



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

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ABSTRACT

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

Location Australia and South Africa.

Methods The red-legged earth mite, *Halotydeus destructor*, 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*, we select populations in both native and invasive ranges that appear to occupy different niches. We characterize thermal tolerance traits and test for acclimation patterns of cold tolerance of these *H. destructor* populations to test for niche shifts.

Results 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 with field-acclimated populations.

Main conclusions 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.

Keywords

Adaptation, Australia, environmental niche models, *Halotydeus destructor*, invasive species, niche shift, plasticity, South Africa, thermal tolerance.

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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 the 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 has also been shown for the invasive mosquito *Aedes albopictus* (Urbanski *et al.*, 2012). Niche shifts through evolution can thus allow improved performance of invasive species 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, 2012; Terblanche *et al.*, 2006; Alford *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 red-legged 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 the 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. Furthermore, 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. We 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 we 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. We also predict that South African populations display more variation in these thermal tolerance traits than do Australian populations, based on the relatively higher genetic diversity in South African populations (Qin, 1997). Further, we 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.

MATERIAL AND METHODS

Sampling and ENM construction

Halotydeus destructor has a wide range of host plant species that are common to both native and invasive ranges. We 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). We added these new locality data, using GPS data recorded at each site, to revise the models (Appendix S1). To determine if there were any significant changes from the models developed by Hill *et al.* (2012), we 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.

Site selection

Using the ENMs, we 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, we sampled across ENM output and extracted monthly minimum and maximum values (Appendix S2). We determined the northern inland limit of *Halotydeus destructor* by regularly sampling along roadsides, about 45 km north of Hay, New South Wales. We sampled four additional sites in New South Wales and Victoria and selected a further population at Conara, Tasmania. In South Africa we 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 collection for earth mites for ecological studies or pesticide efficacy (e.g. Umina & Hoffmann, 2005; Umina *et al.*, 2012). For each population, individuals were acclimated at either 10 or 15 °C (14-h dark, 10-h light cycle) for 2 weeks, with fresh *Trifolium* spp. leaves supplied every few days (see below). These two temperatures reflect the lower limit to development (10 °C) and are close to the optimum development (18 °C – but mites held at this temperature constantly for 2 weeks show mortality, so it was lowered to 15 °C) from mass-rearing methods (see Ridsdill-Smith, 1997).

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). We 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, we selected young adult mites of similar size for each experiment. We did not attempt to determine the sex of the mites, though we avoided the

larger adult mites which are reproductive females (Ridsdill-Smith, 1997). In South Africa *H. 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 by examining coxal setae under 100 × magnification (Qin & Halliday, 1996).

Heat tolerance

To assess how high temperatures affect the voluntary activity levels of *H. destructor*, we constructed an aluminium block with an arena (7.5 mm deep, 25 mm diameter) connected to the waterbath (Hazell *et al.*, 2008). The entire top surface of the block was covered with a Perspex block (50 mm × 100 mm × 10 mm). A small fan was placed next to the arena to prevent condensation. We used Fluon (Blades Biological, Edenbridge, 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 the VLC media player (VideoLAN, version 1.1.10, available at: <http://www.videolan.org/>, accessed July 2011). The temperature of the block was recorded every 10 s with an embedded universal temperature probe (model EI-1034; Electronic Innovations Corp., Lakewood, CO, USA) connected to a LabJack data acquisition device (Model U3; LabJack Corp., Lakewood, CO, 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 5 min at 18 °C (optimal rearing temperature; Ridsdill-Smith, 1997) and then a fast ramp-incline to 28 °C (0.5 °C min⁻¹). A ramp-incline (0.1 °C min⁻¹) was then conducted from 28 °C until complete heat coma (indistinguishable from mortality). We only considered individuals that could be scored for both traits (see Table 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.

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 limits distributions of species such as *Drosophila* spp. (Kellermann *et al.*, 2012). We measured CT_{MIN} by constructing an aluminium block (100 mm × 100 mm × 10 mm) with 19 wells (5 mm 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

Table 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	17.803	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

mites, replicates consisted of around 10 individuals for ease of scoring. A piece of Perspex (100 mm × 100 mm × 3 mm) was placed on top of the arenas. Mites were viewed under 40× magnification and scored directly.

We 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^{−1}) and then taken down further to −5 °C (0.25 °C min^{−1}), to measure the onset of CT_{MIN}. We then held the temperature for 5 min before ramping up (0.25 °C min^{−1}) 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 1.

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, we 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, we performed nested ANOVAs as for HMT/HCT, but added acclimation treatment as a fixed effect to the models and as an interaction term. We 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.

We 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). We obtained 2 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 minimum and maximum values divided by means for time period). To test for population differences we 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. We tested if Australian populations were significantly different from this source population by performing one-way ANOVAs between Stellenbosch and Australian populations for each trait.

RESULTS

Distribution modelling

We 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 Fig. 1. We 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).

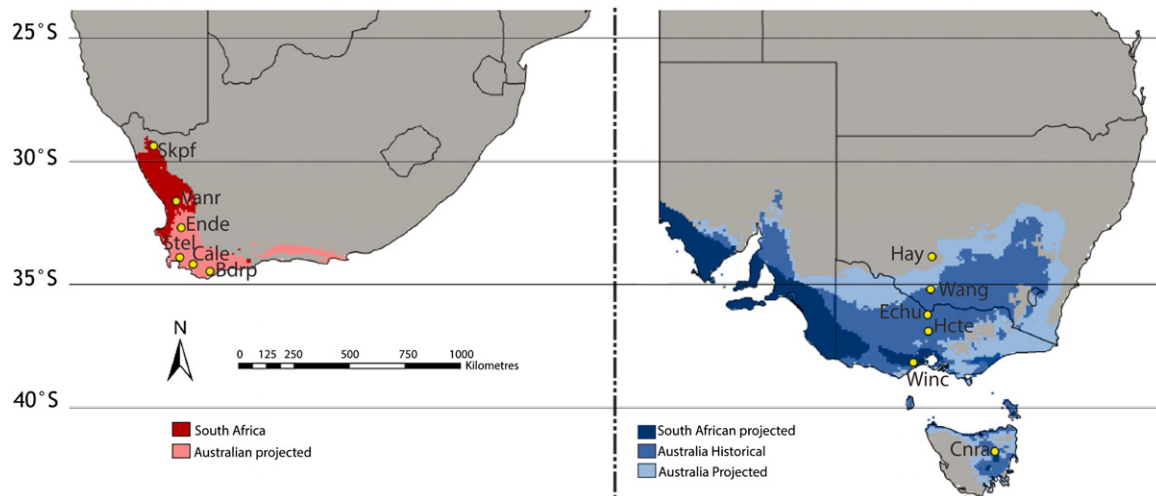


Figure 1 Population sampling of *Halotydeus destructor* in Australia (right) and South Africa (left) on top of the revised environmental niche models of Hill *et al.* (2012). Refer to Table 1 for population names. Shading in South Africa represents model prediction for South Africa (dark shading) and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

Populations sampled

Figure 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 S1). We used six populations in South Africa and six populations in Australia to assess HMT and HCT (Table 1). For CT_{MIN} we only tested four populations in Australia and three in South Africa due to time limitations.

Heat tolerance

For HMT and HCT, we tested 557 individuals of *H. destructor* across the 12 populations (Table 1). South African populations differed significantly for both heat tolerance traits, contributing to an overall population effect (Table 2, Fig. 2). There was no significant difference among populations of *H. destructor* in Australia for either HMT or for HCT (Table 2). Regression analysis indicated that isothermality of temperatures 2 weeks prior to collection was associated with Australian population means of HCT ($t = -3.184$, d.f. = 1, $P = 0.033$), but not for South Africa ($t = 1.138$, d.f. = 1, $P = 0.319$).

For both heat tolerance traits, means were higher in the invasive populations (Fig. 2), and the effect of country was significant for HMT and marginally non-significant for HCT (Table 2). Heat movement threshold (Fig. 2) was lower for South Africa (mean = 31.84 °C) than Australia (mean = 33.58 °C). Heat coma temperature (Fig. 2) was also lower in

South Africa (mean = 34.96 °C) compared with Australia (mean = 35.83 °C).

Cold tolerance

We tested 675 *H. destructor* (Table 1), and Fig. 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 2). There was no interaction between country and acclimation treatment for either trait (Table 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 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 3). Generally, 10 °C acclimation decreased CT_{MIN} onset trait values when compared with 15 °C, and field acclimated mites tended to be intermediate (Fig. 3). However, for one of the Australian populations, Hay, a contrasting acclimation response from the rest of Australian populations was displayed for CT_{MIN} onset, and no acclimation response for recovery. 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). 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.

Table 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 *Halotydeus destructor*. Significant terms ($P < 0.05$) are indicated in bold.

Trait/effect	d.f.	Mean square	<i>F</i>	Error	d.f. (error)	Significance
HMT						
Country	1	18.324	6.009	3.050	10	0.034
Population (country)	10	3.050	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	3.990	1.136	10	0.074
Population (country)	10	1.136	10.378	0.110	12	< 0.001
Australian populations	5	0.377	2.720	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

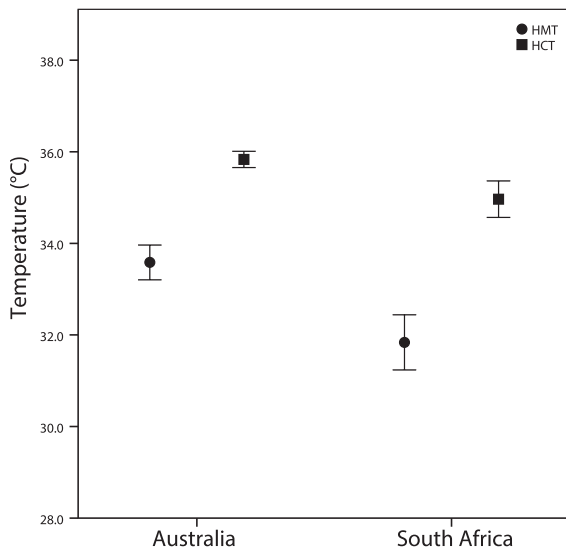


Figure 2 Heat movement threshold (HMT) (circles) and heat coma temperature (HCT) (squares) for Australia and South Africa. Points represent mean of each country. Error bars signify one standard error for means.

Comparing Australian populations to source population

The one-way ANOVAs comparing Stellenbosch with 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.

DISCUSSION

We predicted that Australian populations of *H. destructor* would differ in basal thermal tolerance traits and/or plasticity from South African ones, and that such differences may have contributed to a climatic niche shift. Australian populations of *H. destructor* had a higher trait mean for HMT, a 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. We 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. We 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.*,

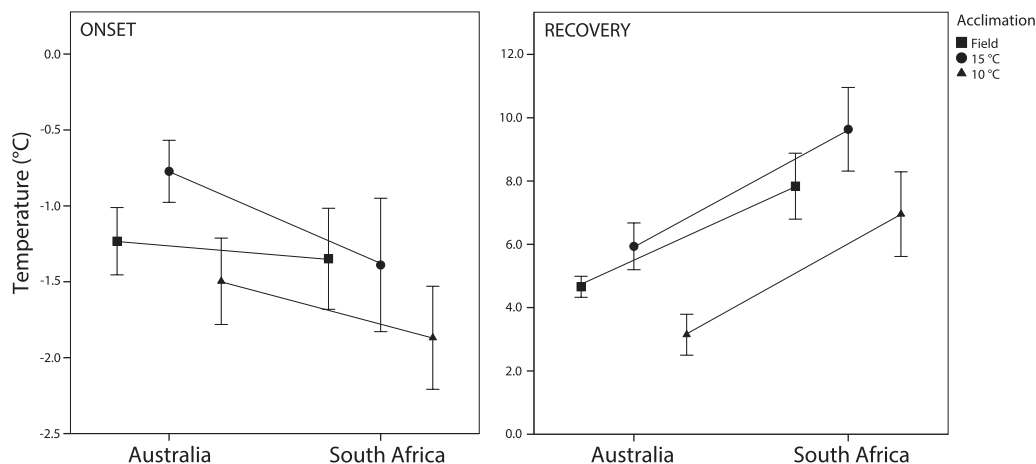


Figure 3 CT_{MIN} onset and recovery for Australia and South Africa. Points represent means of each treatment within country. Acclimation treatments are shown for field (squares), 15 °C (circles) and 10 °C (triangles). Error bars represent one standard error.

Trait/effect	d.f.	Mean square	<i>F</i>	Error	d.f. (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 3 ANOVAs for critical thermal minimum (CT_{MIN}) onset and recovery of 10 °C and 15 °C acclimated populations of *Halotydeus destructor* between Australia and South Africa. Significant terms ($P < 0.05$) are indicated in bold.

pop, population; treat, acclimation treatment.

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. ENMs 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 the 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 (cf. Chown *et al.*, 2009; Mitchell & Hoffmann, 2010). Because there were no significant interactions between acclimation and country, and because the field temperature covariates used added little to explaining observed differences in thermal responses between countries, we suspect that evolutionary adaptation has occurred in basal traits, reflecting adaptive shifts for

Table 4 ANOVAs for critical thermal minimum (CT_{MIN}) onset and recovery of 10 °C and 15 °C acclimated populations of *Halotydeus destructor* within Australia and South Africa, respectively. Significant terms ($P < 0.05$) are indicated in bold.

	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 \times pop	3	1.428	7.597	0.188	23	0.001
South Africa	Treat	1	1.241	4.290	0.289	2.004	0.174
	Pop	2	2.981	10.289	0.290	2	0.089
	Treat \times pop	2	0.290	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 \times 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 \times pop	2	3.281	3.487	0.941	16	0.055

pop, population; treat, acclimation treatment.

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 (Addo-Bediako *et al.*, 2000; Kellermann *et al.*, 2012). 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 substructuring 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 *H. 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 the 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, we 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 the 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.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Appendix S1 Distribution of *Halotydeus destructor* in Australia.

Appendix S2 Characterizing the environments present across the range expansion of *Halotydeus destructor*.

BIOSKETCHES

Matt Hill is a PhD candidate studying pest invertebrates at the University of Melbourne, Australia. His research combines distribution models, thermal physiology and population genetics to test for niche shifts, and how these are important for species invasion and response to climate change.

Steven Chown is at Monash University and has interests in the mechanistic basis of the responses of organisms to environmental change.

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