Host defense or parasite cue: Skin secretions mediate interactions between amphibians and their parasites

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Abstract

Amphibian skin secretions (substances produced by the amphibian plus microbiota) plausibly act as a first line of defense against pathogen attack, but may also provide chemical cues for pathogens. To clarify the role of skin secretions in host-parasite interactions, we conducted experiments using cane toads (Rhinella marina) and their lungworms (Rhabdias pseudosphaerocephala) from the range-core and invasion-front of the introduced anurans' range in Australia. Depending on the geographic area, toad skin secretions can reduce the longevity and infection success of parasite larvae, or attract lungworm larvae and enhance their infection success. These striking differences between the two regions were due both to differential responses of the larvae, and differential effects of the skin secretions. Our data suggest that skin secretions play an important role in host-parasite interactions in anurans, and that the arms race between a host and parasite can rapidly generate spatial variation in critical features of that interaction.

Introduction

Amphibians have suffered immense rates of population declines and extinctions over recent decades (Houlahan *et al.* 2000; McCallum 2007; Grant *et al.* 2019) and are considered the most threatened vertebrate class on the planet (Howard & Bickford 2014). A significant contributor to that mortality has been the invasive chytrid fungus (*Batrachochytrium dendrobatidis*), which attacks the host's epidermis (Lötters *et al.* 2009; Fisher & Garner 2020). The catastrophic impact of chytrid has drawn attention to the vulnerability of anurans to a diverse array of pathogens, such as ranaviruses (Gray*et al.* 2009) and parasites (Hartson *et al.* 2011; Gustafson*et al.* 2018). In response to challenges induced by pathogens, anurans exhibit several lines of defense including immune responses and behavioral avoidance of pathogens (Hossack *et al.* 2013; Koprivnikar *et al.* 2014; McMahon *et al.* 2014). Moreover, amphibian skin secretions consist of secretions produced by the amphibian itself (Clarke 1997) and skin microbiota (mostly bacteria (Federici *et al.* 2015)), hereafter, we refer to this combination as 'skin secretions'. These secretions contain many antimicrobial properties (Gustavo Tempone *et al.* 2007; Govender *et al.*2012), which might help to fight off pathogens (Weitzman *et al.*2019; Christian *et al.* 2021). Simultaneously, pathogens are under selection to overcome those barriers, generating an 'arms race' of adaptations and counter-adaptations in the host and its adversaries (Sorci & Faivre 2008). Those host-parasite interactions may drive the rapid evolution of spatial variation in the attributes of both participants, and selection should favor hosts that can either tolerate infection (limiting the harm caused by a given parasite infection) or reduce infection probability and burden, i.e., resistance (Råberg*et al.* 2009).

Although some studies showed that amphibian skin secretions contain properties to resist chytrid fungus (Rollins-Smith 2009; Niederle*et al.* 2019) and bacteria (Quintana *et al.* 2017), little is known about the role of secretions as parasite defense mechanisms more generally. Similarly, we know little about how parasitic

nematodes find their hosts. Generally, nematodes can utilize olfaction, gustation, thermosensation, and humidity to locate hosts, which they seek via strategies ranging from ambushing to actively crawling toward host-emitted cues (Castelletto *et al.* 2014; Gang & Hallem 2016). If such strategies for infection avoidance (in hosts) and host detection/recognition (in parasites) exist, they are expected to vary geographically between different populations, due to co-evolved local adaptations between host and parasite which occur on small spatial scales (Schmid-Hempel 2011).

Biological invasions provide unparalleled opportunities to investigate arms races between hosts and their parasites, because they create spatial heterogeneity in transmission rates and impose novel selective forces on one or both participants. Recently, we showed that the invasion of cane toads (*Rhinella marina* , Fig. 1) through tropical Australia has generated substantial spatial divergence in host-parasite interactions (Kelehear *et al.* 2012; Brown *et al.* 2016; Mayer *et al.* 2021). The toads have carried with them a native-range lungworm (*Rhabdias pseudosphaerocephala* , Fig. 1) (Dubey & Shine 2008; Selechnik *et al.* 2017) that can reduce viability of the host (Kelehear *et al.* 2011; Finnerty *et al.* 2018). The risk of parasite infection has been modified by the invasion process, with low population densities of hosts at the invasion-front reducing opportunities for parasite transfer among toads (Phillips *et al.* 2010). Apparently as a result, toads at the western invasion-front have evolved a greater resistance to parasite infection (Mayer *et al.* 2021), and nematodes close to the invasion-front have evolved a higher infectiviy (Kelehear *et al.*2012), continuing the arms race. That situation provides an ideal opportunity to investigate the biological role of anuran skin secretions in host-parasite interactions.

Because both parasites and their hosts adapt to local conditions, geographic variation in the skin secretions of cane toads might have both positive and negative consequences for host fitness. Skin secretions might render it more difficult for a parasite to infect their host, if secretions inhibit the parasite's ability to locate and/or enter the host's body or find its way to the target organ where it can grow and mature (here termed the '*host defense* ' hypothesis). Alternatively, the parasite might evolve to use the host's skin secretions as a signal for host location, or as a cloak to hide from the host's immune system during the parasite's migration through the body of the host (here termed the '*parasite cue* ' hypothesis). Notably, these two hypotheses are not entirely exclusive; for example, skin secretions might facilitate host location by the parasite, but still defend against penetration by the parasite. By studying a system where host-parasite interactions have diverged rapidly, we have an opportunity to detect a range of such outcomes. We conducted experiments to (1) clarify the role of toad skin secretions as a host-finding cue for lungworms, and (2) test the function of toad skin secretion as defense mechanism against parasite infection (Table 1).

Material and Methods

Host-parasite system

Cane toads were introduced to Australia in 1935, and carried with them the lung nematode *Rhabdias pseudosphaerocephala* that now occurs throughout most of the toad's Australian range (Barton 1998; Phillips*et al.* 2010). Hermaphroditic adult nematodes live inside the toad's lungs, where they release eggs that hatch into first-stage male and female free-living forms within the host's alimentary tract and are defecated. These larvae mate to produce infective third-stage larvae (L3), which develop for 4-10 days (L2 stage) before breaking out of their mother's body and entering the soil (Baker 1979). When an L3 locates a host, it pierces the epidermis and migrates through tissue to reach the lungs of the toad where it matures and feeds on blood from capillary beds (Kelehear *et al.* 2012). The entire life cycle takes 5-36 days (Kelehear *et al.* 2012). Infection prevalence of lungworms varies seasonally and climatically within Australian cane toads (Barton 1998; Pizzatto *et al.* 2013), but low host densities mean that the parasites do not occur at the forefront of the toad invasion (Phillips *et al.* 2010).

Collection of toads and lungworms

We obtained cane toads from two geographic areas: (1) Mareeba, Queensland (17.03° S, 145.43° E), close to the original introduction site (hereafter 'range-core', toads present >80 years), and (2) Halls Creek, Western Australia (-18.23° S, 127.66° E; hereafter 'invasion-front', toads present <5 years; Fig. 1). We kept toads in

Middle Point, Northern Territory (-12.56° S, 131.32° E; Fig. 1), individually in 20 L containers. To obtain naive, parasite-free toads, two pairs of adult toads from each region were induced to spawn by subcutaneous injection of the gonadotropin-releasing hormone agonist leuprorelin acetate (Lucrin, Abbott Australasia) (Hudson *et al.*2016). Although our sample size of parental toads was low for logistical reasons, low functional genotypic replication is unlikely to have affected our findings because cane toads have very low genetic diversity across Australia, with a consequent high similarity in gene expression among individuals within populations (Rollins *et al.* 2015; Selechnik *et al.* 2019b).

After tadpoles hatched, we raised them in plastic containers (1165 x 1165 x 780 mm; one container per clutch). Emerging metamorphosed toads were housed in plastic containers (375 x 295 x 195 mm) separated by clutch prior to the experimental trials. To obtain parasites, we collected naturally-infected toads from Innisfail, QLD (range-core, n = 61) and Kununurra, WA (close to the invasion-front; toads present [?]10 years, n = 20), because parasites are absent from the invasion-front (Phillips *et al.* 2010). The sites where we collected parasites were about 200 km from the sites where we collected parental toads, to yield similar spatial differences between collection sites for parasites and toads at the range-core and invasion-front. We obtained parasites by collecting toad feces and by sourcing adult *Rhabdias* from toad lungs, which we then cultured in Petri dishes in a mix of aged tap water and toad feces to raise first-stage *Rhabdias* larvae to the L3 stage (Langford & Janovy Jr 2009; Kelehear *et al.* 2012).

Experimental infections

To investigate if skin secretions act as a defense mechanism by the host or as cue for the parasite, or both, we infected toads that had either intact or reduced skin secretions (Table S1, S2). We used 79 captive-raised toads, comprising 20 individuals (10 from each clutch) per treatment group: 20 range-core toads that we infected with range-core L3, 19 range-core toads infected with invasion-front L3, 20 invasion-front toads infected with invasion-front L3, and 20 invasion-front toads infected with range-core L3. Toads were approximately two months old when we commenced the experiment, and were housed individually in plastic boxes (15 x 10 x 7 cm). We assigned individuals to treatment groups such that mean snout-urostyle length (SUL) was similar among treatment groups (ANOVA: $F_{(6, 72)} = 0.30$, $p = 0.94$).

Directly before infection, we reduced skin secretions on half the toads by bathing the animal in water and then swabbing both the dorsal and ventral surface 15 times with a cotton bud (Fig. 1). This process was conducted twice per individual to remove most of the skin secretions (Walker *et al.* 2015). Control toads were also bathed and mock-swabbed to simulate the same handling time and potential stress as the other group. That is, we swabbed the toad with a cotton bud covered with a non-absorptive plastic film (parafilm 'M', Pechiney Plastic Packaging, Chicago, USA), rolling the bud over the body but removing few if any skin secretions (Christian *et al.* 2021). For infection, we placed toads individually into round containers (40 mm diameter) lined with filter paper (Whatman 1, Whatman International Ltd, Maidstone, England) containing 20 L3 in 300 µl water. We left the toads in the infection chamber for 18 h, after which they were placed back in their housing enclosure, and then rinsed the infection chamber with water onto a clean Petri dish to count the number of remaining L3 using a microscope (Leica M60). On the 15^{th} day post-infection, we euthanized the toads by placing them in a tricaine methanesulfonate bath (Crossland & Shine 2011) and dissected them to count the number of established nematodes in the lungs. We calculated the proportion of L3 entering the lung by dividing the number of L3 that established in the lungs by the number of L3 that entered the host (Mayer *et al.* 2021).

Performance of parasite larvae

To investigate if the survival of L3 was affected by exposure to skin secretions of cane toads, or of a native frog (*Litoria caerulea*), we placed <7 day old L3 in a Petri dish containing the treatment solution for 24 h. To obtain skin secretions of cane toads (n = 10 range-core toads and 12 invasion-front toads) and frogs (n = 4), we swabbed individuals as described above. The head of the cotton bud was then diluted in 1 ml water to obtain our treatment solution. We used multiple individuals (but not mixing secretions from different individuals) to avoid pseudoreplication, and used the treatment solution of individual toads and frogs for

L3 originating both from the range-core and invasion-front to avoid biases induced by individual variation of the secretions. Aged tap water was used as control. After keeping L3 in a treatment solution for 24 h, we individually placed them in 96 well microplates (Greiner Bio-One, Kremsmünster, Austria) with 200 µl water. Twice a week we refilled the water level in the microplates and checked whether each L3 was alive by touching it with a human eyelash that was sterilized in 70% ethanol and then washed in sterilized water to minimize the risk of introducing pathogens. If the L3 did not move after 5 attempts, we considered it dead (verified within 3 days as the body began to decompose), and calculated longevity as the number of days from hatching to death. We used a crossed design, testing L3 from different geographic regions with toad skin secretion from different geographic regions as well as frog skin secretion (n = 170 L3; Table S3). L3 for this experiment were sourced from multiple toads (n = 15 range-core toads and 8 invasion-front toads) to avoid pseudoreplication.

To investigate if L3 use cane toad skin secretions as a cue, we obtained skin secretion solutions as described above. The experimental setup consisted of a 15 mm wide and 50 mm long strip of filter paper, in a Petri dish. One end of the strip was used as the 'control' zone and the other end as the 'treatment' zone. We pipetted 20 µl of skin secretion onto the treatment zone and 20 µl water onto the control zone. For procedural controls (i.e., to check that L3 randomly move into both zones), we applied water to both zones. The location of control and treatment zones was assigned randomly. We then pipetted 10 L3 (also contained in 20 µl water) onto the middle of the strip of filter paper. After 2 h, we cut the filter paper in half, washed out the L3 from the paper (same method as in experiment 1), and counted the number of L3 in the control and the treatment zones. We also washed out the Petri dish to count the remaining L3 to estimate the 'recovery rate' (i.e., how many L3 we were able to find). For each scent trial (procedural control, range-core toad skin secretions, invasion-front toad skin secretions) and L3 origin (range-core, invasion-front), we ran 30 replicates, totaling 180 trials using 1,800 L3 (Table S4).

Statistical analysis

To investigate the number of L3 entering the host and the total number of nematodes established in the lungs (dependent variables in separate analyses), we used generalized linear mixed models (GLMM) with a negative binomial response distribution using the R package lme4 (Bates*et al.* 2015) to correct for a non-normal distribution of the count data (O'hara & Kotze 2010). To analyze the proportion of L3 entering the lungs (number of parasites in the lungs in relation to the number that entered the host, dependent variable), we used a GLMM with a binomial response distribution using the R package lme4 (Bates *et al.* 2015). For all three analyses we initially ran a full model, including the initial SUL of the toads, toad origin, L3 origin, and treatment as fixed effects and clutch ID as random intercept (Table 1, S1). Moreover, we divided the analyses into two data sets, one for range-core toads and one for invasion-front toads to avoid higher order interactions (i.e. toad origin x L3 origin x treatment). For these six analyses (Table S1), we included the initial SUL of the toads, L3 origin, treatment, and the interaction of L3 origin x treatment (to test if the reduction of skin secretion had different effects on the infectiveness of L3 from different origin) as fixed effects and clutch ID as random intercept. Finally, we analyzed the size of the nematodes that we had obtained from the toad lungs (dependent variable), using a LMM with a Gaussian link. We included toad SUL, L3 origin, toad origin, and the interaction of treatment x L3 origin as fixed effects and toad ID nested within clutch as random intercept (Table 1, S1).

To investigate differences in L3 survival depending on L3 origin, we used a Kaplan Maier survival analysis in the R package 'survival' (Therneau & Lumley 2014). We then analyzed L3 longevity (dependent variable) using a GLM with a log link, including treatment (separated between range-core and invasion-front toads), L3 origin and their interaction (Table 1). To test if L3 use skin secretions to locate their host, we analyzed the proportion of L3 in the treatment zone (dependent variable) using a GLM with a binomial distribution and a logit link. We included the scent type, L3 origin and their interactions as independent variables (Table 1, S3).

Model selection for all analyses was based on stepwise variable selection using Akaike's Information Criterion corrected for small sample size (AIC_c), selecting the model with the lowest AIC_c (Murtaugh 2009), using

the R package 'MuMIn'(Barton 2016). Parameters that included zero within their 95% CI were considered uninformative (Arnold 2010). We validated the most parsimonious models by plotting the model residuals versus the fitted values (Zuur *et al.* 2010). All statistical analyses were carried out in R 4.0.3 (R Core Team 2013).

Results

Do skin secretions affect the entry and establishment of parasite larvae into the host?

On average, 17.4 L3 (± 2.3 SD) entered the toad. Toad SUL, toad origin, L3 origin, and treatment were uninformative in explaining the number of L3 entering the toad, which was best explained by the intercept-only model (Table S1). Infection prevalence was 92.4% (73 of 79 toads had nematodes in their lungs). The proportion of L3 establishing in the lungs was best explained by toad SUL, toad origin and L3 origin (Table S1), being higher in toads from the range-core *versus* the invasion-front, higher for toads infected with L3 from invasion-front*versus* range-core populations, and higher in larger toads (Table S2, Fig. S1). When we analyzed the data separately for range-core and invasion-front toads, we found that the number of L3 establishing in the lungs was increased by intact skin secretions for range-core L3 but reduced by intact skin secretions for invasion-front L3 (Table S1, S2, Fig. 2). This effect was stronger in toads from the invasion-front compared to the range-core (Fig. 2).

Fifteen days post-infection, toads had between 0 and 19 nematodes in their lungs (mean ± SD: 7.5 ± 4.3), and generally there were more nematodes in the lungs of larger toads, in range-core toads, and when infected with invasion-front L3 (Table S1, S2). Range-core toads had more nematodes in their lungs when the L3 originated from the invasion-front, but the interaction of treatment and L3 origin was uninformative (Fig. 2, Table S2). In invasion-front toads, reduced skin secretions led to a lower number of nematodes in the lungs when the L3 originated from the range-core, but this effect was absent when infected with invasion-front L3 (Fig. 2).

Do skin secretions affect the size of parasites in the host lung?

The size of the adult lungworms that had established in the toads' lungs ranged between 1.6 to 5.2 mm (mean ± SD: 3.5 ± 0.5 mm). Invasion-front lungworms were larger than range-core lungworms, and lungworm size decreased with an increasing number of nematodes in the lungs (Table S2, Fig. S2). Toad SUL, treatment (skin secretions intact versus reduced), and toad origin were uninformative in explaining nematode size.

Effect of skin secretions on the longevity of parasite larvae

L3 lived between 7 and 59 (mean ± SD: 34 ± 10) days. L3 longevity was best explained by parasite origin and treatment (Table S4). L3 from invasion-front populations lived longer than did those from the range-core (mean ± SD: 40 ± 9 versus 29 ± 7 days; log-rank test: $p < 0.001$). Skin secretions from invasion-front toads reduced longevity of both groups of L3 (Fig. 3). In contrast, skin secretions from range-core toads and from frogs did not reduce larval longevity (Fig. 3, Table S5).

Are larvae attracted to skin secretions?

On average, we recovered 90.6% (± 14.2 SD) of the L3 used for this experiment (1,630 of 1,800 L3). The proportion of L3 in the treatment zone was best explained by L3 origin, scent type, and their interaction (Table S4). More L3 (from both populations) were found in the treatment zone than the control area (demonstrating attraction to the scent cue) when skin secretions from range-core toads were used (Fig. 4, Table S5). Conversely, when skin secretions from invasion-front toads were used as the treatment scent, L3 from range-core populations were attracted whereas L3 from invasion-front populations were not (Fig. 4, Table S5).

Discussion

Our data confirm that anuran skin secretions play important and complex roles in host-parasite interactions. In line with the *host defense* hypothesis, skin secretions can decrease a toad's vulnerability to nematodes, by

reducing larval longevity and by reducing the ability of larvae to penetrate to the host's lungs. However, skin secretions also provide chemical cues that can attract infective larvae (*parasite cue* hypothesis) as suggested by other studies (Theodoropoulos *et al.* 2001). Moreover, skin secretions can enhance rather than decrease the ability of parasite larvae to penetrate the toad's body and establish an infection in the lungs. Thus, some effects of skin secretions appear to represent adaptations of the host that reduce vulnerability to parasite attack (Tempone *et al.*2008; Christian *et al.* 2021), whereas other effects are more consistent with adaptations of parasites to exploit the traits of their host (Gang & Hallem 2016). The most striking result from our studies is the magnitude of geographic divergence in host-parasite interactions within our study system. Although cane toads have been spreading through tropical Australia for only 85 years (Urban *et al.* 2008), they appear to have evolved major divergences in the roles that skin secretions play in host-parasite biology.

Broadly, the skin secretions of cane toads appear to act as a defense against lungworms in invasion-front populations in Western Australia (Christian *et al.* 2021), but not in range-core populations in Queensland. Thus, the presence of skin secretions reduced the rate at which invasion-front L3 were able to establish infections in invasion-front hosts, whereas the reverse was true for range-core L3 attacking range-core hosts. In regard to the host, the shift was from comparatively low protection (or the absence thereof) against parasite infection by skin secretions at the range-core, to increased protection at the invasion-front. That geographic divergence fits well with the hypothesis that cane toads in the range-core have low resistance to parasite infection (Mayer *et al.* 2021), because parasites are ubiquitous due to high host densities, and toads thus rely on tolerating rather than resisting pathogens (Adelman & Hawley 2017). Conversely, at the invasion-front, where there is a strong evolutionary pressure for dispersal ability via both natural selection and spatial sorting (Brown*et al.* 2014; Phillips & Perkins 2019), increased host resistance might be favored if parasite infection reduces dispersal ability. Moreover, some sets of immune genes are upregulated at the invasion-front (Selechnik *et al.* 2019a), suggesting that heightened, possibly non-specific, immune responses arising from exposure to other pathogens or conditions at the invasion-front may increase resistance of toads (Brown *et al.* 2018; Mayer *et al.* 2021). Although mechanisms leading to shifts in host immune responses are unclear, our study demonstrates that changes in defense strategies against parasite infection can evolve rapidly.

In regard to the parasite, one surprising result was that in range-core toads, skin secretions enhanced rather than reduced the rate at which larvae were able to reach the lungs and establish an infection. Theodoropoulos *et al.* (2001) suggested that gastro-intestinal helminth parasites express mucin-like molecules to avoid detection by the host's immune system (mucin is the main molecule of the intestinal mucus barrier (Carlisle *et al.* 1991; Sharpe *et al.* 2018)). If so, larvae entering the host's body may benefit by cloaking themselves in host-derived skin secretions that enable them to evade detection by the host's immune system. That tactic apparently does not work at the invasion-front, perhaps because the immune system of invasion-front toads is more active against pathogens (Brown *et al.* 2018) as indicated by the effects of host skin secretion on larval longevity. Additionally, the larger larvae at invasion-front sites potentially cannot be cloaked as effectively, leading to increased susceptibility to the host's immune system. An alternative scenario for the reduced infection success of range-core L3 when skin secretions were reduced might be a reduced ability to detect the host. Range-core L3 used skin secretions as a cue and olfaction is important for host-finding in other species (Gang & Hallem 2016). However, we found no significant difference in the number of L3 entering the host as a function of the presence of skin secretions. Invasion-front L3 also used skin secretions as a cue – but only from range-core toads, not invasion-front toads. This latter finding might have been caused by the negative effect of skin secretions from invasion-front toads on longevity of L3. Thus, invasion-front L3 might still be able to use skin secretions of invasion-front toads as a cue, but avoid exposure to evade detrimental effects. In contrast, the non-coevolved range-core L3 might not detect the detrimental effects of the secretions during the short exposure time of the experiment.

Much remains to be learned about the mechanisms underlying the interactions between amphibian hosts and the organisms that infect them. For example, we do not know which components of the skin secretion function as host defense or parasite cue, respectively. Cane toads from different regions have different proportions of bacteria with antifungal properties (Weitzman et al. 2019), suggesting that microbial properties might

also contribute to defense against parasites. It would be of great interest to tease apart the degree to which the functional attributes of skin secretions (e.g., in reducing or enhancing parasite success) derive from molecules produced by the amphibian *versus* the microbiota that live on its skin. Moreover, the impacts of skin secretions ideally need to be seen in a wider context, as part of a suite of responses that also encompass behavior, morphology and physiology (especially, immune function: Brown *et al.* (2018)). Thus, for example, a highly effective immune response to pathogens that enter the body might relax selection for barriers on the skin. Additionally, it is important to remember that the skin has many other functions, such as respiration, regulating water flow and as the location of toxin-excreting glands (Huang *et al.* 2016; Senzano & Andrade 2018; Blennerhassett*et al.* 2019; Kosmala *et al.* 2020).

The devastating impacts of diseases (especially chytrid-driven) on anuran amphibians worldwide places a high priority on the need to understand factors that render an anuran more or less vulnerable to infection (Rollins-Smith 2009). Strong phylogenetic and geographic variation in the magnitude of disease impacts on amphibians (e.g. Savage & Zamudio 2016; Fisher & Garner 2020) suggest that spatially variable outcomes of host-parasite arms races may be key to understanding – and hopefully, ameliorating – some of those impacts. Our data indicate that at least part of the diversity in host-parasite interactions can involve diversity in the role of skin secretions, and that location-specific selective forces can generate rapid changes in the ways that anuran hosts interact with their pathogens.

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Ethics statement

The study was conducted under the approval of the University of Sydney Animal Care and Ethics Committee (permit number 2019/1489).

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Figures

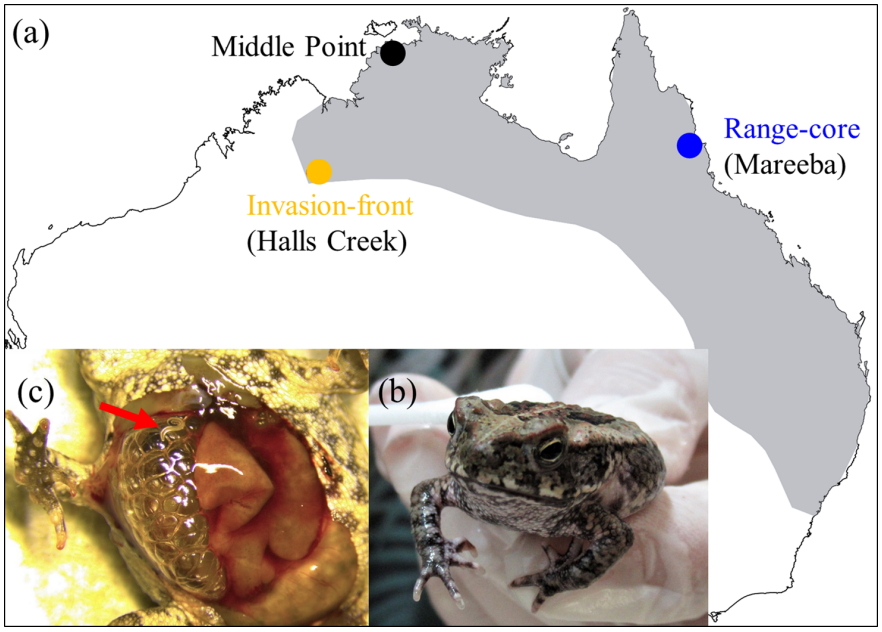

Fig. 1: Map (a) showing the collection locations for cane toads (*Rhinella marina*) from the range-core (Mareeba, QLD) and invasion-front (Halls Creek, WA) that were then bred and their offspring raised in a common garden experiment in Middle Point, NT. Grey shading depicts the approximate cane toad distribution in 2019. Pictures show a cane toad (b) that is being swabbed to remove skin secretions, and the lungworm parasite *Rhabdias pseudosphaerocephala* (c) located within the toads' lung (red arrow indicates location).

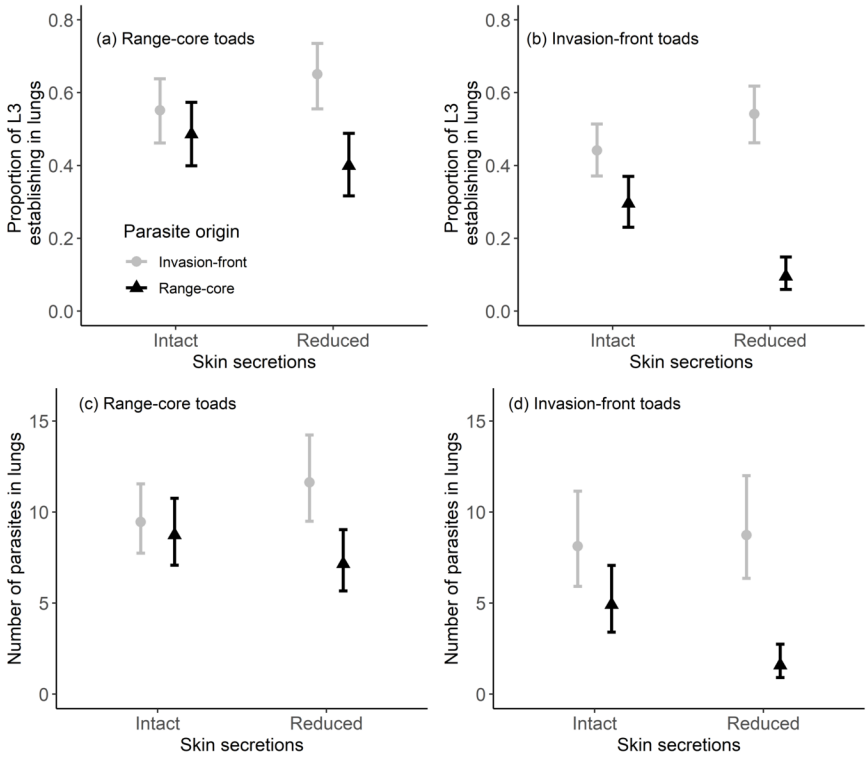

Fig. 2: The effect of the interaction of parasite origin (range-core*versus* invasion-front) and treatment (skin secretions intact*versus* reduced) on the proportion of lungworm larvae (L3) that established in the lungs of cane toads from the range-core (a) and the invasion-front (b). The lower panels show the effect of the interaction of parasite origin and treatment on the number of parasites in the lungs of toads from the range-core (c; note that the interaction was uninformative) and the invasion-front (d). The 95% confidence intervals are given as bars.

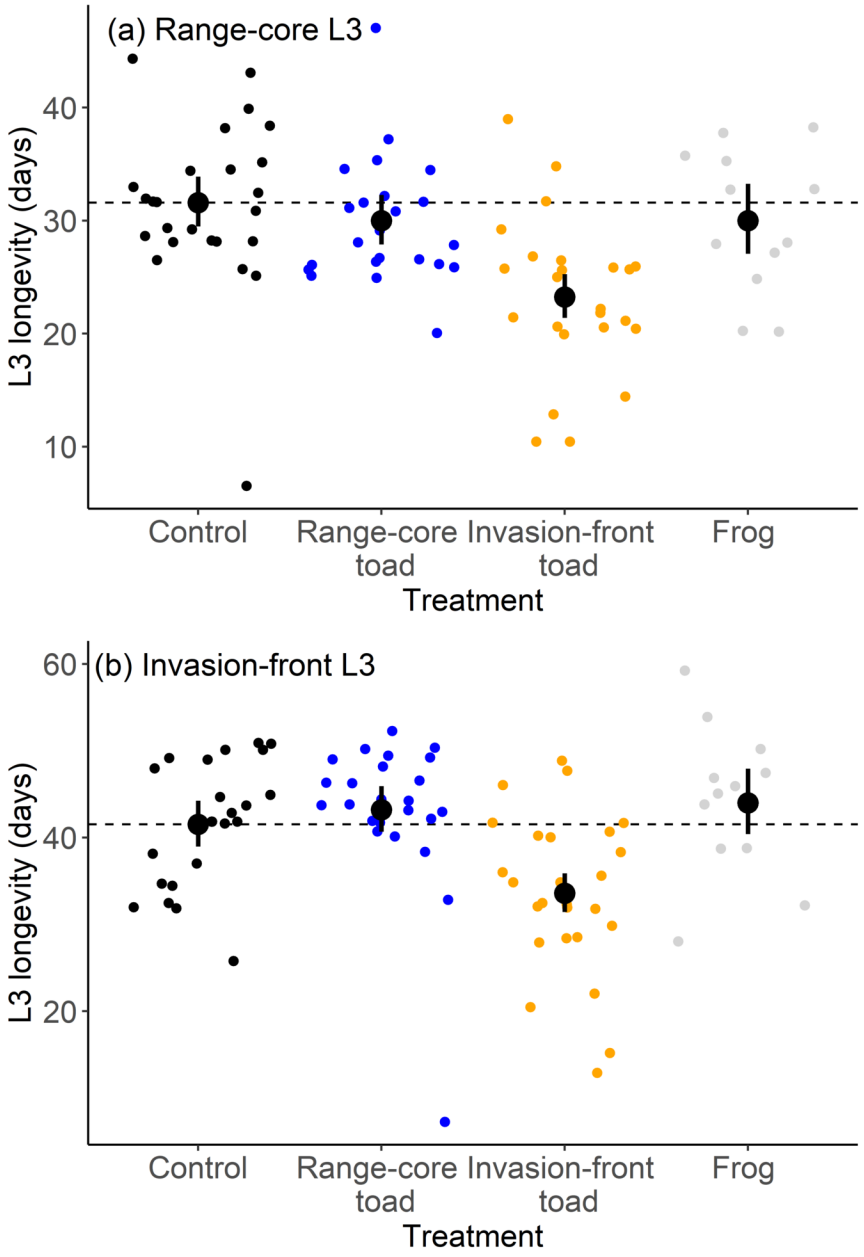

Fig. 3: The predicted longevity (black dots) for lungworm larvae (L3) originating from the range-core (a) and the invasion-front (b) separately for the different skin secretion treatments. Blue dots represent raw data for L3 exposed to skin secretions of range-core toads, orange dots for L3 exposed to secretions of invasion-front toads, and grey dots for exposure to frog skin secretions. The 95% confidence intervals are given as bars and the dashed black line represents the longevity of the control group.

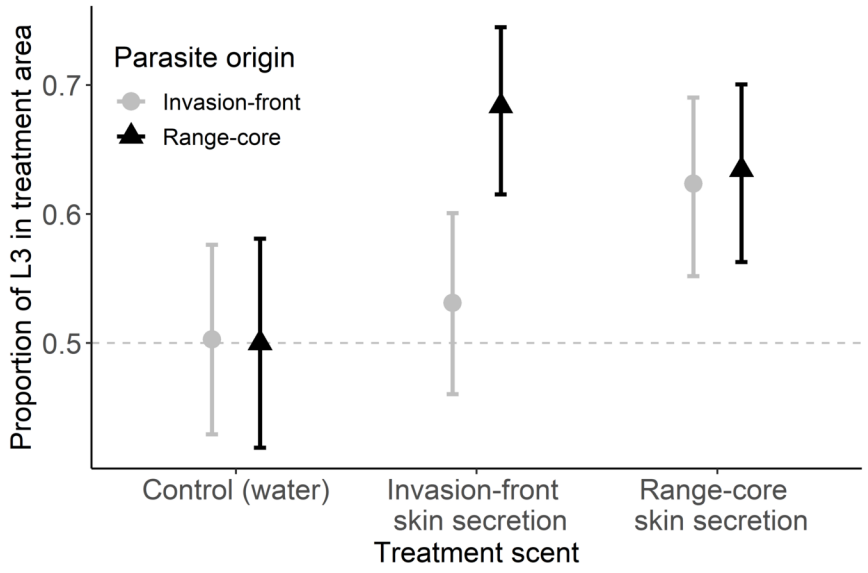

Fig. 4: The effect of treatment scent (water and cane toad skin secretions from the invasion-front and range-core, respectively) on the proportion of lungworm larvae (L3) in the scent treatment area, separately for L3 from the range-core and the invasion-front. The 95% confidence intervals are given as bars and raw data are shown as grey dots.

Tables

Table 1: Overview of the statistical analyses showing the dependent and independent variables, the random intercept, model link, and sample size.

Analysis/Dependent variable	Fixed effects
Infection trial	
Number of L3 entering the toad	Snout-urostyle length + L3 origin + Toad origin + Treatmen
Proportion of L3 establishing in the lung	Snout-urostyle length + L3 origin + Toad origin + Treatmen
Number of nematodes in lungs	Snout-urostyle length + L3 origin + Treatment + L3 origin x
Nematode size	Number of nematodes + Snout-urostyle length + Toad origin
Nematode performance	
L3 longevity	L3 origin + Treatment + L3 origin x Treatment
Scent experiment/ Proportion of L3 in scent treatment area	L3 origin + Scent + L3 origin x Scent