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Predator experience overrides learned aversion to heterospecifics in stickleback species pairs

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Predation risk can alter female mating decisions because the costs of mate searching and selecting attractive mates increase when predators are present. In response to predators, females have been found to plastically adjust mate preference within species, but little is known about how predators alter sexual isolation and hybridization among species. We tested the effects of predator exposure on sexual isolation between benthic and limnetic threespine sticklebacks (*Gasterosteus* spp.). Female discrimination against heterospecific mates was measured before and after females experienced a simulated attack by a trout predator or a control exposure to a harmless object. In the absence of predators, females showed increased aversion to heterospecifics over time. We found that predator exposure made females less discriminating and precluded this learned aversion to heterospecifics. Benthic and limnetic males differ in coloration, and predator exposure also affected sexual isolation by weakening female preferences for colourful males. Predator effects on sexual selection were also tested but predators had few effects on female choosiness among conspecific mates. Our results suggest that predation risk may disrupt the cognitive processes associated with mate choice and lead to fluctuations in the strength of sexual isolation between species.

1. Introduction

How predation alters the process of speciation is still an open question [1–4]. Predators can strengthen divergent natural selection and promote phenotypic evolution [5–7]; however, predation can also cause hybridization and impede speciation [8,9]. Increased hybridization under predation may be mediated by changes in premating isolation between species [10]. Predator presence increases the probability of females being preyed on before mating, effectively increasing the costs associated with mate choice [11–13]. This could alter both how choosy females are and which male traits they prefer. Within species, much previous work has shown that when predators are present and the risk of attack is high, females become less choosy [14–21]. Males and females also avoid detection by predators by decreasing the amount of courtship in the presence of a predator [22–25]. Females may switch to preferring inconspicuous males because these males have reduced risk of predation [26–28]. If predators increase the costs associated with female mate choice within species, they may also increase the costs of discriminating against heterospecific mates.

Currently, we know very little about the effect of predators on sexual isolation between species. Provided that hybrid offspring fitness is non-zero, females who mate with heterospecifics will still have higher fitness than females who are preyed on before mating. Therefore, it may benefit females to discriminate less against heterospecifics when predators are present. Consistent with this idea, female tungara frogs (*Physalaemus pustulosus*) respond more to heterospecific male calls when they are played along with predator calls than when played in the absence of predator calls [29]. However, that study did not use females from a population sympatric with heterospecifics and selection against hybridization in sympatric populations may overwhelm the benefits of flexible

Table 1. Predictions for hypotheses of how predators could alter mating behaviour. We indicate predicted change in predator-exposed females relative to control-exposed under different hypotheses.

(a) predators reduce choosiness within species	change in mating propensity: predator versus control
conspecific male	increase
heterospecific male	no change
(b) predators reduce discrimination between species	
conspecific male	no change/increase
heterospecific male	increase
(c) predators reduce motivation to mate	
conspecific male	decrease
heterospecific male	decrease
(d) predators reduce preference for colourful males	
limnetic female—colourful male	decrease
limnetic female—dull male	no change/increase
benthic female—any male	no change/increase

discrimination against heterospecifics during periods of low predation. To our knowledge, at present, no studies have examined how sympatric females respond to predators in the context of mate choice. One study has tested for predator effects on mate preference in sympatric males and found that male sailfin mollies (*Poecilia latipinna*) were less discriminating against heterospecific Amazon mollies (*Poecilia formosa*) in the presence of a predator than in the absence of a predator [30]. Another study found that distance from a shelter (which may be indirectly related to predation risk) increased the likelihood that swordtail females (*Xiphophorus birchmanni*) would mate with heterospecifics [31]. This dearth of studies on female preference is surprising given that female conspecific mate preference is the main barrier to hybridization in many species [32] and females generally invest more in each mating than males [33].

Predators may also interfere with mate choice and sexual isolation by disrupting underlying cognitive processes. Selecting the best mate out of those available is a cognitively complex task [34,35] because females have to compare multiple males. This complexity will be increased when individuals must also avoid heterospecifics during mating. Stressful experiences are known to impair cognitive processing [36,37]. There is evidence that predation induces similar cognitive deficits [38] and contributes to population differences in learning ability [39]. The effects of predators on the cognitive components of mate choice may be especially important in taxa where conspecific mate preference and discrimination against heterospecifics are shaped by learning. Many species learn aversion to heterospecifics through subadult or recent experience [40] and predation could potentially disrupt this learning process.

Here, we test the effects of predators on female discrimination against heterospecific males in species pairs of threespine sticklebacks. These species are currently sympatric and learning contributes to strong female preference for conspecific over heterospecific mates [41–43]. Benthic and limnetic threespine sticklebacks (*Gasterosteus* spp.) occupy different trophic habitats within the same lakes in British Columbia [44–46]. These species are characterized by a number of morphological, behavioural and genetic differences [44–50]. Although they are not formally named species, they are recognized as different

species because they are strongly reproductively isolated (total reproductive isolation = 0.87) [51]. Sexual isolation between benthics and limnetics is the most important contributor to reproductive isolation and females avoid heterospecific mates using cues such as male size, nuptial colour, shape and odour [41,42,48–54]. Selection against hybrids and reinforcement in sympatry have also contributed to the strength of sexual isolation [48,55–58]. In addition, we know that predators increase the strength of divergent selection on trophic morphology in these species [5], although hybrid survival is low in both the presence and absence of predators [59]. Thus, benthic and limnetic sticklebacks provide an opportunity to test how predators alter discrimination against heterospecifics when there is strong selection against hybridization.

In this study, we test the effects of trout predator attacks on both mate choice within species (sexual selection) and mate choice between species (sexual isolation). Cutthroat trout (*Oncorhynchus clarki*) are a common predator of threespine sticklebacks [46,60,61]. Female preference for conspecific or heterospecific males was measured in an initial mating trial. Following exposure to a model trout predator attack, female mate preference was measured again. We compared preferences of predator-exposed females with those of control females that were tested similarly, but during exposure only viewed the object used to guide the model predator.

We hypothesized that if predator presence increases the costs of mate choice, predator exposure might alter both sexual selection and sexual isolation. Table 1 gives detailed predictions for four alternative hypotheses about how predators may influence sexual selection and isolation. First, we predicted that females would be less choosy and thus, more likely to accept conspecific males as mates after predator exposure. If the costs associated with hybridization are greater than the costs associated with predator presence, predator exposure should only alter sexual selection with no change in sexual isolation (table 1a). However, if the costs of predation are greater than the costs of hybridization, we predicted that females might show increased mating with heterospecific males after predator exposure (table 1b). Our previous work has shown that females learn to discriminate more strongly against heterospecifics through recent courtship experience [43]. We predicted that predator exposure

might alter this process of learned discrimination, generating weaker sexual isolation between species.

Another hypothesis is that predator experience might reduce female motivation to mate. We predicted that this would lead to decreased rates of mating with all males after predator exposure (table 1c). If females reduce mating after predator exposure, it would suggest that the costs of predation lead females to forgo the current mating attempt.

Our final hypothesis is that predators might alter which males females select as mates. Brightly coloured males may be at higher risk of predation because they are more conspicuous to predators and in other species, predators often decrease preference for conspicuous males [17,27]. The red throat and blue body coloration of male sticklebacks are important sexually selected traits [49,50]. If predators increase the costs associated with mating with colourful males, predator exposure could increase mating with less colourful males and decrease mating with brightly coloured males. Previous work has established that the strength of female colour preference differs, with limnetic females having stronger preferences for brightly coloured males than benthic females [49,50]. Thus, we predicted that only limnetics might show this change in colour preference after predator exposure (table 1d). Colour preference in limnetic females strengthens sexual isolation because benthic males tend to have less coloration and limnetic females reject most benthic males because they are not colourful. Thus, we could potentially see predator-induced changes in limnetic female preference for colourful conspecific and heterospecific males, leading to changes in both sexual selection and sexual isolation.

2. Material and methods

We collected reproductive benthic and limnetic sticklebacks from Paxton Lake (49°43' N, 124°31' W), Texada Island, British Columbia in April 2009 using minnow traps. We transported fish to the University of Wisconsin, where the laboratory was maintained at 16°C and 16 h of light. All fish were fed a mixture of frozen brine shrimp (*Artemia* spp.) and bloodworms (*Chironomus* spp.).

All females underwent an initial courtship trial, were given 60 min to rest and then were exposed to a model predator (predator-exposed females) or a wooden dowel identical to the one used to guide the predator (control-exposed females). Approximately 60 min after predator exposure (mean = 61.45 ± 0.32 min, range = 53–76), female mate preference was tested again in a second trial. Males in the first and second trials were different individuals from the same species; each female saw only conspecific or only heterospecific males. Thus, we had four different exposures: (i) predator–conspecific, (ii) control–conspecific, (iii) predator–heterospecific, and (iv) control–heterospecific. We used a pair of stimulus males only once and each male up to three times, although a single male was never used more than twice per treatment (once as a first male, once as a second male). We tested 20 limnetic females per treatment for a total of 79 females (a technical error led to the loss of data for one female in the control–heterospecific treatment). Owing to the low availability of reproductively mature benthic females, we had a reduced sample size of 29 females: control–conspecific = 6, predator–conspecific = 7, control–heterospecific = 8 and predator–heterospecific = 8.

Before testing, individuals were housed in 150 l tanks separately by species and sex. Individual males were transferred to isolated 101 l tanks for nest building. For courtship trials, we transferred the female to a 101 l tank containing a single male

and his nest. Thirty minutes before the initial trial, between trials and during predator exposure, females were individually held in 40 l tanks. Different sets of holding tanks were used for the predator and control groups, because the predator exposure involved some odour cues. For predator exposure, females experienced the visual, olfactory and tactile cues associated with a trout attack. Females were chased by an articulated and realistically painted 25.4 cm long rainbow trout model (Castaic Swimbaits, McCordsville, IN, USA); the head section of the model was coated with trout scent (rainbow trout scent bait, Pro-Cure, Salem, OR, USA). In Paxton Lake, stickleback predators are cutthroat trout (*O. clarki*) rather than the closely related rainbow trout (*Oncorhynchus mykiss*) but these rainbow trout models are a realistic approximation of trout predators and we have previously shown that stickleback respond to them with predator aversion behaviour such as shoaling [62]. For each trial, one rainbow trout model was randomly selected for use from a group of three trout models (including both lightly and darkly coloured trout morphs) [62]. Scent was reapplied before every exposure. The model was attached to a 26.5 cm long wooden dowel (0.7 cm radius). The experimenter held the model by the dowel and by pulling on it was able to guide the model. The model was immersed in the holding tank and 'attacked' the female by rushing towards her and then floating directly over her. After each attack, the experimenter made the model change direction and rush towards and over the female again for a total of 20 attacks per exposure. A single experimenter (G.M.K.) performed all predator exposures. The control exposure consisted of the submersion of a 26 cm wooden dowel into the stimulus tank for 30 s.

The courtship trial began when we released the female from an opaque holding container after 5 min of acclimation and the female moved, the male saw the female, and the male began courtship (these steps were usually simultaneous, but not always). Trials lasted for 20 min or until the female entered the nest. All behaviours of the female and male were recorded using an Observer event recorder (Noldus Technologies, Leesburg, VA, USA). If females produced a second clutch of eggs (more than 1 week after initial testing), they were re-used and considered an independent data point: trout-exposed females were subsequently tested in control exposures (nine of 54 females) and control-exposed females were exposed to trout (five of 55 females). Females were never re-used more than twice. Removing these females from the dataset did not alter our findings ('Reused females removed': electronic supplementary material, tables S1–S3).

We calculated measures of preference that take into account the different stages of stickleback courtship. Females break off courtship early in the sequence when males are unattractive [43,53,63,64]. We calculated female latency to respond as the amount of time from when the male began courting her to when she performed her first behaviour indicating interest in the male. If the female did not respond during the trial, the time value was set at the total trial length (20 min). Thus, longer latencies indicate lower interest in the male. We calculated the number of female follows per male lead to the nest (range 0–1), the number of inspections (the number of times the female examined a male's nest after the male showed it to her, range 0–1) and the female's preference score, which indicates the stage of stickleback courtship reached (from no response to attempted spawning 0–4; actual spawning was prevented so males and females could be re-used). To be included in our dataset, a female had to respond to a male and perform behaviours indicating interest in at least one of her trials (indicating behavioural receptivity to courtship) and have a ripe clutch of eggs (verified by visual inspection after testing). Only a few females were excluded from the dataset owing to lack of any behaviours indicating interest (two per female species).

Male throat and body coloration was scored by a trained observer using standard scores for red throat coloration (sum

of red area and red intensity scores, each ranging from 0 to 5) and intensity of blue–green body coloration (range = 0–5). This allows measurements of these coloration scores, which previous work has established closely correspond to reflectance measurements, to be taken on behaving fish [42,49,65–67]. Males were scored for colour when placed in a nesting tank at the beginning of testing and when removed from the tank after testing. Males were quickly transferred from their tank to a holding container and placed gently by hand on a wet sponge under a full spectrum light. We took the mean of a male's colour scores at the beginning and end of testing as a base colour measure. Each male was also scored for colour in the tank immediately after courting the female; we took the mean of this score and the male's base score as our final measure of colour. We added throat coloration and body coloration scores to get a measure of total coloration, because combining these two measures of colour gives more complete information on male coloration owing to a positive but weak correlation between these measures (limnetic males: Pearson $r = 0.2$, $p = 0.035$, $n = 112$; benthic males: Pearson $r = 0.58$, $p < 0.0001$, $n = 103$).

Statistical analyses were performed in R v. 3.1.2 (R Core Team, 2014). We analysed female preference for conspecific males and for heterospecific males separately because we hypothesized that the predator exposure may have different effects on within- and between-species mate choice and these effects could be obscured in a joint analysis. Preference score was an ordinal variable and was analysed using a cumulative link mixed model in the package 'ordinal' (with a logit link function [68]). For the other three female measures, deviations from normality were assessed with Q–Q plots and the appropriate transformations were performed. Latency to respond was log transformed; follows and inspection were arcsine square root transformed (values of 0 were set to 0.0001 prior to transformation). These measures of preference were analysed using linear mixed models in the R packages 'lme4' [69] and 'afex' (which uses the Kenward–Roger approximation for degrees of freedom). Based on our predictions, we included the following factors in all models: predator treatment (control, trout), trial (first, second), female species (benthic, limnetic) and the interaction between predator treatment and trial. Individual female was included as a random effect. We also included male coloration as a covariate (electronic supplementary material, tables S1–S3). Least-squared means (\pm standard error, s.e.) and pairwise differences were computed using the package 'lsmeans'. In all figures, untransformed values are plotted.

Male colour is a trait used by limnetic females when both selecting conspecific males and rejecting heterospecific males [49,50,66]. Limnetic males are more brightly coloured than benthic males [49], and limnetic females prefer to mate with brightly coloured males. After predator exposure, we hypothesized that limnetics may switch to preferring less colourful males (table 1*d*). We analysed how predators influenced preference in limnetic females for conspecific and heterospecific males by testing for the significance of the three-way interaction between colour, predator exposure and trial (electronic supplementary material, table S4).

Furthermore, if predator exposure weakens preference for colourful males, we predicted that only limnetic females that see dull males and benthic females (who have weak colour preference) would potentially show increased preference for conspecific and heterospecific males (table 1*d*). In the full dataset, predator-exposed limnetics might have a decreased propensity to mate with brightly coloured males and this might mask an increased propensity to mate with dull males. Therefore, to test this prediction, we analysed a dataset that contained all benthic females and only limnetic females that saw dull males on their second trial. We refer to this as the achromatic female dataset because these females should not be strongly influenced by male colour.

We created the achromatic dataset by including only those limnetic females that saw a second male with a colour score lower than both the first male and the male average (colour score < 6.5). This resulted in a dataset of 36 females with conspecific males: 13 benthic females and 23 limnetic females with dull second males (of 40 limnetics tested). For heterospecifics, we had 42 females total: 16 benthic females and 26 limnetic females with dull heterospecific males (of the total 40). These data were analysed using statistical models that were identical to those used for the full dataset. Power analyses indicated that our sample sizes ($N = 18$ – 27 females per predator-exposure treatment) were sufficient to detect significance of moderate effect sizes ($D = 0.5$; power = 0.83 – 0.90).

3. Results

(a) Predator effects on sexual selection and isolation

Predator exposure altered sexual isolation between species but did not influence sexual selection within species. We found no effects of predators on mate choice within species (figure 1; electronic supplementary material, tables S1, S3 and figure S1). Between species, prior experience with heterospecifics generated learned aversion to heterospecifics in the absence of predators (figure 2; electronic supplementary material tables S2 and S3). This learned aversion led to increased sexual isolation in second trials; control females took longer to respond to heterospecifics (mean latency to respond second–first trial = 4.66 ± 0.99 , $t_{52.36} = 4.8$, $p < 0.0001$), followed heterospecific males less (mean second–first = -0.14 ± 0.06 , $t_{52.16} = 2.12$, $p = 0.039$) and had lower preference scores for heterospecifics (mean second–first = -0.46 ± 0.22 , $Z = 2.06$, $p = 0.04$). Female inspection rate did not change between trials (mean second–first = -0.07 ± 0.15 , $t_{52.24} = 0.50$, $p = 0.62$; electronic supplementary material, figure S1). Predator experience undermined this pattern of learned aversion. After predator exposure, females showed weaker discrimination against heterospecifics compared with control females. Predator-exposed females responded quickly to heterospecific males in second trials (mean latency to respond second–first = 0.84 ± 0.97 , $t_{52.43} = 0.40$, $p = 0.69$), which was much faster than control females (second trial predator versus control: $t_{102.65} = 3.63$, $p = 0.0004$). Predator-exposed females also showed slightly higher follow rates and preference scores than control females, although these differences were not significant (follows: predator versus control: $t_{85.42} = 1.78$, $p = 0.08$; preference score: predator versus control: $Z = 1.64$, $p = 0.10$).

(b) Predator effects on preferences for colourful males

For limnetics, predator exposure weakened female preference for colourful heterospecific males; this had further effects on sexual isolation. The interaction between trial, predator exposure and coloration was significant for female follows to heterospecifics ($F_{1,51.61} = 4.27$, $p = 0.04$), but not for any other measure of preference (electronic supplementary material, table S4). Control-exposed females tended to prefer more colourful heterospecific males in second trials ($\beta = 0.071 \pm 0.05$, $t_{37} = 1.31$, $p = 0.21$) but predator exposure changed this preference, inducing females to reject more colourful males and prefer less colourful ones ($\beta = -0.11 \pm 0.04$, $t_{37} = -2.5$, $p = 0.021$; electronic supplementary material, figure S2).

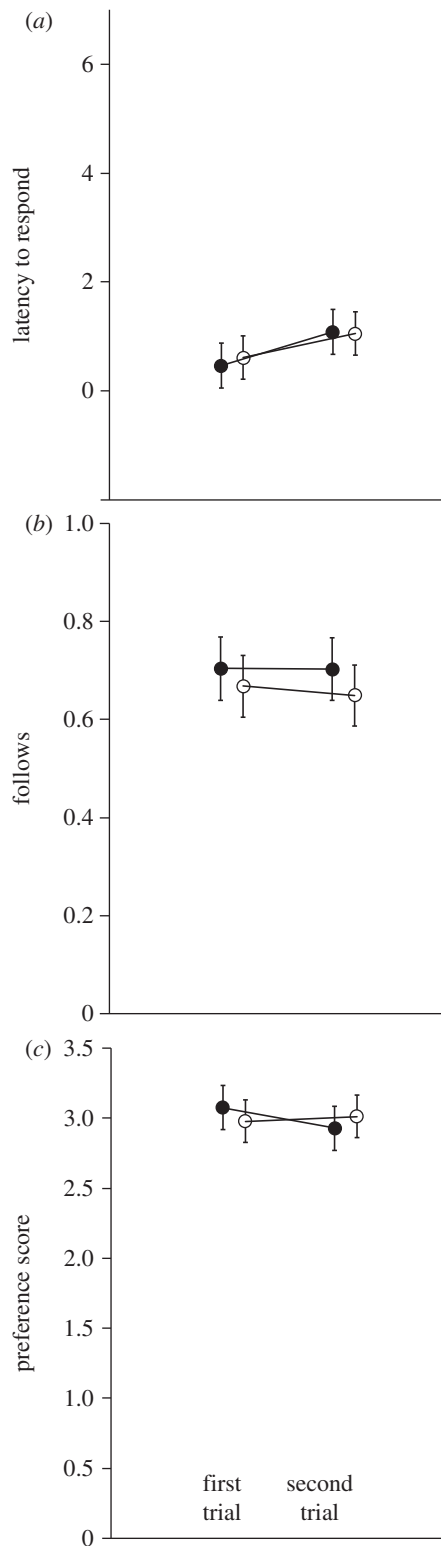


Figure 1. Female mate preference within species and predator exposure. Least-squared means \pm s.e. shown for measure of preference in trials with conspecific mates (sexual selection). Benthic and limnetic female species combined. Filled symbols indicate control exposure, open symbols predator exposure. (a) Latency to respond; (b) follows per lead; and (c) preference score.

However, predator-exposed females did not show altered preferences for conspecific male coloration (electronic supplementary material, table S4).

Analysis of the achromatic female dataset also showed that predator exposure altered limnetic female preference for heterospecific male colour and consequently, sexual isolation. The achromatic dataset included only benthic females and

limnetic females that saw dull males. Preference for conspecifics did not differ between predator- and control-exposed females in the achromatic female dataset (electronic supplementary material, tables S1 and S3). However, predator-exposed females accepted unattractive heterospecifics as mates much more frequently than control-exposed females (figure 2; electronic supplementary material, table S2 and S3), undermining sexual isolation. After exposure, predator-exposed females responded faster to heterospecific males (predator versus control: $t_{76.51} = 2.86$, $p = 0.006$), followed males more (predator versus control: $t_{60.43} = 3.19$, $p = 0.002$) and had higher preference scores (predator versus control: $Z = 2.74$, $p = 0.006$). Again, female inspection rate did not differ between predator and control-exposed females (predator versus control: $t_{70.74} = 1.19$, $p = 0.24$).

4. Discussion

We found that predator exposure weakened sexual isolation in benthic and limnetic sticklebacks. This decrease in the strength of isolation occurs because predators inhibit the expression of learned preferences. Females learned to avoid heterospecific males in the absence of predator attacks, and discriminated more strongly against heterospecifics in their second trial. Experiencing a predator attack overrides this learned aversion: females responded to heterospecifics quickly and strongly, suggesting that predators affect the cognitive processes underlying mate discrimination and choice. Predator exposure also weakened limnetic female colour preference, leading females to mate with dull heterospecific males. This change in colour preference interacts synergistically with the loss of learned aversion to diminish sexual isolation between species. In wild populations, benthic males are much less colourful than limnetic males and limnetic females will mostly encounter dull heterospecific males [49]. In addition, previous work in other populations of sticklebacks found that males respond to predator presence by expressing less colouration [23]. If males are less brightly coloured when predators are present and females lose preference for colourful males, our finding that predators greatly reduce sexual isolation when limnetics encounter less colourful heterospecifics may have major consequences for hybridization in nature.

Our results are the first to show that predators might reduce female discrimination against heterospecifics in sympatric species. These results could explain why periods of high predation can lead to increases in hybridization [8,9]. Previous research has focused on reduced discrimination against heterospecifics in risky situations (lack of refuges) [31] or after predator exposure in allopatric populations [28,29]. Our results, combined with those on male preferences of sympatric sailfin mollies [30], confirm that predators can alter mating decisions and sexual isolation even in sympatric populations with a history of selection against hybridization. These findings suggest that having low quality hybrid offspring may be less costly than forgoing mating and continuing to search for conspecifics when predators are present. Loss of discrimination against heterospecific mates after predator experience should lead to spatial and temporal fluctuations in the strength of sexual isolation in sympatric populations. Therefore, ecological factors such as fluctuations

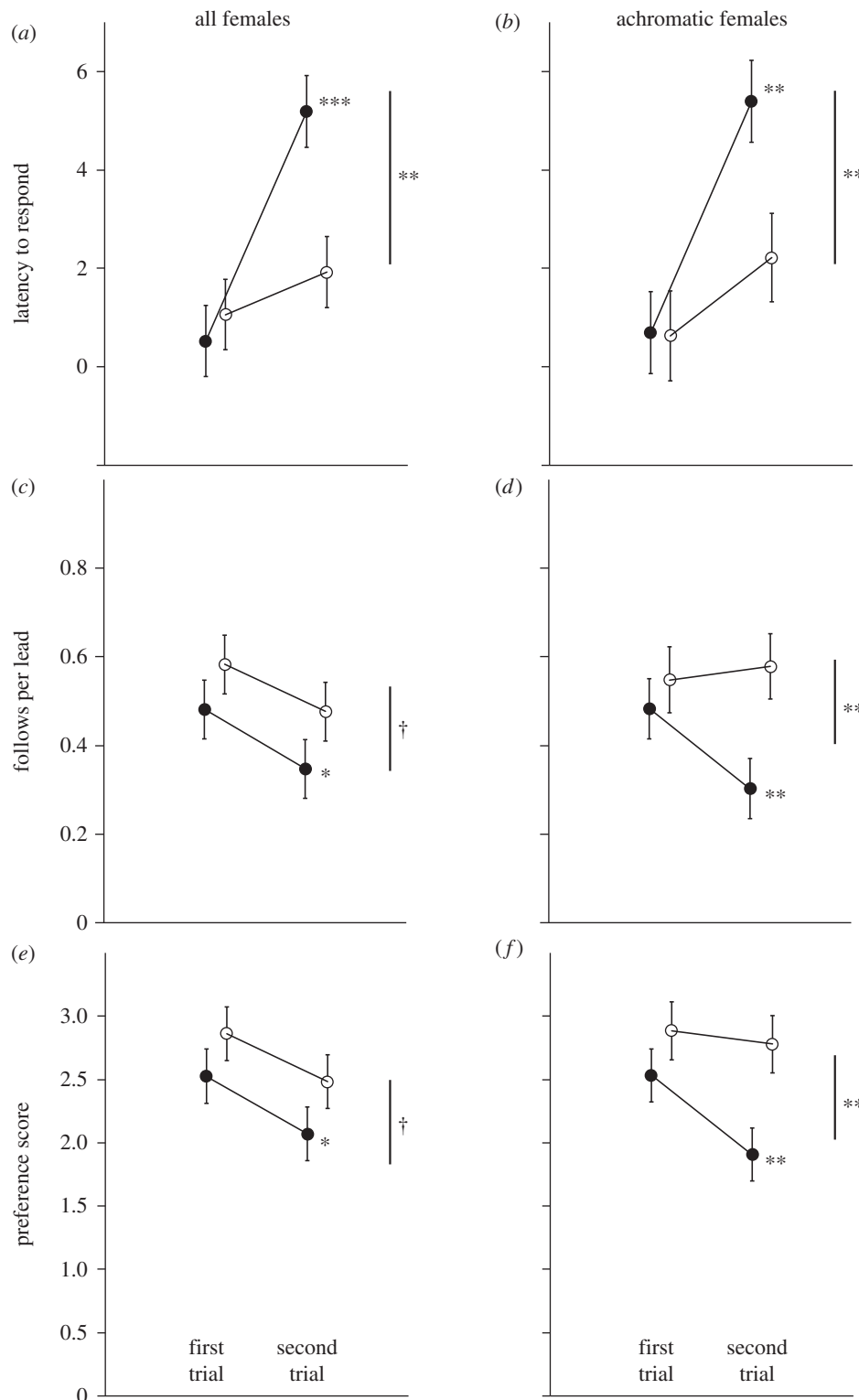


Figure 2. Female mate preference between species and predator exposure. Least-squared means \pm s.e. shown for measures of preference in trials with hetero-specific mates (sexual isolation). Benthic and limnetic female species combined. Filled symbols indicate control exposure, open symbols predator exposure. Left panel shows all females; right panel shows females from the achromatic dataset (benthic females plus limnetics females who saw dull males). (a,b) Latency to respond; (c,d) follows per lead; (e,f) preference score. Significance of comparisons between trials (within treatments) indicated immediately to the right of symbols, significance of comparisons between treatments in second trials indicated next to the vertical line. *** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$, $^{\dagger}p \leq 0.10$.

in predator density can have evolutionary consequences for hybridization and gene flow between species.

Learned aversion contributes to sexual isolation in several sympatric species including damselflies (*Calopteryx splendens*; [70]), fruit flies (*Drosophila melanogaster*; [71]) and sticklebacks [43]. Our finding that predator exposure can disrupt the expression of learned aversion suggests that learned aversion may share some similarities with classical learning tasks

[40,72]. Stressful experiences can inhibit learning and memory in many tasks [36,37]. Recent work suggests that exposure of stickleback mothers to predators can even impair the ability of offspring to learn to associate colour with a reward [73]. Our work suggests that predators can interfere with the normal process of learned aversion during mate discrimination and supports the idea that predators may have substantial effects on cognitive function [39,74].

Predators altered both learned aversion to heterospecifics and which heterospecific males limnetic females preferred. After predator exposure, limnetic females were more willing to mate with less colourful males, perhaps because these males are less conspicuous to predators [14]. This change in colour preference is an important additional way that predators impact sexual isolation in sticklebacks. Male coloration and female preference for bright red coloration contribute to both sexual selection and sexual isolation in this system [49,50,66]. Surprisingly, these predator effects on colour preference did not extend to conspecific males. This is consistent with our general finding that predator effects were most evident in sexual isolation between species rather than sexual selection within species. There are several possible explanations for why predators change preference for heterospecifics, but alter preference for conspecifics very little in these species. We know that stickleback females use multiple traits when selecting mates, including colour, shape, odour and size [50,52,54]. After predator exposure, a conspecific male might be less attractive to a female based on his coloration, but he may compensate via attractiveness in other traits. By contrast, there is no reason why a limnetic female should prefer a colourful heterospecific male if she is no longer attracted to his bright coloration. Another potential explanation is that discriminating between species may be somewhat distinct from within-species mate choice. Consistent with this idea, previous work has found that some mate choice strategies used within species are not applied between species in threespine sticklebacks [43,54] and crickets [75].

Predator effects on sexual isolation may have important and unappreciated consequences for speciation. Much work has focused on how predators alter competition and resource use between species [1]. It has been hypothesized that predators can reduce ecological divergence between

incipient species and decrease extrinsic isolation against intermediate hybrid phenotypes. In addition to altering these traditional components of ecological speciation, if predators influence sexual isolation they could prevent the evolution of assortative mating between populations. Our finding that predators alter female discrimination against heterospecifics strongly suggests that the impact of predators on speciation has been routinely underestimated (including in sticklebacks [5]).

In summary, we found that predator exposure increased the likelihood of mating with heterospecifics in benthic and limnetic threespine sticklebacks. Predator exposure interfered with learned aversion to heterospecifics. Predators also altered female colour preference, further reducing sexual isolation. Thus, predator-induced changes in mate preference can reduce sexual isolation between sympatric species. The potential links between predator-induced effects on mate preference and hybridization deserve to be the subject of future work, particularly in other systems.

Ethics statement. Institutional Animal Care and Use Committee at University of Wisconsin approved all experimental procedures (protocol no. L00317).

Data accessibility. The raw preference data are available in DRYAD: <http://dx.doi.org/10.5061/dryad.50rb3>.

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