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ORIGINAL ARTICLE

Female gray treefrogs maintain mate choice decisions under predation threat, but adjust movements to reduce conspicuousness during mate approach

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Abstract

Female mate choice is remarkably complex and has wide-ranging implications for the strength and direction of male trait evolution. Yet mating decisions can be fickle and inconsistent. Here, we explored predation risk as a source of variation in the effort a female is willing to invest in acquiring a preferred mate type ("choosiness"). We did so by comparing phonotaxis behaviors of female eastern gray treefrogs (*Hyla versicolor*) across trials with and without simulated predators. We tested the *behavioral adjustment hypothesis* (mate choice is unchanged under predation threat, but mate searching behaviors are modified to reduce conspicuousness) against the *mate choice flexibility hypothesis* (mate choice becomes indiscriminate under predation threat). Additionally, effectiveness of evasive behaviors may depend on predator attack strategy, so we incorporated two simulated predator cues (bird model vs predatory ranid call). We found support for the behavioral adjustment hypothesis: choosiness was maintained in the presence of predators, but females reduced conspicuousness of mate searching locomotion. Females approached the conspecific male stimuli slower and more cautiously in both predator treatments. In the ranid frog call treatment (stationary cue), females adjusted movements away from predator location. Females also attempted escape more frequently when predator cues were present. We suggest that focusing exclusively on the final mate decision may overlook nuances in mating decisions and hamper our understanding of the remarkable complexity of mate choice.

Significance statement

The presence of predators is an inherent threat to survival. This leads to the general expectation that higher predation risk results in more indiscriminate mate choice decisions and, hence, a weakening of sexual selection. Yet, discriminating mate choice may be maintained if prudent prey change their approach behavior when detecting the presence of a predator. We conducted playback trials with female treefrogs to test whether their willingness to invest in obtaining a more attractive mate (quantified by "choosiness") differed depending on the presence and type of predation risk. We found that females adjusted their approach behavior in a way that should make them less conspicuous to predators, but that they did not compromise their mate choice decisions. Our results show that strong sexual selection by females' choice can be maintained in high predation environments.

Keywords Sexual selection · Choosiness · Anuran amphibian · Mating decision variation · Behavioral adjustment

Introduction

Mate choice decisions are remarkably complex and have powerful implications for sexual and natural selection (Darwin 1871; West-Eberhard 1983; Rosenthal 2017). Patterns of female mating decisions are expected to affect the strength and direction of male trait evolution with the potential to result in rapid divergence and speciation (Andersson 1994; Coyne and Orr 2004; Rodríguez et al. 2013). However,

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female mate choice decisions are not necessarily fixed. Heritable variation, as well as behavioral flexibility, is prevalent both within individuals and amongst populations (Tobler et al. 2008; Baugh and Ryan 2009) and has the potential to disrupt expected trends by weakening or strengthening selection on male traits (Pomiankowski 1987; Chaine and Lyon 2008; Neelon and Höbel 2017). Exploring sources of variation in mate choice behavior may shed light on the potential adaptive benefits of mate choice plasticity (Bailey and Zuk 2008) and strengthen our understanding of their evolutionary consequences (Jennions and Petrie 1997).

Numerous factors have been identified as possible sources of variation in mate choice (reviewed in Ah-King and Gowaty 2016). Resource availability, social experience, sex ratio, and predator threat can challenge females while making mating decisions, and flexible mate choice may permit the necessary adjustments to maximize fitness (Janetos 1980; Bateson 1983; Partridge and Halliday 1984). Compromising mate choice may be worthwhile when dangers become too serious, as there should be a balance between the fitness benefit of mating with an attractive mate and the risks involved in more extensive mate assessment (Sakaluk and Belwood 1984; Lima and Dill 1990; Gowaty and Hubbell 2009).

The presence of predators is an inherent threat to survival, leading to the general expectation that higher predation risk results in more indiscriminate mate choice decisions (Real 1990; Crowley et al. 1991; Sih 1994; Rosenthal 2017). Indeed, when under predation threat, female guppies (*Poecilia reticulata*) and lesser wax moths (*Achroia grisella*) reverse mate preference and now preferentially approach the less attractive yet less conspicuous male (Godin and Briggs 1996; Gong and Gibson 1996; Edomwande and Barbosa 2020), and female *Gryllus integer* crickets forgo more attractive mates when less attractive ones can be approached under aerial coverage (Hedrick and Dill 1993). In túngara frogs (*Physalaemus pustulosus*), increased perceived predation risk reduces the time spent assessing mates and the overall movement during mate choice (Baugh and Ryan 2010). Alternatively, discriminating mate choice decisions may be maintained in the presence of predators if prudent prey adjusts their behavior in a way that reduces their own conspicuousness. For example, *Ostrinia nubilalis* moths adjust the conspicuousness of their mate-seeking behavior to the degree of predation risk; pheromone release is strongly reduced under high predation risk but only slightly so under low-risk conditions (Acharya and McNeil 1998). Mate choice decisions may similarly be customizable to the degree of predation risk.

Since different predators use distinct hunting tactics (sit-and-wait vs pursuant, spatial planes, directions of attack, threat intensity), prey have the opportunity to assess and uniquely adjust their behavior to reduce detectability or increase escape effectiveness (Lind et al. 2002; Ferrari and Chivers 2009; Weissburg et al. 2014). For example, seeking overhead coverage would be best suited for avoiding an aerial threat but unsuccessful for avoiding a terrestrial threat. To adjust appropriately, prey must exhibit distinct predator recognition strategies and execute custom evasive maneuvers (Howland 1974; Weihs and Webb 1984), which may also vary according to the sensory information provided by the predator (Godin and Briggs 1996) or magnitude of threat (Helfman 1989). There is also evidence of response adaptability of a single prey species to multiple predators (Bulbert et al. 2015) and attack styles (Cooper et al. 2009). Despite the strong evolutionary consequences of adjusting anti-predator strategies to different predators, we know relatively little about whether these avoidance tactics are engaged during mate choice.

Here, we tested if the presence of a predator modifies mate choice behaviors and whether different types of predators elicit different responses. We did so by conducting acoustic playback experiments with female eastern gray treefrogs (*Hyla versicolor*). Females of this species prefer longer duration calls but will approach a shorter call if the perceived distance difference between the alternatives becomes too large. This allows for an assay of the effort each female is willing to invest in obtaining the preferred mate type (i.e., choosiness; Neelon and Höbel 2017; Bastien et al. 2018; Baugh et al. 2021). We tested two hypotheses addressing mate choice behavior under predation threat: (i) the *mate choice flexibility hypothesis* posits that female mate choice decisions become indiscriminate in the presence of a predator. It predicts that females are less choosy during the predator-present treatments. We tested this against (ii) the *behavioral adjustment hypothesis* which posits that female mate choice behaviors are modified but that the mate choice decision itself remains unaffected. This hypothesis predicts that locomotor approaches are modified in a way that reduces conspicuousness during the predator-present treatments. Additionally, we considered predator type by testing the above hypotheses using either a pursuant/aerial predator (flying bird model) or a stationary/terrestrial predator (call of a predatory ranid frog). Since evasive maneuvers may be more effective in response to pursuant/aerial predators, while avoidance behaviors may be more appropriate for stationary/terrestrial predators, we predict that females would show different behavioral adjustments in response to different predator types.

Methods

Study species and site

Eastern gray treefrogs (*Hyla versicolor*) are a common, nocturnal anuran in the eastern USA. Males gather in and around woodland ponds to advertise for mates. In nature, females approach stationary males guided by their calls (phonotaxis), and in acoustic playback trials will repeatedly approach speakers broadcasting synthetic stimuli (Gerhardt 1992; Ryan 2001). Male calls consist of a series of short pulses, and females prefer longer duration calls (Sullivan and Hinshaw 1992; Gerhardt et al. 2000; Reichert and Höbel 2015).

During May/June 2020, we collected 44 females at a pond adjacent to the University of Wisconsin Milwaukee's (UWM) Field Station in Saukville, WI. All individuals were captured while in amplexus to ensure female receptivity to male stimuli. Frogs were transported to the nearby Field Station, where they were kept in a cooler with melting ice to postpone oviposition. Behavioral experiments took place within 3 days of capture. Once started, experiments with the same female were completed within 2 h, and all frogs were released at the site of capture thereafter. Since phonotaxis trials require exact knowledge of the presented call alternatives, it was not possible to record data blindly.

Playback trials

General playback setup

Experiments took place in a dimly lit, temperature-controlled (19–20 °C), semi-anechoic room containing an experimental arena at the UWM Field Station. Dimly lit conditions (0.4 lx; Extech EasyView EA31 Digital Light Meter) are necessary for visual stimuli (Rand et al. 1997), so light within the typical range of nocturnal light was present (Underhill and Höbel 2017). The arena was a 2-m diameter enclosure constructed with wire fencing covered in black fabric. In every trial, there was a small release container in the center of the arena to contain each female before the beginning of her trial and two speakers (JBL Control 1Xtreme) placed outside the arena wall (180° angular separation). In front of each speaker, there was a 10 cm "choice zone" within the arena wall that the female had to enter in order to qualify as a choice.

Females were randomly assigned to one predator treatment condition (ranid frog or predatory bird). Each female received two sets of choosiness trials, one under predator-absent treatment (conspecific call playbacks only) and one under predator-present treatment (one of the two simulated predator threats). Half the females started with the predator-absent treatment, and half started with the predator-present treatment. During the trials, we made detailed sketches of the frogs' movement pathways. In addition, we video-recorded the predator-present trials to later verify that we had not missed subtle behavior changes during live sketches.

Stimulus generation

To create the synthetic *H. versicolor* advertisement calls used to assess choosiness, we used the seewave package (Sueur et al. 2008) in R (Version 3.1.0; R Development Core Team 2014). We created one long, attractive call (18 pulse duration) and one short, unattractive call (6 pulse duration), both of which exist naturally in the study population. These values represent the low end (6 pulse) and the average (18 pulse) call duration in our study population (Reichert and Höbel 2015; OSF pers. obs.). All other call characteristics were maintained at the population average; length of pulse = 25 ms, pulse period = 25 ms, call period = 7750 ms, high frequency peak = 1071 Hz, and low frequency peak = 2142 Hz (which is 10 dB louder in low frequency) (Reichert and Höbel 2015).

Testing choosiness

To test for choosiness, we used a two-speaker design. One speaker broadcast the unattractive (6 pulse) stimulus maintained at 85 dB SPL throughout the experiment. The other speaker broadcast the attractive (18 pulse) stimulus with the amplitude attenuated relative to the unattractive one. The amplitude differences used in these trials ranged from 0 to 24 dB in steps of 3 dB. By modifying amplitude, we manipulated the female's perceived distance to the caller by taking advantage of the inverse square law of sound attenuation: a 6 dB decrease in amplitude is equivalent to a doubling of distance from a sound source. In terms of perceived distance to the sound source, amplitude difference set to 0 dB is equivalent to a perceived 1 m distance from the female in the center of the arena to both male call alternatives, compared to a 24 dB amplitude difference where the attractive call appears 16 m away while the unattractive one still 1 m away. The larger the amplitude difference between the two call alternatives, the more willing the female is to walk further to obtain her preferred mate, and hence, the choosier she is. The amplitude of the stimuli was adjusted using an Extech 407,764 Sound Level Meter (Extech Instruments, RS232/Data logger; C-weighting, fast RMS).

We first tested all frogs at a 12 dB amplitude difference (i.e., the 18 pulse call was 73 dB, and the 6 pulse call was 85 dB), equivalent to intermediate choosiness. The amplitude difference of subsequent trials depended on each female's previous decision; if she chose the 6 pulse call (less attractive but louder or "closer"), we decreased the amplitude difference on her next trial making the task of approaching the more attractive male easier. If the female chose the 18 pulse call (more attractive but softer or "further away"), we increased the amplitude difference in her next trial making the task of approaching the more attractive male more arduous. We repeated this procedure until we had established the highest amplitude difference at which the female still chose the attractive call; this was that female's measure of choosiness. This "adjustable" trial sequence design allowed us to measure choosiness in 3–4 trials per female, ensuring that females remained responsive throughout both the predator-present and predator-absent treatments.

Each trial lasted a maximum of 5.5 min. Between consecutive trials, females received a rest period of approximately 2–5 min; during this time, she was placed in a quiet, dark place inside a small plastic container with pond water. If a female escaped or made no decision in a trial, she was given a repeated trial. If a female escaped or made no decision on multiple trials of the same amplitude, the amplitude difference was decreased to make the decision easier. If a female did not respond in more than three sequential trials, she was removed from the study and not included in the reported sample size.

Assessing locomotor approaches

In addition to quantifying each female's choosiness score, we took four measurements of her locomotor approaches. (i) *Latency* was measured by timing the moment each frog was released from the center container until she hopped into the choice zone. (ii) *Escape* was measured as the total number of trials where the female climbed the arena walls in an attempt to leave the arena entirely. The other two measures, covered approach and angle post-predator, were extracted by reviewing video recordings and notebook sketches that documented the females' movement path during each trial. We overlaid a transparent image that divided the arena into 12 pie slice-shaped arena sections. (iii) *Covered approach* was quantified by the number of arena sections which the frog traversed while moving close to (< 30 cm) the arena wall (illustrated in Fig. 2), excluding regions in front of each speaker since those movements were more indicative of speaker choice. (iv) *Movement angle post-predator* was measured by using the 12 arena sections to determine the angle of initial jump relative to the location of the predator, which was assigned as 0° (hence, a jump angle of 180° would indicate fleeing directly away from the predator).

Predator ecology

North American hylid frogs (such as *H. versicolor*) are preyed upon by a variety of predators, and we chose two typical, yet dissimilar, predator types: ranid frogs and birds. Ranid frogs, such as bullfrogs (*Lithobates catesbeianus*) and green frogs (*Lithobates clamitans*) are large predatory anurans (Werner et al. 1995; Jancowski and Orchard 2013) that have been observed attacking *H. versicolor* in several study sites, including our own (Schwartz et al. 2000) (GH pers. obs.). Ranid frogs are opportunistic sit-and-wait predators. During their breeding season, which overlaps with the breeding season of *H. versicolor*, they emit advertisement calls from inside their territories that may act as predator cues for mate searching females (Schwartz et al. 2000). We classify this ranid predator type as *terrestrial*, *stationary*, and *acoustic*. Wading birds such as herons also feed on frogs and have been observed preying on *H. versicolor* (J. Schumacher; http://vireo.ansp.org/bird_academy/amphibian-eating%20birds.php). These birds typically attack swiftly from an aerial position. This attack behavior likely only creates transient and unpredictable visual cues for prey to respond to at the moment of attack. We therefore classify this bird predator type as *aerial*, *pursuant*, and *visual.*

Predator stimuli

Predatory ranid We created the predatory ranid stimulus by combining a bullfrog (*Lithobates catesbeianus*) call published on the Sounds of North American Frogs CD (Folkways 6166) with 4 s of silence to create a bullfrog playback containing four bullfrog calls (4 s) with 4 s of silence (repeated every 8 s). The call was broadcast from a third speaker placed equidistant from the other two speakers (90° angular separation from each treefrog speaker) at ground level. We adjusted the call amplitude to 85 dB, the same amplitude as the loudest treefrog call, so the bullfrog and nearest potential mate were perceived as equidistant. Eight bullfrog calls (two bouts of four) were played before a female was released to establish predator presence and location. Twenty-four treefrog females were presented with the same ranid predator cue; this was done to standardize signal presentation, but may represent a type of pseudoreplication (Milinski 1997).

Predatory bird To create the bird model, we constructed a cardboard and paper-mâché model of a flying bird with extended wings (43 cm wingspan; 38 cm body length). The model was painted a monochromatic dark gray color with acrylic paint. We used a 1-m string to attach the bird model to the ceiling above the playback arena. Before being deployed, the model was placed on a shelf outside the arena (90° angular separation from each treefrog speaker). Once deployed, the bird model swung (across the entire arena) for approximately 30 s, before gradually slowing down to movements over the center of the arena. Bird movement continued during the maximum time limit of each trial (over 5.5 min). In the trials with a bird predator, we first started the treefrog call playbacks and deployed the bird model the moment the female left the release container. Twenty treefrog females were presented with the same avian predator model; this was done to standardize signal presentation but may represent a type of pseudoreplication (Milinski 1997).

Statistical analysis

We used GLMM models in JMP 15.2.1 (SAS Institute Inc., Cary, NC) to test whether choosiness or locomotor approaches were affected by simulated predator threat. We entered choosiness or one of the locomotor approaches (latency, number of escapes, covered approach, respectively) as response variables and predator treatment (absent/present), predator type (ranid/bird), sequence (if predator-present occurred first or second), and all their two- and three-way interactions as predictor variables. We started the analysis with the full models (Supplementary Tables S1-S4) and then successively removed non-random terms (starting with the three-way and then the two-way interactions) to increase power. Since every female had contributed two measures to the analysis (from predator-absent and predator-present treatments), we also included female ID as a random term in the models.

For the test of our hypotheses, the treatment (predator-absent/predator-present) and the predator type (ranid frog or bird) are the predictor variables of interest since they provide information about whether and how behavior is affected by the presence and type of a simulated predator. The female ID term (included as random term in the models) provides information on how repeatable and individual-specific the behavior is. We estimate repeatability R from the percentage variance component for female ID (Nakagawa and Schielzeth 2010), provided by JMP 15.2.1 (Table 1).

To compare the magnitude of the effect of predator presence and predator type on mate choice behaviors, we calculated effect sizes. We calculated the correlation coefficient r from F-ratios of the GLMM model terms, according to Rosenthal (1991). Values of r range from 0 to 1 and have similar interpretations as r^2 in a simple linear regression.

Table 1 Results of reduced GLMM models examining the effect of predator presence and predator type on choosiness (the effort a female is willing to expend to get a preferred mate) and movement behaviors during mate approach (response latency, covered approach, number of escapes)

Factor	DF	*F*	*p*	Effect size (*r*)[$]
Choosiness[*]				
Treatment	1,42	0.8	0.37	0.14
Predator type	1,42	2.5	0.12	0.25
Treatment × predator type	1,42	0.3	0.58	0.08
Repeatability estimate from female ID term		95% CI = 2.4–20.5	Wald *p* = **0.01**	41.1
Response latency[*]				
Treatment	1,39.2	5.8	**0.02**	0.36
Predator type	1,39.3	1.4	0.24	0.19
Treatment × predator type	1,39.2	0.5	0.49	0.11
Repeatability estimate from female ID term		95% CI = − 1264–1298	Wald *p* = 0.98	0.46
Covered approach[*]				
Treatment	1,54.7	18.3	**< 0.0001**	0.50
Predator type	1,59.3	0.6	0.45	0.10
Treatment × predator type	1,54.7	0.2	0.64	0.06
Repeatability estimate from female ID term		95% CI = 0.15–1.12	Wald *p* = **0.01**	30.4
Number of escapes[**]				
Treatment	1,50.6	16.6	**0.0002**	0.50
Predator type	1,75.8	0.0008	0.98	0.003
Treatment × predator type	1,50.6	0.6	0.49	0.11
Sequence	1,54.8	5.1	**0.03**	0.29
Predator type × sequence	1,54.8	7.9	**0.007**	0.35
Repeatability estimate from female ID term		95% CI = 0.25–1.53	Wald *p* = **0.007**	57.8

[*] GLMM model calculated as [Behavior ~ Treatment + Predator Type + Treatment*Predator Type + (Female ID)]

[**] GLMM model calculated as [Escape frequency ~ Treatment + Predator Type + Treatment*Predator Type + Sequence + Predator Type*Sequence + (Female ID)]

[$] Correlation coefficient (*r*) parameters: 0–0.3 small effect, 0.3–0.5 intermediate effect, > 0.5 large effect

Significant *p*-values indicated with bold font

Correlation values that range from 0 to 0.3 indicate small effect sizes, 0.3–0.5 indicate intermediate effect sizes, and values greater than 0.5 indicate large effect sizes.

To examine whether there was a directional component to the females' locomotor approaches, we computed circular statistics (Batschelet 1981) in the program Oriana (Kovach Computing Services, Anglesey, Wales). Specifically, we tested whether the first movement of the female was directed away from the location of the simulated predator (speaker broadcasting the bullfrog call or the direction the bird model first appeared over the arena). To do this, we computed Rayleigh tests followed by *V*-tests and Watson-William's *F* tests. We tested the null hypothesis that orientation angles were distributed uniformly using Rayleigh tests (hence, a significant Rayleigh test indicates that frog movements show some directionality). If frogs attend to the presented stimuli, orientation angles should not be uniformly distributed but instead should be directed towards or away from a stimulus. In cases where Rayleigh tests indicated nonuniform distribution (i.e., some type of directionality), we used *V*-tests to test for distribution in a specified mean direction. Since the reference angle in our trials was the location of the predator (set to 0°), we set the expected mean for the *V*-tests at either 90° (towards the speaker broadcasting the attractive conspecific call) or 180° (away from the predator stimulus). A significant *V*-test indicates that frog movements are not different from the mean direction that was specified in the *V*-test. Finally, we used Watson-William's *F* tests to test whether the mean angle of movement differed between predator-absent and predator-present treatments.

We provide the angle of the mean vector (μ) and the length of the mean vector (r) for each test. The length of the mean vector is a measure of angular dispersion (similar to standard deviation); its value can range from 0 to 1, where $r=0$ indicates uniform dispersion and $r=1$ indicates complete concentration in one direction. In terms of our study, a high r-value and significant *V*-test would indicate that all females directionally moved away from the location or release site of the predator, while a low r-value indicates that female movement was not impacted by the location of the predator.

Results

Predation threat did not change female choosiness

Females did not become less choosy when confronted with a simulated predator (Table 1). Neither the main effect of predator treatment, the predator type, nor the predator × treatment interaction (Fig. 1a) was significant. However, there was substantial individual variation in choosiness (Fig. 1b), and the significant individual ID term suggests that choosiness is a repeatable female trait (Table 1).

Predation threat changed female approach behavior

The presence of a predator changed all locomotor approaches and the effect was of intermediate to large magnitude (Table 1). Females in the predator-present trials took longer to reach the speaker broadcasting a conspecific male call (Fig. 2a; left panel), they attempted to escape the arena more frequently (Fig. 2b; left panel), and they remained close to the arena wall (which they likely perceived as a cover object) when traveling to the speaker broadcasting a male call (Fig. 2c; left panel).

In general, predator type and trial sequence did not change female locomotor approach; effects were non-significant and of small magnitude (Table 1; Fig. 2 center and right panels). The noteworthy exception was the number of escapes during the bird predator experiment: we found significant sequence and sequence × predator type interaction effects of intermediate magnitude (Table 1). Inspection of the average escape attempts across the different trials showed this was due to frequent escapes during predator-absent trials in the bird predator experiment when females had received the predator exposure trials first (Supplementary Fig. S1). We tentatively interpret this as females remembering the presence of a pursuant predator (approximately > 30 min) and remaining wary in subsequent trials even in the absence of direct predator cues.

Predation threat sometimes changed the directionality of female locomotion

Irrespective of treatment or predator type, the angles of the first jumps of mate searching females were significantly clustered towards the direction of the speaker broadcasting the attractive conspecific call (Table 2; Fig. 3).

In the ranid predator experiment, playback of the bullfrog call during the predator-present treatment provided mate searching females with a cue that indicated the constant presence as well as location of a simulated predator. Here, the mean angles of first jumps were marginally significantly different between predator-absent and predator-present treatments (Watson Williams *F*- test: $F=3.72$, $p=0.06$), with jumps in the predator treatment being directed more strongly away from the location of the predator (Fig. 3a, b). In the bird experiment, there was no directional cue after the initial deployment of the bird model (because the model swung back and forth). Accordingly, we assessed the jumps just before and just after the bird entered the arena. Presentation of a simulated bird predator did not shift jump direction (Watson Williams *F*- test: $F=0.004$, $p=0.95$; Fig. 3c, d).

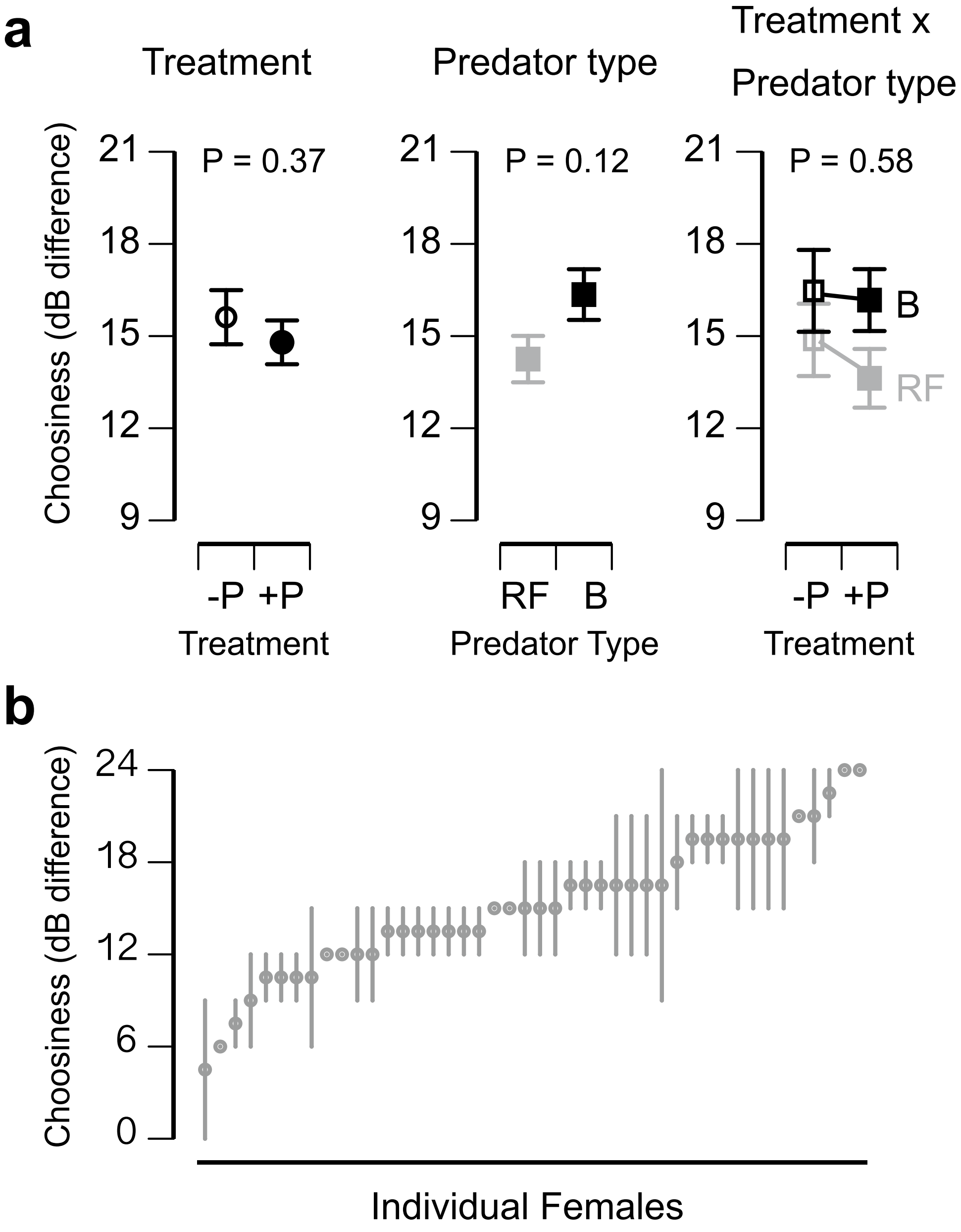

Fig. 1 Predation threat does not affect choosiness. (**a**) Choosiness was not influenced by treatment, by predator type, or by a treatment × predator type interaction. Symbols denote means ± standard error. Data from predator-absent (− P) trials are shown in open symbols and those from predator-present (+ P) trials are shown in filled symbols. Data from the ranid predator trials are shown in gray and labeled RF, and data from the bird predator trials are shown in black and labeled B. Circles denote treatment averages, and squares denote predator type averages. (**b**) Choosiness is highly variable across females but consistent within females: 77% of females varied their two choosiness scores within 6 dB and 59% within 3 dB. Shown is the average (circle) and range (bar) of choosiness scores in each of the two trials a given female completed. Choosiness is measured as the highest amplitude difference (in dB) at which the female still chose the attractive call, hence indicating how much further she would be willing to walk to obtain the preferred mate

Discussion

We tested whether the mate choice behavior of female eastern gray treefrogs was affected by simulated predation risk. We found that choosiness for the longer-duration call did not change when predator cues were present, failing to support the *mate choice flexibility hypothesis.* Rather, the high degree of repeatability of a female's choosiness indicated that it is an individual female trait, which may explain its stability in the face of predation risk. Instead of compromising mate choice when faced with a predator, females adjusted their approach behavior in a way that was consistent with minimizing their conspicuousness. Females took longer to approach a speaker (a possible indicator of frequent immobility between bouts of movement), focused their approach movements to areas of increased cover, and tried more frequently to leave the testing arena altogether. Adjustments of locomotor approaches with no change in choosiness provide strong support for the *behavioral adjustment hypothesis*, demonstrating that females are able to accommodate predation risk while maintaining mating decisions.

Mate choice decisions are the outcome of several components, including preference functions (the ranking of attractiveness of potential mates) and choosiness (the effort invested in obtaining the preferred mate type) (Jennions and Petrie 1997). Recent evidence indicates these are independent traits (Neelon et al. 2019; OSF and GH unpubl. data). This suggests that predation risk may influence each

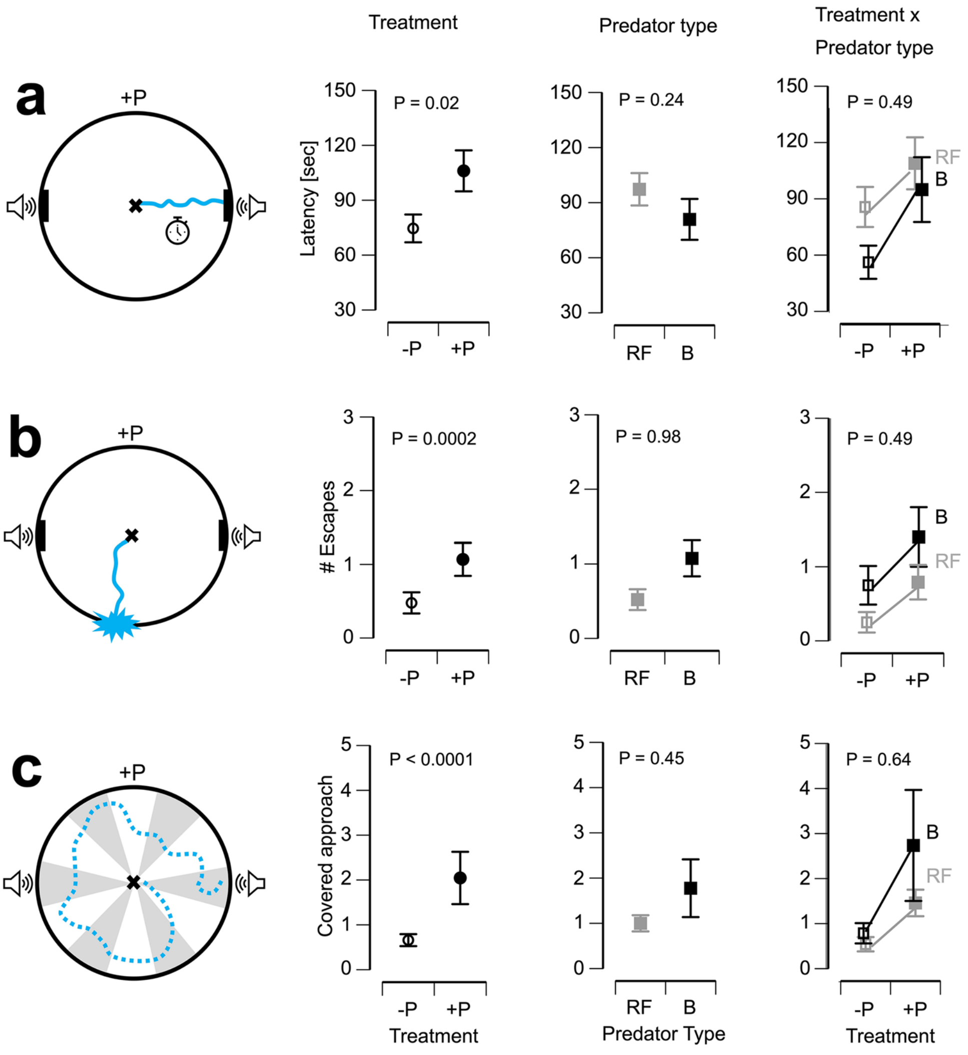

Fig. 2 Predation threat strongly affected locomotor approaches. The first column represents stylized cartoons of the assessed locomotion behaviors. When predator cues were present (+P), females were on average (**a**) slower to reach male stimuli, (**b**) escaped more frequently, and (**c**) moved nearer to the arena wall. Measures derived from averages per female across trials: (**a**) latency (s) to choose a male speaker, (**b**) escape attempts across trials, and (**c**) number of arena sections traversed < 30 cm of wall. Symbols denote means ± standard error. Data from predator-absent (−P) trials are shown in open symbols, and those from predator-present (+P) trials are shown in filled symbols. Data from the ranid predator trials are shown in gray and labeled RF, and data from the bird predator trials are shown in black and labeled B. Circles denote treatment averages, squares denote predator type averages

Table 2 Circular statistics testing the effect of predator type and predator presence on directionality of initial movement

	Ranid frog absent		*Ranid frog present*	
Mean vector*	$\mu = 106$		$\mu = 146$	
Mean vector length*	$r = 0.59$		$r = 0.43$	
Rayleigh test[$]	$Z = 8.4$	**$p < 0.001$**	$Z = 4.35$	**$p = 0.01$**
V-test 90° (towards attractive male)[#]	$u = 3.95$	**$p < 0.001$**	$u = 1.66$	**$p = 0.049$**
V-test 180° (away from predator)[#]	$u = 1.12$	$p = 0.13$	$u = 2.44$	**$p = 0.007$**
	Pre-bird exposure		*Post-bird exposure*	
Mean vector*	$\mu = 112$		$\mu = 113$	
Mean vector length*	$r = 0.54$		$r = 0.8$	
Rayleigh test[$]	$Z = 5.79$	**$p = 0.002$**	$Z = 12.8$	**$p < 0.0001$**
V-test 90° (towards attractive male)[#]	$u = 3.15$	**$p < 0.001$**	$u = 4.65$	**$p < 0.0001$**
V-test 180° (away from predator)[#]	$u = 1.29$	$p = 0.10$	$u = 2.00$	**$p = 0.022$**

[*] Mean vector indicates movement direction and vector length indicates data concentration in this direction

[$] Significant Rayleigh test indicates that movement angles are not random (i.e., directional)

[#] Significant *V*-tests indicate that movement angles were grouped into an expected direction

Significant *p*-values indicated with bold font

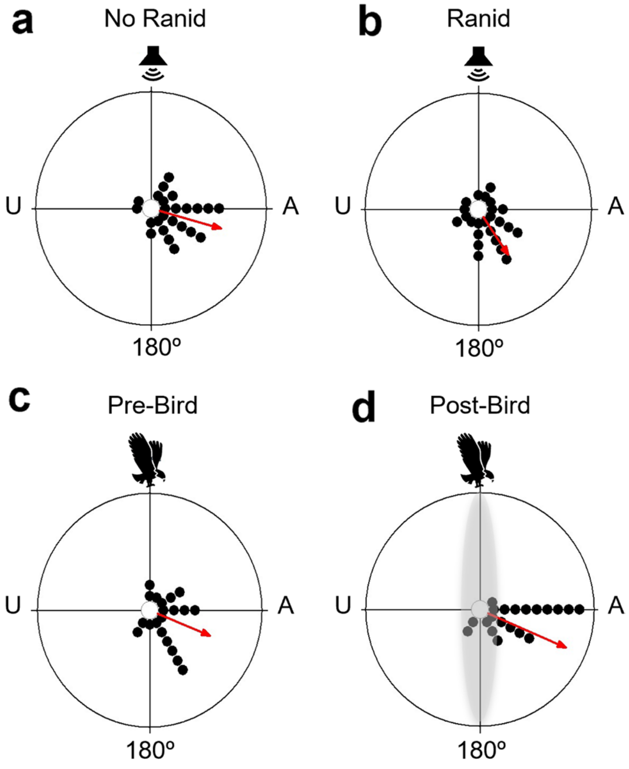

Fig. 3 Polar diagrams showing the direction of initial locomotor approaches. Across all trials, females moved towards the general direction of the speaker broadcasting the attractive call (A). (**a**, **b**) Females did adjust direction away from the location of the speaker broadcasting the predatory ranid call. (**c**, **d**) Females did not change directionality after the bird model was deployed. Degrees standardized across trials: 0° = predator, 90° = attractive call (A), 180° = opposite of predator, and 270° = unattractive call (U). Vector (arrow) directions show mean angle of movement, and vector length indicates strength of directionality

component differently and that a lack of effect on choosiness does not indicate that preferences are unaffected as well (and vice versa). Likewise, support for one hypothesis (say, behavioral adjustment) when examining one mate choice component (say, choosiness) does not indicate that the other hypothesis could not be supported for the second component. With these considerations in mind, it is interesting to note that most of the published research that supports the *mate choice flexibility hypothesis* examined the effect of predation risk on preferences. As predation risk increases, mate preferences are often lost or shifted to less attractive but less conspicuous options. “Less conspicuous” can refer to a variety of scenarios, such as shorter male songs (Edomwande and Barbosa 2020), less elaborate visual displays (Johnson and Basolo 2003), duller ornaments (Godin and Briggs 1996; Gong and Gibson 1996), increased coverage (Hedrick and Dill 1993; Karino et al. 2000; Kim et al. 2009), and avoidance of multimodal signals (Cronin et al. 2019). Interestingly, the few studies that examined choosiness and found evidence in favor of the *mate choice flexibility hypothesis* also report that females seemed to factor in conspicuousness when adjusting their mating decisions. For instance, when predation risk was heightened, female tail-spot wrasse (*Halichoeres melanurus*), a species with male-territory-visiting polygamy, changed mates less frequently and mated more often with the closest male (Karino et al. 2000). Female fiddler crabs (*Uca mjoebergi*) decreased travel distance and sampled primarily the closest males (Booksmythe et al. 2008).

The *behavioral adjustment hypothesis* has received less attention, and, to our knowledge, this study is the first to support it. Published literature, however, contains hints at more widespread occurrence of behavioral adjustments. Schwartz et al. (2000) tested whether female eastern gray treefrogs avoid approaching a conspecific call if it was presented near a bullfrog call. The study concluded that predators did not influence mate discrimination, because female phonotaxis towards conspecific calls did not change. Yet, the authors also describe a high number of uncooperative females that wandered, remained motionless, or attempted to escape (Schwartz et al. 2000). We speculate that, similar to our study, the presence of a predator cue generated the “uncooperative” behavior of these females and that treatment effects may have been uncovered had these behaviors been scored explicitly. Data from pure predator–prey studies highlight how capable prey are in customizing evasive maneuvers in subtle ways such as freeze/flee timing (Eilam 2005; Ilany and Eilam 2008; Nishiumi and Mori 2020), flight distance (Martín et al. 2005; Nishiumi and Mori 2015), escape trajectory (Shifferman and Eilam 2004; reviewed in Domenici et al. 2011), and mirroring the risk magnitude (Helfman 1989; Acharya and McNeil 1998). We consider the same behavioral flexibility expressed when dealing with predators, while foraging may also apply to the process of mate choice.

Additional support for the *mate choice flexibility hypothesis* comes from the differences in behavioral adjustments as a function of predator type. We observed directional avoidance when faced only with a stationary predator (ranid frog) but not with a pursuant one (bird). The sequence effect in the bird predator experiment, where females that were exposed to the bird predator in their first trials maintained a similarly high escape frequency in the subsequent predator-absent trials, also suggests that females attend to differences in predator type. Although not significant across all assessed behavioral measures, we suggest that females make more extreme escape attempts when faced with an unpredictable pursuant predator (bird), while directional avoidance behaviors are sufficient to deal with more predictable stationary threats (ranid frog). Again, pure predator–prey studies document similar behavioral differences: a study assessing escape strategies in túngara frogs (*Physalaemus pustulosus*) also found a difference in directional avoidance between a terrestrial and aerial predator (Bulbert et al. 2015).

Conclusion

Mate choice is a complex behavior, combining components of cognitive decision-making with active locomotion. Our understanding of the causes of variation in mate choice decisions will benefit from expansion into several avenues of research. First, mate choice decisions are the outcome of several, independent components (Jennions and Petrie 1997; Neelon et al. 2019). Understanding whether, and how, they are affected by the same intrinsic or extrinsic variable will inform how the form and speed of sexual selection is affected. Second, we recommend that mate choice studies include more detailed behavioral observations. Had we exclusively focused on the final mating decision (i.e., choosiness score), we would have overlooked enlightening behavioral responses. Third, we encourage the integration of information obtained from correlated avenues of research. For example, behavioral responses in a pure predator avoidance context may point to aspects of prey behavior that should be examined when looking at the effects of predation on mate choice.

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Author contribution All authors conceptualized the study and experimental design. Methods were executed by OSF. OSF prepared the manuscript drafts, and GH critically reviewed and edited subsequent versions. All authors have read and approved the final manuscript and submission for publication.

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Data availability The datasets generated and analyzed during the current study are available as supplementary material.

Code availability Not applicable.

Declarations

Ethics approval All procedures were approved by the Animal Care and Use Committee of the University of Wisconsin Milwaukee (Protocol Number: 19–20#26).

Conflict of interest The authors declare no competing interests.

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