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Divergent male and female mate preferences do not explain incipient speciation between lizard lineages

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Abstract

Diversification in sexual signals is often taken as evidence for the importance of sexual selection in speciation. However, in order for sexual selection to generate reproductive isolation between populations, both signals and mate preferences must diverge together. Furthermore, assortative mating may result from multiple behavioural mechanisms, including female mate preferences, male mate preferences and male-male competition; yet their relative contributions are rarely evaluated. Here, we explored the role of mate preferences and male competitive ability as potential barriers to gene flow between two divergent lineages of the tawny dragon lizard, *Ctenophorus decresii*, which differ in male throat coloration. We found stronger behavioural barriers to pairings between southern lineage males and northern lineage females than between northern males and southern females, indicating incomplete and asymmetric behavioural isolating barriers. These results were driven by both male and female mate preferences rather than lineage differences in male competitive ability. Intrasexual selection is therefore unlikely to drive the outcome of secondary contact in *C. decresii*, despite its widely acknowledged importance in lizards. Our results are consistent with the emerging view that although both male and female mate preferences can diverge alongside sexual signals, speciation is rarely driven by divergent sexual selection alone.

Keywords: *Ctenophorus decresii*, hybridisation, male-male competition, secondary contact, sexual selection

Populations that evolve in allopatry often diverge in sexual signals, which intuitively implicates sexual selection in speciation. However, speciation by sexual selection also requires divergence in mating preferences for signals in order to generate prezygotic isolation should populations come into contact (West-Eberhard 1983; Panhuis et al. 2001; Ritchie 2007). Both signals and preferences can diverge due to drift or local adaptation to different environments rather than, or in addition to, divergent sexual selection (Schluter 2009; Langerhans and Riesch 2013; Mendelson et al. 2016). Thus, sexual signals and mating preferences (or other behaviours involved in acquiring mates, such as male competitive ability) can evolve independently, and several outcomes are possible when populations meet. Behavioural reproductive barriers could be absent, weak, strong, or asymmetrical, resulting in varying levels of admixture, and the potential breakdown of signal and preference differences. For example, song phenotype acts as a barrier to gene flow between subspecies of white-crowned sparrows, but admixed individuals in the contact zone exhibit slightly decreased discrimination, which has the potential to weaken isolation over time (Lipshutz et al. 2019). Consequently, determining the extent to which divergent sexual signals generate premating reproductive isolation upon secondary contact is critical to understand the role of sexual selection in speciation.

Premating isolating barriers between divergent lineages generate patterns of assortative mating. However, assortative mating can arise from multiple behavioural mechanisms such as female mate preference (or rejection of non-preferred mates), male mate preference, and competition between males for access to females. Most studies examining premating isolating barriers focus on female mate preferences but in some species, particularly those that are highly territorial and polygynous, competition between males for access to females can be the primary determinant of male reproductive success (Anderson 1994; Fitzpatrick et al. 2012; Qvarnström et al. 2012; Uller et al. 2013; McDiarmid et al. 2017; Tinghitella et al. 2018). Furthermore, female access to mates may be limited when males dominate territories encompassing the home range of one or more females and/or exhibit mate guarding behaviour (Qvarnström et al. 2012). If males control mating opportunities and do not discriminate between conspecific and heterospecific females, then we may see little evidence of assortative mating. Selection for mate discrimination is expected to be weaker in males than females due to generally lower male *per capita* investment in reproduction, particularly in the absence of parental care (Bateman 1948; Anderson 1994; but see Edward and Chapman 2011). Male mate discrimination between females from divergent lineages may also be limited because females are phenotypically similar (i.e. only male sexual signals have diverged). Our understanding of the relative contributions of male-male competition and male mate discrimination in influencing the outcome of secondary contact remains limited but is of growing interest (Peterson et al. 2005; Johannesson et al. 2008; Vallin et al. 2011; While et al. 2015; Heathcote et al. 2016; Martin and Mendelson 2016).

The tawny dragon lizard *Ctenophorus decresii (*Duméril & Bibron, 1837) is well suited to examining the evolution of behavioural mechanisms that may generate assortative mating upon secondary contact. This small sexually dimorphic agamid lizard comprises two genetically and phenotypically divergent lineages: “northern” and “southern” (Houston and Hutchinson 1998; McLean et al. 2014b; Figure 1). There is evidence that the lineages are incipient species, and the level of divergence between them (3.7% net sequence divergence in mtDNA) is consistent with contraction to, and expansion from, isolated refugia during Pleistocene glacial–interglacial cycles (Byrne 2008; McLean et al. 2014b). The lineages meet in a narrow contact zone (ca. 20 km) where F1 generation hybrids are not present (contact is not recent) and genetic admixture is asymmetrical (McLean et al. 2014b; Dong et al. 2019). Specifically, hybrids backcross to the northern but not the southern lineage, and nearly all hybrid individuals have northern lineage maternally inherited mitochondrial DNA (mtDNA), indicating some degree of reproductive isolation (Dong et al. 2019).

The lineages of *C. decresii* differ in male throat coloration, which they display prominently during both contests and courtship (Gibbons 1979; Stuart-Fox and Johnston 2005; Osborne et al. 2012; Ramos and Peters 2016). Northern lineage males are colour polymorphic, with orange, yellow, orange-yellow (an orange central patch surrounded by yellow) and grey throat morphs co-occurring within populations (Teasdale et al. 2013; McLean et al. 2014b). Conversely, southern lineage males have blue throats with an ultraviolet (UV) reflectance peak (McLean et al. 2014b). Northern lineage colour morphs also exhibit correlated behavioural strategies; grey males are the most cautious, while orange males are the most bold and the most aggressive towards territory intruders (Yewers et al. 2016). Females are cryptically coloured and similar in appearance between lineages (Figure 1; McLean et al. 2013). The species occupies open, rocky habitats and males defend territories using elaborate behavioural displays from elevated rock perches, sometimes engaging in physical contests (Gibbons 1979; Stuart-Fox and Johnston 2005; Osborne et al. 2012; Ramos and Peters 2016). Males are polygynous with territories encompassing the home ranges of one or more females (Yewers et al. 2018); thus, a male’s ability to defend a territory likely influences reproductive success. Parental care is absent and multiple paternity within clutches is rare (4%), though this does not preclude female multiple mating within or between clutches due to the possibility of sperm storage and sperm competition (Hacking et al. 2017). Consequently, the biology as well as the mating system of *C. decresii* suggests strong intra-sexual selection.

In this study, we staged encounters between captive lizards from populations representing the closest genetically pure populations on either side of the contact zone. This enabled us to assess the extent to which divergent mate preferences have evolved in the two lineages, independently of reinforcement subsequent to secondary contact. Doing so is essential to assess the role of sexual selection in the speciation process. We predicted that lizards would display a preference for mates from their own lineage. If this is due to female preference, females should perform more courtship and/or less rejection behaviour during encounters with males from the same lineage. Similarly, if male preference plays a role then males should be more likely to court and/or attempt copulation with females from their own lineage as opposed to the alternate lineage. Additionally, we predicted that northern and southern males would differ in their aggressive behaviour, and that this behaviour may be dependent on the throat colour morph of their opponent.

Materials and Methods

Study species and husbandry

We used 90 adult lizards (> 65mm snout-vent length; SVL) comprising 21 male and 24 female northern lineage *C. decresii* from Caroona Creek Conservation Park, South Australia (-33.4114°S, 139.0945°E), and 21 male and 24 female southern lineage *C. decresii* from private properties around Palmer, South Australia (-34.8223°S, 139.1621°E). Lizards were collected in September in 2015 and 2016, and subsequently kept in captivity at The University of Melbourne, Victoria, Australia, where they were housed individually in 55 × 34 × 38cm (length × width × height) opaque plastic enclosures containing a layer of sand and a crevice between two ceramic tiles for shelter. Housing was maintained at temperatures and lighting cycles that mimicked natural seasonal variation, with UV lights (ZooMed T8 ReptiSun® 10.0 UVB) above each enclosure (30cm), emitting both UVA and UVB radiation. A heat lamp was provided to generate a thermal gradient and allow the lizards to attain their preferred body temperatures (approx. 36°C; Gibbons 1977; S. Walker unpublished data). Lizards were misted with water for hydration and fed live crickets dusted with multi-vitamins three times per week. All behavioural trials were conducted during the breeding seasons (August–December; Gibbons 1977) in 2016 and 2017. Research methods used in this study were reviewed and approved by the Animal Ethics Committee of The University of Melbourne (1413220.3) and the South Australian Wildlife Ethics Committee (25/2015).

Female-male behavioural trials

Females are receptive to mating approximately 2–3 weeks after emergence from hibernation, and after laying their first or second clutch. We conducted mate preference trials during these known receptive periods, when females were in good body condition (average mass of 16.7 g ± 2.9 g), though receptivity cannot be determined with certainty *a priori*. Each female was paired with both a southern and a northern lineage male, with half of the females paired with a southern male first and the other half with a northern male first. Females were placed into the first male's enclosure for a period of 24 hours, and then into the second male's enclosure for the subsequent 24 hours. Both encounters were monitored and recorded using a Swann DVR8-1525 8 channel 960H digital video recorder with a PRO-615 camera attached. We conducted a total of 147 trials, with individual females paired with one southern and one northern male per reproductive cycle, in up to 2 reproductive cycles (average of 3.34 trials, with a range of 2–4 trials, per female).

Videos were analysed using Behavioural Observation Research Interactive Software (BORIS) version 4.1.5 (Friard and Gamba 2016) and both female and male behaviour was scored. For females, we recorded the number of head-bobs (pronounced nodding movement of the head), and combined the number of aggressive behaviours (biting and chasing) and times the female fled from the male as a measure of "rejection". For males, we also recorded the number of head-bobs (courtship behaviour) as well as the number of attempts to copulate, and whether or not copulation was successful. We did not analyse the number of successful copulations as copulation was observed in only 7 of the 147 trials (although more may have taken place under the tile). Lizards were not paired for long enough to ensure mating; rather, we were interested in behaviour during initial contact as an indicator of mate preference.

We tested whether female lineage, male lineage, or their interaction predicted: 1) number of copulation attempts, 2) number of male head-bobs, 3) number of female head-bobs and 4) number of female rejection behaviours using generalised linear mixed models (lme4 package, R; Bates et al. 2015). Female ID, male ID and pairing number (female's first or second trial) were included as random factors in all models to account for repeated use of individuals, and response variables were log transformed to meet model assumptions of normality. We performed pairwise comparisons by calculating least squares means and confidence intervals using the Satterthwaite's approximation for degrees of freedom (lmerTest package, R; Kuznetsova et al. 2017).

Male-male behavioural trials

A previous study investigating aggression levels among morphs of the northern lineage found that orange-throated males were significantly more aggressive towards territory intruders than yellow, orange-yellow or grey-throated males (Yewers et al. 2016). Therefore, we categorised males into three behavioural groups based on lineage and throat colour morph: southern, northern high aggression (orange), or northern low aggression (yellow, orange-yellow, grey). We designed trials such that each focal male was matched with three others, representing each of the behavioural groups, in random order. Pairs were size-matched to minimize the effect of body size on contest outcome, with an average difference of 1.59mm ± 1.16mm snout vent length (SVL) between competing males.

Contest trials were conducted in a neutral 120 × 30 × 60 cm (length × width × height) enclosure (i.e. not the home enclosure of either male). An opaque divider initially separated the enclosure into two equally sized holding areas, each containing a layer of sand, ceramic tile and heat lamp. Just prior to the trial, males were weighed to obtain a measure of body condition as the residuals of a linear model of mass and SVL. The designated "focal" and "opponent" males were then placed into the separate holding areas and allowed to acclimatise for 48 hours to establish residency (Umbers et al. 2012). At the commencement of the trial, the divider was removed and the interaction was recorded from two different angles using Panasonic HC-V770M video cameras. Trials were conducted for a maximum of 25 minutes and monitored to ensure there was no risk of injury to animals (as required under the Animal Ethics permit). Consequently, we did not record contest outcome (i.e. winner, loser) as some trials were stopped before a winner was established. To minimize stress and the potential influence of previous contest outcomes, males were not used in a subsequent trial for at least 48 hours. We conducted a total of 120 trials (involving 42 males), 26 of which were excluded due to no interaction, resulting in 94 trials which were used in the statistical analysis.

We scored focal male behaviour from the video footage using BORIS. *C. decresii* males perform energetic displays during territory defence prior to engaging in physical aggression (Gibbons 1979). Therefore, we recorded the number of head-bobs, tail flicks and push-ups performed by the focal male as a measure of "display behaviour", and combined the duration of chasing and wrestling (involving biting) as a measure of "physical aggression". We also recorded the time between the start of the trial and the focal male's emergence from beneath the tile ("latency"), as this is an indicator of individual boldness. Display behaviour and physical aggression were divided by the total trial duration (minus latency) to account for differences in trial lengths.

We tested whether behavioural group or body condition predicted: 1) focal male latency to emerge, 2) focal male display behaviour and 3) focal male physical aggression using generalised linear mixed models. We included focal male behavioural group, opponent male behavioural group and their interaction, as well as focal male body condition and opponent male body condition as predictor variables in the models. Additionally, focal male ID and focal male trial number were included as random factors in all models to account for repeated use of individuals. For models 2 (display behaviour) and 3 (physical aggression), the response variables were log transformed to meet model assumptions of normality, and we performed *post hoc* pairwise comparisons as detailed above.

Results

Female-male behavioural trials

The interaction between female and male lineage influenced the number of copulation attempts ($F_{1, 103.2} = 6.53$, $P = 0.012$) and male head-bobs (courtship; $F_{1, 91.2} = 6.61$, $P = 0.012$) during trials (Table 1). Males attempted copulation in 68 of the 147 trials and there were more copulation attempts between southern males paired with southern females than southern males paired with northern females ($t_{107.8} = 2.46$, $P = 0.015$) or northern males paired with southern females ($t_{63.9} = 2.00$, $P = 0.050$; Figure 2; Table S1). Similarly, southern males courted southern females (with head-bobs) more than they courted northern females ($t_{91.3} = 3.21$, $P = 0.018$; Figure 2; Table S2). Lineage did not influence female head-bobs; however, both female and male lineage affected female rejection behaviour (Table 1). Specifically, northern females performed more rejection behaviours than southern females ($F_{1, 40.2} = 4.64$, $P = 0.037$) and southern males were rejected more overall (i.e. by females of both lineages) than northern males ($F_{1, 38.9} = 10.06$, $P = 0.003$; Figure 3).

Male-male behavioural trials

Focal males displayed (head-bobs, tail flicks and push-ups) in 73 out of 94 contest trials and 70 trials involved physical aggression (chasing and wrestling). Focal male ID (a random factor) explained a large proportion (57.07%) of the variance in the time taken for the focal male to emerge from beneath the tile (latency). Taking this into account, latency was affected by both the focal male's behavioural category ($F_{2, 41.5} = 4.35$, $P = 0.019$) and opponent male's behavioural category ($F_{2, 58.1} = 5.45$, $P = 0.007$) but neither male's body condition (Table 2). The relationships were such that northern low aggression males (orange-yellow, yellow and grey morph) were slower to emerge than southern males ($t_{39.5} = 2.92$, $P = 0.006$), and the focal male emerged more quickly for a southern ($t_{59.1} = 3.05$, $P = 0.003$) or northern low aggression ($t_{56.9} = 2.26$, $P = 0.028$; Table S3) opponent compared to a northern high aggression (orange morph) opponent (Figure 4; Table S4). Neither male behavioural category, nor body condition, affected display behaviour or physical aggression during trials (Table 2).

Discussion

Speciation via sexual selection requires sexual signals and mating preferences to diverge together, resulting in assortative mating upon secondary contact. Males of the northern and southern lineages of *Ctenophorus decresii* differ markedly in male throat coloration, a signal used in both intra- and inter-sexual interactions. Mating trials between the lineages showed that southern males display a preference for females from their own lineage. Southern males courted and attempted copulation with southern females more than northern females. By contrast, northern males did not court or attempt copulation with northern females more than with southern females. Additionally, northern females displayed more rejection behaviours than southern females and southern males were rejected more (regardless of female lineage) than northern males. These data suggest that both male and female mate preferences may act as behavioural barriers to gene flow upon secondary contact. However, we found no evidence for behavioural barriers to mating between northern males and southern females, suggesting that the evolution of premating isolation prior to secondary contact is incomplete and asymmetrical.

Divergent sexual selection can theoretically drive speciation, even in the absence of reinforcement (Lorch et al. 2003; Reinhold 2004; van Doorn et al. 2009). This is most likely when sexual signals are condition-dependent or directly under natural selection because selection against locally maladapted offspring should generate linkage disequilibrium between trait and preference, ultimately leading to assortative mating (Maan and Seehausen 2011). Male throat coloration in *C. decresii* is locally adapted to increase conspicuousness against the colour of the dominant lichens on rocks in the habitats of the two lineages (McLean et al. 2014a). Southern blue throats are more conspicuous against the orange lichen found extensively on rocks in the south; while northern orange and yellow throats are more conspicuous against the grey-green lichen that dominates in the north (McLean et al. 2014a). Despite local adaptation of the colour signal, we found little evidence for a strong link between signal and preference: southern females did not discriminate between northern and southern males, and northern males did not show a preference for northern females. Thus, the strength of divergent ecological selection on sexual signals in this system appears insufficient to generate strong linkage between sexual trait and preference, and therefore assortative mating upon secondary contact.

Mate discrimination or choosiness is expected to be stronger in the sex that invests more into reproduction per offspring, typically females (Anderson 1994). Thus, premating isolation is generally assumed to be driven by female preferences. In lizards, which mostly lack parental care, females invest more per offspring into reproduction; yet, evidence for female mate choice is scarce (López et al. 2003; Olsson et al. 2003; Martín and López 2006). This is because males largely control female access to mates, either by dominating territories encompassing the home range of one or more females, or by mate guarding (Olsson and Madsen 1995). We do find some evidence suggesting a role for female mate preference in *C. decresii*. Like most lizards, female *C. decresii* mate multiply when receptive, but this does not mean they mate indiscriminately (Elgar et al. 2013), and they may exhibit mate preferences through rejection of non-preferred mates. Our results showed that northern females exhibited more rejection behaviour, and that southern males are rejected more overall, which could have contributed to the lower number of copulation attempts observed between this combination.

Male-male competition is unlikely to drive speciation on its own, but may contribute to reproductive isolation in combination with mate preferences or genetic incompatibilities. For example, in European wall lizards *Podarcis muralis*, asymmetric introgression occurs due to strong differences between lineages in male competitive ability and male mate preferences, which are both linked to body size (While et al. 2015; Heathcote et al. 2016). Correlated evolution of body size and sexual signals is a common pattern (Young et al. 1994; Wirtz 1999; Hagman and Forsman 2003; McGlothlin et al. 2005; reviewed in Bonduriansky 2007). In *C. decresii* males of the two lineages differ very slightly in body size and head shape (McLean et al. 2013), but do not differ in competitive ability in terms of aggression during staged contests. Thus, we find no evidence that male-male competition contributes to incipient speciation in *C. decresii*, despite the importance of intra-sexual selection in this species, and its widely acknowledged importance in lizards more generally.

Although lineages did not differ in aggression, there were differences in boldness between lineages and morphs, consistent with previously described behavioural differences between the northern throat colour morphs (Yewers et al. 2016). Southern males were bolder (faster to emerge from their shelter) than northern low aggression (grey, orange-yellow, yellow) males and equally bold as northern high aggression (orange) males. Overall, focal males were least bold when faced with a northern high aggression (orange) opponent. In other species, higher boldness has been associated with greater reproductive success, foraging ability, dispersal distance and dominance, but may reduce long-term survival (Réale et al. 2007; Ariyomo and Watt 2012). The relationship between boldness and reproductive success in reptiles is relatively unexplored but it has been suggested that boldness may be associated with the ability to defend territories, but is not necessarily correlated with social dominance and aggression (Taylor and Lattanzio 2016).

In summary, we have shown that both male and female mate preferences may potentially influence the nature and extent of reproductive barriers upon secondary contact between lineages of *C. decresii*. We found little evidence of lineage differences in male competitive ability, despite the importance of male-male competition for male reproductive success in *C. decresii* and lizards in general. Furthermore, the marked divergence in male throat coloration is only weakly correlated with divergence in mate preferences, which is also asymmetric. This suggests that divergent sexual selection alone is unlikely to be the primary driver of incipient speciation between the two lineages. Instead, genetic data (asymmetric introgression and northern mtDNA in hybrids) suggest a role for genetic incompatibilities that have arisen during the independent evolution of the lineages (Dong et al. 2019). More broadly, our data are consistent with the emerging view that sexual selection alone rarely drives speciation to completion (Ritchie 2007; Maan and Seehausen 2011; Scordato et al. 2014; Lackey and Boughman 2017; Servedio and Boughman 2017).

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Author Contributions

CAM, RAB and DSF designed the study. CAM, RAB, CMD and KJR contributed to animal collection and maintenance. RAB conducted the experiments and extracted behavioural data. CAM performed statistical analyses. CAM, DSF and CMD drafted the manuscript. All authors contributed to interpretation and revising the manuscript.

Data Accessibility

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Table 1. Results of generalised linear mixed models investigating whether female and male lineage ("southern", "northern") predict behaviour during mate preference trials. Statistically significant relationships are italicised. CI: confidence interval, R^2m: marginal R^2, R^2c: conditional R^2, *df*: degrees of freedom.

Behaviour	Variable	R^2m	R^2c	Estimate (95% CI)	F_{df}	*P*
1) Copulation attempts	Female lineage	0.05	0.12	-0.067 (-0.198, 0.064)	$1.05_{1, 41.5}$	0.311
	Male lineage			-0.018 (-0.139, 0.102)	$0.08_{1, 30.5}$	0.780
	Female lineage × Male lineage			0.149 (0.033, 0.264)	$6.53_{1, 103.2}$	*0.012*
2) Male head-bobs	Female lineage	0.07	0.22	-0.272 (-0.540, 0.006)	$4.12_{1, 31.9}$	0.051
	Male lineage			-0.030 (-0.273, 0.224)	$0.06_{1, 28.8}$	0. 805
	Female lineage × Male lineage			0.293 (0.066, 0.519)	$6.61_{1, 91.2}$	*0.012*
3) Female head-bobs	Female lineage	0.04	0.27	0.189 (-0.033, 0.412)	$2.91_{1, 41.1}$	0.096
	Male lineage			-0.087 (-0.240, 0.067)	$1.26_{1, 101.3}$	0.265
	Female lineage × Male lineage			0.018 (-0.135, 0.171)	$0.05_{1, 100.6}$	0.817
4) Female rejection	Female lineage	0.11	0.22	0.178 (0.009, 0.341)	$4.64_{1, 40.3}$	*0.037*
	Male lineage			-0.279 (-0.457, -0.103)	$10.1_{1, 38.9}$	*0.003*
	Female lineage × Male lineage			-0.074 (-0.227, 0.079)	$0.92_{1, 103.9}$	0.340

Table 2. Results of generalised linear mixed models investigating whether male behavioural category or condition predict behaviour during male-male contests. Behavioural categories are: “southern”, “northern low aggression” (grey, orange-yellow and yellow morph) and “northern high aggression” (orange morph). Statistically significant relationships are italicised. SS: type III sum of squares, R^2m: marginal R^2, R^2c: conditional R^2, df: degrees of freedom, FM: focal male, OM: opponent male

Behaviour	Variable	R^2m	R^2c	SS	F_{df}	*P*
1) Latency	FM behavioural category	0.22	0.73	166.8	$4.35_{2, 41.5}$	*0.019*
	OM behavioural category			209.0	$5.45_{2, 58.1}$	*0.007*
	FM condition			73.4	$3.83_{1, 91.2}$	0.053
	OM condition			63.4	$3.31_{1, 75.7}$	0.073
	FM behavioural category × OM behavioural category			73.0	$0.95_{4, 57.2}$	0.441
2) Display behaviour	FM behavioural category	0.14	0.33	7.7×10^{-5}	$0.84_{2, 32.5}$	0.441
	OM behavioural category			6.1×10^{-5}	$0.66_{2, 58.5}$	0.519
	FM condition			6.1×10^{-6}	$0.13_{1, 65.6}$	0.717
	OM condition			5.0×10^{-5}	$1.09_{1, 92.6}$	0.300
	FM behavioural category × OM behavioural category			4.4×10^{-4}	$2.39_{4, 56.2}$	0.062
3) Physical aggression	FM behavioural category	0.08	0.09	0.061	$1.35_{2, 93.5}$	0.264
	OM behavioural category			0.041	$0.90_{2, 93.6}$	0.410
	FM condition			0.030	$1.33_{1, 92.6}$	0.251
	OM condition			7.2×10^{-6}	$3.0 \times 10^{-4}_{1, 94.0}$	0.986
	FM behavioural category × OM behavioural category			0.011	$0.12_{4, 92.8}$	0.973

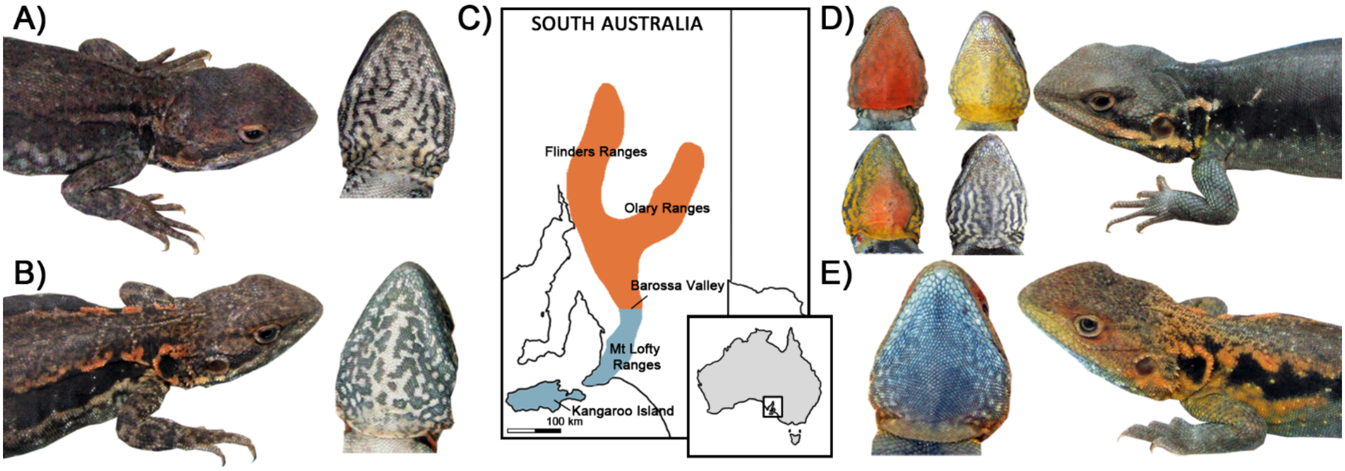

Figure 1. Phenotypic variation between the northern and southern lineages of *C. decresii*. A) and B) show northern and southern lineage females respectively, which are similar in appearance. C) Distribution of the two lineages (northern = orange shading; southern = blue shading). D) and E) show northern and southern lineage males respectively. Northern males are polymorphic for throat coloration.

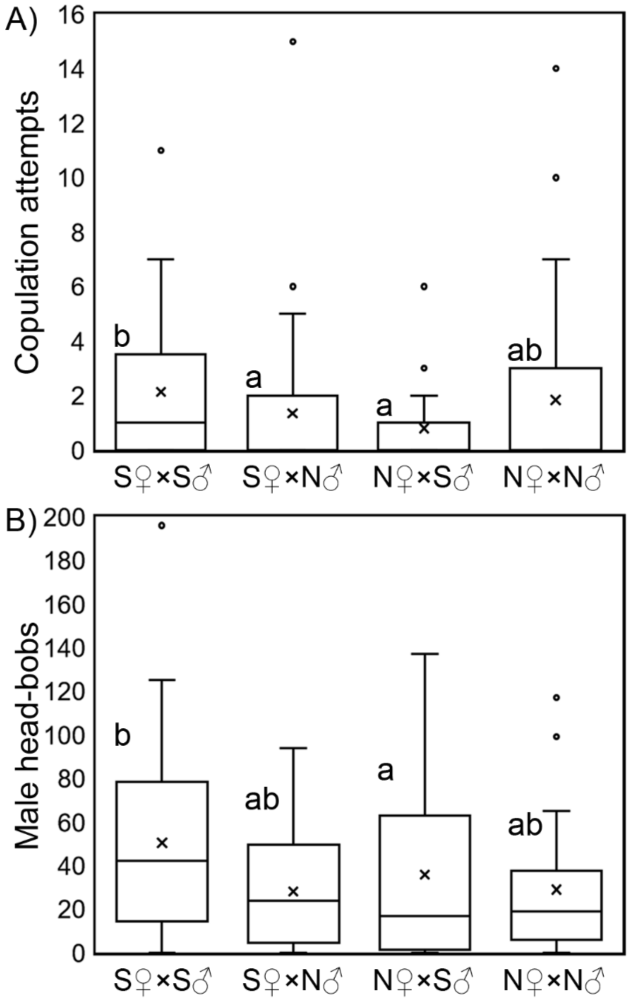

Figure 2. The total number of A) copulation attempts and B) male head-bobs (courtship) performed during mate preference trials between females and males of the southern (S) and northern (N) lineages of *C. decresii*. Letters indicate statistically significant differences between lineage pairings.

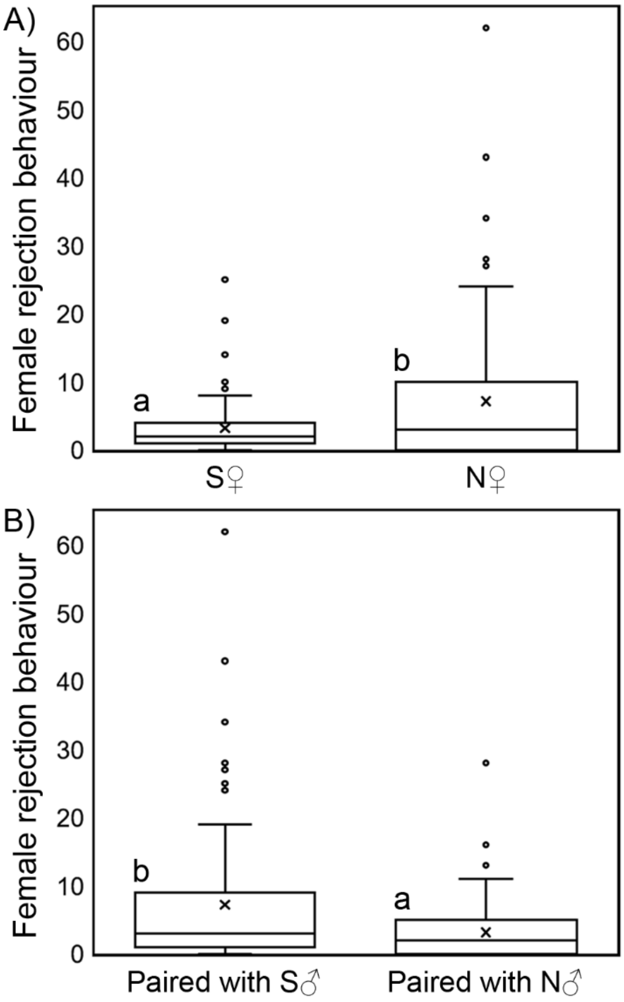

Figure 3. Comparison of A) the total number of rejection behaviours (biting, chasing and fleeing) performed by southern (S) and northern (N) females and B) the number of rejection behaviours performed by females (regardless of lineage) when paired with southern and northern males. Letters indicate statistically significant differences between lineages.

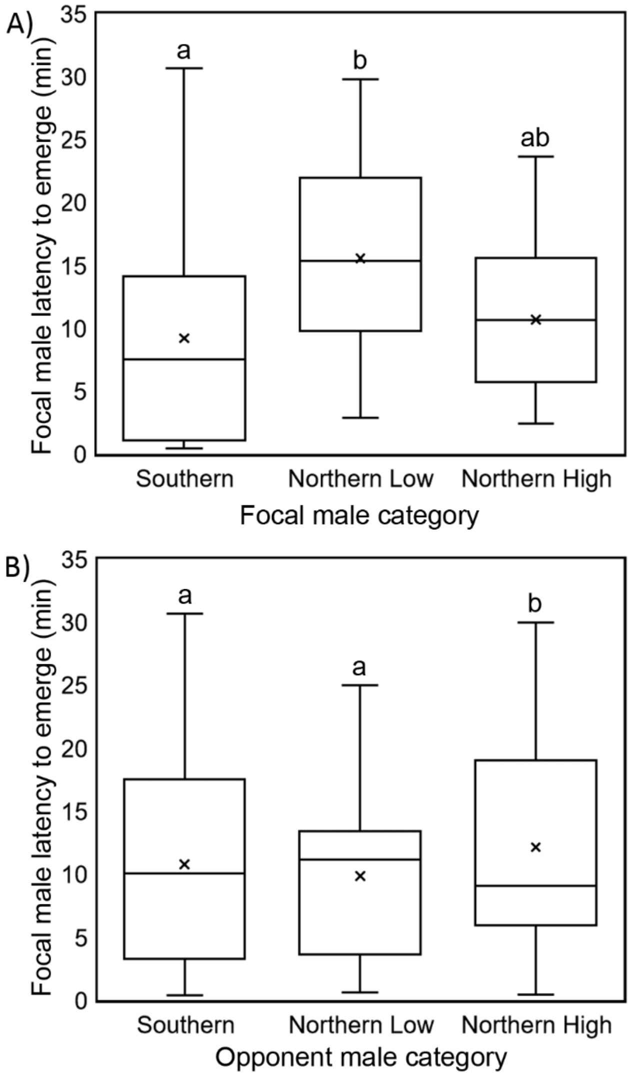

Figure 4. Time taken (in minutes) for A) southern (blue), northern low aggression (grey, orange-yellow, yellow) and northern high aggression (orange) focal males to emerge from beneath the tile and B) focal males to emerge depending on the behavioural category of the opponent male. Lowercase letters indicate significant differences between behavioural categories. Note that focal male ID explains a large proportion of the variance in latency to emerge.