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Animal behaviour

Females alter their mate preferences depending on hybridization risk

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Mating with another species is often maladaptive because it generally results in no or low-fitness offspring. When hybridization is sufficiently costly, individuals should avoid mating with heterospecifics even if it reduces their ability to mate with high-quality conspecifics that resemble heterospecifics. Here, we used spadefoot toads, *Spea multiplicata,* to evaluate whether females alter their preferences for conspecific male sexual signals (call rate) depending on heterospecific presence. When presented with conspecific signals against a background including both conspecific and heterospecific signals, females preferred male traits that were most dissimilar to heterospecifics—even though these signals are potentially associated with lower-quality mates. However, when these same females were presented with a background that included only conspecific signals, some females switched their preferences, choosing conspecific signals that were exaggerated and indicative of high-quality conspecific mates. Because only some females switched their preferences between these two chorus treatments, there was no population-level preference for exaggerated conspecific male signals in the absence of heterospecifics. These results show that hybridization risk can alter patterns of mate choice and, consequently, sexual selection on male signals. Moreover, they emphasize that the strength and expression of reproductive barriers between species (such as mate choice) can be context-dependent.

1. Introduction

When choosing mates from among conspecifics, individuals (often females) are expected to prefer sexual signals that identify those mates that provide them or their offspring with fitness benefits (hereafter 'high-quality' mates) and disfavour sexual signals associated with conspecifics that provide relatively fewer fitness benefits (hereafter 'low-quality' mates) [1,2]. However, if sexual signals of high-quality conspecifics resemble those of heterospecifics, then female preferences for those signals can enhance hybridization risk [3–5]. Thus, a trade-off can arise between the benefits of mating with high-quality conspecific mates versus the risks and costs of hybridization: preferences for signals of high-quality conspecifics enhance hybridization risk, but preferences for signals that differ from heterospecifics enhance the likelihood of mating with relatively low-quality conspecifics [3,6–15].

The evolutionary consequences of this trade-off are threefold. First, if the benefits of choosing high-quality mates are low relative to the costs of hybridization, preference for high-quality conspecifics could be lost. Second, mate preferences might constitutively diverge between populations where heterospecifics are present (sympatric populations) versus absent (allopatric populations), including the refinement of preferences [16] or evolution of preferences for alternative signals in sympatry [3,7,11,15,17,18]. Third, selection could favour the facultative expression of mate preferences in sympatry that depend on whether heterospecifics are physically present at the time of mating (i.e. when hybridization is an immediate risk). Such plasticity in mate preferences could arise if individuals learn to avoid heterospecifics [19–23] or, alternatively, if aspects of the environment

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reliably predict hybridization risk (*sensu* [24–28]). Such context-dependent mate preferences are expected to evolve when the benefits of choosing high-quality conspecifics are high, and an individual's risk of encountering heterospecifics varies from one mating opportunity to another [29,30].

The evolution of context-dependent preferences allows individuals to navigate the fitness trade-off described above because individuals express preferences that optimize the fitness benefits of mate choice given their circumstances [31]. However, context-dependent mate choice has important implications for sexual selection's role in the origins and maintenance of species boundaries. Indeed, facultative mate preferences would cause the direction and strength of selection on sexual signals in sympatry to vary depending on encounters with heterospecifics. If such preferences diminish the level of divergent selection on sexual signals in sympatry versus allopatry, then divergence in sexual signals between sympatric and allopatric populations is less likely relative to what would arise from divergent constitutive preferences [17,32]. Given that divergence between such populations is a proposed mechanism of speciation [17,33–37], factors that reduce divergence also reduce the likelihood of speciation [17,32,35].

We evaluated whether heterospecific presence affects female mate preferences in Mexican spadefoot toads (*Spea multiplicata*) where they potentially co-occur with *Spea bombifrons* in the southwestern USA [38,39]. Female *S. multiplicata* evaluate call rate in choosing a mate [7]. In allopatry, where there is no risk of mating with *S. bombifrons* (or hybrids), females prefer faster-calling conspecifics which are in better condition, have higher fertilization success and produce fitter offspring [7,15,40,41]. However, in sympatry, *S. multiplicata* females risk costly matings with faster-calling *S. bombifrons* (electronic supplementary material, figure S1) or sterile hybrid males [7,42,43]. Thus, sympatric *S. multiplicata* females prefer slower-calling conspecifics whose calls are more dissimilar from heterospecifics' [7,18], even though these slow-calling males might be relatively low-quality [7,15,40,41]. This divergence in constitutive preferences is expected if females trade off the benefits of mating with high-quality mates versus the risks and costs of hybridization.

Yet, facultative mate preferences might also be favoured in this system. Heterospecific and hybrid male frequencies vary across time and space [38,43]; so a given female might encounter heterospecifics during some breeding events but not others. Thus, context-dependent mate preferences could enable *S. multiplicata* females to optimize their mate preferences when cues indicating the current risk of hybridization are available. To test this possibility, we measured female preferences for call rate in two-choice phonotaxis tests under conditions that resembled mating aggregations in nature where hybridization risk is present versus absent. If females alter their preferences depending on the risk of mating with heterospecifics, we predicted that females would prefer slow-calling conspecific calls only when hybridization risk was present.

2. Methods

Using a two-choice phonotaxis experiment, we tested 53 *S. multiplicata* females from sympatric populations in the southwestern USA for preferences of conspecific calls at a fast call rate (37 calls min^{-1}) versus a slow call rate (26 calls min^{-1}) against two background choruses of male calls: (1) a chorus of *S. multiplicata* calls only (i.e. 'pure-species background'), and (2) the same *S. multiplicata* chorus, to which *S. bombifrons* calls had been added (i.e. 'mixed-species background'; electronic supplementary material; [7,18,44]). Females were designated as choosing a call rate stimulus when they came within one body length of the speaker playing that stimulus [7,18,24,44,45]. If 30 min elapsed without a choice, a female was considered unresponsive (electronic supplementary material, figure S3). We recorded each female's time to choose (latency), mass and snout–vent length (SVL; electronic supplementary material). Females were tested with pure versus mixed-species chorus backgrounds on separate days, and whether a female was first tested with the pure-conspecific or mixed-species chorus background was randomized. Call stimuli were switched between speakers after each trial and the leading call stimulus was switched every four trials.

To evaluate the effect of a chorus background (versus no chorus) on mate preference for conspecific call rate, we compared preferences for conspecific call rate from the chorus background experiment described above with preferences by the same females for conspecific call rate in tests without any chorus background (electronic supplementary material). Specifically, prior to the experiment above, we carried out two tests in which some of the same females used above were evaluated for their call rate preference of *S. multiplicata* calls of: (1) 26 calls min^{-1} versus 37 calls min^{-1} (the same stimuli in our chorus background study), and (2) 31 calls min^{-1} versus 37 calls min^{-1} (electronic supplementary material).

Data were analysed in R [46]. We calculated scaled mass index from mass and SVL as a measure of female body condition [47,48]. We used exact binomial tests to test whether females had significant preferences in either chorus treatment. We fitted generalized mixed-effects models using the glmer function (package lme4: [49]) with the predictors chorus treatment, treatment order, body size and condition as fixed effects, and female ID as a random intercept. We modelled how these fixed effects predicted mate choice as a binomially distributed response, and in separate models, how the same set of fixed effects predicted latency (time to choose) as a negative-binomially distributed response (electronic supplementary material). To assess significance of predictors on responses (mate choice or latency), we compared each model with a null model without the predictor, using likelihood ratio tests (LRT; electronic supplementary material).

To compare female preferences for fast versus slow conspecific calls with no background versus each chorus background, we fitted a binomial mixed model of female choice. We included chorus background treatment as a fixed effect and individual female as a random intercept. We tested for differences between the effects of chorus treatment levels, using the glht function (package multcomp: [50]) to correct for multiple comparisons using the joint normal distribution.

3. Results

Female preferences depended on the presence of heterospecifics in the chorus background. In the mixed-chorus background, females preferred the slower call rate (15 chose fast, 29 chose slow; exact binomial test: probability of choosing slow = 0.66, $p = 0.049$). In the pure-conspecific chorus treatment, however, females as a group did not express a preference (22 chose fast, 18 chose slow; exact binomial test: probability of choosing fast = 0.55, $p = 0.64$). Indeed, females' probability of choosing the slow call differed between the chorus background treatments (figure 1, table 1*a*). Neither female size nor condition predicted female choice (table 1*a*). Moreover, females' testing order in each chorus treatment did not predict their choices (LRT: chisq = 1.359, d.f. = 1, $p = 0.24$). Finally, females' latency to choose was not affected by chorus treatments or female phenotype (table 1*b*), though our power to detect a body

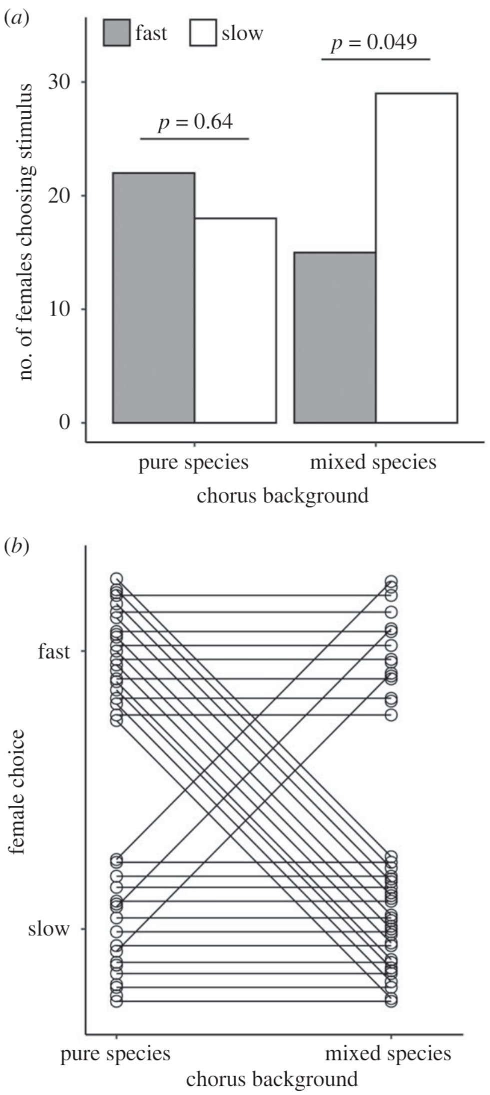

Figure 1. Female choice as a function of background chorus. (*a*) With a background of conspecific calls only, females as a group showed no preference; with a background including heterospecifics, females preferred slower calls. (*b*) Individual female preferences varied across choruses (lines/points (which are offset from each other on the *y*-axis to reveal individual female data) connect each individual's choices between choruses).

size effect might have been insufficient (table 1*b*; electronic supplementary material, figure S4).

Without any chorus background, female preferences were similar to their preferences with a pure-species chorus background ($z = -0.93$, $p = 0.35$, adjusted $p = 0.57$). Thus, a background chorus *per se* did not appear to impact female preferences. However, these females significantly preferred slow calls in the mixed-species chorus treatment. Indeed, preferences in the mixed-species chorus differed from preferences in absence of the chorus, but this difference was not statistically significant at $\alpha = 0.05$ after correction for two *post-hoc* comparisons ($z = -2.10$, $p = 0.04$, adjusted $p = 0.07$).

4. Discussion

When the sexual signals of high-quality conspecifics resemble those of heterospecifics, females face a trade-off between the benefits of identifying high-quality mates versus the risks and costs of hybridization [7]. Although such trade-offs can be resolved in different ways (e.g. using multiple traits [3,7,11]), one solution is for females to facultatively switch their preferences depending on whether heterospecifics are present. Consistent with this possibility, we found that female *S. multiplicata* preferred slower male calls that were more different from heterospecifics, but only when heterospecific calls were present. In the absence of heterospecific calls, females were more likely to choose faster conspecific calls that are more like those of heterospecifics and hybrids (figure 1), but that potentially reflect higher conspecific mate quality.

In the pure *S. multiplicata* chorus background, females as a group did not significantly prefer faster call rates (figure 1; electronic supplementary material). Generally, lack of group-level preference arises either because individual females have no preference for the stimuli and simply choose at random (i.e. females are not 'choosy': [51,52]), or because individuals express preferences, but these preferences vary enough among individuals that no group-level preference is detectable (e.g.: [24,53]). Our data are consistent with the latter explanation. Specifically, some females maintained their preference for slow calls regardless of chorus background whereas others switched to preferring faster calls in the pure-species background (the adaptive switching pattern; figure 1*b*). If females were simply choosing a stimulus at random, those that switched preference across treatments should have been equally likely to switch to preferring slower calls in the pure-species background (the maladaptive switching pattern), which was rarely observed (figure 1*b*).

Previous work found that sympatric females preferred slower calls in the absence of any background [7,18], which differs from our results. One explanation for this difference is that a novel mate choice behaviour might be evolving: rather than constitutively expressing preferences for slow-calling males (as detected in the past [7,18]), females might optimize the fitness benefits of their mate choice decisions by modifying their preferences for call rate in the presence of heterospecifics. Given the fitness benefits of preferring fast call rates [7,15,40,41], selection would favour females that facultatively preferred the fastest-calling males when hybridization was not an immediate risk. If the behaviour is new, it could explain why some females expressed facultative preferences for fast call rates whereas others expressed constitutive preferences for slow call rates (figure 1).

If this behaviour is novel, identifying the mechanism(s) underlying it could provide insight into the behaviour's origins and evolution. In other systems, learning from encounters with heterospecifics can modify female preferences for conspecifics ([2], e.g. [20,21,23]). Spadefoots do not appear to learn mate preferences [54,55], but more work is needed to examine whether and how experience impacts subsequent preferences. A further mechanism for the change in female preferences is differential acoustic interference in the presence versus absence of heterospecifics [56–60]. If females detect slower calls better than faster ones when *S. bombifrons* is present, females could express stronger preferences for slow calls in the mixed-species chorus background. Although our experimental design minimizes interference as an artefact in our choice tests (see electronic supplementary material), we cannot rule out all effects of acoustic masking. Indeed, natural breeding choruses are noisy environments [61], and a preferential response to slower calls mediated by masking would also minimize

Table 1. (*a*) Binomial mixed-effects models of females' choice of call rate stimulus (fast versus slow call rates), and (*b*) negative binomial mixed-effects models of females' latency to choose, for females that made choices. Likelihood ratio tests comparing each model with a null model with only a random intercept of female ID are shown.

model	fixed effect estimate ± s.e.	χ^2	d.f.	*p*
(*a*)				
call rate choice∼chorus + (1|ID)	−1.01 ± 0.54	4.20	1	0.04
call rate choice∼snout–vent length + (1|ID)	−0.06 ± 0.07	0.70	1	0.40
call rate choice∼condition + (1|ID)	0.08 ± 0.10	0.70	1	0.40
(*b*)				
latency∼chorus + (1|ID)	0.04 ± 0.17	0.07	1	0.79
latency∼snout–vent length + (1|ID)	−0.17 ± 0.09	3.14	1	0.076
latency∼condition + (1|ID)	0.05 ± 0.04	1.74	1	0.19

mating with heterospecifics. If so, selection might not favour traits that minimize masking's effects. More work is needed to determine how heterospecific signalling impacts female mate choice.

Our results suggest that the nature and strength of sexual selection vary depending on the immediate presence of heterospecifics. Previous work has focused on how the risk of mating with heterospecifics impacts divergence in sexual signals between sympatric and allopatric populations [9,33,62,63]. Our study indicates that sexual selection exerted by females can become variable *within* populations depending on heterospecifics' actual presence. Temporal variation in mate preferences could reduce the strength of sexual selection on male signals [64], promote polymorphism in these signals [65,66], and/or reduce differentiation in signals between sympatric and allopatric populations. In spadefoots, for example, male calls do not diverge between sympatry and allopatry as expected if females constitutively express divergent preferences [44].

Facultative preferences that depend on the presence of heterospecifics potentially diminish the likelihood that sympatric and allopatric populations will become reproductively isolated if they reduce divergent selection between the populations. Generally, the risk of hybridization in sympatry will drive divergence in sexual signals between sympatric and allopatric populations (e.g. [9,18,33,67]). Consequently, sympatric and allopatric populations are expected to become reproductively isolated, especially when individuals reject potential mates from the alternative population type [17,34,35,37,68,69]. If, however, females facultatively adjust their preferences when heterospecifics are present, the likelihood (and strength) of divergence between sympatric versus allopatric populations is potentially reduced. More broadly, female preferences serve as barriers to gene flow during species formation and maintenance [17,70]. That females might modify their preferences depending on heterospecific mating risk assessed at the time of mating suggests that the strength of these barriers might vary in previously unrecognized ways.

Our finding that female preferences in the presence of a heterospecific background chorus potentially differ from simple two-choice tests suggests that common laboratory assays of behavioural isolation might not accurately measure reproductive isolation in nature if key cues to hybridization risk are absent. This possibility requires further examination and might depend on the specific traits presented. Nevertheless, our work suggests that signals mimicking natural breeding should be provided to clarify how sexual selection and hybridization risk interact to affect speciation.

Ethics. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina-Chapel Hill (protocol 17-073.0-C). Animals were collected with permission under Arizona Game and Fish Department LIC no. SP649270 and New Mexico Department of Game and Fish Authorization no. 1857.

Data accessibility. The data and code are available in Dryad Digital Repository: https://doi.org/10.5061/dryad.p8cz8w9tg [71]. Additional details about data collection and analysis are available in the electronic supplementary material, methods and results [72].

Authors' contributions. G.M.C.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, validation, visualization, writing—original draft, writing—review and editing; K.S.P.: conceptualization, data curation, funding acquisition, methodology, project administration, resources, supervision, writing—original draft, writing—review and editing.

Both authors gave final approval for publication and agreed to be held accountable for the work performed herein.

Conflict of interest declaration. We declare we have no competing interests.

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