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Sexual and geographical divergence in head widths of invasive cane toads, *Rhinella marina* (Anura: Bufonidae), is driven by both rapid evolution and plasticity

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Measurements of > 3700 field-collected cane toads (*Rhinella marina*) show that head width relative to body length differs between the sexes and has become modified during the toad's translocation from French Guiana to Hawai'i and then Australia. Relative head width was highest in the native range, and declined progressively during the invasion. In long-colonized areas (French Guiana through to Queensland) male toads have wider heads than females, but this dimorphism decreases and eventually reverses at invasion fronts in both northern and southern Australia. To explore reasons for that variation, we conducted experiments on captive toads. A toad's head width affected its maximal ingestible prey size and prey-handling ability. Head width relative to body length was highly repeatable, consistent over ontogeny, and exhibited significant heritability ($h^2 = 0.20$). Relative head widths differed between the sexes and populations-of-origin even if offspring were raised under standard conditions in captivity. Nonetheless, relative head width of a cane toad also is affected by prey availability. Captive toads raised on a diet of small prey items developed wider heads than did conspecifics raised on larger prey, partly compensating for the trophic limitations of smaller body size. Sexual and geographical divergences in relative head widths in cane toads are thus the combined result of rapid evolutionary divergence (in < 100 years) and an ability of individual toads to flexibly modify this important morphological feature depending upon local conditions.

ADDITIONAL KEYWORDS: adaptation – *Bufo marinus* – morphology – phenotypic plasticity – sexual dimorphism.

INTRODUCTION

The size of an animal's head strongly influences fitness-relevant traits such as maximal prey size (in gape-limited predators: Shine, 1991a; Forsman & Lindell, 1993), bite force (important for predation and intrasexual rivalry: Vitt & Cooper, 1985) and sensory acuity (by constraining the size of sensory organs: Simon, Machado & Marroig, 2016). Perhaps as a result, head size relative to body size exhibits substantial variation among species, among populations within species, between sexes, and within an individual's lifetime (e.g. Shine, 1986, 1991b; Camilleri & Shine, 1990; Birch, 1999). Divergences in relative head size have been attributed to selective forces but can also arise as phenotypically plastic responses to environmental conditions (Queral-Regil & King, 1998; Bonnet *et al.*, 2001; Aubret, Shine & Bonnet, 2004; Van Kleeck, Chaiaverano & Holland, 2015).

Variation in head size is particularly significant in gape-limited predators, where a larger head may enable an individual to ingest a nutritionally important prey item (Schmitt & Holbrook, 1984; Shine, 1991a). For example, correlations between prey sizes and head widths suggest that head width influences dietary composition in amphibians (Toft, 1980, 1981; de Carvalho Batista *et al.*, 2011; Guimarães *et al.*, 2011). Anurans have wider heads relative to body length than do most other vertebrates (Blair, 1972; Handrigan & Wassersug, 2007), suggesting that head width influences their trophic biology (Emerson, 1985; Deban, O'Reilly & Nishikawa, 2001).

To test ideas about the functional significance of head size, intraspecific comparisons enable more powerful tests than interspecific comparisons (Gonda,

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Herczeg & Merilä, 2013; Rozen-Rechels *et al.*, 2016). Comparisons between populations within a species eliminate extraneous factors, especially if those populations diverged only recently. As a result, biological invasions provide powerful opportunities to explore organismal responses to novel challenges (Kolbe *et al.*, 2004; Dlugosch & Parker, 2008; Prentis *et al.*, 2008) both from the environment and from the process of invasion itself (Lambrinos, 2004; Shine, 2012; Van Kleeck *et al.*, 2015; Laparie *et al.*, 2016).

One intensively studied invasion is that of the cane toad *Rhinella marina* Linnaeus 1758 (formerly *Bufo marinus*) within Australia (Lever, 2001; Phillips *et al.*, 2006). These large anurans have evolved shifts in many traits, despite the brief time span (< 100 years) since they were translocated from their native range (Hudson *et al.*, 2016a). Most traits that have been investigated are functionally linked to the rate of dispersal, which has accelerated within the toad's invaded range (Phillips *et al.*, 2006; Urban *et al.*, 2008; Pizzatto *et al.*, 2017). Less attention has been paid to traits without obvious links to dispersal, but a morphometric analysis documented geographical divergence in head shapes as well as in limb lengths (Hudson *et al.*, 2016a). To explore this divergence, we have examined variation both through space (across the invasion transect) and through time (post-invasion, at one site). To clarify functional consequences of variation in head size, we looked at the effect of head width on prey-handling ability. To clarify the proximate underpinnings of variation in head width, we conducted experiments to measure heritability and developmental plasticity.

MATERIAL AND METHODS

STUDY SPECIES AND SAMPLING

Cane toads are large bufonid anurans native to Latin America (Lever, 2001) but translocated from French Guiana to the Caribbean, then from Puerto Rico to Hawai'i (*n* = 150 toads, in 1932), and from there to north-eastern Australia (*n* = 101 toads, in 1935: Slade & Moritz, 1998; Turvey, 2013). Cane toads consume a variety of prey (Strüssmann *et al.*, 1984; Lever, 2001; Lettoof *et al.*, 2018) including conspecifics (Fig. 1A) (Pizzatto & Shine, 2008). Adult male cane toads are smaller than females and congregate at spawning sites (González-Bernal *et al.*, 2015; Silvester *et al.*, 2017; Lettoof *et al.*, 2018).

We hand-collected mature [> 90 mm snout–vent length (SVL): Alford *et al.*, 1995)] toads from the native range, in Hawai'i and in Australia (Supporting Information, Table S1). We determined sex by dimorphic dorsal colouration, skin rugosity, nuptial pads, and male-specific 'release calls' (Lee, 2001;

Bowcock, Brown & Shine, 2008; Narayan *et al.*, 2008), and one of us (C.M.H.) recorded body length (SVL) and head width (HW) in the field using calipers (Fig. 1B). We chose HW (across the widest part of the head, reflecting the distance between the quadratojugal bones) as our measure of head size because it was more easily, accurately and repeatably measured than other dimensions (such as head length or depth).

At a site in tropical Australia [Fogg Dam, Northern Territory (NT); 12°37′S, 131°18′E] one of us (G.P.B.) also took these measurements across an 11-year period beginning with the arrival of the toad invasion front in 2005. Toads at the invasion front are dispersive (Phillips *et al.*, 2006; Lindström *et al.*, 2013; Pizzatto *et al.*, 2017), and no toad was found at Fogg Dam in more than a single wet-season.

FUNCTIONAL SIGNIFICANCE OF HEAD SIZE FOR FEEDING

First we trained 44 captive toads (approx. 1 year old, 25 females, 19 males) to consume dog biscuits; cane toads are unusual among anurans in eating non-moving items (Lever, 2001), but in our experience do not do so until they are trained by placing live insects in bowls containing immobile food such as dog biscuits. The toads accidentally ingest the biscuits while seizing the insects; and eventually toads will take the biscuits alone. Once training was complete we measured the toads' SVL and HW, offered them a round dog biscuit (13.5 mm diameter, 6.5 mm height, 0.6 g) and scored if they were able to swallow it, how long they held it in their mouths beforehand, and how many swallowing attempts they made. These biscuits were larger than most prey taken by toads, but smaller than the largest beetles we have found inside toad stomachs (unpubl. data). Thus, they provide a standardized challenge appropriate for gape-limited prey-handling in this anuran species.

REPEATABILITY AND HERITABILITY OF RELATIVE HEAD SIZE

We measured repeatability of our measures of SVL and head width by remeasuring five animals (all males, from a single population) three times each (with the observer blind to animal ID). To measure heritability we induced spawning in field-collected cane toads from sites in both eastern and western Australia, and raised the offspring at our field station (for details see Hudson, Brown & Shine, 2016b). Measurements of these 490 common-garden animals, compared to their 62 parents, were used to estimate heritabilities of relative head width with an 'animal model' (Wilson *et al.*, 2010) using ASREML software (VSN International, Hemel

Figure 1. The measurement and functional consequences of relative head width in cane toads (*Rhinella marina*). Cane toads feed primarily on small items, but take occasional prey (like this smaller conspecific) which are so large that they require a wide head to ingest it (A). We used calipers to measure head width (B). Photographs by G. P. Brown (A) and (B).

Hempstead, UK), and to estimate the ontogenetic repeatability of relative head width. We incorporated offspring ID and parental ID as random effects, with HW as the dependent variable, and included SVL as a covariate. We used the R package rptR (Nakagawa & Schielzeth, 2010) to calculate repeatability of measures of HW using individual ID as a random effect and SVL as a covariate.

PLASTICITY IN RELATIVE HEAD SIZE IN RESPONSE TO DIET

Captive-spawned progeny from a single clutch produced by adult toads from Townsville, Queensland; 19 animals) were raised at our field station.

The tadpoles were reared under standard conditions and metamorphs were toe-clipped, then distributed between the two treatments of 'small prey' (2–3 mm long) and 'large prey' (3–15 mm long). Measurements (SVL, HW) were taken at weeks 2, 5 and 7.

STATISTICAL ANALYSES

We used two-factor analyses of covariance (ANCOVAs) to assess the effects of sex, geographical origin and their interaction on head width, with SVL included as a covariate. We refer to measures of head width corrected for SVL as 'relative head width'. For graphical purposes we plot per cent head width (%HW) by dividing head width by SVL and multiplying by 100. As an index of sexual size dimorphism (SSD) we used the difference in mean values of %HW of males and females.

For analyses of the chronoseries data we performed separate multiple regressions to examine effects of sex and time (years since arrival of toads) on (1) SVL, (2) absolute head width and (3) relative head width.

To analyse the effects of experimental diet on SVL, we used a mixed model regression with toad ID as a random effect. Diet treatment (large vs. small prey), day number (as an ordinal variable with three levels: 0, 17 and 28) and their interaction were included as independent variables. To analyse the effects of diet on head width, we added SVL as an independent variable.

Except for the animal model and repeatability calculations, analyses were performed using JMP 11 software (SAS Institute, Cary, NC, USA).

RESULTS

ALLOMETRIC VARIATION IN RELATIVE HEAD WIDTH

HW as a proportion of SVL declined with body size in adult toads of both sexes ($P < 0.0001$), but SVL explained very little variation in %HW ($r^2 = 0.04$ in females, $r^2 = 0.01$ in males; $n = 1835$ and 1946, respectively). The mean shift in %HW from 100 to 130 mm SVL (a size range encompassing > 57% of adults) was 37.1 to 36.4% in females, and 37.3 to 36.9% in males. Separate analyses showed declines in %HW with increasing body size in females from French Guiana (FG), Hawai'i (HI), Queensland (QLD), New South Wales (NSW) and Western Australia (WA), and in males from Hawai'i (HI) and the Northern Territory (NT). In contrast, %HW did not change with increasing SVL in females from the NT or in males from QLD or WA, and increased with body size in males from FG. We ignored these slight and inconsistent effects of allometry in our subsequent analyses.

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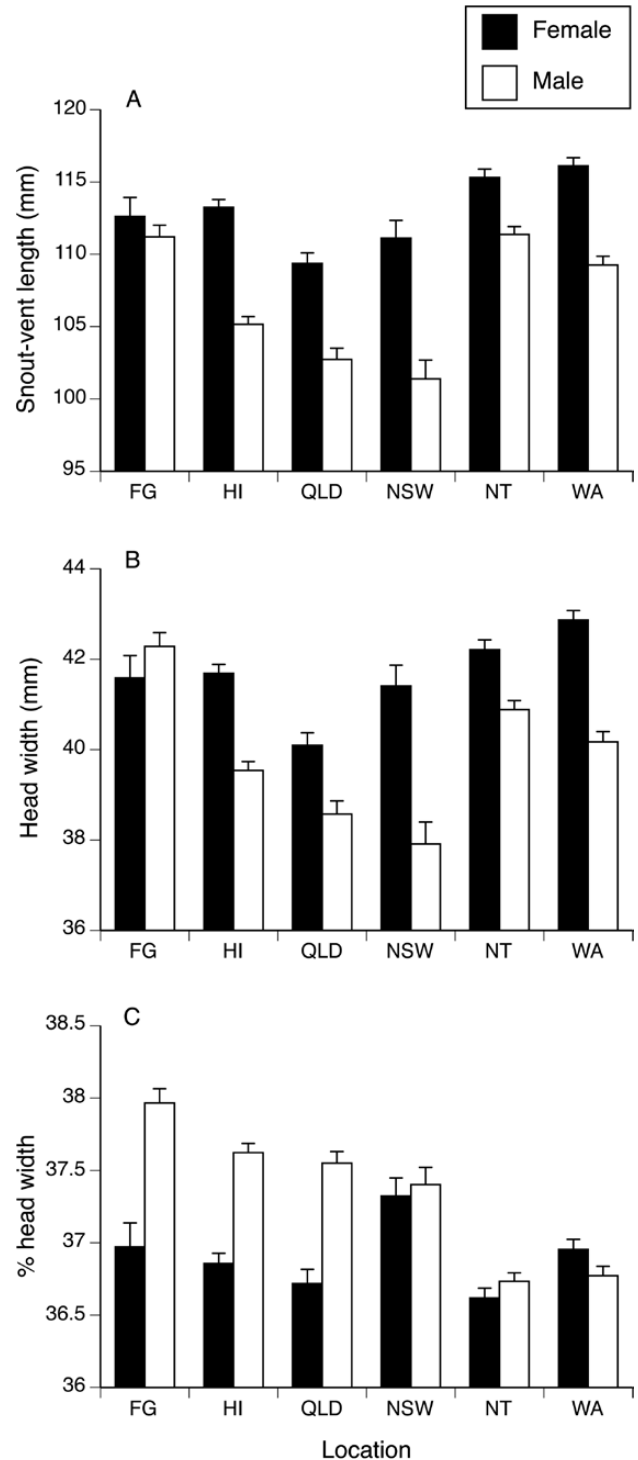## GEOGRAPHICAL AND SEX-BASED VARIATION IN BODY SIZES AND HEAD SIZES

Cane toads exhibit geographical differences both in mean body sizes, and in head widths relative to body size. Mean adult SVL ranged from 109 to 116 mm in females, and from 101 to 111 mm in males (two-factor ANOVA: Population, $F_{5,3769} = 37.1$, $P < 0.0001$; Sex, $F_{1,3769} = 156.1$, $P < 0.0001$; Population*Sex, $F_{5,3769} = 5.7$, $P < 0.0001$; see Fig. 2A). Mean HW of adult toads ranged from 40.1– to 42.9 mm in females, and from 37.9 to 42.3 mm in males (two-factor ANOVA: Population, $F_{5,3769} = 37.11$, $P < 0.0001$; Sex, $F_{1,3769} = 156.09$, $P < 0.0001$; Population*Sex, $F_{5,3769} = 5.74$, $P < 0.0001$; see Fig. 2B). HW relative to SVL also differed between the sexes (multiple regression: SVL, $F_{1,3768} = 27229.50$, $P < 0.0001$; Population, $F_{5,3768} = 19.00$, $P < 0.0001$; Sex, $F_{1,3768} = 36.20$, $P < 0.0001$; Population*Sex, $F_{5,3768} = 17.28$, $P < 0.0001$), and to different degrees (and, even, in opposing directions) in different populations (see Fig. 2C for the same patterns in %HW).

In male cane toads, head width relative to body length was greatest in the native range (French Guiana) and declined monotonically across the invaded range (Fig. 2C). Relative HW in females was less variable, with a modest decline from the native range in French Guiana to Hawai'i to Queensland to the Northern Territory (consistent with the pattern seen in males). However, this pattern was reversed in invasion-front populations (NSW and WA; Fig. 2C shows the same patterns in %HW).
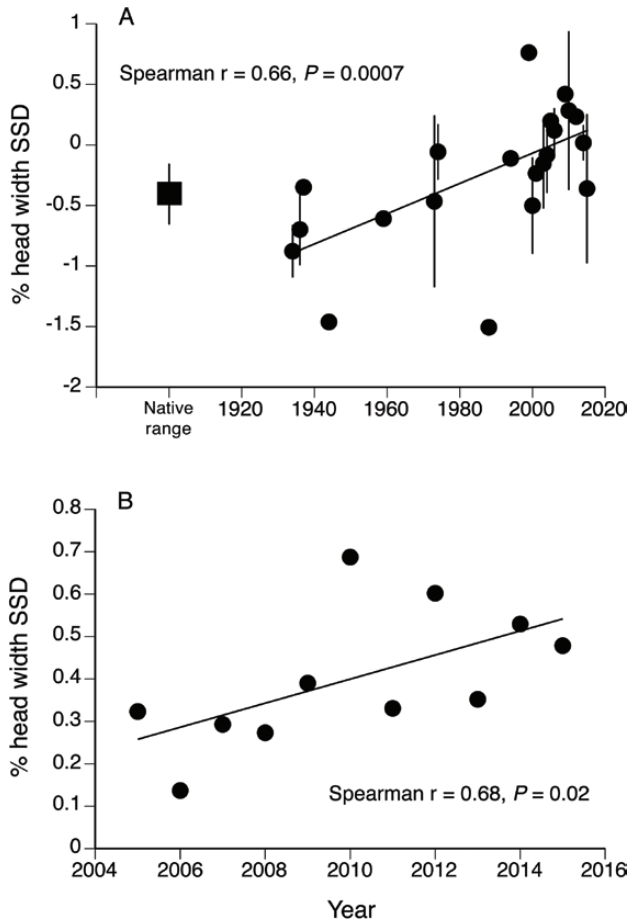
Reflecting those opposing patterns in males and females, sex differences in relative HW differed significantly among toad populations (see above for Population*Sex interaction term). Relative HW was larger in males than females in the native range (French Guiana; and also in HI and QLD, the initial phase of the invasion), but equal in the next location to be invaded (NT); and males had narrower heads (relative to SVL) compared with females in the two invasion-front populations (significantly so in WA; see Figs 2C, 3 for patterns in %HW).

TEMPORAL VARIATION IN RELATIVE HEAD WIDTH DURING AN INVASION

The first female toads to arrive at Fogg Dam (NT) were larger than the first males, but over the next 10 years average size of males increased while average body size of females remained constant (Sex*Year interaction; Fig. 4A, Table 1). Absolute head width was greater in females than in males (Table 1) but mean head width did not shift over time for either sex (Fig. 4B, Table 1). Reflecting these contrasting temporal changes, relative HW of males decreased with time since invasion, while relative HW of females did not (Fig. 4B, Table 1).

Figure 2. Geographical and sex-based variation in (A) adult body size (snout–vent length, SVL), (B) head width and (C) relative head width (head width as % of SVL) in cane toads. Graphs show mean values and associated standard errors. FG = French Guiana, HI = Hawai'i, QLD = Queensland, NSW = New South Wales, NT = Northern Territory, WA = Western Australia.

was highly correlated with absolute HW (Spearman $r = -0.51$, $P = 0.0004$) but not %HW (Spearman $r = 0.20$, $P = 0.19$) and the same was true if we restrict analysis to the 26 trials where the biscuit was successfully ingested (HW vs. swallowing speed, Spearman $r = -0.61$, $P = 0.0008$; %HW vs. swallowing time, Spearman $r = 0.15$, $P = 0.47$).

REPEATABILITY AND HERITABILITY OF RELATIVE HEAD WIDTH

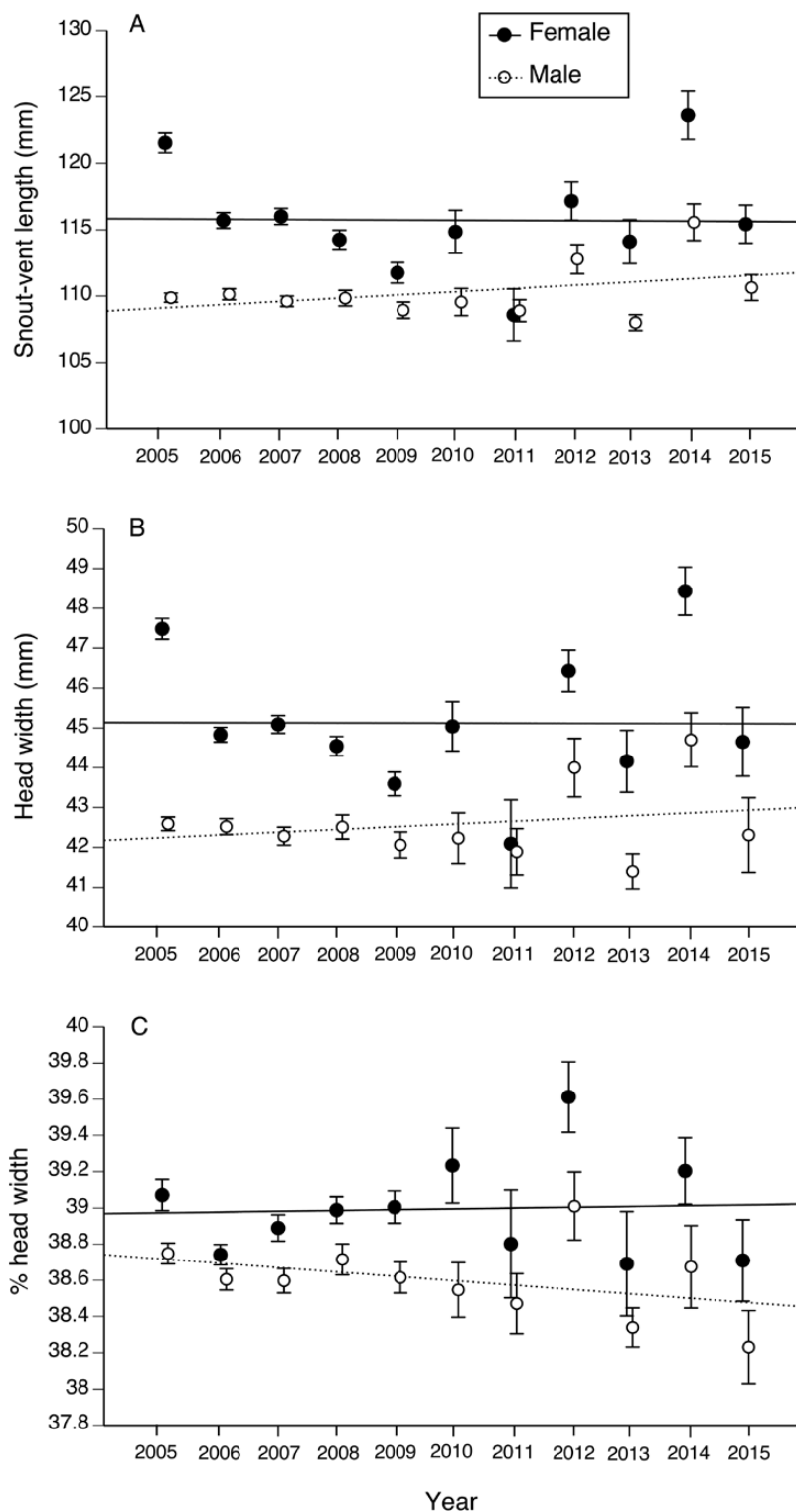Measurement repeatability was high (for SVL, 0.992 ± 0.0364; for HW, 0.966 ± 0.067; for relative HW 0.90 ± 0.19). Our animal-model analysis on wild-caught parents and their common-garden-raised progeny showed that relative HW was significantly heritable ($h^2 = 0.20 \pm 0.05$ if sex is included as a fixed effect; 0.16 ± 0.04 without inclusion of sex). No sexual divergence in relative HW was evident among the wild-caught parents of the common-garden offspring, from eastern (QLD) versus western Australia (Sex*State, $F_{1,56} = 0.14$, $P = 0.71$; Fig. 5A). Among common-garden offspring, sexual divergence in relative HW was evident among the progeny of QLD toads (relative head size larger in males than in females) but not WA toads (Sex*State, $F_{1,167} = 4.11$, $P = 0.044$; Fig. 5B).

DIET-INDUCED PHENOTYPIC PLASTICITY IN RELATIVE HEAD WIDTH

The young toads increased about four-fold in mass (from an average of 0.6 to 2.6 g) over the course of this 7-week experiment. Although we attempted to standardize prey volume, toads on the 'large-prey' treatment were slightly larger at the end of the experiment than were toads on the 'small-prey' treatment (mean mass of 2.83 vs. 2.25 g; $F_{1,17} = 2.60$, $P = 0.13$). Prey size strongly affected temporal changes in relative HW, with toads on the 'small-prey' diet developing wider heads compared with conspecifics given larger prey (Table 2, Fig. 6).

DISCUSSION

In many species, much of the variation in relative head size is driven by absolute body size (Vitt & Cooper, 1985). Such allometry complicates the analysis of divergences among populations and between sexes, but is not a significant problem with cane toads. The heads of this species are functionally isometric (Birch, 1999), and we saw only minor deviations in relative head width associated with body size of adult toads. The broad similarity in mean body sizes of toads in different populations further reduces any confounding effect of allometry on geographical comparisons.

Figure 3. Sex differences in head size through space (geographical comparison) and through time (at a single site; Fogg Dam, Northern Territory). (A) Changes in sex disparities in relative head widths (head width as % of snout–vent length) through the invasion history. Because multiple sites were invaded at the same time, some invasion date values are represented by more than one site and hence have error bars (SEM). (■) Native range, (●) invasive populations. (B) Shifts in sex disparities in relative head widths through time at Fogg Dam, over the 11 years following the first arrival of invasive cane toads at that location. SSD = sexual size dimorphism.

Because of the sex-based divergence in relative HW, our index of sexual dimorphism (difference in %HW of female minus male) increased significantly over time (see Fig. 3B; $N = 11$ years, Spearman $r = 0.68$, $P = 0.021$).

CONSEQUENCES OF HEAD WIDTH FOR PREY-HANDLING

Of 44 captive toads, 26 swallowed the biscuit and 18 did not. Swallowing success was related to absolute HW ($\chi^2 = 6.93$, $P = 0.009$), but not to %HW ($\chi^2 = 0.002$, $P = 0.96$). Handling time (including all trials) also

Figure 4. Shifts in (A) body length (snout–vent length, SVL), (B) head width and (C) relative head width (head width as % of SVL) of female and male cane toads at Fogg Dam, Northern Territory, as a function of time since initial colonization. Graphs show mean values and associated standard errors.

Future work could usefully explore other dimensions of shape variation within the heads of toads, although skull length and width appear to be isometrically linked (Birch, 1999; Hudson *et al.*, 2016a).

In their native range, cane toads have large heads that are wider (relative to body length) in males than in females. That pattern persists in samples from sites colonized more than 80 years ago (HI and QLD). As the toads began to spread out across Australia, however, they evolved a reduction both in relative head width and in the sex disparity in head width. At the Northern Territory sites (midway through the invasion transect), relative head widths were similar in males and females. The more rapid reduction of head widths in males

Table 1. Effects of time (years since initial arrival of toads at a site) and sex on cane toad morphology

Trait	Effect	d.f.	F	P
SVL	Sex	1,3459	347.60	**<0.0001**
	Year	1,3459	4.12	**0.0426**
	Sex*Year	1,3459	6.18	**0.0130**
Head width	Sex	1,3458	396.55	**<0.0001**
	Year	1,3458	3.04	0.0813
	Sex*Year	1,3458	1.29	0.2553
Relative head width	SVL	1,3457	25972.04	**<0.0001**
	Sex	1,3457	44.62	**<0.0001**
	Year	1,3457	0.22	0.6380
	Sex*Year	1,3457	12.18	**0.0005**

The table shows results of multiple regression analyses on effects of time since invasion and sex on the mean body sizes, head widths and relative head widths (head width corrected for covariate SVL) of cane toads (*Rhinella marina*) at Fogg Dam, Northern Territory, Australia. SVL = snout–vent length. Significant values ($P < 0.05$) are indicated in bold.

than in females continued as the invasion progressed, such that males had narrower heads (relative to SVL) than did females in recently colonized sites at both the western and the southern edges of the cane toad's current distribution in Australia. Thus, over the course of their Australian invasion, the ancestral condition of wider heads in males than in females was reversed.

What pressures have driven this rapid (80-year) decrease in relative head widths of male cane toads? That question is difficult to answer unambiguously. In relation to its body size, a cane toad has a larger head than most other vertebrates: a toad's body is less than three times longer than its head is wide (see Fig. 2C). As a result, relative head width may influence trophic biology (gape-limitation and prey-handling: Shine, 1991a), sensory abilities (space available for sensory structures; degree of binocular overlap in vision: Kraatz *et al.*, 2015; Kraatz & Sherratt, 2016; Simon *et al.*, 2016), burrowing efficiency (Engbrecht *et al.*, 2011), and/or acoustic communication (call propagation or localization: Wilczynski, McClelland & Rand, 1993; Griddi-Papp, 2008). Those functional correlates suggest that variation in head width is unlikely to arise from genetic drift or founder effects (because we would expect selection to counter such processes). Although the multiple functional roles of head size make it difficult to tease apart selective forces, we suggest the following evolutionary scenario.

The ancestral condition of relatively wider heads in male than in female toads may reflect sex differences in ecology and reproductive biology. Broadly, female cane toads concentrate on finding food to fuel reproduction, moving through the wider habitat matrix and returning to water only to rehydrate; clutches are produced once
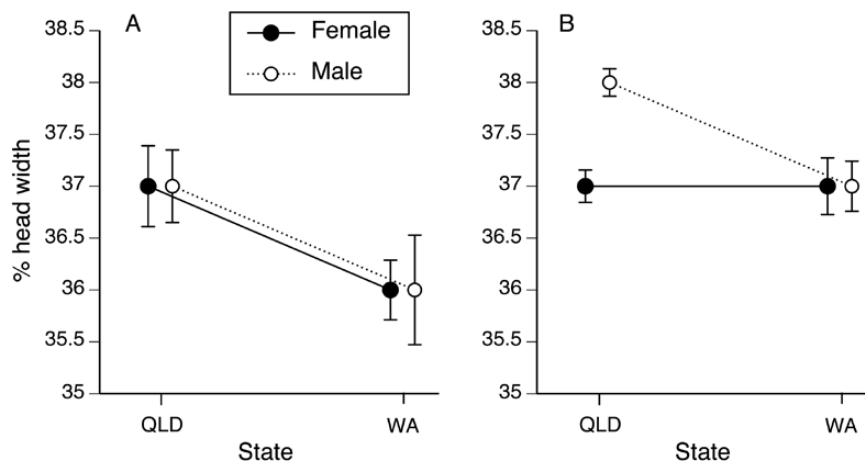
Figure 5. Relative head widths (head width as a % of snout–vent length) of (A) adult cane toads caught from the wild from eastern Australia (Queensland, QLD) and Western Australia (WA), and (B) in the captive-raised progeny of toads from these two populations. See text for details. Graphs show mean values and associated standard errors.

Table 2. Effects of prey size on cane toad morphology

Trait	Effect	d.f.	*F*	*P*
SVL	Prey size	1,24	0.42	0.5246
	Time	2,34	412.43	**<0.0001**
	Time*Prey size	2,34	7.11	**0.0026**
Head width	Prey size	1,25	0.02	0.8962
	Time	2,34	347.29	**<0.0001**
	Time*Prey size	2,34	0.16	0.8545
Relative head width	SVL	1,29	239.79	**<0.0001**
	Prey size	1,45	3.96	0.0528
	Time	2,40	0.68	0.5110
	Time*Prey size	2,36	9.43	**0.0005**

The table shows the results of repeated-measures analysis of the effect of prey size on body size, head width and relative head width (head width corrected for covariate SVL) of captive-reared cane toads. SVL = snout–vent length. Significant values (*P* < 0.05) are indicated in bold.

or twice a year (González-Bernal *et al.*, 2015; Silvester *et al.*, 2017; Lettoof *et al.*, 2018). In contrast, male cane toads spend much of their time at the edges of water bodies, producing advertisement calls that attract reproductive females (González-Bernal *et al.*, 2015). That habitat difference may influence the spectra of available prey (e.g. da Silva *et al.*, 2016). For example, metamorph cane toads are restricted to the margins of water bodies in dry conditions (Child, Phillips & Shine, 2008), increasing opportunities for cannibalism (Fig. 1A) in the sex that spends more time near the water. Additionally, relative head width may play a role in sexual selection, perhaps by modifying the advertisement call (Wilczynski *et al.*, 1993; Griddi-Papp, 2008).

The situation in Hawai'i and Queensland probably resembles that in the native range, favouring retention of the ancestral state of wider heads in males. But as cane toads dispersed westwards and southwards from north-eastern Australia, the selective forces on head morphology changed. The rate of dispersal of individuals at the invasion front accelerated (Urban *et al.*, 2008), such that those animals began to disperse farther and faster than has been recorded in any other anuran (Phillips *et al.*, 2006; Pizzatto *et al.*, 2017). Invasion-front toads focus on travelling, and feed as they disperse (Phillips *et al.*, 2007), eliminating any sex-based divergence in feeding habitats. Reproduction is rare among invasion-front toads (Hudson *et al.*, 2015), further reducing sex differences in habitat use. Competition for mates may be lower under the low densities at the invasion front, reducing selection on male-specific cranial attributes that influence the advertisement call. At the same time, the biomechanical stresses of prolonged long-distance dispersal impose novel selection on the anuran body plan. Many invasion-front cane toads exhibit spinal
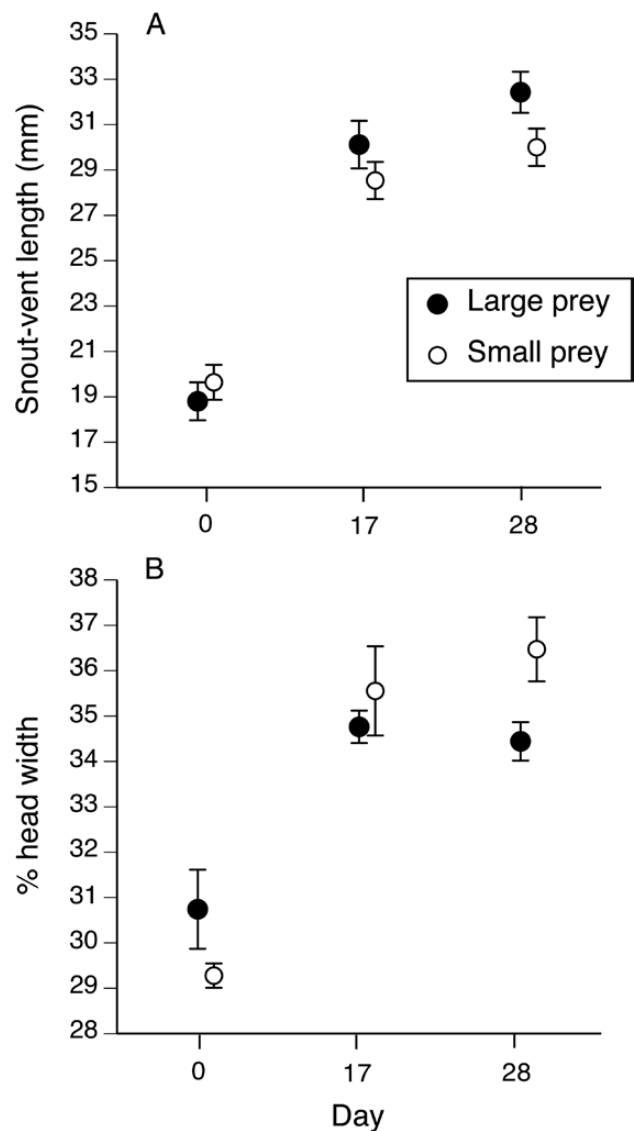
Figure 6. Effects of experimental treatment (small vs. large prey size) on (A) body size (snout–vent length) and (B) relative head width of juvenile cane toads. Graphs show mean values and associated standard errors.

arthritis, reflecting intense stress on the spinal column due to frequent movement (Brown *et al.*, 2007).

We have no data on the impact of relative head width on the speed or efficiency of locomotion in cane toads, but a larger head plausibly increases stress on the spinal column during hopping (Handrigan & Wassersug, 2007). If so, the central role of dispersal in the lives of invasion-front toads may have favoured convergence of the sexes towards a relative head width that facilitates sustained movement. Smaller head size also may allow limited resources (such as calcium) to be directed to the locomotor apparatus rather than the head. Consistent with the latter

hypothesis, calcium supplementation of the diet of young toads increased relative head sizes in range-core but not invasion-front toads (Stuart, 2016). Additionally, changes in cranial morphology might allow more effective placement of sense organs such as eyes; for example, interspecific variation in skull shape in rabbits may be due to selection for placement of the eyes to maximize the ability to visualize the substrate during rapid hopping locomotion (Kraatz *et al.*, 2015; Kraatz & Sherratt, 2016).

Both sexes of cane toads exhibited a reduction in relative head width over the course of the invasion, but females in the two widely separated invasion-front populations in Australia show a reversion towards wider heads. Also, relative head widths of male toads decreased rapidly through time in the chronoseries from a single site following invasion. Those rapid shifts at invasion fronts may have been driven by the non-adaptive process of spatial sorting (Shine, Brown & Phillips, 2011) and/or by developmental plasticity rather than by natural selection.

Spatial sorting is an evolutionary mechanism that generates novel phenotypes through space rather than time, by accumulating dispersal-enhancing traits in individuals at the leading edge of an expanding range front (Shine *et al.*, 2011). For example, if a larger head increased dispersal rate of females, despite conferring no fitness advantage, we would expect that trait to be common in the invasion vanguard. We have no data to show any such functional link, but it is straightforward to imagine possibilities. For example, a wider mouth may allow ingestion of larger prey, providing more energy to fuel continued dispersal, but at the cost of spinal stress and, ultimately, a shorter life. Intriguingly, a similar reversal of morphological clines at the toad invasion front (plus a reduction in sexual dimorphism) occurs also in limb dimensions. Relative to body size, limb length declined in cane toads as they dispersed away from the native range and through the Australian tropics, but that decline was reversed at the leading edge of the invasion (Hudson *et al.*, 2016b).

The rapid decline in relative head width of male toads immediately following initial colonization may be due to developmental plasticity rather than genetically driven changes. Although relative head widths exhibited significant heritability, the unexplained variation in this trait among captive-raised progeny probably reflects environmental impacts. In support of this interpretation, captive toads fed on smaller prey developed wider heads. Experiments on snakes (another group of gape-limited predators) have revealed similarly plastic responses of jaw dimensions to prey size – but in the opposite direction. In snakes, exposure to large prey induces an increase in jaw size, allowing the young snakes to swallow prey items that would otherwise be too large (Queral-Regil & King, 1998; Bonnet *et al.*, 2001; Aubret *et al.*, 2004).

Why should access to larger prey increase jaw size in snakes but reduce jaw size in toads? Juvenile snakes in an environment where all prey items are almost too large to ingest (as occurs in offshore islands) are under 'hard selection' (Wallace, 1975) to increase maximum prey size; a snake that is unable to swallow large prey cannot survive (Aubret *et al.*, 2004). In contrast, most of the insects consumed by cane toads are so small that the predator's head width does not constrain ingestion, and a longer handling time might disadvantage a toad but is unlikely to cause starvation. Under this 'soft selection' (Wallace, 1975), a slow-growing toad that increases its head width relative to its body size may benefit from the resultant increase in absolute head size.

Hence, a developmentally plastic increase in relative head width in response to encountering small prey may be adaptive, by compensating for slow growth in absolute body size. Our data on prey-handling ability indicate that absolute head width, not head width relative to body length, determines a toad's ability to manipulate and ingest prey items. Accordingly, a young toad that encounters small prey, and hence grows slowly, may benefit from developing a wider head than would be optimal for other (non-trophic) functions. We have no data on prey sizes of male and female toads, and note that the head-width dimorphism (as well as population-level variation) was evident even in captive-reared toads exposed to identical spectra of prey sizes. Thus, these divergences are at least partly due to heritability rather than developmental plasticity. The situation is complex, however; for example, sex divergences in relative head width appear to be genetically determined in Queensland toads but not Western Australian conspecifics (Fig. 5). Future work could usefully expose growing toads to a wider range of conditions (including of feeding rates as well as prey sizes) and explore impacts of larval environments also [although Blouin & Brown (2000) reported that larval temperatures did not affect head widths of anurans].

In summary, the morphology of the head in cane toads has been substantially modified by the species' translocation from South America and its subsequent spread across Australia. Head width relative to body length decreased, and the sex-based disparity declined and, eventually, reversed. The ancestral condition probably reflected ecological and reproductive divergence between the sexes, but the shift from sedentary habits to sustained dispersal imposed novel selective forces. The result was a reduction in relative head width, especially in males (initially the larger-headed sex), and a transition from sexual dimorphism to monomorphism. Some of the variation in relative head width is underpinned by genetic and/

or epigenetic factors, despite the brief time frame of the toad invasion, and some by developmental plasticity. Shifts in prey size (perhaps enforced by geographical variation in insect diversity, and by the lessened opportunities for sex-based habitat segregation at a rapidly expanding range front) may have generated variation in the relative head widths of toads among populations and between sexes.

Our results add to examples of rapid shifts in organismal morphology during invasions (Whittier, Macrokanis & Mason, 2000; Herrel *et al.*, 2008; Forsman, Merilä & Ebenhard, 2011; Berthouly-Salazar *et al.*, 2012; Therry *et al.*, 2014; Bitton & Graham, 2015; Pergams *et al.*, 2015; Renaud *et al.*, 2015). However, the probable causal mechanisms underlying this divergence in cane toads do not relate to novel challenges in the newly invaded landscape. Instead, the shift in head morphology of toads appears to involve sex-specific selective forces imposed by sustained range expansion. A priori, one would imagine that a change in dispersal rates would impose evolutionary pressures on traits such as limb bones, muscles and circulatory systems, rather than a trait such as head width. Nonetheless, shifts in head morphology during the cane toad invasion suggest that responses to invasional acceleration may extend to parts of the phenotype not overtly linked to locomotor ability. Future work should look more broadly at multiple phenotypic traits of range-core versus range-front individuals, to assess the generality of such wider impacts on morphology.

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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.

Table S1. Sampling locations and sample sizes of male and female cane toads measured for this study