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Cite this article: Pfennig KS, Pfennig DW, Porter C, Martin RA. 2015 Sexual selection's impacts on ecological specialization: an experimental test. *Proc. R. Soc. B* **282**: 20150217.
<http://dx.doi.org/10.1098/rspb.2015.0217>

Received: 30 January 2015
 Accepted: 2 April 2015

Subject Areas:
 evolution, ecology

Keywords:
 competition, mate choice, disruptive selection, resource polymorphism, speciation

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2015.0217> or via <http://rspb.royalsocietypublishing.org>.

Sexual selection's impacts on ecological specialization: an experimental test

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In many species, individuals specialize on different resources, thereby reducing competition. Such ecological specialization can promote the evolution of alternative ecomorphs—distinct phenotypes adapted for particular resources. Elucidating whether and how this process is influenced by sexual selection is crucial for understanding how ecological specialization promotes the evolution of novel traits and, potentially, speciation between ecomorphs. We evaluated the population-level effects of sexual selection (as mediated by mate choice) on ecological specialization in spadefoot toad tadpoles that express alternative ecomorphs. We manipulated whether sexual selection was present or reversed by mating females to their preferred versus non-preferred males, respectively. We then exposed their tadpoles to resource competition in experimental mesocosms. The resulting distribution of ecomorphs was similar between treatments, but sexual selection generated poorer trait integration in, and lower fitness of, the more specialized carnivore morph. Moreover, disruptive and directional *natural* selection were weaker in the sexual selection present treatment. Nevertheless, this effect on disruptive selection was smaller than previously documented effects of ecological opportunity and competitor density. Thus, sexual selection can inhibit adaptation to resource competition and thereby hinder ecological specialization, particularly when females obtain fitness benefits from mate choice that offset the cost of producing competitively inferior offspring.

1. Introduction

Many populations contain distinct resource-use phenotypes, each of which uses a narrower range of resources than the population as whole [1,2]. In extreme cases, this ecological specialization can take the form of alternative phenotypes showing differential resource use; i.e. ecomorphs. The existence of such ecomorphs within the same population (resource polymorphism (*sensu* [3])) has long fascinated evolutionary biologists, because the evolution of a resource polymorphism potentially represents a crucial, early stage in the origin of novel, resource-use traits and possibly even new species [3–10]. Additionally, from an ecological standpoint, alternative ecomorphs might function as separate species [11], and their presence within a population can alter the outcome of interactions with other species [12]. Thus, identifying the factors that promote (or inhibit) the evolution of resource polymorphism is vital for understanding the origins and maintenance of biodiversity [4].

Much is known about natural selection's role in fostering resource polymorphism: resource polymorphism evolves when intraspecific competition for resources generates disruptive natural selection [3,13,14]. However, *sexual* selection's contribution to the evolution of resource polymorphism remains unclear. Sexual selection could either facilitate or inhibit the evolution of ecological specialization depending on whether or not it generates phenotypes that could be favoured by disruptive natural selection [15–22].

Female mate choice is a key driver of sexual selection [23], and a female's choice of mate has the potential to impact her offspring's resource-use phenotypes and competitive ability for resources [15,16,20]. Female mate choice can thereby influence the evolution of resource polymorphism in particular and ecological specialization in general. Indeed, females could preferentially mate with those

males that sire competitively superior offspring with specialized phenotypes, especially if male sexual signals are directly linked to male resource use; i.e. ‘magic traits’ [16,17,24,25]. Alternatively, females might prefer males for any number of other reasons [23], with female mate choice exerting indirect effects on offspring phenotypes and ecological performance [15,16,25]. Regardless, female mate choice has the potential to impact both the distribution of phenotypes and ecological performance among a female’s offspring [15–17,20,25].

At the population level, the effects of mate choice (and, more generally, those of sexual selection) on ecological specialization, if any, should be manifest by altering the distribution of resource-use phenotypes and, concomitantly, the patterns of natural selection on those phenotypes. If mate choice facilitates (versus inhibits) the evolution of ecological specialization, then the population should come to contain fewer intermediate phenotypes [15,18]. Moreover, if mate choice facilitates specialization, it should enhance both a population’s mean fitness in the presence of resource competition as well as the fitness of those individuals within the population that express more specialized (as opposed to intermediate) phenotypes.

We evaluated these predicted population-level effects of mate choice using spadefoot toads, *Spea multiplicata*, in which female mate choice mediates sexual selection [26–29]. We experimentally manipulated whether the natural pattern of mate choice was either present or reversed and reared the tadpole offspring in replicate experimental populations that experienced resource competition. Our goals were to evaluate how sexual selection (as mediated by female mate choice) affects: (i) the extent of divergence between ecomorphs and the coordinated expression of traits critical to resource acquisition; (ii) population and ecomorph fitness and (iii) the strength and form of natural selection in response to resource competition. Additionally, we also evaluated the relative effect size of sexual selection’s impact on the distribution of phenotypes and patterns of selection on those phenotypes. We used the same experimental design as a previous study [30], which evaluated the effects of resource quality and intensity of resource competition on the distribution of, and selection favouring, alternative ecomorphs. We could therefore directly contrast sexual selection’s effects, specifically those of mate choice, with two key drivers of ecological specialization: ecological opportunity and competitor density [30–33]. Such contrasts are needed to evaluate the importance of sexual selection in ecological specialization [21,31].

2. Material and methods

(a) Study system

Mexican spadefoot toad tadpoles, *S. multiplicata*, express a resource polymorphism consisting of an ‘omnivore’ ecomorph and a ‘carnivore’ ecomorph [34]. Omnivores are round-bodied with a long gut, small jaw muscles, numerous rows of keratinized denticles and smooth keratinized mouthparts (MP). They are dietary generalists, feeding on detritus, algae and small crustaceans [35]. Carnivores, by contrast, are narrow-bodied with a short gut, greatly enlarged jaw muscles, few denticle rows (DR) and serrated MP. They are dietary specialists, feeding mostly on anostracan fairy shrimp [35].

Although environmental cues (e.g. diet) affect which morph an individual adopts (reviewed in [36]), families differ in propensity to produce carnivores, suggesting the presence of underlying heritable variation in ecomorph production [37,38]. Moreover,

the resource polymorphism has been lost in populations that are sympatric with a congener, *S. bombifrons*, with which it competes for resources. The individuals in these populations produce mostly omnivores, and thereby reduce competition with *S. bombifrons*, the superior competitor for shrimp [39–42]. We therefore used an allopatric (pure) *S. multiplicata* population, in which both ecomorphs occur.

Previous work in allopatric pure *S. multiplicata* populations has demonstrated that intermediate phenotypes are disfavoured by disruptive selection [43–45]. Moreover, in all populations sampled, significant bimodality in resource-use phenotypes exists, indicating that all populations exhibit the resource polymorphism, albeit to a varying extent [30].

How mate choice impacts this resource polymorphism is unclear. In *S. multiplicata*, females choose their mates: males call to attract mates, and females initiate pair-formation by closely approaching and touching the male [26]. In the allopatric populations, *S. multiplicata* females prefer males with faster call rates that are in better condition; that is, they exert condition-dependent mate choice [26–29]. By preferring such males, females choose mates that provide them with higher fertilization success and enhanced quality offspring [26,27].

Because *S. multiplicata* in allopatric populations exhibit both resource specialization and condition-dependent mate choice, they are well suited to evaluate how sexual selection, as mediated by mate choice, affects ecological specialization.

(b) Experimental manipulation of sexual selection

We experimentally manipulated sexual selection to determine its population-level effects on ecological specialization. To do so, we collected 10 male–female amplexed pairs as they formed at a pure *S. multiplicata* breeding aggregation near Portal, AZ, USA. The pairs were numbered consecutively as they were collected (to control for temporal effects, see below), broken apart and the male found with the female was designated that female’s ‘preferred’ mate [26,27]. As noted above (and in previous studies [26,27]), females choose their mates: males do not force copulations with females nor do they break apart existing pairs. At the end of night, we collected unmated, calling males from the breeding aggregation. These males were designated ‘non-preferred’ males, because they were calling, but had not been chosen as a mate [26,27]. This preferred/non-preferred design is identical to that previously used in this system to measure the fitness consequences of mate choice [26,27].

We returned the animals to the nearby Southwestern Research Station (SWRS). We generated male–female pairings that fell into two different treatment groups: a ‘sexual selection present’ treatment (hereafter SS+) and a ‘sexual selection reversed’ treatment (hereafter SS–). In the SS+ pairings, females were mated to their original preferred male. In SS– pairings, females were mated to randomly chosen non-preferred males. In the SS– treatment, we mated females to only non-preferred males (rather than randomly chosen males) to remove any possible effects of quality of the preferred males, which tend to be larger and/or in better condition than non-preferred males [26–28]. Thus, our SS– treatment was the antithesis of the SS+ treatment in that it reversed the mate choice pattern observed in natural allopatric populations. By contrasting the two treatments, we could thereby ascertain how the natural patterns of mate choice impacted the expression and performance of the ecomorphs.

To control for the possibility that females choosing later in the evening were less likely to obtain higher quality males (if such males are the first to be mated), we mated the female in the first pair to her preferred mate and then paired all remaining alternately numbered females (i.e. those from odd-numbered pairs) with their preferred mates. The females from even-numbered collected pairs were mated to randomly chosen non-preferred males. That the

first female was mated to her preferred male (as opposed to a non-preferred male) was randomly determined.

For all pairings, we placed a female with the male in a water-filled tank. The resulting eggs were aerated until tadpoles hatched. Three days after hatching, we removed a subset of the tadpoles from each clutch and placed them in mesocosms as described below.

(c) Mesocosms

Our mesocosm design was similar to that used previously to measure the effects of intraspecific competition and ecological opportunity on ecological specialization [30]. In our experiment, we held ecological opportunity and competition constant, while manipulating sexual selection as described above. Because our design was similar to that used previously, we directly compared the effect size of sexual selection with that of ecological opportunity and competitor density (see below).

We reared tadpoles from SS+ and SS− treatments in replicate wading pools—‘mesocosms’—that resembled natural ponds in which *Spina* tadpoles develop. We arranged 12 wading pools (1.8-m diameter) in an outdoor array at SWRS. Wading pools were filled with dechlorinated water and substrate from a natural pond. We replicated each treatment six times.

From each of the above 10 families, we randomly chose 1116 tadpoles. Then, 186 tadpoles from each of the five families per treatment were randomly assigned to each of six replicate mesocosms per treatment. Thus, each mesocosm contained 930 tadpoles (186 tadpoles/per family × five families) of a given treatment type (SS+ or SS−).

Tadpoles were fed live shrimp daily (collected daily from natural ponds). Tadpoles could also eat natural detritus from the substrate, algae that grew and any invertebrates that colonized the pools (e.g. mosquito larvae). After two weeks, we removed all tadpoles (verified by draining the pools entirely), then euthanized and preserved them in ethanol-filled jars. A jar from one mesocosm dried out, and so the tadpoles from this replicate were not usable. We therefore had six replicates of the SS+ and five of the SS− treatments.

(d) Tadpole measurements

We measured a randomly chosen subset of tadpoles from each mesocosm (88–152 tadpoles/mesocosm). Tadpoles were measured using previously published methods [30,38]. Briefly, we measured each tadpole’s snout–vent length (SVL) and Gosner developmental stage (GS; [46]). We then measured the width of its orbitohyoideus muscle (OH; carnivores have a larger OH) and scored its MP on an ordinal scale that ranged from 1 (most omnivorous) to 5 (most carnivorous). Finally, we counted the number of DR and gut coils (GC; carnivores have fewer DR and GC than omnivores).

We combined the above measures into a single ‘morphological index’ (e.g. see [38] and references therein). To calculate this index, we used a principal component (PC) analysis. The morphological index consisted of the first PC (the only PC with an eigenvalue greater than 1), which explained 49.6% of the variation.

(e) Sexual selection’s impact on the distribution of trophic morphology

We next determined whether trophic morphology within each mesocosm was distributed unimodally or bimodally by using previously published methods [30]. Briefly, we tested whether the distribution of the morphological index was better described by a single normal distribution or by a mixture of two normal distributions. We used both maximum likelihood and Bayesian approaches. We evaluated the fit of a single versus a mixed model using ΔAICc criteria ($\Delta\text{AICc} \geq 4$). The maximum-likelihood

approach was done in R (v. 3.1.0) and the Bayesian approach used software described in Brewer [47].

The Bayesian and ML analyses found strong support for bimodality in 10 mesocosms and weak support for bimodality in one mesocosm (see electronic supplementary material, table S1). Based on these results, we estimated the degree of bimodality and the divergence between trophic phenotypes in each mesocosm using the distributions fit by the ML analysis.

After fitting the distributions, we used Mann–Whitney *U*-tests (using R v. 3.0.1) to evaluate whether the SS+ and SS− treatments differed in: (i) the degree of bimodality, as measured by 1 minus the proportion of values falling between the two modes for each pond; (ii) divergence in trophic phenotype, as measured by the distance between the modes of the ML fitted distribution; (iii) the proportion of carnivores produced, as measured by the proportion of individuals that fell under the probability density function for the more carnivorous phenotype; and (iv) the variance in the morphological index.

(f) Sexual selection’s impact on fitness and patterns of natural selection

We determined whether SS+ and SS− treatments differed in the pattern of natural selection on alternative ecomorphs as follows. Using SVL and GS as fitness proxies (see [43,44]), we independently evaluated overall differences in linear and quadratic selection on the morphological index between the two treatments by fitting separate generalized linear mixed models (using nlme in R v. 3.0.1). To estimate linear selection, our models for each fitness proxy included the linear morphological index term, sexual selection treatment, and their interaction as fixed effects. Mesocosm ID was included as a random effect. To estimate quadratic selection, our model included both linear and quadratic morphological index terms, treatment, and the interaction between the quadratic term and treatment as fixed effects. Again, mesocosm ID was included as a random effect. For all models, a significant interaction between the treatment and morphological index term would indicate that selection on trophic morphology differs between the two treatments. Additionally, we used cubic spline analyses to visually verify the presence of disruptive selection (i.e. the existence of a fitness minimum in each treatment; see [48]).

Finally, we determined whether fitness proxies (as measured by SVL, GS and % survival) differed for the SS+ and SS− treatments. To contrast SVL and GS, we fit generalized linear mixed models (using nlme in R v. 3.0.1). In each model, sexual selection treatment was included as a fixed effect, mesocosm ID was included as a random effect, and SVL or GS was our response variable. We evaluated whether per cent survival differed between treatments with a Mann–Whitney *U*-test.

(g) Sexual selection’s effects on trait integration

The above analyses ascertained: (i) whether sexual selection enhances or decreases the production of intermediate composite phenotypes (as measured by our morphological index) and (ii) the pattern of selection on those composite phenotypes. However, ecological specialization might also depend on the *integration* of individual aspects of morphology. We therefore evaluated whether trait integration differed between SS+ and SS− treatments by calculating Pearson correlation coefficients between each trophic character of the morphological index. We performed Steiger’s *Z* test [49] to evaluate whether correlations for each trait differed between each treatment. We controlled for multiple comparisons using the false discovery rate correction [50]. Differences in direction and magnitude of trait correlations between our treatments would suggest that sexual selection affected trait integration.

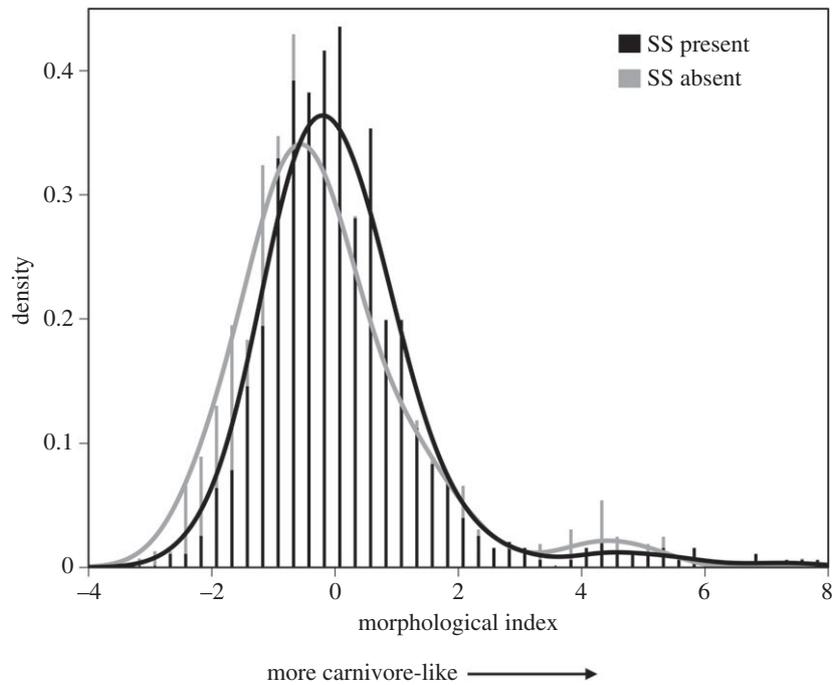


Figure 1. Distribution of combined measure of morphology for mesocosms where sexual selection was present (SS+) versus mesocosms where sexual selection was reversed (SS−). Higher values of morphological index are more carnivore-like, whereas lower values are more omnivore-like.

Table 1. Contrast between the SS+ and SS− treatments in measures of bimodality and divergence of resource-use morphology.

contrast	mean SS+ value (s.d.)	mean SS− value (s.d.)	Z statistic	p-value
bimodality	0.99 (0.02)	0.99 (0.01)	−0.64	0.57
distance between peaks	4.38 (1.02)	4.48 (0.60)	−0.55	0.66
proportion carnivores produced	0.05 (0.02)	0.07 (0.02)	1.83	0.08
variance in trophic morphology	1.76 (0.68)	1.99 (0.67)	0.37	0.79

(h) Contrasting the effects of sexual selection with those of ecological opportunity and strength of competition

In a previous study, using an identical design to that here, Martin & Pfennig [30] evaluated the impact of ecological opportunity and strength of resource competition on the degree of bimodality, divergence in trophic phenotype and the strength of disruptive selection experienced by a population. Because they used the same design, we visually compared the effect sizes we obtained by manipulating sexual selection with the effect sizes they obtained by manipulating ecological opportunity and strength of competition by manipulating conspecific density. (Note, however, that we analysed the selection data differently from the earlier experiment: because we analysed the selection data in a single mixed model in the current study, we had more power, and smaller confidence intervals, than in the earlier study, where selection data were analysed on the individual mesocosm level.) We calculated Cohen's *d* and associated 95% CIs as our measure of effect size for each comparison.

3. Results

(a) Sexual selection's impact on the distribution of trophic morphology

We found strong support for phenotypic bimodality in 10 mesocosms and weak support for bimodality in one (electronic

supplementary material, table S1). When we contrasted SS+ and SS− treatments, we found no statistically significant differences between treatments in: degree of bimodality; extent of divergence in trophic phenotype; or variance in trophic morphology (figure 1 and table 1). Although the SS− treatment tended to have a higher proportion of carnivores than the SS+ treatment, the difference was not significant at the 0.05 level (table 1). Thus, we found no clear evidence that our manipulation of sexual selection either enhanced—or reduced—the extent of bimodality or trophic variation.

(b) Sexual selection's impact on fitness and patterns of natural selection

We found no significant difference between the SS+ and SS− treatments in our fitness proxies at the 0.05 level: SVL (SS−, mean (s.e.): 7.99 ± 0.21 ; SS+, mean (s.e.): 7.39 ± 0.19 ; $F_{1,9} = 4.57$, $p = 0.061$); GS (SS−, mean (s.e.): 26.88 ± 0.13 ; SS+, mean (s.e.): 26.68 ± 0.12 ; $F_{1,9} = 1.20$, $p = 0.302$); % survival (SS−, mean = 0.74; SS+ = 0.77; $Z = -0.73$, $p = 0.537$).

Nevertheless, we found that, overall, both treatments experienced disruptive selection (SVL: $F_{1,1498} = 723.98$, $p < 0.0001$; GS: $F_{1,1498} = 758.17$, $p < 0.0001$) along with a linear component of selection favouring carnivore phenotypes (SVL: $F_{1,1499} = 99.50$, $p < 0.0001$; GS: $F_{1,1499} = 333.30$, $p < 0.0001$). Visual inspection of cubic spline plots confirmed the presence of fitness minima and the directional component of selection

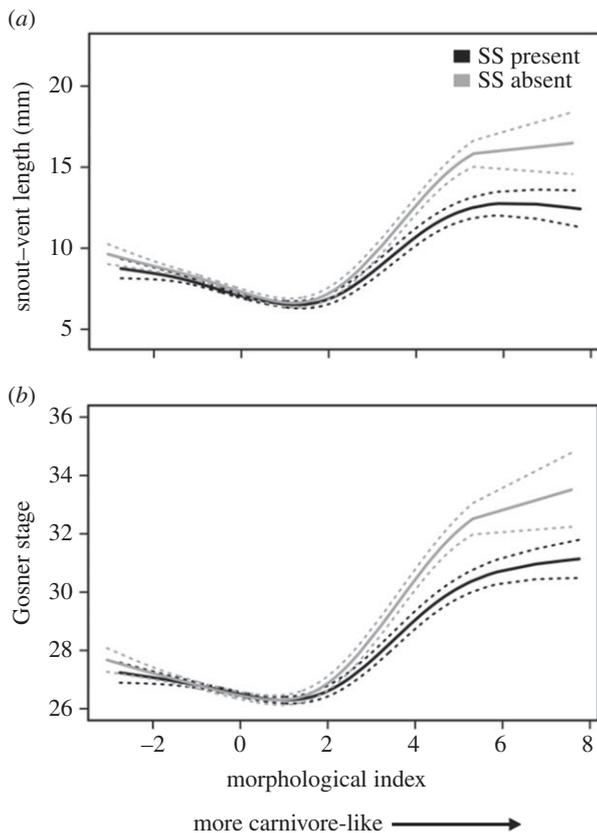


Figure 2. Spline analysis (with 95% CIs) showing fitness minimum for intermediate phenotypes in both SS+ and SS− treatments for two fitness proxies: (a) SVL and (b) GS. Although both treatments experienced disruptive selection, the SS− treatment exhibited significantly stronger disruptive selection. Linear selection favouring the carnivore ecomorph was also significantly stronger in the SS− treatment, with SS− carnivores obtaining higher fitness than SS+ carnivores.

(figure 2). When we contrasted our treatments, we found stronger directional selection favouring carnivores in the SS− treatment than in the SS+ treatment (SVL: $F_{1,1498} = 9.60$, $p = 0.002$; figure 2; GS: $F_{1,1498} = 16.31$, $p = 0.0001$). Indeed, the greatest difference in fitness between the SS− and SS+ treatments stemmed from differences in carnivore size and development rate (figure 2). Furthermore, we also found that the SS− treatment experienced stronger disruptive selection than the SS+ treatment (SVL: $F_{1,1497} = 93.30$, $p \leq 0.0001$; figure 2; GS: $F_{1,1497} = 116.64$, $p \leq 0.0001$).

(c) Sexual selection's impact on trait integration

Although bimodality was unaffected by sexual selection, sexual selection significantly affected trait integration. In particular, we found a significant difference between SS+ and SS− treatments in the relationship between size-corrected OH and the number of GC ($Z = 7.11$; $p < 0.001$). Namely, after controlling for multiple tests, size-corrected OH and the number of GC showed a stronger, more negative correlation in the SS− treatment than in the SS+ treatment (figure 3).

(d) Contrasting sexual selection's effects with those of ecological opportunity and strength of competition

We visually compared the effects observed here with those observed in an identical design, which measured the effects

of ecological opportunity and competitor density on the degree of bimodality, divergence in trophic phenotype and strength of disruptive selection (figure 4). We found that the non-significant effect of manipulating sexual selection on the degree of bimodality was not significantly different from the effects of conspecific density or the (lack of) effect of ecological opportunity. Likewise, the non-significant effect of manipulating sexual selection on trophic divergence was not significantly different from the effect of ecological opportunity or the (lack of) effect of conspecific density. However, sexual selection's negative effect on the strength disruptive selection (for the fitness proxy SVL) was weaker than the effects of either conspecific density or ecological opportunity (figure 4). Comparisons of the effect sizes obtained from measures of disruptive selection using GS as the measure of fitness were qualitatively similar (not shown).

4. Discussion

Explaining the origin and maintenance of resource polymorphism (and, hence, ecological specialization) requires clarifying sexual selection's impacts, particularly those of mate choice, on traits that are subject to disruptive natural selection [15,16,25]. We addressed this problem by evaluating the population-level effects of sexual selection (as mediated by condition-dependent mate choice) on ecological specialization.

We experimentally generated replicated populations of spadefoot toad tadpoles that were produced by parents for whom the natural pattern of mate choice (and, therefore, sexual selection) was either present (SS+) or reversed (SS−). We found that experimental populations in which naturally occurring sexual selection was present were no more likely to express bimodal—as opposed to unimodal—distributions of phenotypes than were populations in which naturally occurring sexual selection was reversed (figure 1). Thus, mate choice does not appear to significantly affect the distribution of ecomorphs in this system.

Conversely, naturally occurring sexual selection (via mate choice) resulted in reduced correlation of individual traits that are critical to the fitness of the more specialized carnivore ecomorph (figure 3). Indeed, carnivore fitness was lower in experimental populations where sexual selection was present than in those populations where sexual selection was reversed (figure 2). Moreover, both directional and disruptive natural selection were weaker in the sexual selection present treatment (figure 2). One might contend that, because survival was less than 100% in our mesocosms, we potentially underestimated the strength of selection due to the 'invisible fraction' of unmeasured individuals [51,52]. However, it is unlikely that our results were biased by the omission of unmeasured tadpoles from the analysis for at least two reasons. First, any effect of the 'invisible fraction' was likely similar across treatments because tadpole survival did not significantly differ between them. Second, the mode and direction of competitively mediated selection in *Spea* is congruent among different fitness proxies (i.e. SVL, GS, and survival; [30]), indicating that these parameters would not be biased by the omission of unmeasured tadpoles from the analysis. Thus, taken together, our results suggest that sexual selection, mediated by female mate choice, can reduce both ecological specialization (by reducing trait integration) and the strength of natural selection favouring alternative ecomorphs.

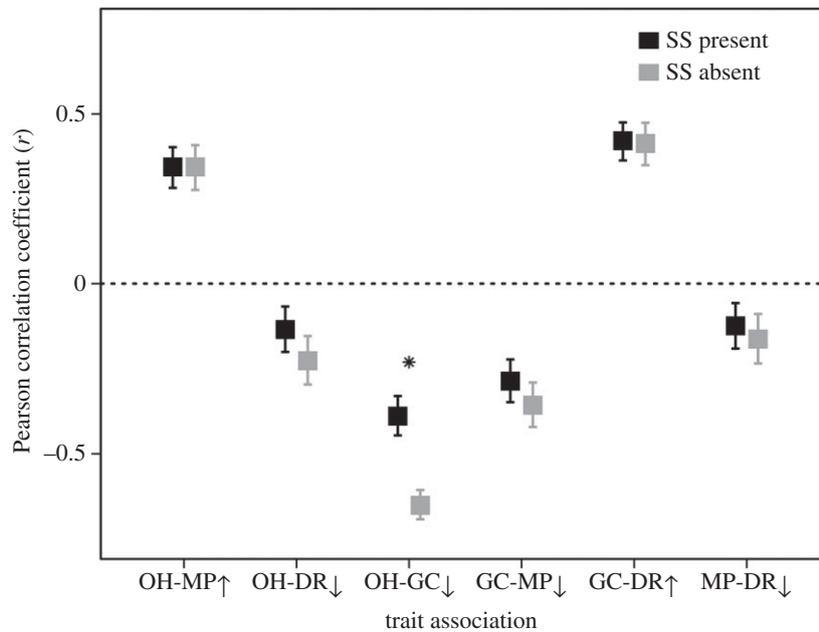


Figure 3. Comparison of the correlation coefficients between each pair of morphological traits in the SS+ versus the SS- treatment. Greater integration of these traits (as indicated by a stronger correlation between them) is favoured for more successful resource acquisition (arrows indicate direction of correlation that is favoured). Error bars show 95% CIs. Asterisk indicates significantly different correlation between treatments. Trait abbreviations: OH: size of the orbitohyoideus muscle (larger OH favoured in carnivores); MP: mouthparts score (higher MP favoured in carnivores); DR: number of denticle rows (fewer DR favoured in carnivores); GC: number of gut coils (fewer GC favoured in carnivores).

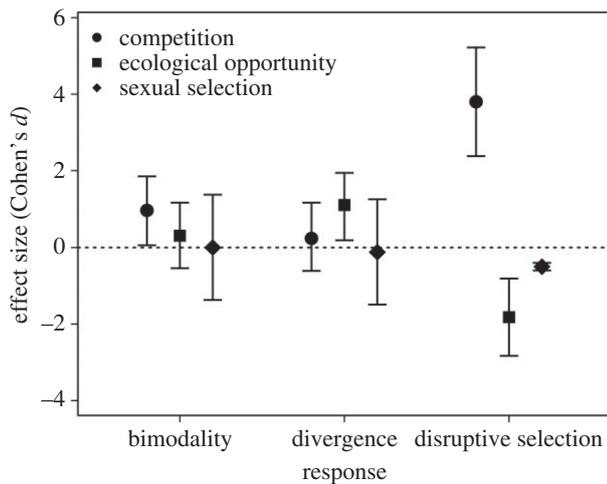


Figure 4. Comparison of the effects (Cohen's $d \pm 95\%$ CIs) of manipulating conspecific density, ecological opportunity and sexual selection on the degree of bimodality, trophic divergence and strength of disruptive selection (for the fitness proxy SVL) in experimental mesocosms. Effect sizes represent the magnitude and direction of change in the response variable in response to the presence of (i.e. sexual selection), or increase in (i.e. conspecific density and ecological opportunity) the manipulated treatments.

Despite these differences in natural selection between treatments, we found no evidence of an overall reduction in mean fitness where sexual selection was present versus reversed (although our ability to detect an effect of SVL might have been a power issue; see Results). Generally, whether sexual selection enhances or reduces population fitness, and the ability of populations to evolve adaptations in response to natural selection, remains unclear: previous empirical work suggests that sexual selection can impede adaptation [53–55], whereas contrasting theoretical work suggests that sexual selection can either inhibit [56,57] or facilitate adaptation [15,20,58]. Although mean fitness did not differ between treatments, our

finding that carnivores in the SS+ treatment had lower fitness is consistent with the hypothesis that mate choice and (more generally) sexual selection can inhibit adaptive ecological specialization in response to resource competition.

Nevertheless, the negative effect of sexual selection on the strength of disruptive natural selection was weaker than the previously documented effects caused by variation in resource availability (i.e. ecological opportunity) or competitor density (figure 4), both of which are potentially key drivers of ecological specialization [30–33]. By contrast, sexual selection's effects on measures of polymorphism, bimodality and divergence between morphs, did not differ significantly from those of ecological opportunity and competitor density. However, for bimodality, the effects of ecological opportunity and sexual selection did not differ from zero, whereas competitor density had a significant positive effect. Likewise, for divergence between morphs, the effects of competitor density and sexual selection did not differ from zero, whereas ecological opportunity had a significant positive effect (figure 4). Thus, taken together, sexual selection's impact on competitively mediated natural selection appears to be weak compared with ecological factors that more directly impact the incidence and strength of resource competition.

The effects of manipulating the natural patterns of mate choice were relatively modest. Nevertheless, our results indicate that female mate-choice decisions can result in reduced fitness for their offspring in the context of resource competition. For the carnivore ecomorphs in particular, natural mate choice (SS+) resulted in fitness reductions (figure 2). Among carnivores, we found a reduction in mean body size and development rate in the sexual selection present treatment. Such a reduction is seemingly attributable to the breakdown of the correlation between two key traits: number of GC and size of the OH muscle (figure 3), which are crucial to resource acquisition by carnivores [38]. Why this relationship breaks down in the presence of sexual selection requires additional study.

Why should females prefer males that sire competitively inferior carnivore offspring, as our data suggest females in this population do (figure 2)? One possible explanation is that increased growth and development of carnivores in the SS− treatment might come with survival costs that were not measured and are therefore actually less fit. However, this possibility does not adequately explain the finding that carnivores in the SS+ treatment exhibited poorer integration of key traits, which could affect performance and competitive ability (figure 3) [38]. Moreover, spadefoots develop in ephemeral desert pools, where faster development and larger size are generally favoured [28,59,60].

A more likely explanation is that, by engaging in condition-dependent mate choice (i.e. by preferring males in good condition [26,27]), females obtain a direct fitness benefit: enhanced fertilization success of their eggs and hence more offspring [26]. Moreover, in the absence of resource competition, male condition predicts offspring size at metamorphosis: better condition males sire larger offspring [27]. These fitness benefits of condition-dependent mate choice likely offset the costs of sexual selection that we observed here (figure 2). Generally, the relative costs and benefits of mate choice are unlikely to be fixed: they can vary both spatially and temporally [61]. In spadefoots, the relative costs and benefits of condition-dependent mate choice will potentially depend on ecological opportunity and the strength of resource competition.

Our results therefore suggest that a more thorough understanding of sexual selection's role in ecological specialization requires knowing: (i) how ecological opportunity and resource competition impact the evolution of sexual traits (e.g. female mate preferences) by dictating the relative costs and benefits of such traits and (ii) if the evolution of such sexual traits in turn alters natural selection favouring resource specialization. Knowledge of these factors promises to help establish whether and how sexual selection impacts ecological specialization in response to competitively mediated natural selection. Such information is critical for identifying the origins of novel traits used in resource acquisition as well as how new species might arise through such divergence.

Ethics statement. The University of North Carolina's IACUC approved all the procedures.

Data accessibility. Data are available as the electronic supplementary material.

Acknowledgements. We are grateful to Jeff Conner, Joel Kingsolver, Jack Weiss and the UNC Biology R group for statistical advice; Katrina Pfennig, Elsa Pfennig, Emily Schmidt, Jeff Paull, Aaron Leichty, Dawn Wilson, and the staff and volunteers at the Southwestern Research Station for field assistance; and Rebecca Safran and three anonymous reviewers for comments that improved the manuscript.

Funding statement. This work was supported by a New Innovator Award from the Office of the Director, National Institutes of Health (1 DP2 OD004436-01) to K.P. and a grant from the National Science Foundation (DEB-1019479) to D.P.

References

- Bolnick DI, Svanbäck R, Yang LH, Davis JM, Hulsey CD, Forister ML. 2003 The ecology of individuals: incidence and implications of individual specialization. *Am. Nat.* **161**, 1–28. (doi:10.1086/343878)
- Lomnicki A. 1988 *Population ecology of individuals*. Princeton, NJ: Princeton University Press.
- Smith TB, Skúlason S. 1996 Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annu. Rev. Ecol. Syst.* **27**, 111–133. (doi:10.1146/annurev.ecolsys.27.1.111)
- Pfennig DW, Pfennig KS. 2012 *Evolution's wedge: competition and the origins of diversity*. Berkeley, CA: University of California Press.
- West-Eberhard MJ. 2003 *Developmental plasticity and evolution*. New York, NY: Oxford University Press.
- Hendry AP, Grant PR, Grant BR, Ford HA, Brewer MJ, Podos J. 2006 Possible human impacts on adaptive radiation: beak size bimodality in Darwin's finches. *Proc. R. Soc. B* **273**, 1887–1894. (doi:10.1098/rspb.2006.3534)
- Calsbeek R, Smith TB, Bardeleben C. 2007 Intraspecific variation in *Anolis sagrei* mirrors the adaptive radiation of Greater Antillean anoles. *Biol. J. Linn. Soc.* **90**, 189–199. (doi:10.1111/j.1095-8312.2007.00700.x)
- Liem KF, Kaufman LS. 1984 Intraspecific macroevolution: functional biology of the polymorphic cichlid species *Cichlasoma minckleyi*. In *Evolution of fish species flocks* (eds AA Echelle, I Kornfield), pp. 203–215. Orono, ME: University of Maine Press.
- Meyer A. 1993 Trophic polymorphisms in cichlid fish: do they represent intermediate steps during sympatric speciation and explain their rapid adaptive radiation? In *New trends in ichthyology* (eds J-H Schröder, J Bauer, M Schartl), pp. 257–266. London, UK: Blackwell.
- Wund MA, Baker JA, Clancy B, Golub JL, Foster SA. 2008 A test of the 'flexible stem' model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. *Am. Nat.* **172**, 449–462. (doi:10.1086/590966)
- Harmon LJ, Matthews B, Roches SD, Chase JM, Shurin JB, Schluter D. 2009 Evolutionary diversification in stickleback affects ecosystem functioning. *Nature* **458**, 1167–1170. (doi:10.1038/nature07974)
- Bolnick DI *et al.* 2011 Why intraspecific trait variation matters in community ecology. *Trends Ecol. Evol.* **26**, 183–192. (doi:10.1016/j.tree.2011.01.009)
- Rueffler C, Van Dooren TJM, Leimar O, Abrams PA. 2006 Disruptive selection and then what? *Trends Ecol. Evol.* **21**, 238–245. (doi:10.1016/j.tree.2006.03.003)
- Bolnick DI. 2004 Can intraspecific competition drive disruptive selection? An experimental test in natural populations of sticklebacks. *Evolution* **58**, 608–618. (doi:10.1111/j.0014-3820.2004.tb01683.x)
- van Doorn GS, Edelaar P, Weissing FJ. 2009 On the origin of species by natural and sexual selection. *Science* **326**, 1704–1707. (doi:10.1126/science.1181661)
- Maan ME, Seehausen O. 2011 Ecology, sexual selection and speciation. *Ecol. Lett.* **14**, 519–602. (doi:10.1111/j.1461-0248.2011.01606.x)
- Bolnick DI, Fitzpatrick BM. 2007 Sympatric speciation: models and empirical evidence. *Annu. Rev. Eco. Evol. Syst.* **38**, 459–487. (doi:10.1146/annurev.ecolsys.38.091206.095804)
- Kirkpatrick M, Nuismer SL. 2004 Sexual selection can constrain sympatric speciation. *Proc. R. Soc. Lond. B* **271**, 687–693. (doi:10.1098/rspb.2003.2645)
- Labonne J, Hendry AP. 2010 Natural and sexual selection giveth and taketh away reproductive barriers: models of population divergence in guppies. *Am. Nat.* **176**, 26–39. (doi:10.1086/652992)
- Bonduriansky R. 2011 Sexual selection and conflict as engines of ecological diversification. *Am. Nat.* **178**, 729–745. (doi:10.1086/662665)
- Thibert-Plante X, Hendry AP. 2011 Factors influencing progress toward sympatric speciation. *J. Evol. Biol.* **24**, 2186–2196. (doi:10.1111/j.1420-9101.2011.02348.x)
- Weissing FJ, Edelaar P, van Doorn GS. 2011 Adaptive speciation theory: a conceptual review. *Behav. Ecol. Sociobiol.* **65**, 461–480. (doi:10.1007/s00265-010-1125-7)
- Andersson M. 1994 *Sexual selection*. Princeton, NJ: Princeton University Press.
- Servedio MR, Van Doorn GS, Kopp M, Frame AM, Nosil P. 2011 Magic traits in speciation: 'magic' but not rare? *Trends Ecol. Evol.* **26**, 389–397. (doi:10.1016/j.tree.2011.04.005)

25. Safran RJ, Scordato ES, Symes LB, Rodriguez RL, Mendelson TC. 2013 Contributions of natural and sexual selection to the evolution of premating reproductive isolation: a research agenda. *Trends Ecol. Evol.* **28**, 643–650. (doi:10.1016/j.tree.2013.08.004)
26. Pfennig KS. 2000 Female spadefoot toads compromise on mate quality to ensure conspecific matings. *Behav. Ecol.* **11**, 220–227. (doi:10.1093/beheco/11.2.220)
27. Pfennig KS. 2008 Population differences in condition-dependent sexual selection may promote divergence in non-sexual traits. *Evol. Ecol. Res.* **10**, 763–773.
28. Pfennig KS, Pfennig DW. 2005 Character displacement as the 'best of a bad situation': fitness trade-offs resulting from selection to minimize resource and mate competition. *Evolution* **59**, 2200–2208. (doi:10.1554/05-263.1)
29. Pfennig KS, Rice AM. 2014 Reinforcement generates reproductive isolation between neighbouring conspecific populations of spadefoot toads. *Proc. R. Soc. B* **281**, 20140949. (doi:10.1098/rspb.2014.0949)
30. Martin RA, Pfennig DW. 2010 Field and experimental evidence that competition and ecological opportunity promote resource polymorphism. *Biol. J. Linn. Soc.* **100**, 73–88. (doi:10.1111/j.1095-8312.2010.01380.x)
31. Wagner CE, Harmon LJ, Seehausen O. 2012 Ecological opportunity and sexual selection together predict adaptive radiation. *Nature* **487**, 366–369. (doi:10.1038/nature11144)
32. Schluter D. 2000 *The ecology of adaptive radiation*. Oxford, UK: Oxford University Press.
33. Losos JB. 2010 Adaptive radiation, ecological opportunity, and evolutionary determinism. American Society of Naturalists E. O. Wilson award address. *Am. Nat.* **175**, 623–639. (doi:10.1086/652433)
34. Pomeroy LV. 1981 Developmental polymorphism in the tadpoles of the spadefoot toad *Scaphiopus multiplicatus*. PhD dissertation, University of California, Riverside, CA, USA.
35. Paull JS, Martin RA, Pfennig DW. 2012 Increased competition as a cost of specialization during the evolution of resource polymorphism. *Biol. J. Linn. Soc.* **107**, 845–853. (doi:10.1111/j.1095-8312.2012.01982.x)
36. Ledón-Rettig CC, Pfennig DW. 2011 Emerging model systems in eco-evo-devo: the environmentally responsive spadefoot toad. *Evol. Dev.* **13**, 391–400. (doi:10.1111/j.1525-142X.2011.00494.x)
37. Pfennig DW. 1999 Cannibalistic tadpoles that pose the greatest threat to kin are most likely to discriminate kin. *Proc. R. Soc. Lond. B* **266**, 57–81. (doi:10.1098/rspb.1999.0604)
38. Martin RA, Pfennig DW. 2011 Evaluating the targets of selection during character displacement. *Evolution* **65**, 2946–2958. (doi:10.1111/j.1558-5646.2011.01357.x)
39. Pfennig DW, Martin RA. 2009 A maternal effect mediates rapid population divergence and character displacement in spadefoot toads. *Evolution* **63**, 898–909. (doi:10.1111/j.1558-5646.2008.00544.x)
40. Pfennig DW, Martin RA. 2010 Evolution of character displacement in spadefoot toads: different proximate mechanisms in different species. *Evolution* **64**, 2331–2341.
41. Pfennig DW, Murphy PJ. 2000 Character displacement in polyphenic tadpoles. *Evolution* **54**, 1738–1749. (doi:10.1111/j.0014-3820.2000.tb00717.x)
42. Pfennig DW, Murphy PJ. 2002 How fluctuating competition and phenotypic plasticity mediate species divergence. *Evolution* **56**, 1217–1228. (doi:10.1111/j.0014-3820.2002.tb01433.x)
43. Martin RA, Pfennig DW. 2009 Disruptive selection in natural populations: the roles of ecological specialization and resource competition. *Am. Nat.* **174**, 268–281. (doi:10.1086/600090)
44. Pfennig DW, Rice AM, Martin RA. 2007 Field and experimental evidence for competition's role in phenotypic divergence. *Evolution* **61**, 257–271. (doi:10.1111/j.1558-5646.2007.00034.x)
45. Martin RA, Pfennig DW. 2012 Widespread disruptive selection in the wild is associated with intense resource competition. *BMC Evol. Biol.* **12**, 136. (doi:10.1186/1471-2148-12-136)
46. Gosner KL. 1960 A simplified table for staging anuran embryos with notes on identification. *Herpetologica* **16**, 183–190.
47. Brewer MJ. 2003 Discretisation for inference on normal mixture models. *Stat. Comput.* **13**, 209–219. (doi:10.1023/A:1024214615828)
48. Schluter D, Nychka D. 1994 Exploring fitness surfaces. *Am. Nat.* **143**, 597–616. (doi:10.1086/285622)
49. Steiger JH. 1980 Tests for comparing elements of a correlation matrix. *Psychol. Bull.* **87**, 245–251. (doi:10.1037/0033-2909.87.2.245)
50. Benjamini Y, Hochberg Y. 1995 Controlling the false discovery rate—a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B Methodol.* **57**, 289–300.
51. Grafen A. 1988 On the uses of data on lifetime reproductive success. In *Reproductive success* (ed. TH Clutton-Brock), pp. 454–471. Chicago, IL: University of Chicago Press.
52. Hadfield JD. 2008 Estimating evolutionary parameters when viability selection is operating. *Proc. R. Soc. B* **275**, 723–734. (doi:10.1098/rspb.2007.1013)
53. Behm JE, Ives AR, Boughman JW. 2010 Breakdown in postmating isolation and the collapse of a species pair through hybridization. *Am. Nat.* **175**, 11–26. (doi:10.1086/648559)
54. Seehausen OLE, Takimoto G, Roy D, Jokela J. 2008 Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Mol. Ecol.* **17**, 30–44. (doi:10.1111/j.1365-294X.2007.03529.x)
55. Rundle HD, Chenoweth SF, Blows MW. 2006 The roles of natural and sexual selection during adaptation to a novel environment. *Evolution* **60**, 2218–2225. (doi:10.1111/j.0014-3820.2006.tb01859.x)
56. Webb C. 2003 A complete classification of Darwinian extinction in ecological interactions. *Am. Nat.* **161**, 181–205. (doi:10.1086/345858)
57. Kokko H, Brooks R. 2003 Sexy to die for? Sexual selection and the risk of extinction. *Ann. Zool. Fennici* **40**, 207–219.
58. Lorch PD, Proulx S, Rowe L, Day T. 2003 Condition-dependent sexual selection can accelerate adaptation. *Evol. Ecol. Res.* **5**, 867–881.
59. Pfennig DW, Mabry A, Orange D. 1991 Environmental causes of correlations between age and size at metamorphosis in *Scaphiopus multiplicatus*. *Ecology* **72**, 2240–2248. (doi:10.2307/1941574)
60. Pfennig DW. 1992 Polyphenism in spadefoot toads as a locally adjusted evolutionarily stable strategy. *Evolution* **46**, 1408–1420. (doi:10.2307/2409946)
61. Robinson MR, van Doorn GS, Gustafsson L, Qvarnstrom A. 2012 Environment-dependent selection on mate choice in a natural population of birds. *Ecol. Lett.* **15**, 611–618. (doi:10.1111/j.1461-0248.2012.01780.x)