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The loneliness of the long-distance toad: invasion history and social attraction in cane toads (*Rhinella marina*)

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Individuals at the leading edge of a biological invasion constantly encounter novel environments. These pioneers may benefit from increased social attraction, because low population densities reduce competition and risks of pathogen transfer, and increase benefits of information transfer. In standardized trials, cane toads (*Rhinella marina*) from invasion-front populations approached conspecifics more often, and spent more time close to them, than did conspecifics from high-density, long-colonized populations.

1. Introduction

Biological invasions may impose selection on behavioural traits, generating differences between individuals from long-colonized regions compared to individuals from populations at the expanding edge of the species' range. Compared with conspecifics from long-colonized populations, invasion-front individuals may be more exploratory and have a higher propensity to take risks [1] and consume novel foods [2]. Social behaviour also may diverge across an invasion range if populations differ in density, resource availability or climate [3]. Because population densities are lower at expanding range edges than in long-established populations, invasion-front pioneers will encounter conspecifics only rarely [4]. The cost of associating with a conspecific may be reduced at low densities, due to reduced competition for scarce resources and a scarcity of parasites and pathogens at the range-edge (due to reduced transmission rates, enforced by low encounter rates with conspecifics [5,6]). Social attraction may be beneficial at the range-edge because it allows individuals to exchange information (e.g. about the location of resources) and find mates; and grouping may provide protection from predators and from abiotic challenges such as extreme temperatures and desiccation [7].

Through social learning, individuals gain information by observation or interaction with other animals [8]. Such learning may be important at invasion fronts because individuals constantly encounter novel, unpredictable environments in which they must learn the location of food, water, shelter and mates [3]. Seeking out conspecifics and observing their activities may provide information about the location of resources [9]. For example, foraging conspecifics may indicate successful feeding sites and novel food acceptability while sheltering conspecifics may reveal the location of safe havens [8,10].

To test for geographical divergence in social behaviour, we quantified social attraction in standardized trials of cane toads (*Rhinella marina*) from long-colonized populations in Hawai'i and Australia, and from invasion-front populations in Australia. We predicted that invasion-front toads would be more likely to approach a conspecific, and to spend time with it, than would toads from long-colonized areas.

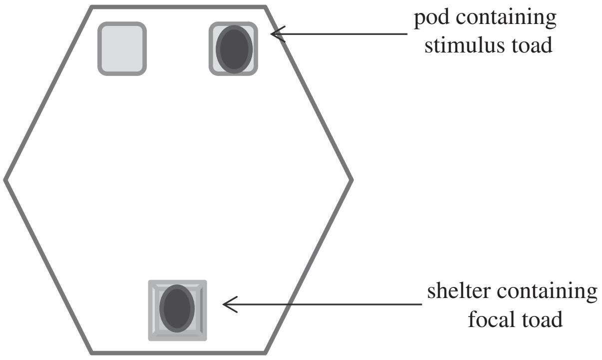

Figure 1. Arena testing social attraction in cane toads (*Rhinella marina*). The arena contained a transparent pod housing a stimulus toad and an empty transparent pod opposite the focal toad's start point.

2. Material and methods

Cane toads are anurans native to Latin America [11]. They were translocated to Hawai'i (via Puerto Rico) in 1932, and from there to Australia in 1935, to control insect pests in sugarcane plantations [11]. Populations in Hawai'i and eastern Australia (Queensland) thus represent long-colonized areas (greater than 80 years since colonization). Toads spread out across tropical Australia after their release; the invasion front is now greater than 3000 km from its point of origin [12]. In 2016 (when we collected our samples), the front was moving through the Kimberley region of northwestern Australia.

We collected 68 adult cane toads comprising 34 individuals (17 male, 17 female) from each of two locations across their Australian invasion range: Cairns, Queensland (greater than 80 years since colonization) and Oombulgurri, Western Australia (less than 3 years since colonization). In 2015, we collected 119 adult toads from two Hawai'ian islands (greater than 80 years since colonization) comprising 59 (29 male, 30 female) from Hawai'i (Big Island hereafter) and 60 from O'ahu (30 male, 30 female). Toads were collected by hand, measured (SUL, ±0.01 mm) and weighed (±0.1 g). Toads greater than 90 mm SUL were classed as adults and sex was determined by male-specific vocalizations and nuptial pads [13].

Toads were transported to animal-holding facilities (in the Northern Territory (12°37′ S, 131°18′ E) for the Australian toads; and near Hilo (19°20′ N, 155°9′ W) for the Hawai'ian toads). Two to four toads were housed together, fed live mealworms or crickets three times per week and water was provided ad libitum. Details of toad husbandry are described elsewhere [1,14].

Social attraction trials consisted of a 'stimulus' female toad secured within a transparent pod inside an (120 × 120 × 83 cm) arena (figure 1). To ensure that approaches to the stimulus toad were motivated by social factors rather than the search for shelter, we also provided an empty transparent pod (figure 1). The focal toad was introduced beneath a shelter at the opposite end of the arena to the stimulus toad (figure 1). Five minutes later this shelter was removed and the focal toad was free to explore the arena for 30 min. Order of testing was randomized among toads from different populations. However, during the Hawai'ian trials, the two island populations were assayed consecutively rather than simultaneously due to space constraints.

Because adult toads are active at night [11] trials were carried out in low-level red-lighting. Trials were filmed using CCTV cameras and we scored videos (with the scorer blind to toad origin and sex) using Ethovision XT10 software. Arenas and pods were wiped with diluted ethanol (10%) between trials to eliminate scent cues.

We used generalized linear models ('glm', R Core Team, 2017) to compare populations with respect to (i) whether or not a toad approached the stimulus toad to within 100 mm (the average body length of toads used in the study) and (ii) the amount of time spent within 100 mm of the stimulus toad. For the data on approach (Yes versus No) we used a model with a binomial logit link, and for data on time (log-transformed) we used a Gaussian identity link function. We included the potentially confounding factors mass, arena and trial number in both models. In preliminary analyses, we also included an invasion history × sex interaction term to assess the hypothesis that sex differences in behaviour may vary among populations. In both cases the interaction term was non-significant (both $p > 0.12$), and its inclusion did not significantly improve the fit over that of a model that included only main effects (likelihood ratio test; both $\chi^2 < 4.22$, both $p > 0.12$). Thus, we retained main effects only in our final models. We used Tukey's post hoc tests for pairwise comparisons. We pooled data from the two Hawai'ian islands as previous work revealed no significant behavioural differences between toads from these populations [14].

Table 1. Results of multiple regression analyses on factors affecting (i) whether or not cane toads (*Rhinella marina*) approached a conspecific and (ii) the amount of time cane toads spent within 100 mm of a conspecific in standardized behavioural trials. Origin refers to whether toads were from long-established populations (in Hawai'i and in, Queensland, Australia) or invasion-front populations (in Western Australia). Bold text indicates *p*-values <0.05.

variable	effect	d.f.	χ^2	*p*-value
(i) approach (Y/N)	origin	2	7.93	**0.019**
	sex	1	8.49	**0.004**
	arena	3	3.44	0.327
	trial	5	5.64	0.342
	mass	1	0.09	0.763
(ii) time (s)	origin	2	10.25	**0.006**
	sex	1	8.29	**0.004**
	arena	3	3.66	0.300
	trial	5	4.60	0.466
	mass	1	0.02	0.872

3. Results

Invasion-front individuals were more likely to approach the stimulus toad, and to spend more time with it, than were conspecifics from long-colonized areas (table 1; figure 2). Males were more likely to approach the stimulus toad and stay close to it, than were females (table 1; figure 2). Effects of body mass, arena and trial were non-significant in both analyses (table 1).

4. Discussion

As predicted, invasion-front toads were more likely to approach conspecifics and spent more time with them than did toads from long-colonized populations. We also found a sex effect; males were more likely to approach conspecifics and spent more time with them than did females. Because our stimulus toads were female, that sex bias might reflect sexual rather than social attraction. However, the greater sociality of male than female toads was seen in both long-colonized and range-edge populations, suggesting that the effect of invasion history

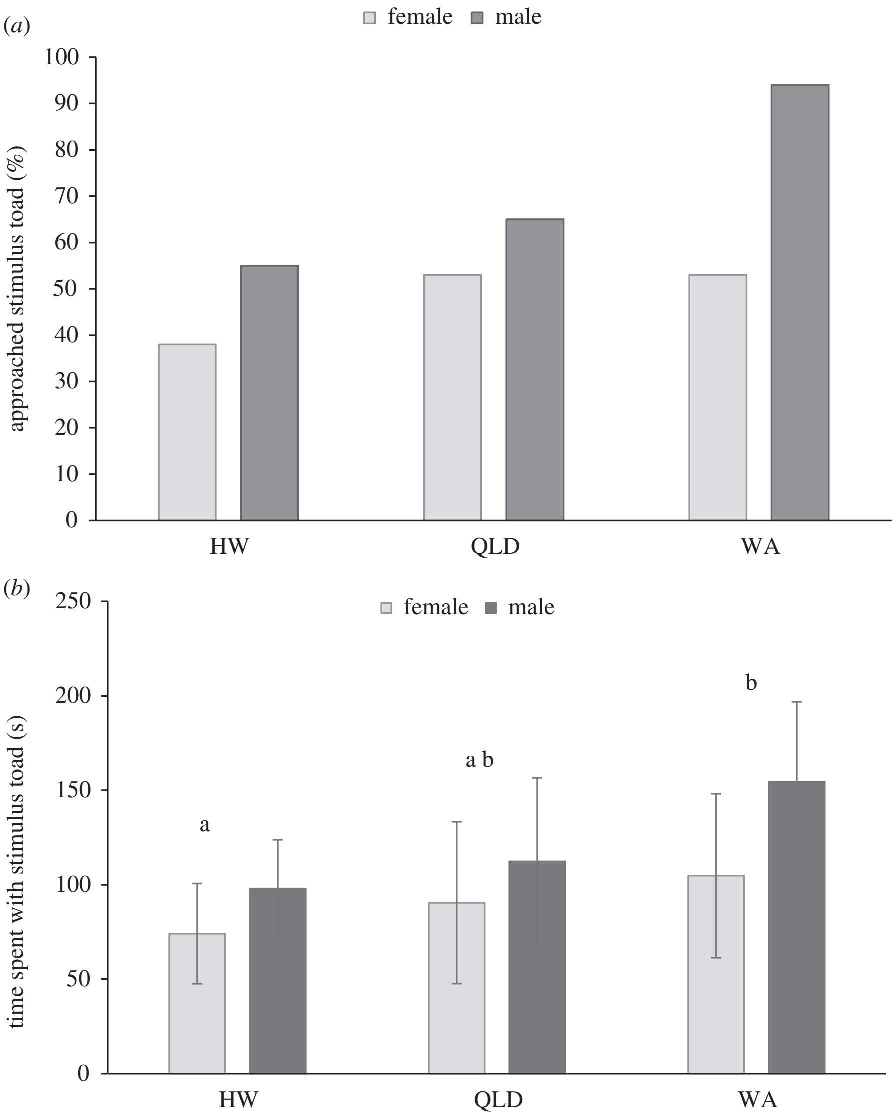

Figure 2. In standardized trials, cane toads (*Rhinella marina*) from three areas with different invasion histories (WA = invasion front, Western Australia; QLD = long-colonized, Queensland, Australia; HW = long-colonized, Hawai'i) and of different sexes (males = dark grey bars, females = light grey bars) exhibited different levels of social attraction (as quantified by the proportion of individuals that approached the stimulus toad (*a*) and the amount of time spent within 100 mm of a stimulus toad (*b*)). Letters above error bars represent significant (α = less than 0.05) differences between populations calculated from a Tukey's post hoc test.

on social attraction was not due to differences in sexual selection among populations. Below, we consider possible explanations for the divergence in social attraction between long-colonized and invasion-front populations.

First, the costs and benefits of social attraction may differ at high versus low population densities, which in turn differ predictably across the invasion range. For example, low densities at the invasion front may reduce ecological costs of proximity to conspecifics, such as competition for food or transfer of parasites or pathogens. Such costs likely are greater in high density (i.e. long-colonized populations [5]).

Second, organisms at the invasion front encounter novel and unpredictable conditions, conferring a selective advantage on any mechanism (including social learning) that enables individuals to find resources such as food, shelter and mates [8]. By contrast, individuals in long-colonized areas already know resource locations, and may benefit from avoiding conspecifics through reduced competition and pathogen transfer [5,6]. Studies of guppies (*Poecilia reticulata* [15]) and nine-spined sticklebacks (*Pungitius pungitius* [16]) support this prediction as individuals with up-to-date information on resource locations tend to ignore social cues. In novel environments, social learning may be less costly than innovation [8]. For example, seeking out conspecifics may facilitate finding safe and reliable food patches [9]. Cane toads are attracted to foraging areas occupied by already-feeding conspecifics [17], as has also been documented in lizards [18] and crab spiders [19].

Thirdly, harsh environmental conditions may favour social attraction at the invasion front because grouping with conspecifics acts as a physiological buffer. For example, grouping reduces the surface area exposed to the external environment, thereby reducing rates of heating and cooling (as in Andean toads *R. spinulosa* [20]) and/or reducing evaporative water loss. Thermal and hydric conditions vary substantially across the cane toads' invasion range from tropical areas in Queensland and Hawai'i to the seasonally dry, monsoonal invasion front in Western Australia. Therefore, invasion-front toads may benefit from social attraction because grouping protects them from intense thermal and hydric stress [21].

Finally, vanguard toads may benefit from social attraction if aggregation protects against novel invasion-front predators. Under the 'selfish herd' hypothesis, animals can reduce individual predation-risk by forming groups [22]. Aggregation also might reduce the risk of infection by novel parasites [23].

Our data document geographical variation in social attraction in cane toads, but do not reveal the proximate basis for that divergence. An individual toad's level of social attraction may be influenced both by its genetics, and by phenotypically plastic responses to environmental conditions. Behavioural traits are heritable in many species (reviewed in [24]), including cane toads [1]; but plasticity is also widespread [25]. To quantify the relative roles of those two mechanisms, we would need to raise toads in standardized (common-garden) conditions [5].

Ethics. Research conducted under Approval Number 2014/628 from the University of Sydney Ethics Committee.

Data accessibility. Data available from the Dryad Digital Repository: (https://dx.doi.org/10.5061/dryad.g72j0) [26].

Authors' contributions. J.G. co-designed experiments, conducted animal collection, experiments, data analyses and drafted the manuscript; R.S. co-designed experiments and contributed to all stages of the manuscript. G.B. assisted with experimental design, data analyses and drafting the manuscript. M.J.W. assisted with experimental design and drafting the manuscript. All authors reviewed, edited and approved the final version of the manuscript for publication and agreed to be held accountable for the content therein.

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