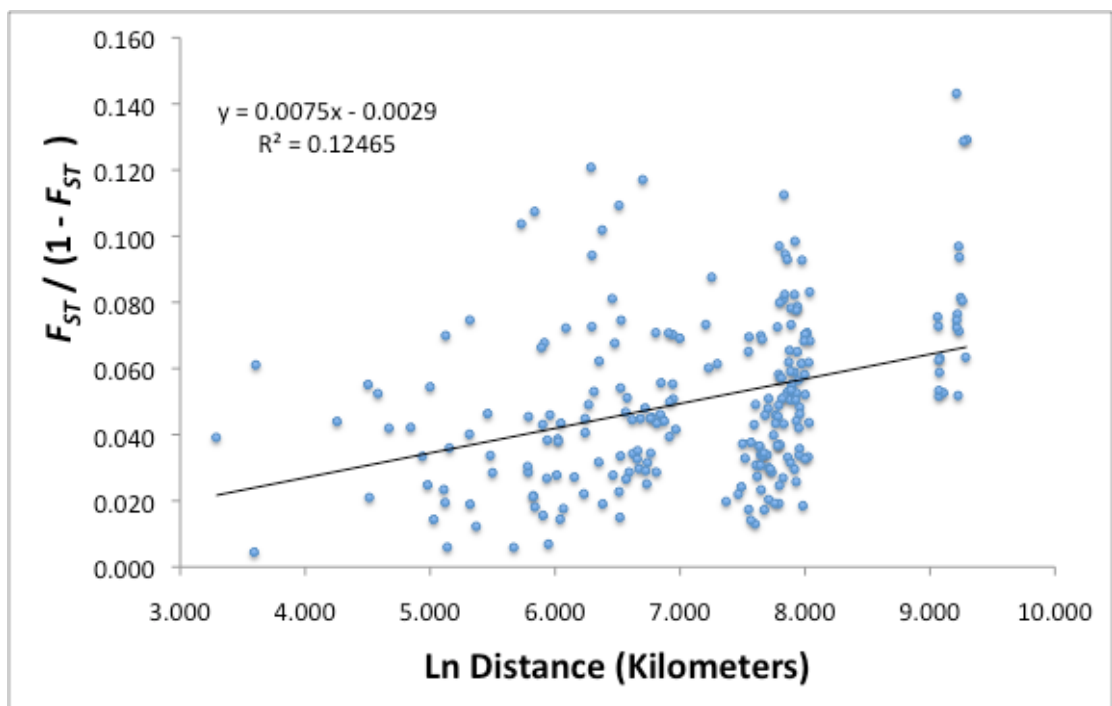
Table 5.3. Indices of genetic variation calculated for *H. destructor* populations. Mean values over loci are presented for number of alleles (a), allelic richness (r), observed (HO) and expected (HE) heterozygosities, inbreeding (F_{IS}), Hardy-Weinberg equilibrium P values.

Population	a	r	HE	HO	HW P-value	FIS
WAOR	8.50	8.201875	0.7557	0.6819	0.1	0.1
WARI	8.63	8.08475	0.7489	0.7022	0.6605	0.063
WAES	9.38	8.565625	0.7604	0.6707	0.1802	0.12
WABL	8.00	7.476	0.7337	0.7213	0.6025	0.017
WABX	8.88	8.017125	0.7328	0.7312	0.5356	0.002
WAEI	9.38	8.320875	0.7331	0.7066	$P < 0.001$	0.037
WAFO	9.50	8.77375	0.7774	0.7070	0.1546	0.092
WAON	7.63	6.83275	0.7016	0.6322	0.0023	0.1
TRWG	9.13	8.293125	0.7695	0.7478	0.796	0.029
NYG1	8.50	7.76075	0.7542	0.7226	0.1482	0.043
TRBG	7.75	7.036125	0.7131	0.6825	0.3127	0.044
NNC1	8.88	7.956625	0.6845	0.7022	0.6079	-0.026
MG01	8.50	7.75625	0.7273	0.6551	$P < 0.001$	0.101
AST7	9.25	8.239625	0.7691	0.7882	0.3033	-0.025
SA44	8.13	7.56025	0.7613	0.7458	0.6688	0.021
AR01	9.25	8.44725	0.7711	0.7663	0.2269	0.006
TRHC	7.25	6.575125	0.6988	0.6680	0.3841	0.045
NH01	8.63	7.68875	0.7414	0.6708	0.01	0.097
TATW	8.00	7.234125	0.6867	0.7044	$P < 0.001$	-0.026
TATF	8.25	7.543875	0.7705	0.7706	0.0773	0
TAFO	8.25	7.375375	0.6824	0.6486	0.125	0.05
SM19	9.00	8.095875	0.7367	0.6743	0.0962	0.086

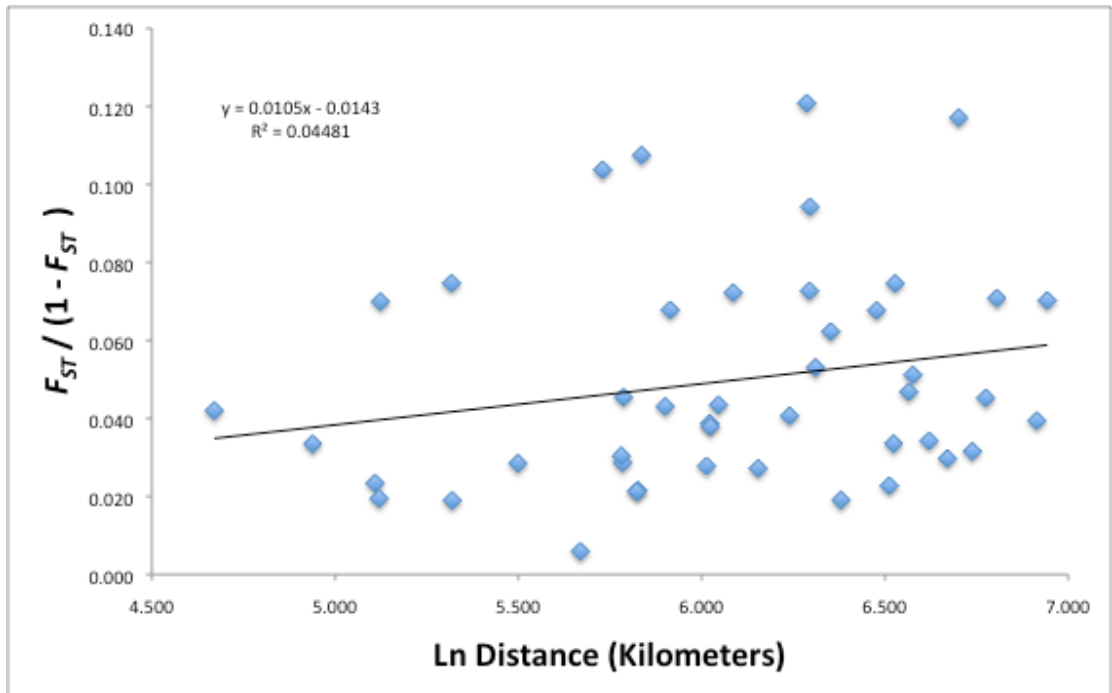
Global $F_{ST} = 0.048$, 95% confidence interval = 0.041-0.056. Where $P < 0.01$ this was based on only one or two markers.

Table 5.4. Pairwise F_{ST} between populations of *H. destructor* used in this study (bold is non-significant)

	WAOR	WARI	WAES	WABL	WABX	WAEI	WAFO	WAON	TRWG	NYG1	TRBG	NNC1	MG01	AST7	SA44	AR01	TRHC	NH01	TATW	TATF	TAFO	SM19
WAOR	0																					
WARI	0.0042	0																				
WAES	0.0175	0.0259	0																			
WABL	0.0384	0.0346	0.0215	0																		
WABX	0.0241	0.0404	0.0142	0.0205	0																	
WAEI	0.0058	0.0141	0.0173	0.0522	0.0377	0																
WAFO	0.0066	0.0153	0.0305	0.0442	0.0326	0.0121	0															
WAON	0.0297	0.0299	0.0137	0.0186	0.0196	0.0426	0.0276	0														
TRWG	0.0434	0.0406	0.0483	0.0345	0.0423	0.0603	0.05	0.0496	0													
NYG1	0.0358	0.0317	0.0578	0.0412	0.0611	0.065	0.0469	0.0461	0.0322	0												
TRBG	0.0741	0.054	0.0644	0.0584	0.0761	0.0864	0.0685	0.0373	0.0733	0.0693	0											
NNC1	0.0285	0.0251	0.024	0.0182	0.0327	0.0461	0.0316	0.0197	0.0302	0.0379	0.0295	0										
MG01	0.0326	0.0324	0.0235	0.0278	0.0327	0.0457	0.044	0.022	0.0513	0.0503	0.0416	0.0222	0									
AST7	0.0264	0.0351	0.0214	0.0199	0.0169	0.0438	0.0382	0.0185	0.0519	0.0633	0.0586	0.0288	0.019	0								
SA44	0.0505	0.0677	0.05	0.0703	0.0588	0.0491	0.0554	0.0695	0.0588	0.0857	0.1139	0.0596	0.0883	0.0675	0							
AR01	0.0175	0.0355	0.0206	0.0308	0.0136	0.03	0.0333	0.0276	0.05	0.0445	0.0686	0.0325	0.029	0.0287	0.0505	0						
TRHC	0.0337	0.0326	0.0213	0.0291	0.0378	0.0459	0.0277	0.0053	0.0453	0.0458	0.031	0.0178	0.0196	0.0251	0.0695	0.0374	0					
NH01	0.0731	0.0535	0.0483	0.0495	0.0579	0.0849	0.0663	0.0325	0.0613	0.0656	0.0413	0.0403	0.0331	0.0432	0.1144	0.0686	0.0353	0				
TATW	0.0614	0.0507	0.0354	0.0403	0.048	0.072	0.064	0.0153	0.0552	0.0567	0.0422	0.0279	0.0458	0.0426	0.0694	0.0407	0.0269	0.0475	0			
TATF	0.0678	0.0884	0.0654	0.0851	0.0747	0.1011	0.0727	0.0664	0.0867	0.1048	0.0969	0.0674	0.0939	0.0635	0.0753	0.0884	0.0676	0.1079	0.075	0		
TAFO	0.0559	0.0525	0.0466	0.0441	0.05	0.073	0.066	0.0255	0.0725	0.0805	0.0661	0.0441	0.0429	0.034	0.1253	0.0661	0.0334	0.0484	0.0575	0.0985	0	
SM19	0.048	0.0516	0.0433	0.0344	0.0422	0.061	0.055	0.0281	0.0399	0.0682	0.0416	0.0244	0.0428	0.0277	0.0709	0.0541	0.0282	0.0526	0.0421	0.0924	0.0497	0



A



B

Figure 5.2. A. Association between linearised F_{ST} against the logarithm of geographic distance (km) in all populations of *Halotydeus destructor* ($n = 22$). Scores for the accompanying Mantel test is $r = 0.35$, $P = 0.01$; B. Association between linearised F_{ST} against the logarithm of geographic distance (km) in eastern Australian populations of *Halotydeus destructor* ($n = 10$). Scores for the accompanying Mantel test is $r = 0.21$, $P = 0.18$. Lines (and R^2) reflect linear regression analyses.

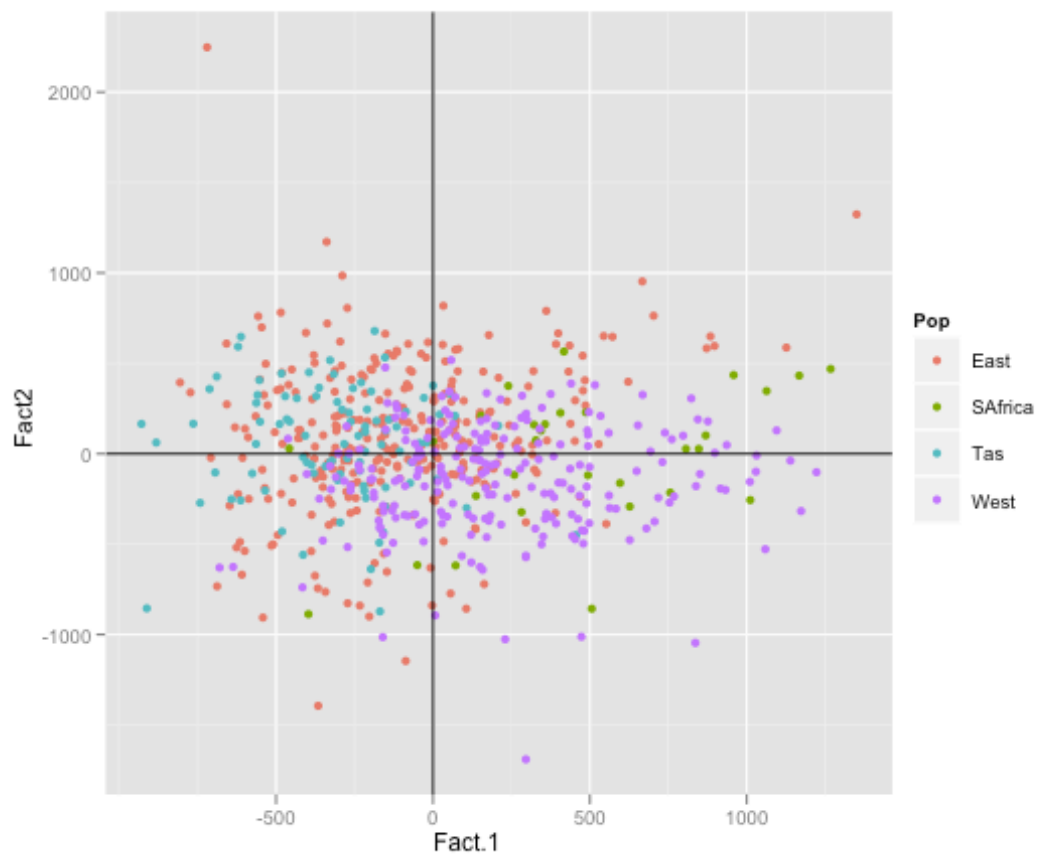


Figure 5.3. Two-dimensional scatter plot showing relationships between *H. destructor* sample locales based on a factorial correspondence analysis (FCA) of microsatellite loci for 8 sites. The first factor (x axis) explains 1.91% of the variance, whilst the second factor (y axis) explains 1.72%. East = Eastern Australia, SAfrica = South Africa, Tas = Tasmania, West = Western Australia.

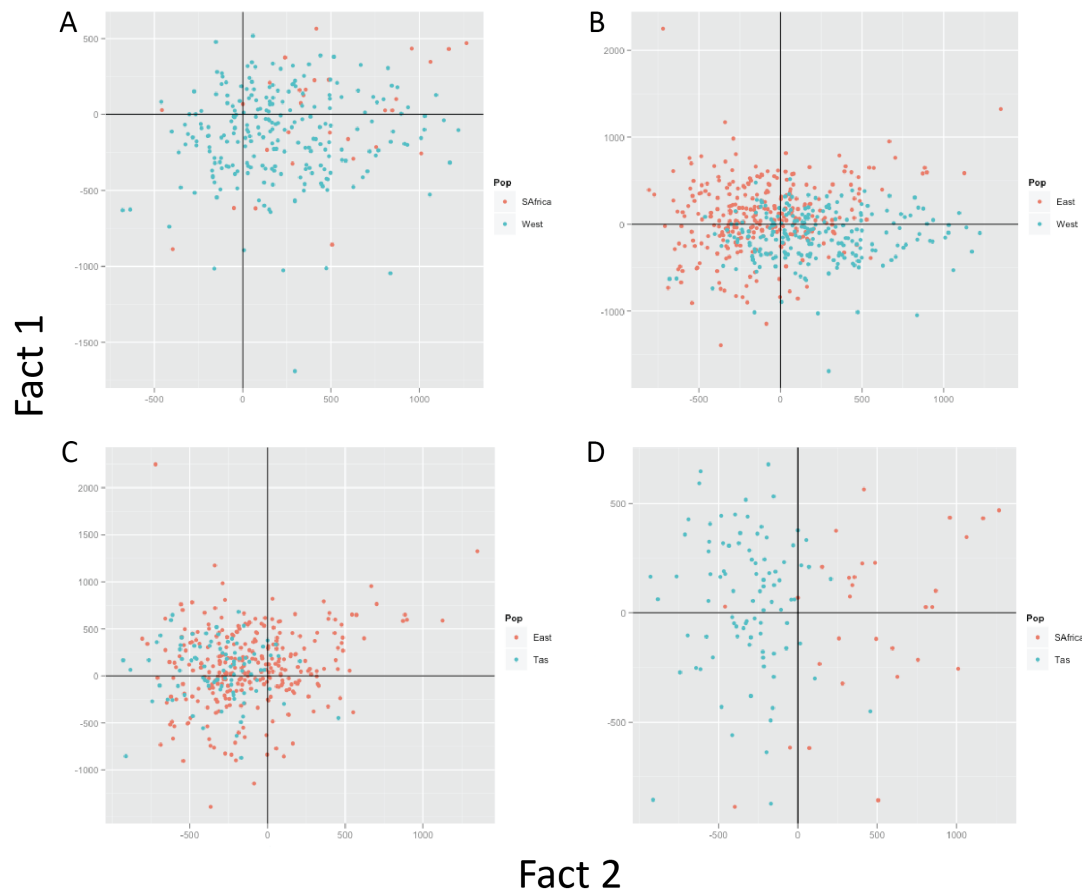


Figure 5.4. Factorial Correspondence Analysis replotted for selected pairwise comparisons between regions. A: South Africa (red) and Western Australia (blue); B: eastern Australia (red) and Western Australia (blue); C: eastern Australia (red) and Tasmania (blue); D: South Africa (red) and Tasmania (blue).

The relationships between sample locales are depicted by the two-dimensional factorial correspondence analysis (FCA) of the microsatellite variation (Figure 5.3). The two factors that explain the majority of the variation were very low (factor 1 = 1.91%, factor 2 = 1.72%). When all multilocus genotypes are plotted against each other, there is no clear separation of samples (Figure 5.3). To examine patterns between regions I plotted pairs of regions, and found South African and Western Australian populations overlap (Figure 5.4a), whereas for Western Australia and eastern Australia some separation is suggested (Figure 5.4b). Tasmania and eastern Australia also largely overlap (Figure 5.4c). Locales that are furthest apart (South Africa and Tasmania) are separated on different sides of the y-axis (Figure 5.4d). This may indicate that populations become more differentiated the further they are east of the South African and Western Australian populations,

Using the Evanno *et al.* (2005) method for determining the true K , STRUCTURE identified five genetic clusters ($K = 5$). Like the FCA, the STRUCTURE analysis indicates some divergence between eastern Australia and Western Australian population, as evidenced by the higher proportion of yellow ancestral genotype in Western Australia (Figure 5.5). South Africa appears to be marginally closer to Western Australia. The Heathcote (HCTE) population also appears to be different from the other eastern Australian populations. The GENELAND analysis determined four genetic clusters ($K = 4$), with the South African population clustering with Western Australian populations, most eastern Australian populations clustering together, and the Heathcote population in Victoria being the sole member of its cluster (Figure 5.6).

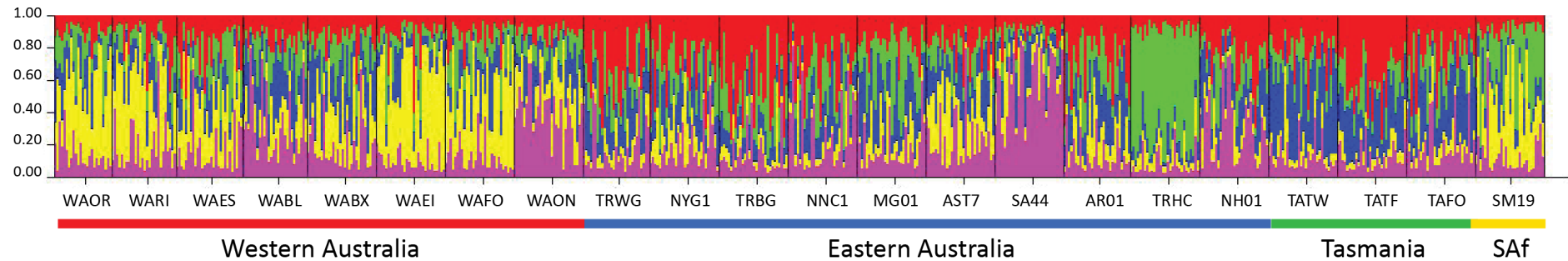


Figure 5.5. STRUCTURE summary plot of the estimated membership coefficient (y axis) for each individual in 5 population clusters. Each individual is represented by a single vertical line broken into segments, where segments are proportional to the membership coefficient for each of the population clusters. Individuals are arranged into populations from which they were sampled following the order given in Table 5.2.

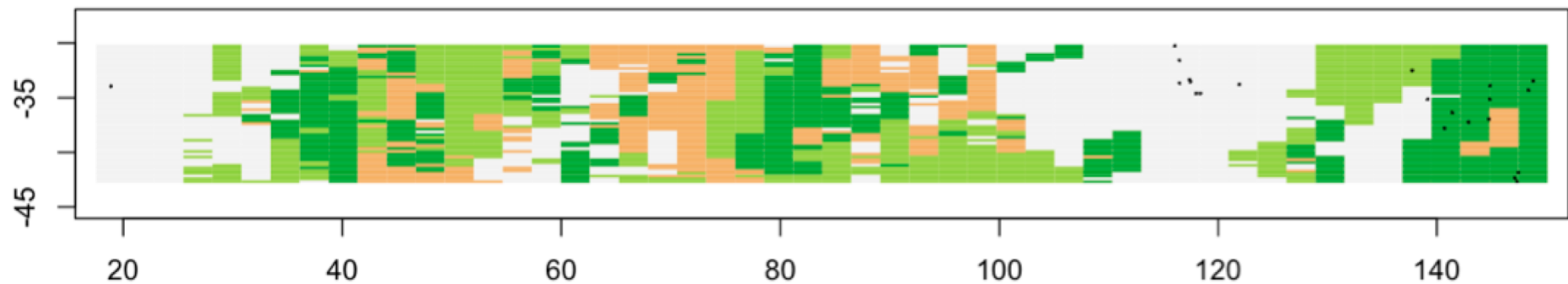


Figure 5.6. GENELAND map of estimated cluster membership and spatial representation of population genetic structure for *Halotydeus destructor*. Colours reflect putative populations ($K = 4$) and points reflect the spatial coordinates (latitude(y-axis)-longitude(x-axis)) of each sampling locale.

5.5 Discussion

As I only characterized one South African population it was not possible to explicitly test the hypothesis that Australian populations are from a single origin in South Africa. However, the population at Stellenbosch is 50 km from Cape Town and, based on the eight loci used in this study, this population seems genetically similar to the Australian populations, particularly those in Western Australia. The hypothesis that South African populations have higher genetic variability than Australian populations could also not be tested for the same reason; however, genetic variability across Australia is low, indicated by low F_{ST} values. Global F_{ST} in this study is very low (0.048), consistent with the findings of Weeks *et al.* (1995), who estimated a global F_{ST} value of 0.015 (based on allozymes). STRUCTURE, GENELAND and FCA results suggest Western Australian and eastern Australian populations are marginally differentiated, although this is not strongly supported (i.e. no congruence with K estimates between Bayesian analyses).

It is not clear why the South African populations failed to amplify for the microsatellite markers developed here. Due to the specificity of the primers it is possible that there exists a high level of genetic divergence among the South African populations, perhaps similar to that found between closely related species. All South African populations appear to belong to a single morphospecies, however, there does appear to be high variation within populations, as demonstrated across the five polymorphic allozyme loci used by Qin (1997). It would be worthwhile investigating mitochondrial genetic divergence in regions such as cytochrome oxidase *I* (COI) to test for high genetic differentiation and allow a comparison with microsatellite variation as done for other pests (e.g. Yang *et al.*, 2012; Zhang *et al.*, 2012). It is possible that South African mite populations comprise a cryptic species complex.

The high number of alleles for the microsatellites characterized here may be indicative of large colonizing population(s) reaching Western Australia around 1917. The invasion route is likely to be linked to ship's ballast, with soil being offloaded in both Cape Town and Bunbury ports (Newman, 1925; Baker, 1995). This gives the potential for multiple introductions from the same source population and the movement of thousands of *H. destructor* eggs. Assuming a west to east movement

across Australia, the genetic similarity of mites from across the country and high level of allele richness also suggest that large numbers of individuals of *H. destructor* have moved to eastern Australia and also into Tasmania. The GENELAND and STRUCTURE analyses suggest higher differentiation of eastern Australian populations from South Africa than Western Australian populations. The FCA plot also suggests that South Africa and Tasmania have the highest level of differentiation. These data are consistent with a pattern of movement from west to east and the historical data for *H. destructor* (Newman, 1923; 1925; Qin, 1997).

Between Australian populations of *H. destructor*, I found low levels of genetic differentiation although this was significant. However, in the absence of historical collections of *H. destructor* and sequence data it is difficult to attribute this to either high levels of contemporary gene flow or the historical effects of invasion and rapid expansion. Other studies of invasive pest invertebrates within Australia have found some species exist as large panmictic populations, with no genetic structure among populations (e.g. Endersby *et al.*, 2006; Endersby *et al.*, 2007). Species like the lepidopterans *Plutella xylostella* (Endersby *et al.*, 2006) and *Helicoverpa amigera* (Endersby *et al.*, 2007) have large migratory phases in their life histories which likely contributes to the homogeneity of allele frequencies across populations. *Halotydeus destructor* populations persist in a given area across seasons, but perhaps with colonizing events from other populations reintroducing new individuals each season. This is mainly through wind dispersal of diapause eggs and human mediated transport of farm machinery, allowing for great distances to be covered. The presence of weak genetic structure across populations suggests that there may be repeated colonization events followed by periods of partial isolation that lead to some genetic differentiation being maintained. Colonization events have previously been implicated in accounting for the genetic structure of *Penthaleus* spp. (which are asexual) (Robinson *et al.*, 2002). Consistent with the findings of Weeks *et al.* (1995), there was no isolation by distance in eastern Australian populations. This suggests that there is long distance colonization in *Halotydeus destructor* rather than events that always involve movement to adjacent populations. Local adaptations, such as increased thermal resistance, are likely to be able to spread between populations through colonization events, perhaps providing widespread tolerance to environmental stressors such as climate change. Alternatively, high levels of gene flow could also constrain

adaptation in local populations.

These findings also have implications for the management of *H. destructor* in Australia. Firstly, the lack of strong differentiation between eastern and western Australia suggest that there is a high potential for genetic exchange between these regions. This means that pesticide resistance to insecticides present in Western Australia (Umina, 2007; Umina *et al.*, 2012) may be able to spread rapidly to eastern Australian states. Umina *et al.* (2012) have already shown resistance within Western Australia has spread quite quickly, and over large geographic distances. Further, the presence of some genetic differentiation among nearby populations points to the feasibility of local management actions in controlling these mites. Suppression of mite numbers in one region may be effective for some time rather than areas becoming continuously reinvaded, as might be the case for more mobile species such as the aerially dispersed wheat curl mite, *Aceria tosichella* (Miller *et al.*, 2012b).

This work needs to be extended in several ways. As already mentioned the issue of cryptic species in South Africa must be examined in order to understand the levels of variation that exist in the native range. It would also be beneficial to examine the populations with more loci, particularly the six additional microsatellite described here (Table 1). Differences between environments and climate space across the genetic clusters proposed by the Bayesian analyses could also be tested, to determine whether there are any clear environmental differences between regions or populations. New Zealand also has *H. destructor* populations and it would be interesting to know if these are genetically similar to Australian populations, or represent a different introduction pathway. Such information may be useful in understanding if New Zealand populations will respond to climate change in a similar way to Australian populations. With more South African samples and additional loci, genetic differences could also be compared with thermal tolerance trait differences to test for local adaptation. The results here should also be contrasted to adaptive genetic variation- this would depend on studies being undertaken to develop markers that target shifts in physiological traits and their underlying genes.

CHAPTER 6: General Discussion

6.1 Overview

In this thesis I aimed to build a predictive framework to assess climate change response for pest invertebrate species of grains growing in Australia. I used an interdisciplinary approach, combining modern species distribution models, niche limiting traits and genetic variability data. The combination of these different avenues of investigation formed a useful and transferrable analytical framework. While there is no single “magic-bullet” for predicting pest response of climate change, the tools presented in this framework present different lines of inquiry to predict climate response for different species, and isolate hypotheses that can then be used to test specific attributes of an individual pest. Such information will certainly aid in future management decisions of invertebrate pests of grains growing and other agricultural systems. A summary of the four areas of experimental work undertaken during this study is presented below. Implications for future research and questions raised during this thesis are also discussed.

I first demonstrated how Environmental Niche Models (ENMs) can be used to make preliminary assessments of climate space for cryptic *Penthaleus* species and how climate space for these species may shift under different climate change scenarios. The ENMs were highly effective at discriminating different climate responses between cryptic mite species. These ENM results and other work (e.g. varying ecology such as host plant preferences (Umina & Hoffmann, 2004) and pesticide tolerances (Umina & Hoffmann, 1999)) suggest that *Penthaleus* species are not likely to respond to climate change in the same way. Outbreak frequency and prevalence are likely to shift for these species, leading to changes in risk for growers and potentially control failures.

Both *H. destructor* and *Penthaleus* spp. have comprehensive distribution datasets, compiled from targeted surveys and reports from across Australia. However, such information is limited for most pest invertebrate species of agriculture and thus ENMs are not always going to provide an appropriate modeling tool for these species. For

invasive species it is usually impossible to know whether a species has reached the full extent of its distribution (under current climate conditions) and thus assumptions based on time since arrival are made. In order to characterize the complete range of species-environment relationships for an invasive species, it is important to understand what other geographical regions the organism has reached. For *H. destructor* this information was readily available (chapter three), as the species has only invaded Australia and New Zealand. However, *Penthaleus* spp., as well as many other pest invertebrate species, are “worldwide” invaders with unknown origins, and have established across suitable climatic zones, often in both hemispheres of the world. This issue will be problematic for many invasive pest species, due to incomplete sampling in all geographic regions, misidentification and taxonomy discrepancies, or restricted access to survey records. For example, the *Penthaleus* species complex has only been identified confidently within Australia (Qin & Halliday, 1996a; Halliday, 2006), with most global records only reporting *P. major*. This makes any validation methods of the models outside of Australia problematic.

By then moving on to an integrated approach (chapters three to five), combining ENMs, niche limiting traits and information on genetic variability data, I was able to determine traits that limit the distribution of *H. destructor*, and how these niche-limiting traits appear to have shifted. In chapter three, the ENMs suggested niche-shift for *H. destructor* and how this species may have undergone adaptive shifts in limiting traits to allow for a wider geographic distribution in Australia than is predictable from native range data alone. While distribution data alone could report a range shift for *H. destructor*, the use of ENMs to identify shifts in important variables that limit the distribution provides valuable insight into processes that will mediate response to climate change. Of course there are also other reasons for niche-shift to occur, such as changes in farming practices and release from competitors in the native range, however, I was able to devise clear hypotheses to test in the subsequent chapters.

In chapter four I then took the hypotheses generated by the ENMs to select populations to characterize thermal tolerance traits, and identified a putative adaptive shift for *H. destructor* following invasion. Chapter four demonstrated that characterizing niche-limiting traits in both native and invasive ranges could point to likely processes underlying species adapting to new environments and range

expansions. There are few other studies that have provided such evidence (or a test of such shifts) (two recently published examples are Urbanski *et al.*, 2012 for the mosquito *Aedes albopictus*, and Rey *et al.*, 2012 for the tropical ant *Wasmannia auropunctata*). The results from chapter four also have implications for the response of this species to climate change. A wider thermal activity window may allow for Australian *H. destructor* populations to remain in the same geographic regions, even with hotter and drier conditions forecasted. Given the availability of suitable host-plants, milder winters may also increase numbers earlier in the establishment phase of crops. This hypothesis could be tested by a phenological or a mechanistic model to examine how the thermal “activity window” characterized in chapter four will impact on growth, feeding and fecundity under climate change (see 6.3 Future Directions).

Chapter five looked to find genetic structure between populations of *H. destructor*. The results suggest that Australian populations of *H. destructor* do not comprise one large, panmictic population; genetic differentiation was low, indicating high gene flow and limited genetic structuring. Any adaptive response to changes in climate is therefore likely to spread rapidly between Australian populations. Unfortunately these data do not allow for tests of environmental niche differences between populations or regions, as it is difficult to ascertain any clear geographic patterning to *H. destructor* populations. For other species it may be possible to delineate clear geographical boundaries and use this information in this framework. By understanding differences in environmental response between populations, and how much gene flow is evident between populations, it will allow for predictions of response to climate change to be made at the level of populations rather than species (see 6.3 Future Directions).

6.2 Limitations of framework

While the framework used in this study allowed me to predict the response of *H. destructor* to climate change, there are some limitations to the framework. One of the main limitations is that each species is considered in isolation and as a single population. Species do not exist free from interactions with other species, nor are they homogenous in response to environmental stressors. Interactions such as competition, predation and parasitism are all likely to be impacted by climate change, resulting in

species composition shifts and biocontrol failures (Sutherst *et al.*, 2007; Thomson *et al.*, 2010).

Biotic interactions may restrict the spread of invasive invertebrate pests (i.e. a new predator encountered in invasive range, or competitive interactions between species) under present climatic conditions, but these interactions may shift under climate change, resulting in altered pest invertebrate distributions. Biotic interactions can also impact on population abundance across time, limiting species distributions spatially. In terms of biocontrol, beneficial (predators and parasitoids) species may respond to climate change in a completely different way than the pest invertebrate they attack (Thomson *et al.*, 2010). While it is important to understand how beneficial invertebrates can help control pests under present climate conditions, their success under a changing climate also needs to be considered. For *H. destructor*, biotic processes are not likely to enhance ENM resolution. Although there are a few predatory mites that can suppress *H. destructor* (Ridsdill-Smith, 1997; Halliday & Paull, 2004), none are significant predators at the scale of the broad climatic variables used. However, especially for *Penthaeus* spp., it would be useful to determine if shifts in climate could alter competitive interactions, with outcomes on distributions and abundance. This will have important management implications, as competition between species on pasture and crops may shift, making control of specific species-complexes challenging (Umina & Hoffmann, 2005).

Another limitation of this framework is that it does not incorporate any seasonal dispersal parameters directly, but rather assumes dispersal in a broader sense, as a measure of genetic variability. While there is evidence of gene flow between *H. destructor* populations, there is little understanding of how the mites move across the landscape in terms of direction and spread. This may not be as important for species like earth mites that generally remain in a given area, but imperative for pest species that seasonally migrate into crops, such as native budworm (*Helicoverpa punctigera*) and diamondback moth (*Plutella xylostella*) (see Mazzi & Dorn (2012) for a review).

ENMs also inherently assume that species exist as one large population and do not incorporate differences in phenotypes between populations. If this framework is to be applied to a species where this clearer structure between populations (for example,

multiple and distinct introductions) then it would be important to examine the differences in environments occupied by populations or lineages (e.g. Arteaga *et al.*, 2011; Newman & Rissler, 2011). While it is possible to measure population structure and divergence using microsatellites, neutral genetic diversity and adaptive genetic diversity are often not correlated (Holderegger *et al.*, 2006), so at this stage it is not possible to test for adaptive shifts directly.

6.3 Future directions

In this thesis I demonstrated a framework to understand the response of *Halotydeus destructor* to climate and predict the response of this species to future climate change. The methods outlined in chapters of this thesis provided understanding of different aspects of *H. destructor* biology and ecology, and complemented each other in an overall framework. However, there are several directions for future research in order to understand the response of *H. destructor* to climate change in finer detail and to also make this framework more broadly applicable to other pest invertebrate species. In this final section, I suggest ways to address the limitations described above and discuss how new tools might allow for these processes to be incorporated into this framework.

In chapter two, differences in predictor variables influencing *Penthaleus* spp. drove hypotheses about physiological variation between *Penthaleus* spp. and these could be investigated in the same way that such traits were characterized for *H. destructor* (chapter three). By characterizing thermal responses of sympatric *Penthaleus* spp., differences in physiology and how these may mediate competition, range shifts and future outbreaks under climate change can be assessed. Having some thermal tolerance or desiccation resistance information for the three *Penthaleus* species, CLIMEX models (Robinson & Hoffmann, 2001) that were initially built on distribution data alone, could be further developed to try and discriminate species-climate relationships. This may be especially beneficial for *P. tectus*, as the initial CLIMEX approach did not work for this species (Robinson & Hoffmann, 2001). These data could also be implemented into a more mechanistic approach to examine climate change response at a finer scale. Such models could then be compared to the

ones from chapter two, focusing on areas of congruence between the modelling methods.

Environmental Niche Models, like those in chapters two and three, could be expanded on by incorporating other ecological processes like biotic interactions and dispersal. Species interactions are currently incorporated in ENMs at a basic level, including covariates of competitive interactions (Pellissier *et al.*, 2010; Meineri *et al.*, 2012) and available prey items (Hof *et al.*, 2012), to look at how these interactions affect modelling outcomes. Another approach that looks promising is to spatially nest a community of species within a modelling framework that incorporates co-occurrence indices (Boulangeat *et al.*, 2012). Population processes such as dynamic ranges and dispersal parameters can also be incorporated into ENMs. Developments in this area include dynamic range models (DRMs) to estimate spatial population dynamics (Pagel & Shurr, 2012; Shurr *et al.* in press) and other dynamic species distribution models that can incorporate stochastic processes such as dispersal, growth and competition within a Bayesian framework (Marion *et al.* in press). Processes such as biotic interactions and dispersal could also be explicitly linked within a mechanistic modelling framework and there are simple interaction parameters available for CLIMEX models to examine changes in the Ecoclimatic Index under climate change (Sutherst *et al.*, 2007).

The thermal tolerance traits characterized in this thesis (chapter four) could be expanded to characterize traits for other life stages, such as diapause, and look at intergenerational effects. It would also be beneficial to characterize populations in Western Australia, to determine if increased thermal resistance is confined to eastern Australia. Thermal resistance data could be combined with egg development rates (e.g. James & O'Malley, 1991; Ridsdill-Smith & Annells, 1997) and used to build a CLIMEX model for *H. destructor*. This could form a comparison to ENMs and be projected onto the same future climate surfaces (e.g. the CliMond dataset) to examine areas of model congruence. It would also be of great interest to reciprocally project CLIMEX models constructed with Australian and South African thermal tolerance parameters between ranges, to test how greater thermal resistance of Australian *H. destructor* populations may affect model projections. The Insect Life Cycle Modeling (ILCYM) software (Sporleder *et al.*, 2009) can be used to determine number of

generations in a given geographical area under different climatic conditions (Kroschel *et al.* in press) and would provide an ideal comparison to the ENMs built here. A phenology model like ILCYM could determine if suitable climate space determined by the ENMs will translate into faster population growth for *H. destructor*.

A clear future direction for *H. destructor* research is to construct a mechanistic model. Mechanistic models that incorporate trait variability (e.g. Kearney *et al.* 2009) could provide an alternative to using an ENM-centered framework. However, while mechanistic models that characterize the thermodynamic niche (e.g. Kearney *et al.*, in review) provide comprehensive detail, these require many parameters and extensive empirical research. A simpler mechanistic model could use the characterized thermal resistance from chapter four combined with development parameters from the literature and relate these to the microclimate of the mites. This could be used to examine fine-scale processes that influence life-history traits of *H. destructor* and determine conditions suitable for feeding and reproduction. By having a mechanistic understanding of how climate affects life-history traits such as emergence (e.g. Kearney *et al.*, 2010b), and number of generations, it may be possible to translate these into guidelines for control measures.

Future work for genetic data could focus on how to incorporate population substructuring into niche analysis and climate change predictions. Neutral genetic variation such as microsatellite information can also be used to geographically partition landscapes to corresponding regions of genetic structure, so to compare the environmental space of the partitions (e.g. Wellenreuther *et al.*, 2011). This approach has also been successful in correlating climatic boundaries with differences in morphological traits and genetic divergence for the southern leopard frog, *Rana sphenocephala* (Newman & Rissler, 2011). Similarly, niche similarity and gene flow was estimated between two lineages of the nine-banded armadillo by using a combination of microsatellites and climate data through principle component analysis (Arteaga *et al.*, 2011). Spatial output from population genetic analysis software such as GENELAND (Guillot *et al.*, 2005) can provide maps of a landscape and probability of population membership to different groups, or *K*. By taking known distribution points within each spatial division of genetic structure it is possible to compare differences in climate space, and cross-validate ENMs through data-

partitioning. As microsatellite markers measure neutral genetic diversity and not adaptive genetic diversity, the microsatellite library developed herein could potentially be used in future studies to contrast against adaptive markers. Such data could be used to examine how *H. destructor* may respond to climate change at the population level. There are also six other candidate loci that could be revisited (chapter five) and used in future studies, including those looking at spread of resistance genes for *H. destructor*. Other future directions could include investigating thermal tolerance traits at the genetic level and further contrasting the microsatellite library against any adaptive markers developed in future studies.

The framework presented in my thesis provides a sound platform to make informed predictions of pest invertebrate climate change response. As climate change is likely to affect more than any one modelling process can capture, it may be important to combine multiple modelling methods (e.g. ENM, semi-mechanistic, phenological and thermodynamic mechanistic models). Using combinations of models to assess the response of pest invertebrates, such as *H. destructor* to climate change, may translate into more targeted management decisions. This framework should now be applied to other invertebrate pests, such as (in grains) the lucerne flea (*Sminthurus viridis*), and mite species including Balaustium mites (*Balaustium medicagoense*), Bryobia mites (*Bryobia* spp.), wheat curl mites (*Aceria tosichella*) and aphid species such as bird-cherry oat aphid (*Rhopalosiphum padi*) and green peach aphid (*Myzus persicae*). Applying the methods outlined in my thesis to more species will test the robustness of this framework, while generating important information to aid in pest management and crop protection under climate change.

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APPENDICES

Appendix 1.1 – Testing data collection bias

MAXENT models rely on unbiased datasets. I performed the following two analyses to ensure the datasets were unbiased and allowed for error in the methods used to compile the distribution information. First, as I extracted locality data from the published literature, I needed to confirm that any error in point locality data will not affect model performance. To do this I created a 25km buffer around each point and then randomly sampling within this buffer – or jittering. The jittered points were added to the points that had GPS coordinates (when available) to ‘anchor’ them with highly accurate locality data. This was repeated to give 10 replicates which all included random error surrounding each point extracted from the literature. Running these as separate models in MAXENT and then quantifying niche overlap using ENMtools revealed no significant difference between the models built on the extracted points versus the models built on the extracted points with random error (t -test: Aust.hist $p = 0.379$; Aust.curr $p = 0.376$).

Secondly, to test whether the datasets I used in the models were unbiased from roadside sampling, I built datasets that randomly sampled both near to and away from roads in southern Australia. I first obtained a shapefile of the roads for Australia from Geoscience Australia (www.ga.gov.au). An area was defined with a northern border that stretched across Western Australia, South Australia, Victoria and New South Wales. This border represents the possible extent of the distribution of *Halotydeus destructor*, including available area between known localities. I created a 1.5km buffer around each road and randomly sampled 5000 points restricted to this buffer (‘inside’). I then randomly sampled 5000 random points outside of this buffer (‘outside’).



Figure 1. Polygon used to mask southern Australia, with roads shapefile.

I considered the inside and outside datasets as separate ‘species’ and analysed them accordingly with the *dismo* package in R (version 2.10.1; R Development Core Team 2009) to determine if they were drawn from the same environments. I extracted variable information for the suite used in this study (*arid*, *bio4*, *bio8*, *bio18*, *bio19*) for each of the 5000 points in each dataset. I then performed a Principle Components Analysis (PCA) to determine how overlapping the climate space was for each ‘species’.

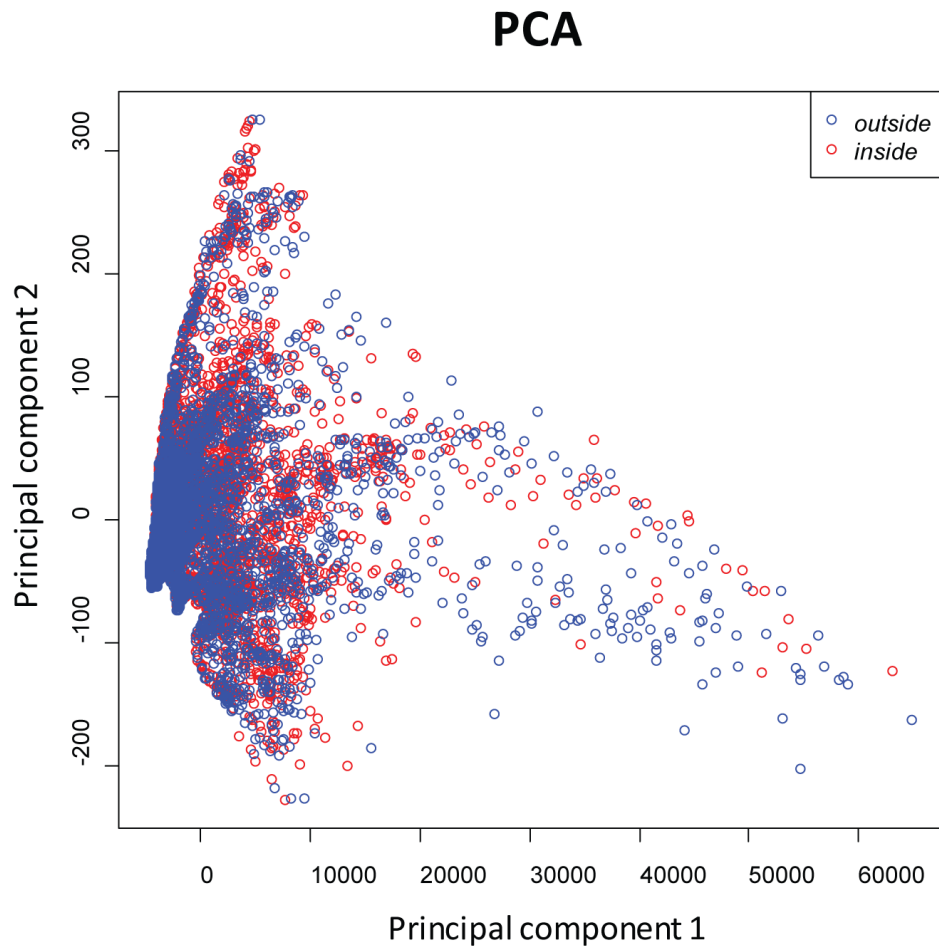
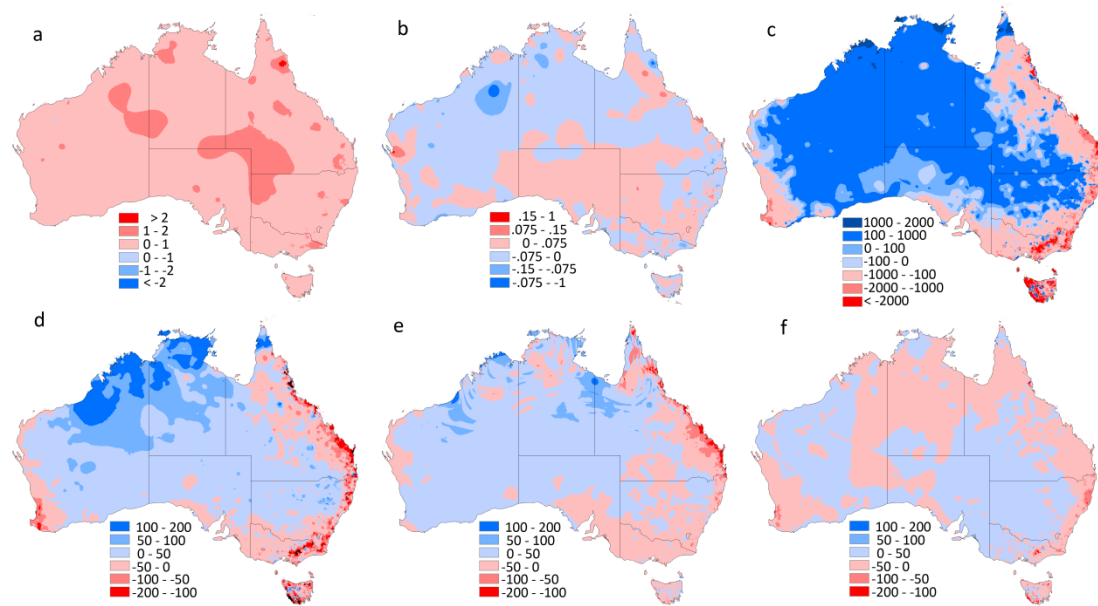


Figure 2. Principle Component Analysis of randomly sampled datasets, inside (red circles) and outside (blue circles) a road-buffer zone across southern Australia.

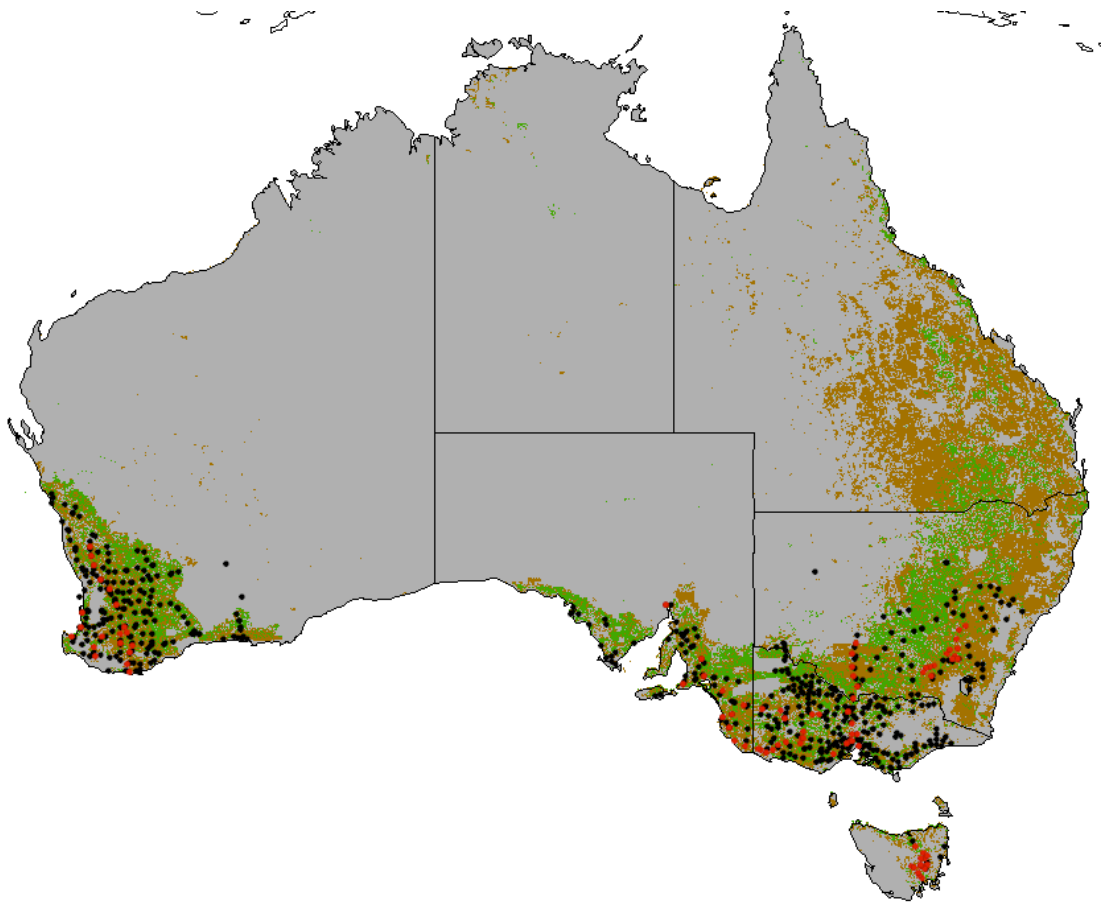
The datasets almost entirely overlap one another, suggesting that they are drawn from the same climate space; the axes hold the same explanation power for both. I can conclude from this analysis that the sampling from roadsides themselves do not provide bias in the climate variables chosen for this study.

Appendix 1.2 – Differences between climate datasets



These figures show differences between climate datasets (Current [1975-2010] – Historical [1921-1995]) for the variables used in this study, and mean temperature and precipitation. (a) “Mean Annual Temperature” (bio1), legend = temperature (°C); (b) “Temperature Seasonality (Coefficient of Variation)” (bio4), legend = coefficient of variation; (c) Aridity index (arid), legend = change in aridity index (The aridity index quantifies the ratio of precipitation availability over atmospheric water demand, with low values indicating arid conditions, and high ones humid climates); (d) “Annual Precipitation” (bio12), legend = precipitation (mm); (e) “Precipitation of Warmest Quarter” (bio18), legend = precipitation (mm); (f) “Precipitation of Coldest Quarter” (bio19), legend = precipitation (mm).

Appendix 2.1 - Distribution of *H. destructor* in Australia.

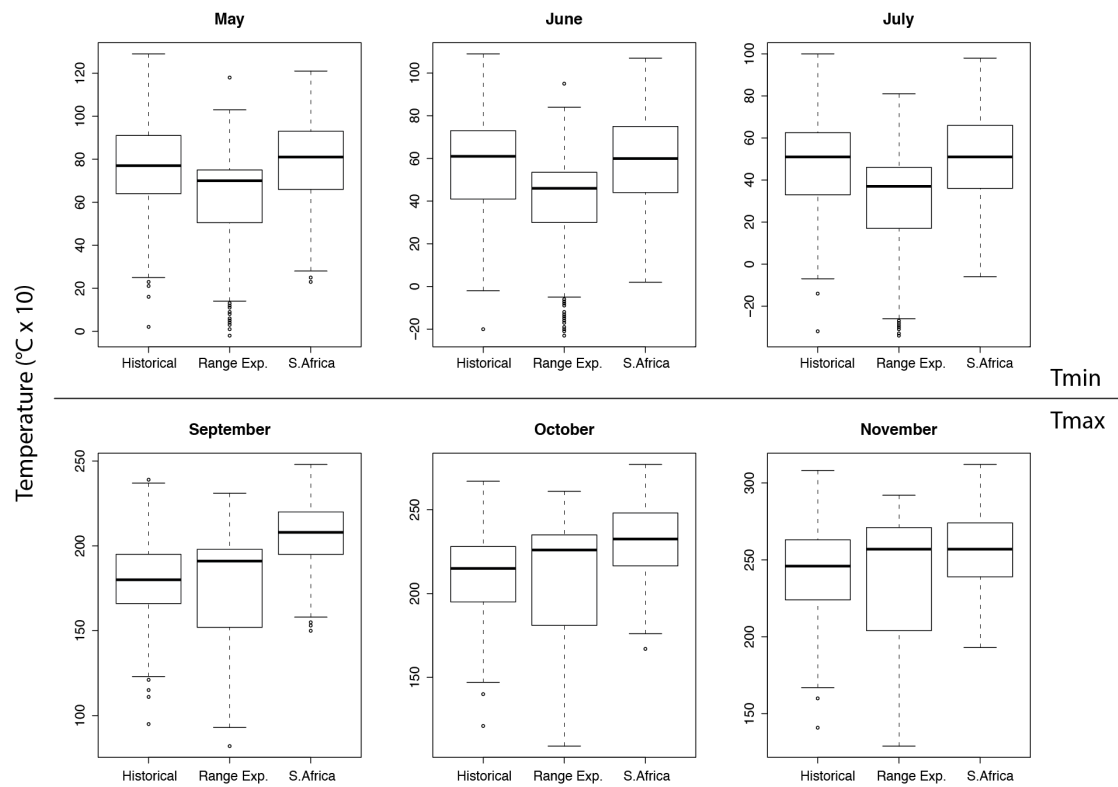


This figure shows the current distribution of *Halotydeus destructor* in Australia. With 530 distribution points from Hill *et al.* (2012) (black circles) plus 91 additional points added in this study (red circles) (see Methods). The distribution is plotted over cropping (green shading) and modified pastoral regions (brown shading) of Australia (Land Use of Australia, Version 4, 2005-06, Australian Bureau of Agricultural and Resource Economics – Bureau of Rural Sciences (ABARE–BRS). Available from <http://www.daff.gov.au/abares/>, accessed December 2012).

Appendix 2.2 Characterizing the environments present across the range expansion of *Halotydeus destructor*.

I aimed to determine differences in climatic variables across the core distribution and recent range expansion of *H. destructor*. Such information is essential to develop relevant hypotheses surrounding adaptive shifts for this species.

I thresholded model output from Hill *et al.* (2012), for all three models (South Africa, and both Australian historic and Australian current models) using the LTP-E (5) (5% of model scores at all training records removed) technique (Pearson *et al.*, 2007; Donalisio & Peterson, 2011). These thresholded outputs represented geographic areas for a) the South African native range, b) the historic Australian distribution of *H. destructor* in the 1960s and c) the area of range expansion beyond the historic distribution in Australia. I then generated 1000 random points across each of these areas. I obtained monthly temperature data from Worldclim (<http://www.worldclim.org> accessed October 2012; Hijmans *et al.*, 2005), and for each randomly generated point I extracted minimum and maximum temperatures of each month (averaged 1950-2000). As South Africa and Australia share Mediterranean type climates with mild, cool winters and hot, dry summers, I plotted minimum temperature for May, June, and July (beginning and middle of active season for *H. destructor*) and maximum temperature mainly for September, October and November (end of active season for *H. destructor*).



The area of range expansion for *H. destructor* in Australia has lower minimum temperature values than the area of the historic (and core) distribution, based on ENMs. For the months May, June, July (top three panels). For September and October, South Africa has higher temperature values than Australia, in November both South Africa and the Range expansion area are higher than the current Australian (bottom three panels).