

Original Article

A trade-off between natural and sexual selection underlies diversification of a sexual signal

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A longstanding hypothesis in evolutionary biology is that trade-offs between natural and sexual selection often underlie the diversification of sexual signals in the wild. A classic example of this “selection trade-off hypothesis” proposes that males evolve elaborate and conspicuous ornamentation in low-risk environments where female preferences dominate selection on sexual traits, but they evolve muted and relatively cryptic sexual traits in high-risk environments where selection from predators acts against conspicuous sexual traits and female preferences potentially weaken or reverse. However, little direct empirical evidence supports this notion. Using the model system of Bahamas mosquitofish (*Gambusia hubbsi*)—where males have recently evolved greater orange coloration in their dorsal fins in blue holes lacking predatory fish relative to populations with fish predators—we tested this hypothesis using fish replicas differing only in dorsal-fin color. Specifically, we employed plastic fish models in a combination of field and lab experiments to directly examine conspicuity to predators and female preferences for dorsal-fin color. We found that orange-shifted dorsal fins resembling the color exhibited in predator-free populations appeared more conspicuous to predatory bigmouth sleepers (*Gobiomorus dormitor*) that are evolutionarily naive to mosquitofish. Wild-caught female mosquitofish preferred the orange-shifted dorsal-fin model during dichotomous choice tests; evolutionary history with predators did not affect female preferences. Similar mate-choice trials with lab-born virgin females also found preferences for the orange-shifted dorsal-fin model and revealed significant genetic variation for female preferences. Our study provides direct empirical evidence documenting a trade-off between natural and sexual selection in a colorful sexual signal.

Key words: female mate choice, natural selection, Poeciliidae, predation, secondary sexual characters, sexual selection.

INTRODUCTION

One major, ongoing goal of evolutionary biology is to understand the causes of sexual signal diversification, as secondary sexual traits comprise some of the most conspicuous and elaborate aspects of phenotypic diversity (Boughman 2002; Andersson and Simmons 2006; Safran et al. 2013; Mendelson et al. 2014). Sexual selection by female mate choice represents one major evolutionary driver of trait elaboration. However, the same traits that render males more attractive to females can simultaneously attract the attention of predators and parasites (Andersson 1994; Wagner 1996; Zuk and Kolluru 1998; Rosenthal et al. 2001). As such, sexual signals are often assumed to represent an evolutionary balance between

natural and sexual selection. Although sexual selection tends to favor greater elaboration, natural selection frequently favors dull and less conspicuous traits that allow the bearer to avoid detection and enjoy improved survival prospects (Endler 1980, 1982; Zuk et al. 1993; Kotiaho et al. 1998). Indeed, animals exhibiting greater ornamentation or more elaborate courtship displays have been shown to suffer increased risk of predation (Godin and McDonough 2003; Husak et al. 2006; Woods et al. 2007; Hernandez-Jimenez and Rios-Cardenas 2012). Trinidadian guppies (*Poecilia reticulata*) represent one especially well-documented example of this phenomenon, where these fish have evolved greater coloration when inhabiting natural, or experimentally manipulated, low-predation risk environments (Endler 1980, 1983).

Although this “selection trade-off hypothesis” is widely accepted, directly testing it has proved challenging. Correlations among both traits and selective agents are pervasive in nature, making it

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difficult to assess both natural and sexual selection on the trait of interest while controlling for other potentially confounding factors. Moreover, to understand how sexual signals diversify, studies should perform such tests within natural systems where the sexual trait has undergone evolutionary diversification. Thus, a powerful way to test for a trade-off between natural and sexual selection is to experimentally manipulate only the male sexual signal and assess its conspicuity to predators or parasites (natural selection) and its attractiveness to females (sexual selection) across multiple populations within a study system characterized by sexual signal diversification. Although such an experiment would provide one of the strongest tests of the selection trade-off hypothesis, no study to date has employed this approach.

Variation in ecological conditions can alter both natural and sexual selection on sexual signals, leading to signal diversification across ecologically different environments. For instance, environments with higher predator densities likely confer greater viability costs for bearing elaborated traits due to reduced locomotor ability or increased detectability (Godin and McDonough 2003; Langerhans et al. 2005). Thus, variation in predation risk can clearly result in modified natural selection on sexual signals. However, high-risk environments can also affect the magnitude or direction of sexual selection by increasing costs associated with searching for and assessing potential mates, reducing the availability or quality of territories, or altering the information content of sexual signals (Schluter and Price 1993; Jennions and Petrie 1997). As a result, females inhabiting different environments may exhibit different preferences for male traits due to behavioral plasticity or genetically based differences (Schluter and Price 1993; Godin and Briggs 1996; Johnson and Basolo 2003; Eraly et al. 2009; Fuller and Noa 2010). In particular, geographic variation in predation risk can result in vastly different mating environments, often selecting for reduced choosiness and mate assessment in populations experiencing high threat of predation (Magnhagen 1991; Jennions and Petrie 1997). For example, guppies from high-predation stream regions show weakened preference for bright coloration in males (Stoner and Breden 1988; Endler and Houde 1995; Schwartz and Hendry 2007). Variation in female preferences between different environments can have important implications for population divergence and speciation (Maan and Seehausen 2011).

Here, we directly test whether a trade-off between natural and sexual selection underlies diversification of a conspicuous sexual signal—orange dorsal-fin coloration—in Bahamas mosquitofish inhabiting blue holes. Inland blue holes (water-filled vertical caves) on Andros Island, The Bahamas, harbor simple fish communities of typically just 1–3 species that colonized these isolated environments during the last ~15 000 years (Heinen et al. 2013). Two common inhabitants include a small livebearer, the Bahamas mosquitofish (*Gambusia hubbsi*), and a larger piscivore, the bigmouth sleeper (*Gobiomorus dormitor*). Across Andros Island, all possible community compositions of these 2 fishes occur in different blue holes: mosquitofish without sleepers (low-predation risk for mosquitofish), both mosquitofish and sleepers (high-predation risk for mosquitofish), and sleepers without mosquitofish (naive sleepers). Neither environmental factors (e.g., water color, turbidity, pH, depth, and resources) nor genetic relatedness of both mosquitofish and sleeper populations systematically covaries with fish community (Heinen et al. 2013; Heinen-Kay and Langerhans 2013; Martin et al. forthcoming). However, low-predation mosquitofish populations exhibit far greater population densities than

high-predation populations, presumably resulting in more intense intraspecific competition (Heinen et al. 2013). Sleeper population density and size structure do not differ with mosquitofish presence, and no other fish species are present in sleeper-only blue holes (Martin et al. forthcoming). Sleepers represent the top predator in all blue holes where they are present. This unique “natural experiment” allows us to test multiple questions about how natural selection, sexual selection, and their interaction shapes sexual signal evolution in the wild.

Although Bahamas mosquitofish males have evolved differences in multiple sexual signals between predation regimes (Martin et al. 2014), we focus here on one of the most obvious secondary sexual traits, the bright orange dorsal fin. Males display their dorsal fin to females during courtship and to other males when competing for potential mates (all authors, personal observation). Mosquitofish inhabiting low-predation blue holes have repeatedly evolved dorsal fins exhibiting greater orange coloration relative to counterparts in high-predation populations (Martin et al. 2014). The carotenoid-based orange dorsal-fin coloration has a genetic basis and covaries with body condition and testis size, suggesting that this trait might serve as an honest indicator of male quality (Martin et al. 2014; Giery ST, unpublished data). Previous work has also demonstrated that Bahamas mosquitofish populations in different predation regimes exhibit strong sexual isolation, placing this investigation of sexual signal divergence within the context of ongoing speciation (Langerhans et al. 2007; Langerhans and Makowicz 2013).

We employ a promising method for directly testing predictions about the processes underlying sexual signal diversification: presenting physical models of the study organism that differ only in the secondary sexual trait of interest to both natural predators and female conspecifics. First, to assess the influence of natural selection via predation on dorsal-fin coloration, we used fish replicas differing only in dorsal-fin color to determine whether the orange-shifted dorsal fins characteristic of low-predation mosquitofish populations are more conspicuous to predatory bigmouth sleepers and whether sleeper response depends on evolutionary history (presence or absence of mosquitofish). Testing fin conspicuity with both coevolved and naive sleepers allows us to evaluate both current natural selection and selection at the time of colonization. Second, to assess the influence of sexual selection on dorsal-fin coloration, we used the models to test whether female mosquitofish prefer greater orange coloration in male dorsal fins and whether any observed female preference is genetically based. Given that wild low-predation males consistently possess more orange-shifted fins than high-predation males (Martin et al. 2014), we predict that overall, orange-shifted dorsal fins are simultaneously more conspicuous to predatory bigmouth sleepers and more attractive to female mosquitofish. We additionally examine whether the strength or direction of female preference depends on evolutionary history with sleepers. Due to potential viability costs in males bearing more orange fins, combined with possibly elevated costs of mate preferences, female mosquitofish from high-predation environments might express a reduced preference for orange fin coloration or even a preference for the less orange fin typical in high-predation populations. Considering that prior work has demonstrated that females from different predation regimes exhibit divergent preferences for other traits in this system (male body shape: Langerhans and Makowicz 2013), it is possible that female preference for fin color might have also diverged.

MATERIALS AND METHODS

Model construction

The ability to manipulate a single trait while holding all others constant offers clear advantages for studying the effects of the focal trait on performance and fitness. Compared with phenotypically modified live animals or video playback methods, physical models are easily implemented in both lab and field situations and have been previously used in a number of studies to test predictions about prey conspicuity or susceptibility to predation (e.g., Brodie 1993; Caley and Schluter 2003; Husak et al. 2006) and female mating preferences (e.g., MacLaren et al. 2004; Speares et al. 2007; Kozak et al. 2008; Williams and Mendelson 2013; Williams et al. 2013). To our knowledge, no previous research has used the same models to test both natural and sexual selection in the same system.

We created plastic Bahamas mosquitofish models using 3D scanning and 3D printing technologies. First, we molded a mosquitofish prototype using modeling clay to reflect a male with an intermediate body shape and a large body size within the range of natural variation. Using a NextEngine 3D laser scanner (NextEngine, Santa Monica, CA), we obtained digital shape data that were printed with a Stratasys uPrint 3D printer (Stratasys, Eden Prairie, MN). Because we wished to reduce potential sources of variation between models while maximizing biological realism, we hand-painted models using artist-grade acrylic paint to exhibit some, but not all, physical features including the eyes, pupils, subocular bar, caudal-fin spots, and the pale abdominal region (Figure 1a,b). The color of paint applied on the dorsal fins was mixed by hand to match the average low- and high-predation dorsal-fin color values exhibited in the wild as measured by CIE L^*A^*B and RGB colorspaces in Adobe Photoshop (Martin et al. 2014) and reflectance spectrometry using an Ocean Optics Jaz spectrometer (Ocean Optics, Dunedin, FL) (Figure 1c). Using average fin color values from each predation regime provides a more realistic test of selection on fin color than using extreme color values that may be rare in the wild. Because the caudal fin and gonopodium are translucent, we painted them to mimic the background color of the experimental aquaria used in mate-choice trials (Figure 1b; see below) in an attempt to resemble their appearance in the wild. For the in situ predator trials, these 2 fins were instead painted the same color as the body. We painted all models during a single session using the same batch of paint to minimize variation between models. We used a total of 4 model sets (each set comprised a low- and high-predation color model) in 3 experiments—multiple model sets were employed to avoid biases resulting from any subtle, unintended differences between models. Methods employed in the 3 experiments described below adhered to the Animal Behavior Society's guidelines for ethical animal treatment and were approved by the Institutional Animal Care and Use Committee at North Carolina State University (protocol #13-101-O).

Experiment 1: Conspicuity to predators

Experimental design and procedure

To test whether dorsal fins exhibiting greater orange coloration are more conspicuous to bigmouth sleepers, we conducted trials examining sleeper responses to the models in 5 blue holes (3 high-predation, 2 predator-only; see Supplementary Table S1) from 12 July 2013 to 17 July 2013. Sleepers are continually observed swimming and actively foraging in blue holes throughout daylight hours (Langerhans et al. 2007; Martin et al. forthcoming), and we

conducted all trials between 10:00 and 16:00. We deployed model pairs in situ using a floating T-shaped control bar constructed of PVC pipe. The control bar consisted of a long bar (3 m) connected to a crossbar (0.7 m) from which a low-predation and high-predation models were suspended from opposite ends with fishing line (Figure 1d). Models were suspended at a depth of 1 m, with small fishing weights suspended 30 cm below each model to ensure model stability and prevent underwater drift. At each blue hole, we conducted 20 five-min trials at equally spaced locales around the perimeter of the blue hole within 1 m of the shoreline. For each trial, 2 snorkelers monitored each model from ~3 m away, whereas a fifth snorkeler held the control bar in place. During each 5-min trial, we measured the number of inspections, inspection duration, and number of attacks by sleepers on each model. We defined an inspection as the entry of a sleeper within a 30 cm radius of a model, where the sleeper clearly oriented toward the model. Inspection duration included the time in which at least 1 sleeper was present within 30 cm of a model. Attacks described cases where a sleeper bit or mouthed a model. We alternated the left–right position of the models between each trial (this also alternated the observers for each model type) and conducted 10 trials with each of 2 model sets in each blue hole. We excluded 3 trials due to logistical problems (models resting on ledge or impaired visibility caused by disturbed substrate from blue-hole wall), leaving a total sample size of 97 trials (Supplementary Table S1).

Statistical analyses

To test whether the average low-predation dorsal-fin color was more conspicuous to bigmouth sleepers, and whether evolutionary history with mosquitofish affected sleeper responses, we conducted separate general linear mixed models (LMMs) using the 3 response variables (number of inspections, inspection duration, and number of attacks) as dependent variables. Independent variables included model fin color, mosquitofish presence, and their interaction. We also included blue hole as a random term and trial number nested within blue hole as a random blocking term, thereby treating trials as the unit of replication for tests of model fin color and blue holes as the unit of replication for the test of mosquitofish presence. Time of day, model set, and left–right model position were excluded from final analyses due to nonsignificance. We followed up significant interaction terms with Tukey's honestly significant difference (HSD) tests. We conducted all analyses in JMP (SAS Institute, Cary, NC); data met assumptions of general linear models.

Because we conducted multiple significance tests based on the same set of trials, we can experience inflated Type I error rates. To correct for this without suffering the substantial increase in Type II error rates (reduction of statistical power) associated with Bonferroni correction procedures (e.g., Garcia 2004; Nakagawa 2004; Verhoeven et al. 2005), we controlled the false discovery rate (FDR; Benjamini and Hochberg 1995; Storey 2003; Storey and Tibshirani 2003). FDR describes the proportion of significant tests that are actually null. We used the program QValue (Storey 2002) to control the FDR at 5% using the bootstrap procedure to estimate π_0 (probability of a true null hypothesis). We interpreted P values ≤ 0.05 as significant if the q value (FDR equivalent of P value) determined by QValue for that particular test was also ≤ 0.05 .

Experiment 2: Wild-caught female preferences

Experimental fish

To test whether sexual selection currently favors greater orange coloration in male dorsal fins, we conducted dichotomous choice tests

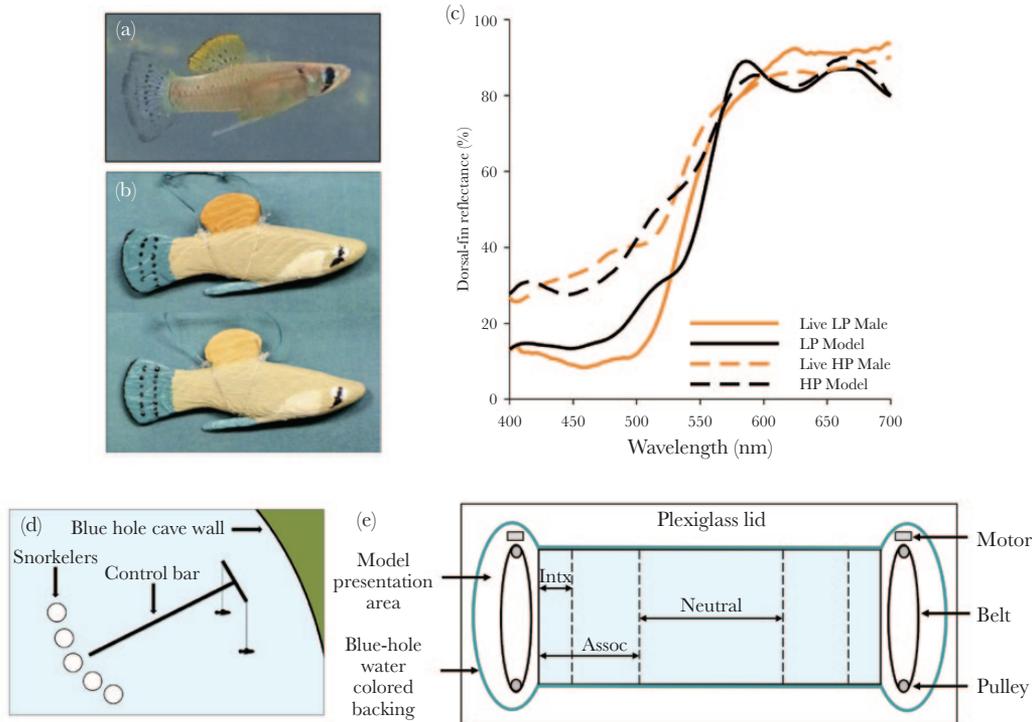


Figure 1

Illustration of study organism and methodological details. (a) Underwater photograph of a male Bahamas mosquitofish in a blue hole. (b) Photograph of mosquitofish models exhibiting average low-predation (LP, top) and high-predation (HP, bottom) dorsal-fin coloration. (c) Reflectance spectra of dorsal fins for the painted models and representative live Bahamas mosquitofish males. Top view of experimental apparatus for (d) predator trials in blue holes and (e) mate-choice trials for both wild-caught and lab-born mosquitofish. Panel (e) depicts the interaction zones (Intx), association zones (Assoc), and neutral zone within the experimental tank (light blue), as well as the motors, belts, and pulleys outside the tank, which were attached to the plexiglass lid that rested on top of the experimental tank. Mosquitofish models were suspended from the belt with fishing line.

with wild-caught females ($n = 70$; Supplementary Table S1) using the models as stimuli. We captured adult female Bahamas mosquitofish between 11 July 2013 and 18 July 2013 from 6 blue holes (3 low-predation, 3 high-predation) using handheld dip nets and transported the fish to Forfar Field Station on Andros Island for experimental trials. Female mosquitofish were isolated from males for at least 2 days prior to mate-choice trials and tested within 2 weeks of capture. Fish were fed a diet of Tetra-min Pro fish flakes while in captivity.

Dichotomous choice trials

Dichotomous choice tests were conducted in a plexiglass aquarium (interior dimensions: 51 cm long \times 20 cm wide \times 14 cm deep) filled to a depth of 8 cm with a combination of water from the female's original holding tank and tap water aged at least 24 h and treated with Amquel Plus and Fish Protector. Water temperature ranged from 27.0 to 29.7 °C (average water temperature in blue holes at the time of capture was 30 °C). Models were attached by fishing line to belts positioned at opposite ends of the experimental tank, outside and above the aquarium (low-predation model at one end, high-predation model at the other). The belts attached to a motor and pulley system (all out of view of the female) so that each model "swam" through the air in 22-cm-long ellipses parallel to the tank sidewalls (Figure 1e; Kozak et al. 2008). The entire aquarium and model presentation area were lined with paper printed with the average color of water in blue

holes (Martin et al. 2014) with the exception of the top, which had clear plexiglass for video recording of trials from above (Figure 1e). This served to mimic an average blue-hole environment background and prevent the fish from witnessing any motion outside the tank other than the models. The fishing line that attached each model to the belt was painted a similar blue color to minimize its visibility to the female. Black lines on the paper lining the aquarium bottom visually divided the tank into 5 segments (the experimental female could move freely about the tank): interaction zones (5 cm long) were at opposite ends of the tank adjacent to the model presentation area; association zones (15 cm long) included each interaction zone and an additional 10 cm length of the tank; a neutral zone (21 cm long) comprised the center of the tank (Figure 1e). We used 2 different model sets in dichotomous choice trials to ensure fish responses did not result from subtle variation between models.

Each trial commenced with a 5-min acclimation period where we turned the motors on and placed a single female into the experimental tank. Pieces of blue-hole water color paper ("curtains") blocked the model viewing area, which prevented the female from seeing the models during the acclimation period. After 5 min, we pulled away the paper curtains to expose the models to the female and began video recording the tank from above using a Sony DCR-SR68 camcorder (Sony, New York, NY) for the first 5-min observation period. Following this observation period, we allowed the motors to continue running for a 2-min intermission

period where we again blocked the female's view of the models with paper curtains and reversed the left–right positioning of the models, allowing us to account for any potential side biases exhibited by experimental females. After the intermission, we removed the paper curtains and video recorded the tank from above for an additional 5-min observation period. Thus, an entire trial lasted 17 min, 10 of which contained behavioral observations. Initial right–left presentation of models was alternated between each trial. We conducted trials during normal activity hours of these fish (all trials between 7:00 and 19:30) and tested individuals from each population on at least 2 different days to avoid confounding trial day with population. We sacrificed females immediately following each trial with an overdose of MS222 and individually preserved females in 95% ethanol. Because female body size may covary with choosiness in livebearing fishes (Kahn et al. 2010), we used tpsDig2 software (Rohlf 2010) to measure standard length (tip of rostrum to apex of the hypural plate) from lateral photographs captured with a Canon Rebel XS digital camera (Canon, Melville, NY).

Using the video recordings of mate-choice trials, we measured the total amount of time a female spent in the tank zones corresponding to each model, summed across the entire 10-min observation period. We defined occurrence within each zone as the presence of the entire head of the female (anterior to pectoral fins). Because it is unclear how to best quantify female preference, we used 3 different “mating response” metrics: 1) interaction zone time (5 cm proximity to model presentation area), 2) association zone time (15 cm proximity to model presentation area), and 3) proportional interaction zone time (interaction zone time/association zone time). Interaction zone time likely reflects a conservative estimate of female preference because a fish within a given interaction zone is quite close to the model presentation area relative to the size of the experimental tank (~10% of the tank area) and their own body size (average body length of wild-caught experimental females: 37.8 mm \pm 0.58 standard error [SE]). Prior work in livebearing fishes has demonstrated that female association time during mate-choice experiments predicts whether a female will subsequently mate with a given male (Walling et al. 2010). Proportional interaction time reflects the time a female spent in very close proximity to a model (interaction zone) relative to the time she had the opportunity to interact with the model (association zone). Previous work has found that proportional interaction time provides a useful indicator of how attractive a female finds a male stimulus given her overall interest in associating with the stimulus (Johnson and Basolo 2003; Langerhans et al. 2007; Langerhans and Makowicz 2013).

Statistical analyses

We tested whether wild-caught female mosquitofish preferred to associate with the low-predation model and whether this preference differed between predation regimes by conducting separate LMMs using the 3 estimates of mating response (interaction zone time, association zone time, proportional interaction zone time) as dependent variables. Model fin color, predation regime, and their interaction served as predictors. We also included blue hole and female ID as random effects; individuals served as the unit of replication for tests of model fin color, whereas blue holes served as the unit of replication for the test of predation regime. Standard length and model set were excluded from final analyses due to nonsignificance. We followed up significant interaction terms with Tukey's HSD tests. Because we conducted multiple tests for the

same trials, we again controlled the FDR at 5% using methods described above.

Experiment 3: Lab-born female preferences

Experimental fish

To test for a genetic basis and heritability of female preferences for male dorsal-fin color, we conducted dichotomous choice trials with lab-born F1 generation virgin female Bahamas mosquitofish ($n = 34$). Experimental fish represent the offspring of 8 different females captured as adults from Cousteau's blue hole (high-predation). Thus, we did not assess the genetic basis for any possible differences in female preference among populations—we were specifically interested in testing whether preferences observed in wild-caught fish had a genetic basis (i.e., did not require mating experience or native environmental cues) and whether genetic variation for female mating preference exists within a population (i.e., heritability), indicating that preferences could evolve. Fish were raised in sibling groups under common lab conditions in 7-L tanks, fed a diet of live brine shrimp, Tetra-min Pro fish flakes, and freeze-dried *Daphnia* and bloodworms, and had never experienced nor witnessed any copulations. Male siblings were removed from each tank prior to reaching sexual maturity. Six of the wild-caught mothers produced multiple daughters in the lab, permitting a test of heritability of mate preference ($n = 32$). We conducted dichotomous choice tests using methods identical to those described above for wild-caught fish, except that experimental females were not sacrificed at the end of the trial. Instead, we photographed fish alive for standard length measurement. Water temperature in the experimental tank ranged from 23 to 27 °C (24.4 ± 0.19 SE), similar to the rearing temperature of the fish (24–26 °C).

From the video recordings of mate-choice trials, we measured the same 3 estimates of mating response as described above but additionally calculated estimates of mating preference associated with each of the 3 response types to evaluate individual variation in model preference for heritability analyses (following methods in Langerhans and Makowicz 2013). For interaction and association zone times, we separately calculated mating preference as the time associating near the low-predation model minus the time near the high-predation model divided by the sum of time spent associating with both models. For proportional interaction time, we calculated mating preference simply as the low-predation model value minus the high-predation model value. These estimates of mating preference can range from -1 (absolute preference for the low-predation model) to 1 (absolute preference for the high-predation model), with 0 representing no preference.

Statistical analyses

Because mating response data exhibited nonnormality, we tested whether lab-born virgin females possessed a preference for the low-predation model using nonparametric Wilcoxon signed-rank tests. We separately tested for differences between model types in interaction zone time, association zone time, and proportional interaction zone time. Using the mate preference scores, we conducted separate analyses of variance (Anovas; data met assumptions for Anova) to test for heritability of mate preference based on full-sib family differences. Due to our modest sample size, we do not report heritability estimates but rather focus exclusively on the significance tests for genetic variation in female preference. To avoid inflated Type I error rates due to conducting multiple tests for the same trials, we again controlled the FDR at 5% using methods described above.

RESULTS

Experiment 1

For the number of inspections and inspection duration, the significant interaction term indicated that the effect of model color depended on the presence of mosquitofish within the blue hole (Table 1). Post hoc tests revealed that sleepers inhabiting mosquitofish-free blue holes more frequently inspected and spent more time inspecting the orange-shifted low-predation model than the high-predation model (Figure 2). Naive sleepers also exhibited higher overall responsiveness to the models, inspecting both models significantly more than coevolved sleepers. Number of attacks did not differ between the models or in association with mosquitofish presence (Table 1). On average, the number of attacks per trial on either model type was low within all blue holes (least-squares means: 1.0–1.4).

Experiment 2

As predicted, wild-caught female mosquitofish preferred the low-predation model with the more orange dorsal fin. Females spent significantly more time in the association zone of the low-predation model compared with the high-predation model (Table 2 and Figure 3a). Although not significant using $\alpha = 0.05$ with 2-tailed tests, we observed a trend in the same direction for both proportional interaction zone time and interaction zone time (the latter being significant with a 1-tailed test, matching our a priori prediction). Females originating from different predation environments did not differ in fin color preferences (Table 2).

Experiment 3

Wilcoxon signed-rank tests revealed that lab-born virgin females from Cousteau's blue hole spent significantly more time associating with the orange-shifted low-predation model as indicated by the interaction zone and proportional interaction zone times during dichotomous choice tests; association zone time did not meet statistical significance although the pattern was in the predicted direction (Table 3 and Figure 3b). Furthermore, our Anova testing for differences among families indicated significant heritability for female preference for more orange dorsal fins based on interaction zone time and proportional interaction zone time; association zone time exhibited a suggestive trend in the same direction (Table 4).

DISCUSSION

To our knowledge, this is the first study to empirically examine both natural and sexual selection using the same experimentally manipulated stimuli across multiple populations within the context of ongoing sexual signal divergence. Using physical models

of male Bahamas mosquitofish that mimicked the average dorsal-fin color observed in either low- or high-predation populations, our findings suggest that the orange-shifted low-predation dorsal fins are simultaneously more conspicuous to naive predators and more attractive to both experienced and virgin female conspecifics. These results bolster the selection trade-off hypothesis as a compelling mechanism that can explain evolutionary diversification of secondary sexual traits while also uncovering system-specific nuances. For instance, female preference for orange coloration in males did not weaken in the presence of predatory bigmouth sleepers despite the apparent viability cost of colorful fins in that environment.

We had the remarkable opportunity in this study to examine naive predators in an attempt to uncover how selection likely operated at the time of blue-hole colonization. This provides a strong test of whether natural selection via sleeper predation might have driven the reduced orange fin coloration in high-predation mosquitofish, as it avoids confounding factors such as the subsequent evolution of refined search images for prey. Evidence from naive sleepers confirmed that greater orange coloration of mosquitofish dorsal fins indeed draws more attention from these predators.

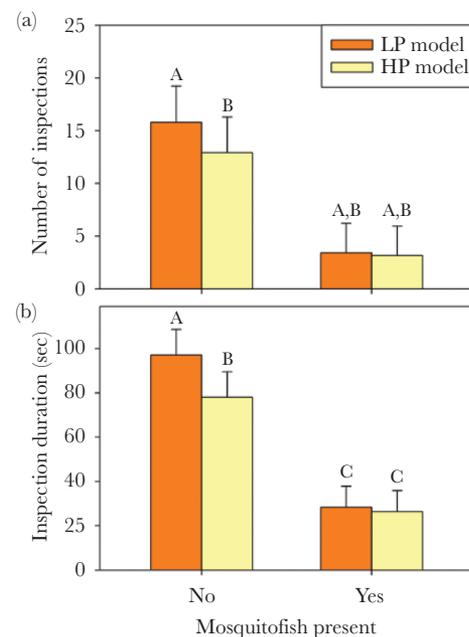


Figure 2

(a) Number of inspections and (b) inspection duration for the low-predation (LP) and high-predation (HP) mosquitofish models exhibited by bigmouth sleepers living in the presence and absence of mosquitofish. Least-squares means \pm SE depicted. Letters indicate significant differences.

Table 1

Results of general LMMs examining variation in inspection behaviors of predatory bigmouth sleepers in response to model Bahamas mosquitofish stimuli in blue holes

Source	Number of inspections			Inspection duration			Number of attacks		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Model fin color	10.45	1, 95	0.0017	6.31	1, 95	0.0137	0.06	1, 95	0.8019
Mosquitofish presence (MP)	6.36	1, 3.02	0.0855	17.67	1, 3.04	0.0240	0	1, 2.87	0.9808
Model fin color \times MP	7.26	1, 95	0.0083	4.15	1, 95	0.0445	0.65	1, 95	0.4213

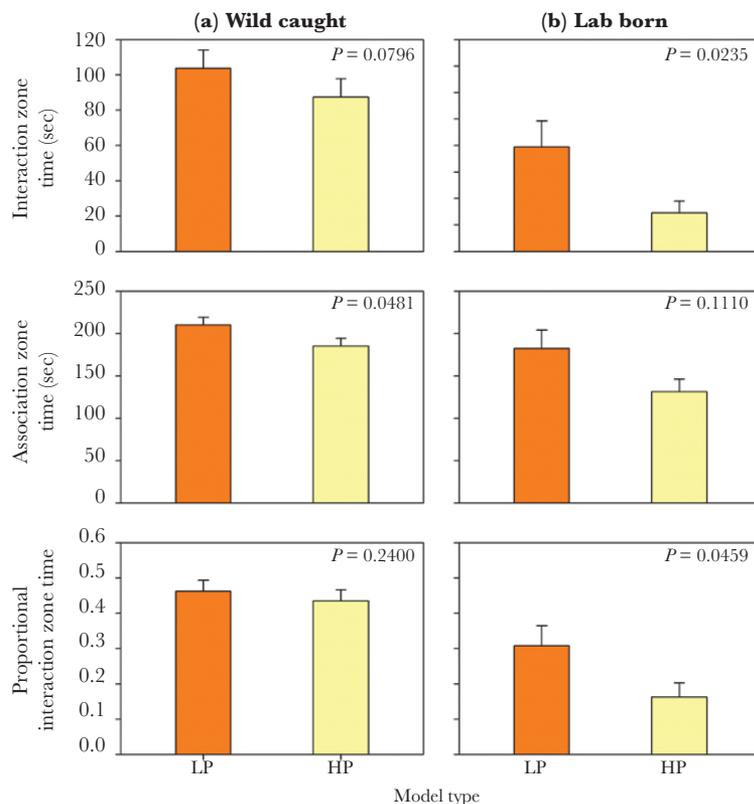
P values ≤ 0.05 that remained significant after controlling for a FDR of 5% are bolded. df, degrees of freedom.

Table 2

Results of general LMMs examining variation in mating response of wild-caught female Bahamas mosquitofish for low- and high-predation models during dichotomous choice trials

Source	Interaction zone			Association zone			Proportional interaction zone		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Model fin color	3.1665	1, 68	0.0796	4.0515	1, 68	0.0481	1.4051	1, 68	0.2400
Sleeper presence (SP)	0.8522	1, 4,081	0.4072	1.5110	1, 3.94	0.2873	0.3348	1, 4,124	0.3348
Model fin color × SP	0.0071	1, 68	0.9333	0.5380	1, 68	0.4658	0.4727	1, 68	0.4727

P value ≤ 0.05 that remained significant after controlling for a FDR of 5% is bolded. df, degrees of freedom.

**Figure 3**

Three estimates of mating responses of (a) wild-caught and (b) virgin lab-born female Bahamas mosquitofish during dichotomous choice tests between low-predation (LP) and high-predation (HP) male mosquitofish models. Least-squares means ± SE depicted.

Table 3

Results of Wilcoxon signed-rank tests investigating preference for the orange-shifted dorsal-fin coloration in the low-predation model compared with the high-predation model in lab-born female Bahamas mosquitofish

Mating response	<i>z</i>	<i>P</i>
Interaction zone	-113.50	0.0235
Association zone	-93.50	0.1110
Proportional interaction zone	-101.0	0.0459

P values ≤ 0.05 that remained significant after controlling for a FDR of 5% are bolded.

Although these results support the notion that male Bahamas mosquitofish have evolved reduced sexual ornamentation in high-risk environments because bright orange fins attract the notice of predatory sleepers and decrease survivorship, we found that the effect

of dorsal-fin color on predatory inspections largely depended on whether sleepers co-occurred with mosquitofish.

Larger diet breadth and less refined prey search images may explain why naive sleepers exhibited both a stronger response toward the low-predation model and more responsiveness overall to the experimental mosquitofish models than sleepers that coevolved with mosquitofish. Naive sleepers consume a greater diversity of prey items (including at least 8 orders of insects, as well as crustaceans, gastropods, and arachnids) than coevolved sleepers that primarily consume mosquitofish and large insects (Martin et al. forthcoming). With a broad array of potential prey items, naive sleepers may be more likely to approach a novel item to assess its suitability as prey compared with coevolved sleepers. Moreover, naive sleepers may lack a search image for Bahamas mosquitofish, allowing the model dorsal-fin color to greatly affect its perceived conspicuousness and attractiveness as prey. Meanwhile, a history of intimate predator-prey coevolution in high-predation blue holes

Table 4
Summary of results from Model II Anovas examining heritable variation in female preferences for male Bahamas mosquitofish dorsal-fin coloration

Mating response	<i>F</i>	df	<i>P</i>
Interaction zone	2.73	5, 26	0.0411
Association zone	2.24	5, 26	0.0804
Proportional interaction zone	4.83	5, 26	0.0029

P values ≤ 0.05 that remained significant after controlling for a FDR of 5% are bolded. df, degrees of freedom.

may have resulted in sleepers that possess a highly refined search image for mosquitofish prey. Owing to this strong search image and their primarily piscivorous diet, coevolved sleepers may rapidly approach motionless mosquitofish (models) from a distance but quickly realize without close inspection that the models are not actually live fish (i.e., not profitable prey items). This efficient rejection of plastic models as potential fish prey could mask the occurrence of greater conspicuity or attractiveness of the low-predation model.

Alternatively, selection against orange dorsal-fin coloration in high-predation environments might primarily involve correlated traits and not color per se—for example, males with orange-shifted dorsal fins might exhibit larger body size, more risky courtship behaviors, and less vigilance (Cordes et al. 2014; Martin et al. 2014). If true, then our results suggest that during early stages of predator–prey coevolution, selection might act directly against orange coloration but later act indirectly against orange coloration via selection on correlated traits. Sensory bias seems unlikely to explain differences in sleeper responses to mosquitofish models: although sleeper morphology and diet differ between blue holes with and without Bahamas mosquitofish (Martin et al. forthcoming), no evidence supports differences in sensory bias for orange perception or response between these environments—sleepers lack orange coloration in all sites, and no other orange-colored diet items are known.

Matching our prediction, female mosquitofish preferred to associate with the low-predation model during dichotomous choice trials. Therefore, sexual selection by female mate choice indeed favors greater orange dorsal-fin coloration in males in this system, consistent with ample evidence from other systems where females prefer mates with greater sexual ornamentation (Andersson 1994; Andersson and Simmons 2006). Combined with viability costs of orange dorsal fins in the presence of sleepers, the mating advantage of more colorful males in Bahamas mosquitofish presumably explains the evolution of greater orange coloration in the absence of bigmouth sleepers (Martin et al. 2014).

The most prominent models of sexual selection require genetically based female preferences (Andersson and Simmons 2006), but few studies have investigated heritability of preferences compared with those examining heritability of sexual trait expression (Bakker and Pomiankowski 1995; Mead and Arnold 2004; Sharma et al. 2010). We confirmed here that female preferences for dorsal-fin coloration have a genetic basis, with significant heritability within at least one high-predation blue hole. Thus, this preference for greater ornamentation does not solely reflect environmental effects or learning in female Bahamas mosquitofish. But why might female Bahamas mosquitofish prefer greater orange coloration in male dorsal fins?

We suggest that this carotenoid-based trait may serve as an indicator trait, relaying information about a potential mate's ability to

provide indirect (genetic) benefits, with female preference evolving through a “good genes” mechanism (Kirkpatrick 1987; Maynard Smith 1991; Andersson 1994). Low-predation blue holes possess high mosquitofish densities, likely resulting in intense resource competition (Heinen et al. 2013). Given that carotenoids must be obtained from the environment, more orange fins might signal superior foraging efficiency. Though high-predation environments harbor less dense mosquitofish populations (Heinen et al. 2013), high-predation females may still value foraging ability or condition/health—carotenoid-based traits sometimes reflect immune response or parasite resistance (Lozano 1994; Hill and Farmer 2005). Although foraging ability should not prove critically important in high-predation environments, parasite infection is common across all blue holes (Langerhans RB, Johnson PTJ, unpublished data), thus parasite resistance could potentially explain indirect benefits associated with carotenoid signals in both environments. It is also possible that fin color signals different information in different environments: for example, fin color might indicate predator evasion ability, rather than foraging efficiency, in high-predation populations. A signal advertising this ability could provide useful information to both females—who should indirectly benefit from mating with locally successful males by having offspring that inherit this trait (Lorch et al. 2003; Badyaev 2004)—and predators about viability and ability to deter predatory attacks by indicating high escape potential (Godin and Dugatkin 1996; Leal 1999). In this case, females prefer males with greater orange coloration in their dorsal fins because they suffer greater conspicuity to predators but have nonetheless survived. Future work should attempt to elucidate the mechanism(s) underlying female preference for, and the information content of, orange dorsal fins.

Although trade-offs between natural and sexual selection are commonly invoked to explain patterns of sexual signal diversification, other mechanisms can produce patterns consistent with this interpretation. Thus, we must empirically test specific hypotheses of how both aspects of selection act on sexual signals if we wish to uncover the importance of the selection trade-off hypothesis in the wild. For instance, differences in resource availability can lead to sexual trait differences across environments, especially for carotenoid-based sexual signals (Grether et al. 1999). Sensory environments (e.g., background color) can also drive population divergence in sexual traits to maximize signal detectability in different transmission environments (Boughman 2002; Leal and Fleishman 2004). These factors can also covary with predation risk in nature, obscuring the causes of signal diversification. In this study system, previous work has demonstrated the importance of resource availability and background light environment in among-population variation in dorsal-fin color, in addition to independent divergence of fin color between predation regimes (Martin et al. 2014). With multiple possible selective agents responsible for the evolution of sexual signal diversity, experimental approaches provide an especially powerful means of revealing the importance of the selection trade-off hypothesis compared with other mechanisms. Our results indicate that a trade-off between natural and sexual selection can indeed drive major patterns of signal diversity during an adaptive radiation.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.beheco.oxfordjournals.org/>

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