Modeling the consequences of thermal trait variation for the cane toad invasion of Australia

Jason J. Kolbe,[1,3] Michael Kearney,[2] and Richard Shine[1]

[1]*School of Biological Sciences A08, University of Sydney, Sydney, New South Wales 2006 Australia*
[2]*Department of Zoology, University of Melbourne, Victoria 3010 Australia*

Abstract. Mechanistic species distribution models (SDMs) are ideally suited for predicting the nonnative distributions of invasive species, but require accurate parameterization of key functional traits. Importantly, any ability of the invader to acclimate or adapt rapidly to local conditions must be incorporated. Our field and laboratory studies measured phenotypic variation and tested for plasticity in the thermal sensitivity of locomotor performance and low-temperature tolerance of adult cane toads *Bufo marinus* in eastern Australia. We used a biophysical model to explore the adaptive significance of this variation and how it affected distribution predictions. Laboratory trials showed that geographic differences in low-temperature tolerance (i.e., the critical thermal minimum; CTMin) of field-caught toads reflect thermal acclimation, whereas populations differed in the thermal dependence of locomotor performance even after acclimation. Incorporating low-temperature tolerance as a dimension of the fundamental niche reduced the predicted southern distribution. To test whether these factors predicted to be range limiting were consistent with reduced performance for individuals, we used the biophysical model and daily climate data to conduct "virtual transplants." These models predicted that acclimation reduced cold stress by 32–100% for toads sheltering near the ground surface; toads inside burrows could remain above their CTMin, but the required burrow depth increased with latitude. Low-temperature tolerance of the adult phase may constrain the southern range limit of the cane toad in Australia, and plasticity in this trait may have facilitated the southward range expansion.

Key words: anuran; Australia; Bufo marinus; cane toad; distributional modeling; nonnative range; phenotypic plasticity; thermal tolerances.

Introduction

Predicting the establishment, spread, and impact of potential invaders are primary goals of invasion biology (Drake et al. 1989, Williamson 1996, Kolar and Lodge 2001, Lockwood et al. 2007). Studies often compare life-history characteristics to predict successful invaders (e.g., Rejmanek and Richardson 1996, Kolar and Lodge 2002), and more recently, have used a variety of modeling approaches to predict nonnative distributions (e.g., Peterson 2003, Broennimann et al. 2007, Kearney et al. 2008, Phillips et al. 2008a). Correlative species distribution models (SDMs) use the association between point occurrences or abundance measures and environmental data to predict geographic distributions, whereas more rarely used mechanistic SDMs combine phenotypic measurements of organisms (i.e., functional traits), biophysical models, and fine-scale climatic data to predict distributional limits independent of localities currently occupied (Kearney and Porter 2009). Both approaches typically assume that phenotypic traits and physiological tolerances do not change during invasion. This is a troubling assumption because many examples show that phenotypes can change rapidly during introductions due to evolution or phenotypic plasticity (e.g., Sexton et al. 2002, Stockwell et al. 2003, Yeh and Price 2004, Ghalambor et al. 2007, Hendry et al. 2008).

In the course of an invasion, the fundamental niche (Hutchinson 1957) of an introduced species will restrict the environmental space it can occupy, ultimately limiting its geographic range. Because of biotic interactions, dispersal limitations, and the availability of particular environmental conditions, native-range populations may not exist in all geographic areas within a species' fundamental niche (Jackson and Overpeck 2000). Consequently, when introduced outside of its native range, a species may experience novel environmental space that differs from any conditions experienced in its native range. Similarly, as an invading species expands its range, it may encounter environmental conditions that differ from those in the regions already invaded. Two mechanisms could confer an ability to tolerate these novel conditions. First, pheno-

[3] Present address: Department of Organismic and Evolutionary Biology and Museum of Comparative Zoology, 26 Oxford St., Harvard University, Cambridge, Massachusetts 02138 USA. E-mail: jjkolbe@gmail.com
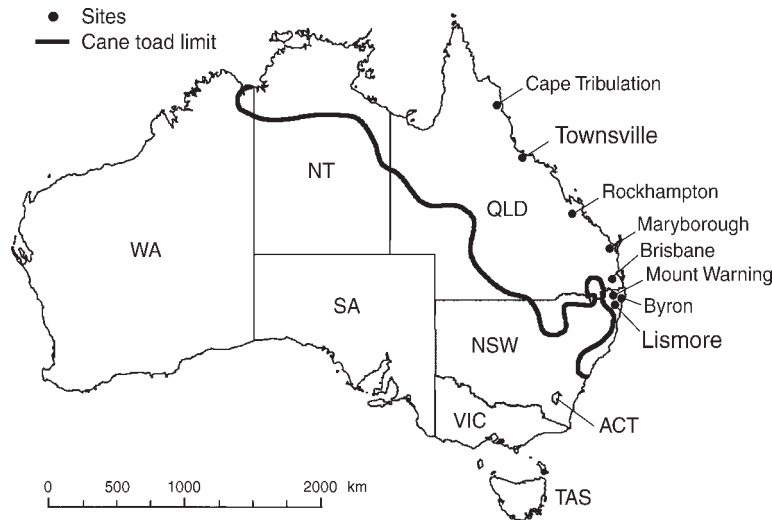
Fig. 1. Map showing the cane toad (*Bufo marinus*) sampling sites for this study in Queensland and New South Wales, Australia. The heavy black line defines the approximate spatial limit of all cane toad locality records. Abbreviations for states and territories are as follows: Australian Capital Territory (ACT), New South Wales (NSW), Northern Territory (NT), Queensland (QLD), South Australia (SA), Tasmania (TAS), Victoria (VIC), and Western Australia (WA). In bold are the two sites sampled for the laboratory acclimation experiment, Townsville, QLD (near the original site of introduction in Gordonvale) and Lismore, NSW. Site names correspond to those in Table 1.

typic plasticity in response to environmental conditions may allow an expansion of realized niche space, without any evolution of phenotypic values or reaction norms during the invasion. Second, novel selective pressures may result in evolution of trait values or reaction norms, thereby expanding the fundamental niche and allowing range expansion (Sexton et al. 2009). Thus, phenotypic variability may accompany establishment and expansion during invasion, confounding range predictions based on the assumption of static phenotypes.

Mechanistic SDMs explicitly incorporate the relationship between functional traits and climatic variation (e.g., Kearney and Porter 2004, Gilman et al. 2006, Buckley 2008). They therefore have an advantage over correlative SDMs in that questions about trait variation (either evolved or plastic) can be evaluated directly, although the implications of trait variation on distribution predictions using mechanistic SDMs are beginning to be explored only recently (see Buckley 2008). Here we utilize a set of biophysical/behavioral models in conjunction with field and laboratory data on lower thermal tolerances and thermal sensitivity of locomotor performance to assess the implications of trait variation for the predicted distribution of a widespread and devastating invader of Australia.

Since its introduction near Cairns in tropical northeast Queensland (QLD) from Hawaii in 1935 (originally from South America; Lever 2001), the cane toad *Bufo marinus* has expanded both westward (in northern Australia) and southward (in eastern Australia) to occupy >15% of continental Australia (Fig. 1). Toads continue to spread rapidly west through the tropical

Northern Territory (NT) into Western Australia (WA). In contrast, current range expansion southward along the east coast into temperate northern New South Wales (NSW) is markedly slower (Seabrook 1991, Lever 2001, Estoup et al. 2004, Urban et al. 2007, 2008). The cane toad invasion is having negative effects on Australian fauna (e.g., Crossland 1998, 2000, Phillips and Shine 2004, Shine 2010). No less than five efforts to predict the cane toad's range in Australia exist, using both correlative and mechanistic SDMs (van Beurden 1981, Floyd 1983, Sutherst et al. 1996, Urban et al. 2007, Kearney et al. 2008, reviewed in Phillips et al. 2008b). These models suggest that thermal constraints on breeding, development, foraging, and metabolism may limit the southern spread of cane toads in Australia (see Appendix A for a review of previous distribution predictions). We test two predictions derived from previous studies of the cane toad distribution in Australia: that cold stress due to insufficient warmth for daily metabolism limits southern expansion (Sutherst et al. 1996) and that cold temperatures limit adult foraging activity on the southern invasion front (Kearney et al. 2008). By isolating these dimensions of the fundamental niche, we can evaluate how variation in a functional trait affects the distribution prediction.

We focus on four questions to identify phenotypic variation related to thermal constraints and its implications for the distribution of the cane toad in Australia. First, do populations vary in lower thermal tolerance or thermal sensitivity of locomotor performance (Floyd 1983, Kearney et al. 2008)? We predict that southern toads will tolerate lower body temperatures and will hop

TABLE 1. Sites from which cane toads (*Bufo marinus*) were sampled for critical thermal minimum (CTMin) in New South Wales (NSW) and Queensland (QLD), Australia.

Site	Latitude (°S)	Elevation (m)	Mean July minimum temperature (°C)	Mean CTMin (°C)	N
Cape Tribulation, QLD	16.1	0	15.5	8.2	20
Townsville, QLD	19.3	10	13.1	7.5	21
Rockhampton, QLD	23.4	10	9.7	7.6	20
Maryborough, QLD	25.5	10	10.5	7.3	21
Brisbane, QLD	27.4	20	8.5	6.0	21
Mount Warning, NSW	28.4	200-400	7.4	4.9	8
Byron, NSW	28.7	10	9.9	6.2	21
Lismore, NSW	28.8	35	7.5	5.8	17

Notes: In bold are the two sites sampled for the laboratory acclimation experiment, Townsville (near the original site of introduction in Gordonvale, QLD) and Lismore. Site names correspond to those in Fig. 1.

faster at low body temperatures than northern toads, an ability that will extend the predicted southern distributional limit. Second, has the southward expansion of toads in eastern Australia been accompanied by low-temperature acclimatization? If so, then northern and southern populations should not differ in thermal sensitivity after short-term laboratory acclimation.

Third, does this phenotypic variation affect the distribution prediction? We use a recently published mechanistic SDM for the cane toad in Australia (Kearney et al. 2008) to evaluate how variation in thermal sensitivity of locomotor performance influences the distribution prediction. This approach attempts to map a species' fundamental niche onto a landscape, and assumes a species cannot survive or reproduce outside of this geographic area (i.e., population growth equals zero). We build upon previous work by incorporating lower thermal tolerances (measured in our study) into the model. Finally, are factors predicted to be range limiting consistent with reduced performance for individuals? Using the traits-based, biophysical/behavioral model and daily climatic data, we ask how toads respond to latitudinal clines in climatic conditions using empirically measured values for locomotor performance and low-temperature tolerance, both before and after acclimation. Daily data are critical in this respect because we are interested in extreme cold stress events, which are not captured by monthly or annual mean temperatures typically used in SDMs. These "virtual transplants" are modeled after experiments that assess the costs and benefits of laboratory acclimation for field-released individuals (Loeschcke and Hoffmann 2007, Kristensen et al. 2008). By explicitly modeling trait–environment interactions, we can explore the functional demands imposed by environmental gradients and potential benefits of acclimation during range expansion.

MATERIALS AND METHODS

Latitudinal variation in critical thermal minimum

We examined variation in the lower thermal tolerance of cane toads from north of the original point of introduction to the southern extent of the range expansion in northern NSW (Fig. 1), sampling eight populations along a 1500 km-latitudinal transect (Table 1). We sampled 8–21 adult toads per population (mean = 18.6) between 16 February and 7 March 2007. To look for short-term changes in lower thermal tolerances, we sampled toads from the same pond in Lismore, NSW again eight weeks later on 12 April 2007. Our measure of lower thermal tolerance was the critical thermal minimum (CTMin), a widely used index of cold tolerance in ectotherms (Cowles and Bogert 1944, Spellerberg 1972) defined as the lower temperature at which an animal loses its ability to right itself.

We measured CTMin on field-caught toads within 12 hours of capture as follows. Starting from a temperature of 22–24°C, we cooled toads by placing them individually in small plastic boxes inside a cooler with ice, and every 10 minutes we tested the righting response of each toad by flipping it on its back and stimulating its pelvic region with a small brush. We took body temperature (T_b) at this time by inserting a thermocouple probe 1 cm into the toad's cloaca. If a toad was unable to right itself after 30 seconds, the T_b was recorded as its CTMin. If a toad righted itself, it was cooled for another 10 minutes and retested. Methodological issues such as start temperature or rate of temperature change can affect measurement of critical thermal limits in insects (Terblanche et al. 2007). Although our method for measuring CTMin was consistent, toads cooled at rates ranging from 0.02° to 0.31°C/minute. Therefore, we included cooling rate as a covariate in the statistical analysis (Chown et al. 2009).

We used analysis of covariance (ANCOVA) with mass and cooling rate as covariates to test for a difference among populations in field-measured CTMin. We used a *t* test to determine if CTMin values differed after eight weeks for the Lismore population. Given a significant difference among populations in CTMin, we tested for a relationship between CTMin and both latitude and mean July (winter) minimum temperature using linear regression. Temperatures were extracted from the WorldClim (Hijmans et al. 2005) layer for mean July minimum temperature using the

latitude/longitude of each population. All statistical analyses were performed with JMP software (SAS Institute 2003).

Low-temperature acclimation experiment

We brought adult toads (mass = 86.5 ± 58.9 g, range = 19.5–383.5 g, mean ± SD) from Townsville ($n = 22$) and Lismore ($n = 27$) into the laboratory to test for an effect of low-temperature acclimation on CTMin. Toads were maintained on a 12L:12D photoperiod, fed crickets twice weekly, and had access to both water and dry substrate within their cages. They were held at 32°C for four weeks (Lismore) or eight weeks (Townsville) prior to the start of low-temperature acclimation and then transferred to 18°C for eight weeks. Because we measured CTMin on the same individuals over time, we used repeated-measures ANCOVA with mass as a covariate to test for a between-subjects population effect, and within-subject effects of acclimation time (i.e., capture, four weeks at 18°C, and eight weeks at 18°C) and the population by acclimation time interaction. We tested for a simple effect of acclimation time on CTMin for each population separately and used Tukey's hsd post hoc test to determine which acclimation times were significantly different. We also tested for a significant difference in CTMin between populations at each acclimation stage, adjusting for multiple comparisons using a Bonferroni correction (Rice 1989).

Locomotor performance differences between populations

We measured hopping speed of adults at four temperatures (15°, 22°, 29°, and 35°C) for the Townsville ($n = 22$) and Lismore ($n = 26$) populations after the eight-week acclimation at 18°C. Toads were maintained for at least 12 hours at the test temperature prior to a trial. Toads were weighed and T_b taken, then placed at the start of the racetrack and encouraged to hop by stimulating their hindlimbs with a paintbrush. The racetrack was 2 m long × 20 cm wide with 15-cm walls to discourage toads from hopping off the racetrack, and the substrate was cage carpet. We recorded times with a handheld stopwatch and toads were run twice at each temperature.

We analyzed the average hopping speed (meters per second) over the 2 m for each toad using ANCOVA with T_b and mass as covariates to test for a difference among populations. In addition to the two populations in this study, we also included hopping speeds for toads from the QLD and NT populations used in Kearney et al. (2008). We extrapolated from the temperature vs. hopping speed curve generated from these trials to estimate the low-temperature zero-activity point for each population. This was done by fitting a quadratic equation to each set of points and solving for speed = 0. This minor extrapolation was necessary to generate performance curves to be used in the biophysical modeling.

Biophysical modeling

We used a suite of biophysical/behavioral models (Niche Mapper) to predict potential distributions (Porter et al. 1973, Tracy 1976, Porter 1989, Kearney and Porter 2004, Porter and Mitchell 2006). We refer readers to Kearney et al. (2008) for applying this approach to the cane toad's distribution in Australia (see Appendix B for the details of its application to the distribution predictions and virtual transplants in this study). Here we briefly describe elements critical to this study. Niche Mapper constructs hourly estimates of microclimatic conditions directly relevant to organisms interacting with their environment, and in conjunction with phenotypic data from organisms solves coupled mass- and energy-balance equations to predict core body temperatures at hourly intervals throughout the day. Our modeling allowed only nocturnal activity in adult toads and assumed that toads were active whenever thermal conditions permitted (although in nature, toads may cease activity if the desiccation cost is high). We ran separate simulations that either forced toads to remain on the surface in the shade (e.g., in dense vegetation) or allowed toads to use underground burrows if surface conditions were unsuitable. Cane toads often use shelter sites that buffer their exposure to ambient conditions (Schwarzkopf and Alford 1996, Seebacher and Alford 1999, 2002).

Effect of functional trait variation on distribution predictions

The prediction of Kearney et al. (2008) for the spatial extent of the fundamental niche for cane toads in Australia used constraints on egg, larval, and adult stages of the life cycle, calculating whether conditions were suitable for breeding in each month and summing for a total length of the breeding season. We explored the effect of functional trait variation on the distribution predictions, focusing on two dimensions of the fundamental niche. First, we substituted the empirically measured thermal performance curves for Townsville and Lismore toads, to explore the effect of variation in hopping speed on two key components of distribution predictions (adult activity and movement potential). We present Australia-wide surfaces showing the difference in annual activity and movement potential between estimates from Kearney et al. (2008) and those using Townsville and Lismore performance curves.

Second, Kearney et al. (2008) did not consider the thermal tolerance of inactive toads as a constraint on the spatial extent of the fundamental niche in their model. We used the biophysical/behavioral model to make Australia-wide estimates of the cumulative time surface-restricted toads would spend at or below their CTMin. We then asked to what extent the previous distribution predictions changed when these lower thermal limits were included as a constraint, under the assumption that toads experience serious declines in fitness when core body temperature drops below the

CTMin (see *Discussion* for a justification relating to oxygen supply and demand at low temperatures). We used the high and low CTMin values measured in our study to illustrate the effect of acclimation on the distribution predictions. For simulations allowing toads to burrow, we generated Australia-wide estimates of the depth required to avoid exposure to CTMin. These simulations established areas where burrow use may enable toads to avoid lower thermal limits. These Australia-wide simulations were based on long-term monthly climatic averages.

Virtual transplants

To test whether factors predicted to be range limiting are consistent with reduced performance for individuals, we used our biophysical/behavioral modeling framework to ask how toads with the different hopping speeds and CTMin values would perform under the daily weather conditions observed at points along a latitudinal transect in eastern Australia. To conduct these virtual transplants, we used daily weather data for 2006 (the year prior to CTMin data collection) instead of the long-term monthly averages used for continent-wide simulations; this enabled us to capture extremes in climatic variation relevant for events happening on a daily time scale. These simulations generated site-specific values for potential nightly movement and cold stress (cumulative hours that core body temperatures were at or below CTMin). This procedure allowed us to determine the potential benefit of phenotypic acclimation and population-level differences for toads encountering thermally challenging environments during range expansion in eastern Australia.

We used paired t tests to explore differences in accumulated cold stress among the simulation scenarios. Because burrow-using toads always found a position above their CTMin, we used Wilcoxon signed-rank tests to evaluate differences in burrow depth needed to avoid the CTMin. By mapping the range of CTMin values from before and after acclimation, we explored the extent that acclimation of the CTMin could facilitate southward range expansion in eastern Australia. We also calculated the time spent below 18°C (to determine if our laboratory acclimation temperature was ecologically relevant) and the lowest T_b predicted for toads (to measure exposure to extreme temperatures, in addition to cumulative cold stress).

RESULTS

Latitudinal variation in critical thermal minimum

After adjustment by covariates, field-collected cane toads from populations along our latitudinal transect differed significantly in CTMin (Table 2a; Appendix C: Fig. C1). Interactions between population and each covariate were nonsignificant and removed from the final model. The mass of a toad did not affect its CTMin, but toads that cooled more slowly had lower CTMin values (Table 2a). For Lismore, CTMin

TABLE 2. Results of ANCOVAs exploring factors affecting critical thermal minimum (CTMin) and hopping speed among populations of cane toads.

Factor	F	df	P
a) Field-measured CTMin ($R^2 = 0.602$)			
Population	13.40	7, 139	<0.0001
Mass	0.05	1, 139	0.8163
Cooling rate	14.25	1, 139	0.0002
b) CTMin during acclimation			
Between subjects			
Population	8.35	1, 46	0.006
Mass	0.65	1, 46	0.424
Within-subject			
Acclimation time	31.34	2, 45	<0.001
Population by acclimation time	27.08	2, 45	<0.001
c) Hopping speed ($R^2 = 0.602$)			
Population	10.02	2, 378	<0.0001
Body temperature	523.91	1, 378	<0.0001
Mass	4.53	1, 378	0.0339
Population × body temperature	6.81	2, 378	0.0012

Notes: ANCOVAs are with (a) mass and cooling rate as covariates testing for a difference among eight cane toad populations in field-measured critical thermal minimum (CTMin); (b) mass as a covariate testing for a difference in CTMin between two populations of cane toads measured at three acclimation times: capture, 4 weeks at 18°C, and 8 weeks at 18°C (repeated-measures); and (c) body temperature as a covariate testing for a difference among the three cane toad populations in hopping speed (m/s) measured across 2 m with test temperatures ranging from 15° to 35°C.

decreased 0.8°C from February (late summer) to April (late autumn; $t = 2.34$, df $= 26$, $P = 0.01$). Both latitude (linear regression, $r = -0.86$, $P < 0.01$, $n = 8$) and mean July minimum temperature (linear regression, $r = 0.85$, $P < 0.01$, $n = 8$) showed significant relationships with CTMin.

Low-temperature acclimation experiment

In the laboratory acclimation experiment, repeated-measures ANCOVA revealed a significant difference in CTMin between populations, acclimation times, and their interaction (Table 2b). The (nonsignificant) population by mass interaction was removed from the final model. Mean CTMin values decreased over time due to acclimation for both Townsville ($F_{2,20} = 92.84$, $P < 0.0001$) and Lismore ($F_{2,25} = 92.84$, $P < 0.0001$), across all three acclimation times for Townsville but only from capture to four weeks for Lismore (Tukey's hsd tests; Appendix C: Fig. C2). The CTMin for Townsville was 2.1°C higher than Lismore at capture ($F_{1,50} = 73.33$, $P < 0.0001$), similar between sites after four weeks of acclimation at 18°C ($F_{1,47} = 1.37$, $P = 0.249$), and 0.6°C lower in Townsville toads after eight weeks ($F_{1,47} = 6.30$, $P = 0.016$). Acclimation induced a greater decrease in CTMin for Townsville toads (3.9°C) than Lismore toads (1.2°C). This population by acclimation time interaction may have arisen in part due to the difference in holding time at 32°C between

Lismore difference

a

Annual
activity (h)

□ 0–60
▨ −100
▨ −200
▨ −300
▨ −400
■ −500

Townsville difference

b

Annual
activity (h)

■ >1200
■ 1200
▨ 1000
▨ 800
▨ 600
▨ 400
□ 200
□ 0

c

Movement
(km/yr)

□ 0
▨ −5
▨ −10
▨ −15
■ −20
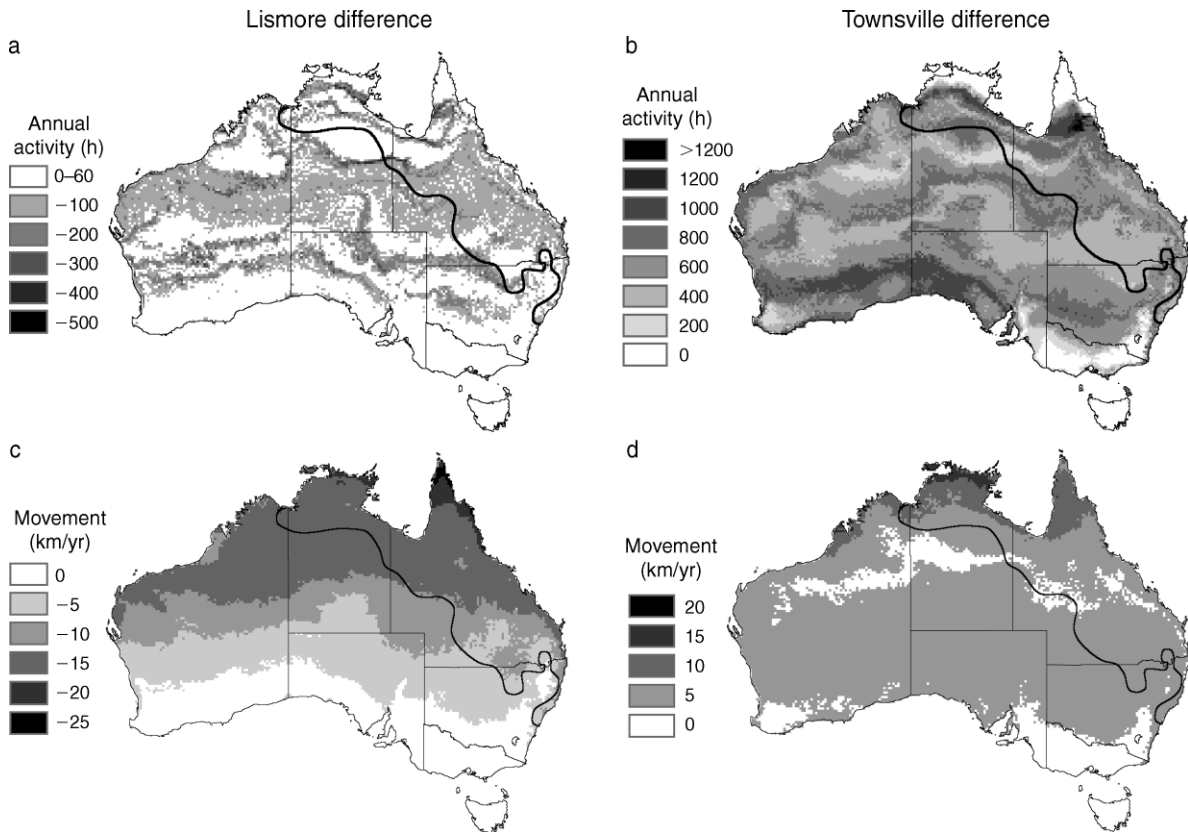■ −25

d

Movement
(km/yr)

■ 20
■ 15
▨ 10
▨ 5
□ 0

FIG. 2. Biophysical model predictions for key components of the fundamental niche and their effect on the distribution of the cane toad in Australia. Panels (a)–(d) show differences for each cell between predictions using low-temperature tolerance and thermal performance curves measured for Lismore or Townsville, and the original Kearney et al. (2008) predictions for annual activity (a, b) and potential movement (c, d). Values for each cell are dependent on the performance data (from Lismore or Townsville) and the local environmental conditions at each cell, creating a surface that varies continuously over all of Australia. Panels (e) and (f) show the breeding implications of excluding areas where surface-restricted toads would spend time at or below their critical thermal minimum (CTMin) from the potential distribution predicted by Kearney et al. (2008) using the high (8.2°C) and low (3.6°C) observed CTMin values, respectively. Similarly, panels (g) and (h) show differences in predictions of required burrow depths that toads must use to avoid spending time with a body temperature (T_b) at or below their CTMin using the high (8.2°C) and low (3.6°C) observed CTMin, respectively. Estimates of the number of breeding months per year in panels (e) and (f) are based on monthly assessments of (1) the ability of a toad to move >5 m per night in that month, (2) the availability of water for breeding, and (3) the potential for the eggs and tadpoles to develop and mature from a thermal perspective. The heavy black line in all panels defines the spatial limit of all cane toad locality records, and the dotted line in some panels defines the full extent of the original predictions from Kearney et al. (2008).

populations prior to the start of the 18°C acclimation treatment.

Differences in locomotor performance among populations

The hopping speed of a toad was affected by its population, trial temperature, mass, and the population by trial temperature interaction (Table 2c). The (nonsignificant) population by mass interaction was removed from the final model. Mean hopping speeds from 15° to 25°C were similar among populations, but Townsville toads hopped fastest at 30°C (Appendix C: Fig. C3). Unlike QLD and NT toads from Kearney et al. (2008), hopping speeds of Townsville and Lismore toads did not decline at 35°C. Quadratic fits of trial

temperature vs. hopping speed curves yielded zero-activity temperatures of 11.3°C for Townsville and 13.8°C for Lismore.

Effect of functional trait variation on distribution predictions

Continent-wide simulations, using long-term average monthly maximum and minimum climatic data, illustrated the landscape-scale consequences of different performance curves for annual activity and potential movement. Compared to the Kearney et al. (2008) prediction, annual activity was predicted to decrease (by 20 days) using the Lismore performance curve, but increase (by up to 50 days) using the Townsville data
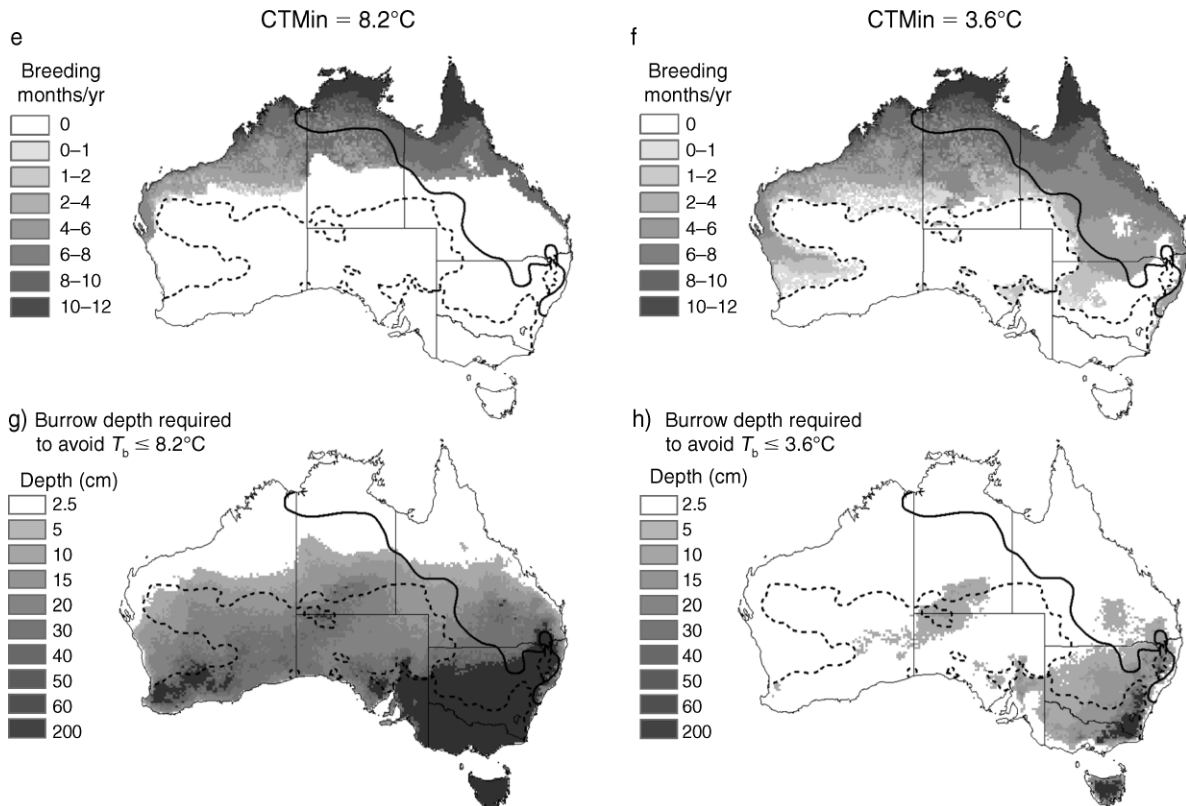
FIG. 2. Continued.

(Fig. 2a, b). Our simulations thus suggested large (up to 20 km/yr) differences in annual potential movement between the Kearney et al. (2008) estimate and those using Lismore and Townsville performance curves (Fig. 2c, d). Using the hopping speed data for Townsville toads, potential movement was predicted to be up to 5 km/yr more over most of Australia (Fig. 2d), whereas when using the Lismore data potential movement was predicted to be between 5 and 20 km/yr less than the previous prediction (Fig. 2c). Incorporating this variation into the predicted number of breeding months showed a correspondingly slight decrease using the Lismore performance data, but a substantial increase in the potential for breeding using the Townsville data (Appendix D: Fig. D1).

Continent-wide simulations also illustrated the landscape-scale consequences of different lower thermal tolerances for cold stress (Fig. 3a). Considering surface-restricted toads first, we found that with a CTMin of 8.2°C (the mean value observed for the most northern population: Cape Tribulation, QLD), toads in Australia north of the Tropic of Capricorn (~23°) would rarely be cold stressed. This region extends down the eastern coast to a latitude of 29° near Byron, NSW. All collecting sites are within the region of zero cold stress if the CTMin reduces to 5.3°C, close to the lowest field-measured CTMin value (Mount Warning, NSW, 4.9°C). If CTMin

is <7.4°C, sites in the south of mainland Australia have zero cold stress. At a CTMin of 3.6°C (the lowest mean value recorded after laboratory acclimation), cold stress is predicted only in inland southern regions, the island of Tasmania, and central Australia.

Kearney et al. (2008) predicted 59% of Australia to lie within the cane toads' fundamental niche. The highest CTMin observed in our study (8.2°C) reduces this estimate to 29% (Fig. 2e), whereas the lowest CTMin observed (3.6°C) reduces it to 54% (Fig. 2f). Although similar to the Kearney et al. (2008) prediction over much of Australia, incorporating a CTMin constraint of one month at 3.6°C reduces the predicted distribution in NSW and matches well the spatial limit of cane toad locality records for this area. Burrow-using toads could always avoid their CTMin, but this would necessitate finding shelters at least 5 cm below ground at the inland regions near their present southern range limit if they were to avoid the lowest CTMin of 3.6°C (Fig. 2g, h).

Virtual transplants

Predictions for average nightly movements by adult toads ranged from 0 to 500 m depending on the month and the population (Townsville or Lismore) used to parameterize the simulation (Appendix D: Fig. D2). All populations except the northernmost (Cape Tribulation, QLD) showed little or no predicted movement during

a) CTMin thresholds

T_b (°C)

- >8.2
- >7.4
- >5.3
- >3.6
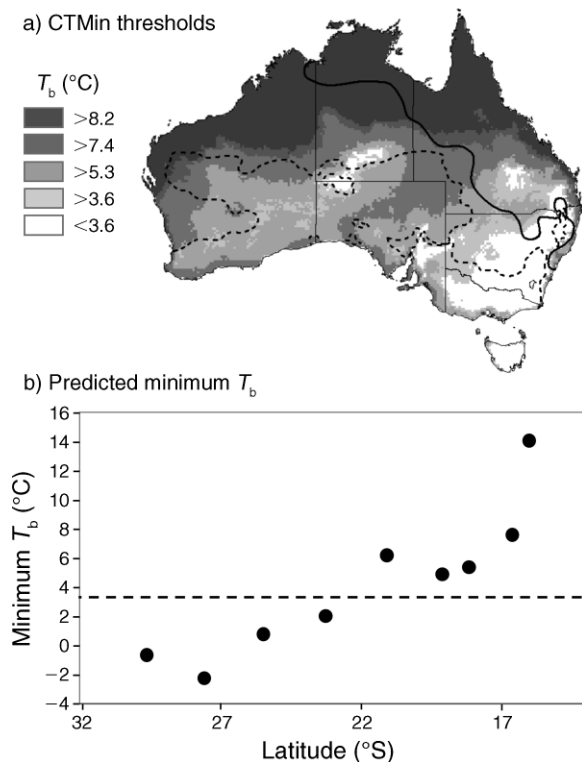- <3.6

b) Predicted minimum T_b

FIG. 3. Biophysical model predictions of (a) regions where cane toads would spend time at or above different thresholds of the CTMin, assuming that toads were restricted to superficial shelter sites above ground, and (b) the minimum body temperature predicted using daily climate data for toads along the latitudinal transect in eastern Australia. In panel (a), the dotted line defines the full extent of the original predictions from Kearney et al. (2008), and the heavy black line defines the spatial limit of all cane toad locality records. In panel (b), the dashed line indicates the lowest observed CTMin value of 3.6°C.

cooler months (June to September). Differences in the thermal sensitivity of hopping speed among populations and climatic differences resulted in greater potential movements per night at northern than southern sites, especially in summer (Appendix C: Fig. C3 and Appendix D: Fig. D2). Relative to Lismore models, predictions using the Townsville data predict a greater reduction in potential movement in warmer months (18–23%) rather than cooler months (6–11%). Overall, the greatest reduction in potential movement was at the Townsville site (23%) and the lowest at the Mount Warning site (12%). Total annual potential movement, averaged across sites, was 19% higher using Townsville than Lismore data. For the higher elevation and high latitude Mount Warning site, this resulted in predicted potential movement of 536 m/yr, close to the threshold for persistence (500 m) suggested by Kearney et al. (2008).

Simulations based on daily weather records predicted that toads restricted to shelters on the surface (thick grass, leaf litter, or small rocks) would spend from 0

hours to >30 days at or below their CTMin (Fig. 4a, b) depending on their location. All populations except those at the lowest latitudes in this study would experience body temperatures below 18°C for at least eight consecutive weeks (Appendix D: Fig. D3), suggesting that our laboratory acclimation conditions were biologically realistic. A reduction in cold stress due to acclimation was observed south of 22°S (between Townsville and Rockhampton in QLD: Figs. 1 and 4a, b; Appendix D: Table D1). The Townsville population realized a 66–100% reduction in cold stress, whereas the Lismore population reduced cold stress by 32–93% for non-burrowing simulations. When simulated toads were allowed to use burrows up to 200 cm deep, they could always remain above their CTMin. However, cold protection required increasing burrow depths with latitude with one of the southern sites requiring a 200 cm depth and many sites requiring >10 cm depth (Fig. 4c, d; Appendix D: Table D1). Acclimation reduced the required burrow depths, particularly for sites south of 22°S. The lowest body temperatures predicted for toads were below the lowest CTMin of 3.6°C for sites south of 22°S and fell below 0°C at the most southerly sites (Fig. 3b).

DISCUSSION

Although species distribution models have become an important tool for predicting nonnative ranges of invasive species (e.g., Broennimann et al. 2007), few studies have explicitly incorporated the possibility of geographic variation in phenotypes among nonnative populations, or whether this variation affects the distribution prediction (but see Terblanche et al. 2006). We found differences among populations in both the thermal sensitivity of hopping speed and CTMin (Table 2a, c; Appendix C: Figs. C1 and C3), and the CTMin exhibited rapid acclimation (Table 2b; Appendix C: Fig. C2). Variation in empirically measured performance curves (Appendix C: Fig. C3) led to differences in biophysical model predictions of annual activity and potential movement (Fig. 2a–d), which translated into changes in the number of breeding months and reduced the predicted cane toad distribution compared to previous predictions (Fig. 2; Appendix D: Fig. D1). Continent-wide simulations using long-term averages did not detect a change in the ability of a toad to move >5 m per night in that month (one component used to assess breeding potential); however, simulations with daily climate data show variation in potential nightly movement that may have consequences for breeding movement, dispersal, or local foraging (Appendix D: Fig. D2). In contrast, incorporating CTMin (and acclimation of this trait) as a dimension of the fundamental niche had a large effect on the predicted distribution. Our virtual transplants suggest that CTMin acclimation may benefit toads during range expansion in eastern Australia by reducing cold stress at higher latitudes (Fig. 4).
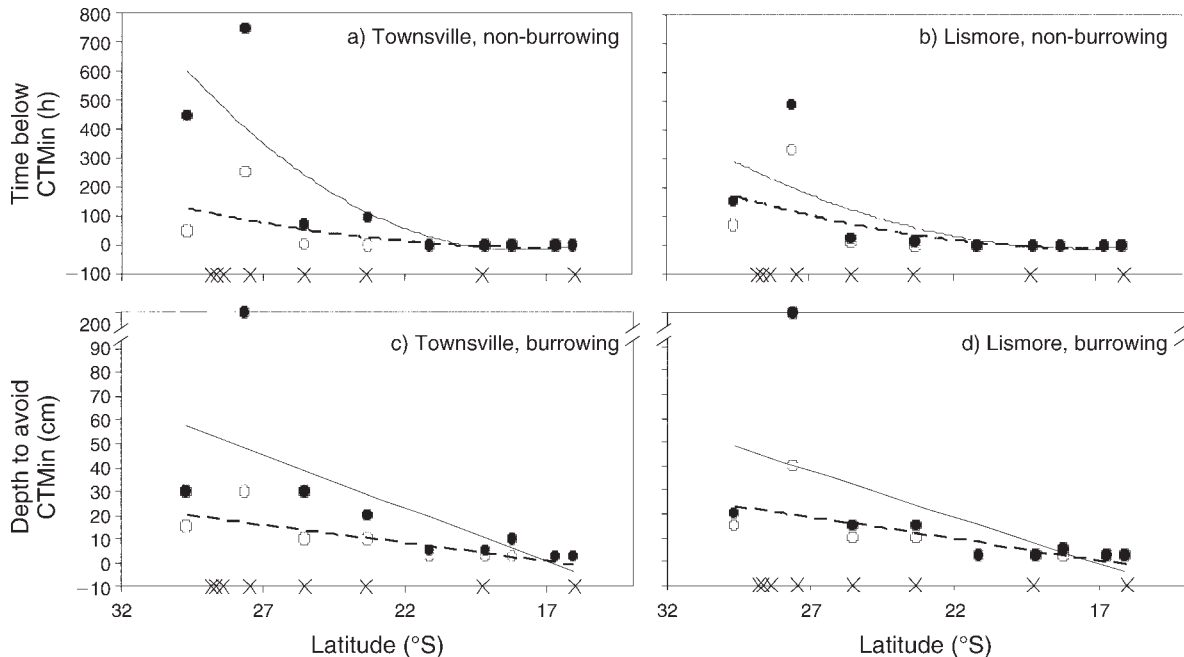
FIG. 4. Cold stress (the cumulative hours at or below the critical thermal minimum, CTMin), calculated using the ectotherm model and microclimate data modeled using observed daily climate data from 2006 for nine sites along a latitudinal transect in eastern Australia. (a, b) The non-burrowing simulations forced toads to remain on the surface in deep shade (90%) and low wind (0.1 m/s), whereas (c, d) the burrowing simulations allowed toads to use burrows up to 200 cm. Burrow-using toads were always able to find a position in the soil profile above their CTMin, so we report the maximum depth the toads must have located themselves rather than cumulative hours below the CTMin. Solid circles and solid lines represent results from simulations using CTMin values before acclimation (Townsville, 7.4°C; Lismore, 5.3°C), and open circles and dashed lines use CTMin values after 18°C acclimation for 8 weeks (Townsville, 3.6°C; Lismore, 4.2°C). The "×" symbols along the x-axis give the latitude of sites sampled for CTMin using field-caught toads (see Fig. 1 and Table 1).

The southward expansion of cane toads in eastern Australia has been accompanied by an increased tolerance to low temperatures (3.4°C difference in CTMin across our 1500-km transect; Appendix C: Fig. C1). Many other anurans show a similar decrease in CTMin with increased latitude (Snyder and Weathers 1975, John-Alder et al. 1988), but it is often unclear whether such differences are plastic responses, a result of fixed genetic differentiation, or a combination of both. Laboratory acclimation of eight weeks at 18°C caused the mean CTMin of the Townsville population to decrease by 3.8°C (Appendix C: Fig. C2), and over two months the mean CTMin of the Lismore population decreased from 5.8° to 5.0°C. These results suggest that the latitudinal range of CTMin values observed among transect populations is obtainable through acclimatization.

Although acclimation to low temperatures is known in some temperate amphibians (e.g., Layne and Claussen 1982, Menke and Claussen 1982), it is thought to be limited in tropical species. The low-elevation, tropical origin of introduced cane toads (Janzen 1967, Brattstrom 1968, Snyder and Weathers 1975, Huey 1978, Rome et al. 1992, Ghalambor et al. 2006, Deutsch et al. 2008) would lead us to expect a narrow range of thermal tolerances, limited acclimation ability, and high

CTMin, but our data do not support this prediction. Instead, cane toads in Australia can acclimate to low temperatures and exhibit a low CTMin. Have studies mischaracterized the thermal limits and acclimation ability of tropical species or is this a case of rapid post-introduction adaptation? A comparison of acclimation reaction norms for CTMin between Townsville (19°S), the source population for the Australian invasion in Hawaii, USA (20°N), and the native-range source for all introductions (French Guiana and Guyana, South America, 5°N; Slade and Moritz 1998, Lever 2001) is needed to clarify thermal acclimation abilities of cane toads prior to introduction in Australia. However, the Townsville population (near the original introduction site) acclimated to a CTMin value lower than northern NSW populations (Appendix C: Figs. C1 and C2), despite never naturally experiencing the colder climatic conditions typical of the latter region (Appendix D: Fig. D3). Toads occupy colder areas in Australia than expected based on their native distribution (Sutherst et al.1996, Urban et al. 2007), including areas with a mean minimum July temperature of <5°C in northern NSW (Urban et al. 2007). Still, the Lismore population could not tolerate lower temperatures than Townsville after acclimation, which would be predicted for adaptation to

colder conditions. Thus, we have no evidence for post-introduction evolution of lower thermal tolerances.

Hopping speed at low temperatures did not differ among populations, but reaction norms differed such that the Townsville population performed better at higher temperatures (Appendix C: Fig. C3). Given the persistence of this difference between Townsville and Lismore after an eight-week 18°C-acclimation period, and the lack of evidence for an acclimation effect on adult locomotor performance in other anurans (Wilson and Franklin 2000, Wells 2007), this may reflect a genetic difference between populations or developmental plasticity. These hypotheses need to be tested by raising toads in a common environment and determining if differences in hopping speed persist.

How might CTMin and the acclimation of this trait affect the distribution of cane toads across Australia? Our landscape-level simulations are based on monthly maximum and minimum temperatures, rendering them conservative for predictions of toad body temperatures near the ground surface because they do not capture extremes like daily weather data would. For these analyses, we tentatively assume than any time spent below the CTMin would prevent the occupation of a site. Where toads are restricted to superficial retreats near the surface (such as leaf litter, dense vegetation, or small rocks), body temperatures below the CTMin are predicted across much of southern Australia, ranging from 79% of the continent (CTMin = 8.2°C, unacclimated Cape Tribulation toads) to 35% (CTMin = 3.6°C, acclimated Townsville toads; Fig. 3a). Acclimation of lower thermal tolerance would greatly expand the potential distribution of the cane toad in Australia (Fig. 2e, f). The distribution prediction constrained by a CTMin value of 3.6°C is similar to that of Kearney et al. (2008) in western and central Australia, but reduced and more similar to the current distribution limit in eastern Australia (Fig. 2f). The southward expansion in NSW has slowed considerably (Seabrook 1991, Estoup et al. 2004), and the climatic envelope within the range of the cane toad has not expanded with respect to cold in the last 30 years (Urban et al. 2007).

In contrast, toads with access to underground shelter-sites could always avoid their CTMin in our biophysical simulations. For a CTMin of 8.2°C, this would require burrows >2.5 cm depth for non-coastal regions south of 21°30′, including depths up to 2 m in the southern extremes of the present range (Fig. 2g). For a CTMin of 3.6°C (acclimated Townsville toads), burrows >2.5 cm depth (maximum 15 cm) were only required in the most southerly parts of their present range, including only 1.5% of known presence sites (Fig. 2h). Thus, tolerance to extreme temperature should depend not only on physiological tolerance, but also the availability of suitable shelter sites. These analyses suggest that the ability of toads to invade southern parts of Australia is likely to have been strongly facilitated by acclimation of the CTmin and the extent to which it is constrained by shelter-site availability. To penetrate farther south than their present range, including areas predicted as marginally suitable by Kearney et al. (2008), access to deep burrows is required.

The performance limit induced by low temperatures likely depends on an interaction between biochemical, cellular, and systemic processes (Hochochka and Somero 2002, Angilletta 2009). For example, tolerance to thermal extremes may result from a mismatch between the demand for oxygen and the ability to supply oxygen to tissues (Pörtner 2001, Pörtner and Knust 2007). Indeed, at 5°C (i.e., close to the CTMin values measured in this study), isolated cane toad brain and liver tissue is unable to maintain intracellular sodium and potassium homeostasis (A. J. Hulbert, *unpublished data*). A disruption of brain function provides a mechanistic explanation for the loss of the righting response; breathing also ceases at this point forcing toads into anaerobic glycolysis and ultimately causing death if such temperatures are sustained. However, we do not know how much cold stress accumulation is required to kill a toad. There are few data on cold-induced mortality in cane toads, but the mortality rate of metamorph cane toads from northern NSW at 4.5°C was 50% after 4 h (van Beurden 1978), and adult toads were unable to tolerate >1 h at 0°C (van Beurden 1979). Mass die-offs of free-ranging cane toads were observed in southeastern QLD in the winter of 2006 coincident with record low daily temperatures ranging from −0.6° to 4.2°C (G. Vickery, *personal communication*; Fig. 3b), suggesting a possible direct or indirect effect of low temperatures on toad survival. Lower thermal tolerance in cane toads may be correlated with lower lethal temperature, as observed in other taxa (Hori and Kimura 1998, Das et al. 2004), and a decrease in CTMin via acclimation may confer a direct survival advantage at lower temperatures. We thus assume that body temperatures below the CTMin are deleterious and that survival is not possible for extended periods below this threshold. If toads use more superficial cover, they may experience cooler body temperatures more briefly, and it would be important to know the duration of cold stress that would be fatal.

What are the implications of our results for studies of range expansion in invasive species? Our results suggest that cane toads in Australia are expanding their range southward through plasticity in low-temperature tolerance, an unexpected result given the tropical origin of this species. The key implication of this finding is that methods not measuring and incorporating plasticity into distribution predictions will likely underestimate the expansion capabilities of invasive species. We show that toads would experience the environmental stimulus for inducing acclimation that we used in the laboratory (Appendix D: Fig. D3) and that the acclimation effect decreases exposure to temperatures otherwise below their thermal limits (Fig. 4). This plasticity may increase the likelihood of persistence as cane toads move south

into colder areas, and influence the pattern of natural selection on low-temperature tolerance in these newly established populations (Yeh and Price 2004). That plasticity facilitates range expansion in an invasion is not surprising (Richards et al. 2006); however, by utilizing a biophysical model that predicts body temperatures, we were able to extend our laboratory-based finding of low-temperature acclimation to realistic climatic and weather scenarios across the latitudinal span of the cane toad invasion. Our method of virtual transplants offers a novel way to quantify how divergent phenotypes perform under varying environmental conditions, providing a mechanistic understanding of trait variation that goes beyond typical field or laboratory assessment of plasticity in invasive species.

In addition to the effects of adult activity and potential movement on the distribution prediction, greater potential movement (as predicted by models using the thermal performance curve from Townsville toads) may result in increased dispersal and foraging opportunities. This difference is greater in warmer months and lower latitudes because the performance curves differ more at higher temperatures (Appendix C: Fig. C3). Cane toads are primarily visual predators, are relatively unselective in prey type or size, and their food intake is related to the abundance of food (Zug and Zug 1979). Toads covering a greater distance per night likely will encounter more food and have higher intake rates. If this translates into higher survival or reproduction, then faster toads may have an advantage. Potential trade-offs, such as greater susceptibility to predation, also need to be considered in the overall effect of variation in potential movement on fitness (Loeschcke and Hoffmann 2007). Predation may be a less important factor for cane toads in Australia due to the lack of toxin resistance in many native fauna, although data suggest toads that move farther have a higher probability of predation (Phillips et al. 2008a).

Our approach isolates the abiotic aspects of the niche and simulates the ability of individuals interacting with their environment to maintain optimal body temperatures (Kearney and Porter 2004, 2009), ultimately revealing areas where survival and reproduction are not possible. Further extensions that explicitly consider population dynamics in areas with nonzero fitness may reveal additional regions where cane toad populations cannot be sustained, providing another constraint on the distribution (e.g., Crozier and Dwyer 2006, Buckley 2008). Use of observed daily weather conditions rather than the monthly averages used in most climate-based analyses allows us to more accurately detect temperature extremes and quantify the effect on body temperatures. Climate surfaces for Australia based on daily thermal extremes rather than monthly averages, when available, will permit continental-scale prediction of where toads sheltering close to the surface would accumulate a lethal amount of cold stress. Presumably this refinement would detect exposure to low temperatures more often than do

predictions based on monthly averages. Biotic interactions may provide an additional constraint on the ability of a toad to achieve its optimal temperature, meaning that calculations of cold stress based solely on abiotic conditions are likely to overestimate the distribution. The close association of toads with human habitation may buffer exposure to temperature extremes, by providing more shelter sites and perhaps extending the range.

Mechanistic SDMs explicitly model the relationship between phenotypic measurements from organisms and climatic data, but previous applications have not incorporated phenotypic variation due to phenotypic plasticity or daily climate data as in our virtual transplants. This mechanistic approach to evaluating trait-based hypotheses for range limits can be expanded to include other traits such as water loss, larval development rates, or high temperature tolerance, and has broad application to both static and dynamic ranges. Such analyses can also identify traits and localities where research effort should be focused to obtain refined predictions of invasion potential. Integrating predictive modeling with targeted phenotypic data collection will produce greater insight into the causal mechanisms of range expansion in biological invasions.

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APPENDIX A

A description of previous species distribution modeling for cane toads in Australia (*Ecological Archives* A020-086-A1).

APPENDIX B

A description of the methodological details for the biophysical modeling used to predict the distribution of the cane toad in Australia (*Ecological Archives* A020-086-A2).

APPENDIX C

Figures showing population means for critical thermal minimum temperatures measured in the field and during the acclimation experiment, and hopping speeds at different temperatures for cane toads in Australia (*Ecological Archives* A020-086-A3).

APPENDIX D

Table and figures showing results of biophysical modeling for the cane toad in Australia (*Ecological Archives* A020-086-A4).