Habituation underpins preference for mates with novel phenotypes in the guppy

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Abstract

Populations harbor enormous genetic diversity in ecologically important traits. Understanding the processes that maintain this variation is a long-standing challenge in evolutionary biology. Recent evidence indicates that a mating preference for novel sexual signals can be a powerful force maintaining genetic diversity. However, the proximate underpinnings of this preference, and its generality, remain unclear. Here, we test the hypothesis that preference for novel sexual signals is underpinned by habituation, a nearly ubiquitous form of learning whereby individuals become less responsive to repetitive stimuli. We use the Trinidadian guppy (*Poecilia reticulata*), in which male colour patterns are diverse yet heritable. We show that repeated exposure to males with a given colour pattern reduces female interest in males with that pattern, and that interest recovers following brief isolation. These results fulfil two core criteria of habituation: responsiveness decline, and spontaneous recovery. To distinguish habituation from sensory adaptation and fatigue, we also demonstrate stimulus specificity and dishabituation. These results provide the first evidence that habituation causes preference for novel sexual signals, addressing the mechanistic underpinnings of this mating preference. Given the pervasiveness of habituation among taxa and sensory contexts, our findings suggest that preference for novelty may play an underappreciated role in mate choice and the maintenance of genetic variation.

Keywords: Habituation, Sexual selection, Mate choice, Sensory bias, Genetic diversity, Frequency dependence

Introduction

To understand the processes that maintain genetic diversity is a central goal in evolutionary biology [1,2]. Populations of organisms are often observed to have higher levels of genetic variation than can be explained by standard population genetic models that incorporate directional selection, mutation, and genetic drift [3,4]. Negative frequency-dependent selection, in which the fitness of a genotype is greater when that genotype is rare, provides one possible explanation for this diversity because it can maintain high levels of variation without genetic load [5,6]. One ecological process capable of generating negative frequency-dependent selection is a rare male mating advantage, wherein males with rare phenotypes garner higher mating success. A recent field experiment confirmed that male guppies (*Poecilia reticulata*) with rare sexual signal variants had greater mating success than common variants, and implicated a female mating preference for suitors with novel phenotypes as the cause [7]. Intriguingly, a mating preference for novel sexual signals has been reported for several taxa, including three species of poeciliid fishes [8-10], fruit flies [11], and humans [12]. However, the proximate mechanism(s) responsible for preference for novel phenotypes remain elusive, as do its evolutionary origins. Addressing these gaps is important for predicting the generality of this preference, and for better understanding its consequences for sexual selection and the maintenance of genetic diversity.

Here, we investigate a potential mechanism underlying preference for novel phenotypes during mate choice. We test the hypothesis that this preference is underpinned by habituation to familiar sexual signals. Habituation is formally defined as the process whereby repeated exposure to a given stimulus (e.g., a sexual signal) causes a decline in the responsiveness of an individual (e.g., mating interest) to that stimulus, beyond the effects of sensory adaptation or fatigue [13,14].

Importantly, habituation involves a degree of stimulus specificity, meaning that the decline in responsiveness does not extend to other stimuli of the same sensory modality [14]. It is this stimulus specificity that could generate a preference for mates with novel phenotypes: if the decline in responsiveness is specific to the phenotype variants that an individual repeatedly encounters, then interest in unfamiliar or rare variants should not decline to the same extent, resulting in preference for novel phenotypes.

Indeed, there is some evidence that animals habituate to components of sexual signals. For example, in grackles, males that had their repertoires artificially enhanced to have multiple syllables were preferred to control males that repeated a single syllable [15]. This may be because females habituated to repeated songs. Similarly, zebra finches habituate to same-song notes but responsiveness is restored with transitions to different notes [16]. These examples suggest that habituation may lead animals to favour more complex or variable signals [17]. However, we are not aware of any studies investigating whether habituation generates a preference for novel phenotypes. This distinction is important because it is preference for novel phenotypes that can result in negative frequency-dependent selection on sexual signals, thereby maintaining genetic variation within populations.

Intriguingly, habituation is considered the simplest form of learning, and is believed to be ubiquitous among animals [13,14]. Furthermore, evidence of habituation has been found for a wide range of ecologically relevant behaviours [14,18], including anti-predator responses (e.g. [19,20]), foraging behaviours (e.g. [21]), and exploration (e.g. [22]). Given its pervasiveness, it seems likely that habituation is a general process that shapes responses to sexual signals.

Determining whether habituation underpins preference for mates with novel phenotypes could therefore provide insight into the potential generality and evolutionary origins of preference for novel phenotypes.

We used the Trinidadian guppy to ask whether habituation can generate preference for males with novel colour patterns. The guppy is an excellent study system because it has well-characterized reproductive behaviours, and because the role of preference for novel phenotypes in generating a rare male mating advantage has been extensively documented in this species. The colour patterns of adult male guppies are diverse yet heritable, providing one of the most extreme examples of morphological polymorphism known [23,24]. Females exhibit robust preferences for males with colour patterns that are unfamiliar (i.e. dissimilar to those they have previously seen) [8,25-27], and also prefer males with rare colour patterns over those with common patterns [28,29]. Furthermore, a field experiment has demonstrated a mating advantage for males with rare colour patterns in natural guppy populations [7]. Lastly, past studies on habituation to sexual signals have focused exclusively on the auditory system [15,16]; the guppy provides an excellent opportunity to provide the first test, to our knowledge, of habituation to visual patterns.

To determine whether preference for novel colour patterns is underpinned by habituation, we exposed female guppies to a series of stimulus males, and then assayed their mating interest in males with colour patterns that were either very similar to, or very different from, the stimulus males. These data allowed us to test criteria that are widely used to demonstrate habituation [13,14].

Methods

Overview

We tested four key characteristics of short-term habituation. The first of these criteria is responsiveness decline: repeated exposure to males with a given colour pattern should reduce a female's mating interest in males with that colour pattern. Second, we tested for spontaneous recovery: following responsiveness decline, temporarily withholding the stimulus should cause the response to recover. Third, responsiveness to the habituated stimulus should recover following exposure to a novel stimulus, a phenomenon called dishabituation. The fourth characteristic is that the responsiveness decline should involve a degree of stimulus specificity. Importantly, the latter two criteria – dishabituation and stimulus specificity – are unique to habituation learning, and are therefore commonly used to distinguish between habituation and alternative explanations such as other forms of learning, sensory adaptation, and fatigue [13,14]. Fatigue occurs when a stimulus energetically taxes an organism's sensory and/or behavioural systems, reducing its responsiveness to future stimuli. Because fatigue is not limited to the systems that process and respond to a single type of stimulus, stimulus specificity is not observed. Additionally, fatigue is not reversed by the presentation of a novel stimulus, meaning that dishabituation does not occur. Sensory adaptation refers to an organism's sensory peripheries becoming less sensitive as a result of extended stimulus exposure, causing perception of the stimulus to fade over time. One example of this is "nose blindness", in which an organism becomes unable to detect a repeatedly encountered scent. This differs from habituation, in which the decline in responsiveness is caused by an attentional shift away from the repetitive stimulus, due to changes in the central nervous system. The higher order level of processing involved in habituation means that habituation (but not sensory adaptation) is characterized by dishabituation

and stimulus specificity. We also asked whether guppies exhibit long-term habituation to male colour patterns, in which extended exposure results in responsiveness decline that persists for long time periods (e.g., hours or days) without spontaneous recovery. The protocols used to test each of these criteria are detailed below.

Study system and husbandry

The guppy is a live-bearing species with a promiscuous mating system in which males are persistent in their pursuit of females, and females are choosy [30]. The guppies in our experiment were lab-reared descendants of the "Houde" tributary of the Paria river in Trinidad. At sexual maturity, males develop complex colour patterns that are heritable and at least partially Y-linked, yet also extremely polymorphic. Consequently, within natural populations a given male will typically have a colour pattern similar to a few other males, but different from the rest [7,30]. We used males derived from two Iso-Y lines, which differ from one another in the non-recombining region of the Y chromosome but otherwise share the same (non-inbred) genetic background [27,31]. Consequently, patterns are similar among males from a given line, and differ substantially between lines (Figure 1), allowing us to discretely categorize males as having similar patterns (i.e. same Iso-Y line) or different patterns (i.e. different lines). The origin and maintenance of these lines is described in Supplementary Methods.

Males and females were removed from their parents within 12 h after their birth, and placed in rearing tanks. As they matured, we sorted these fish into single-sex tanks where they were held until they were used in the experiment (at approx. 114 to 156 days old). Rearing and single-sex tanks were visually isolated from adult males so that females were naïve to male colour patterns

at the start of the experiment. Additional information on fish rearing and husbandry is in Supplementary Methods. All procedures involving live animals were reviewed and approved by the FSU Animal Care and Use Committee (protocol #1740).

Stimulus exposure

We exposed virgin females to a series of stimulus males with similar colour patterns, and then assayed female preference for males with colour patterns that were either familiar (i.e. similar to those stimulus males) or unfamiliar (i.e. dissimilar to those stimulus males). Our experimental design included treatments in which each female was exposed to either 3, 6, 9, 12, or 15 different males with the same colour pattern. We designed a divided tank setup that allowed us to expose females to males in a controlled manner, and with minimal stress to females. This divided tank consisted of a female compartment on one side, separated from a male compartment on the other side by three acrylic dividers. The outer two dividers were clear and watertight, preventing olfactory communication and minimizing disturbance to the female caused by netting males into and out of the male compartment. The middle divider was opaque blue and attached to a pulley system that allowed us to raise and lower it. Prior to the experiment, we habituated males and females to the movement of the opaque divider, eliminating behavioural stress responses elicited by the divider's movement (Supplementary Methods). We excluded a small number of fish (7 females and 2 males) that exhibited these responses after 6 days of habituation to the divider.

During the experiment, we placed a single focal female in the female compartment and exposed her to one stimulus male at a time by placing the male in the male compartment and slowly raising the opaque divider. At the end of the exposure period, the opaque divider was lowered so

185 that the male could be replaced with another male from the same line with minimal disturbance
186 to the female. This process was repeated following the habituation paradigm outlined below.
187 Each exposure lasted 2 min, and the opaque divider was lowered for 1 min between exposures.
188 Traditionally, in habituation experiments, behavioural responses of the individual are measured
189 each time the stimulus is applied. However, female guppy mating interest can be most reliably
190 measured when fish are allowed to freely interact. Therefore, we tested female mating interest by
191 conducting mating trials subsequent to these exposure periods. Mating interest assays consisted
192 of allowing the female to freely interact with a single male while we scored their reproductive
193 behaviours. Because a female's experiences during a mating trial could bias her subsequent
194 mating interest, we tested each female only once, immediately after exposure (except where
195 otherwise indicated). A female was never exposed to the same male more than once throughout
196 exposure and testing. This decoupled habituation to colour patterns from familiarity with
197 individual males. Females were all virgins at the start of the experiment, in order to avoid
198 variation in mating history or exposure to males that could have affected mating interest. Further
199 information on procedures for stimulus exposures are in the Supplementary Methods. Prior to the
200 experiment, we screened males based on their mating effort (Supplementary Methods). We only
201 used males that exhibited relatively high levels of sexual behaviours, to ensure that the males in
202 our experiment actively solicited the attention of females.
203
204 *Criterion 1: Responsiveness decline*
205 The first criterion we tested is responsiveness decline: repeated exposure to males that all had
206 similar colour patterns should reduce a female's mating interest in males with that colour pattern.
207 To provide a baseline, we included a treatment in which females were naïve (i.e. had not been

exposed to any males prior to the mating interest assay). We compared the mating interest of these naïve females against that of females exposed to either 3, 6, 9, 12, or 15 stimulus males that had the same colour pattern as the male used to test female mating interest. We hereafter refer to these responsiveness decline treatments as "naïve" "3", "6", "9", "12", and "15". Females in these and all other treatments experienced otherwise similar rearing and husbandry conditions. If female exhibited responsiveness decline, we predicted that female mating interest should be lower for females that were exposed to the 15 stimulus males than for naïve females. To determine how many exposures were required to elicit responsiveness decline, we compared naïve females against the treatments in which females were exposed to either 3, 6, 9, or 12 stimulus males before testing. Treatments used to test responsiveness decline and other criteria of habituation are summarized in Figure 2. Predictions associated with each criterion are depicted in Supplementary Figure S1.

Criterion 2: Spontaneous recovery

We tested for spontaneous recovery by testing whether withholding the stimulus caused the response to recover. That is, females should show increased mating interest in males with the familiar pattern if they are briefly isolated from males after exposure. For this treatment, after presenting the female with 15 stimulus males (as above), we held them in isolation for 30 min before testing their mating interest in a male with the same colour pattern as the stimulus males. During the isolation period, females were left within the divided tank with the opaque barrier lowered such that they were not exposed to any males. We predicted that the mating interest of females that were isolated before testing should be higher than in the "15" treatment, in which females were also exposed to 15 stimulus males but were tested immediately.

231

232 *Criterion 3: Dishabituation*

233 Responsiveness to the habituated stimulus should recover following exposure to a novel
234 stimulus, a phenomenon called dishabituation. That is, after exposure to a series of stimulus
235 males with similar patterns, exposure to a male with a novel colour pattern should renew interest
236 in males with the familiar pattern. For this treatment, after presenting the female with 15
237 stimulus males, we exposed her to a 16th male with a different colour pattern and then
238 immediately assayed her mating interest in a male with a colour pattern similar to the original
239 stimulus males. We predicted that female mating interest should be greater than for females in
240 the "15" treatment, who were not exposed to the novel colour pattern prior to testing.

241

242 *Criterion 4: Stimulus specificity*

243 The fourth characteristic of habituation is that the responsiveness decline should exhibit stimulus
244 specificity. This means that exposure to a series of stimulus males with similar colouration
245 should reduce interest in males with the same colour pattern more than it reduces interest in
246 males with a different colour patterns. We tested for stimulus specificity by exposing the female
247 to 15 stimulus males, and then testing her mating interest in males with a different colour pattern
248 than the stimulus males. We made two predictions. First, female mating interest in the stimulus
249 specificity treatment should be higher than for females in the "15" treatment, who were tested
250 with males that had the familiar colour pattern. Second, the mating interest of females in the
251 stimulus specificity treatment should be similar to that of naïve females. Importantly,
252 dishabituation and stimulus specificity distinguish habituation from sensory adaptation, fatigue,

253 or other forms of learning [14]. Therefore, results fulfilling all four of the criteria described thus
254 far are diagnostic of habituation.
255
256 *Criterion 5: Long-term responsiveness decline*
257 Habituation can operate over both short and long timescales [14,32], so we also asked whether
258 guppies exhibit long-term habituation. Long-term habituation can occur after many stimulus
259 exposures, resulting in responsiveness decline that persists over a long timescale–typically days
260 or weeks–without spontaneous recovery [13,14,32]. We tested two criteria that are indicative of
261 long-term habituation. First, we tested for long-term responsiveness decline. We did this by
262 exposing females to 15 similar stimulus males each day, for 4 consecutive days. Females were
263 then isolated from males for 24 h, and on the fifth day were tested with a male that had the same
264 colour pattern as the stimulus males. We predicted that if females in the long-term
265 responsiveness decline treatment did not exhibit spontaneous recovery, then their mating interest
266 would not be significantly different from that of the females in the "15" treatment who were
267 tested immediately.
268
269 *Criterion 6: Long-term stimulus specificity*
270 To determine whether any long-term responsiveness decline was attributable to habituation
271 (rather than sensory adaptation, fatigue, or other forms of learning), we included a long-term
272 stimulus specificity treatment. Females in this treatment were treated the same as in the long-
273 term responsiveness decline treatment, except that they were tested with a male that had a
274 different colour pattern from the stimulus males. We predicted that their mating interest would
275 be higher than that of females in the long-term responsiveness decline treatment.

Each of our 11 treatments (naïve, 3, 6, 9, 12, 15, spontaneous recovery, dishabituation, stimulus specificity, long-term responsiveness decline, and long-term stimulus specificity) had a sample size of n = 20 females (220 females tested in total). Male lines were used in a counterbalanced manner; for each treatment, half of stimulus males were from Line 9, and the other half were from Line 10.

Behavioural trials

To measure female mating interest, we placed the female and test male together in an observation tank and allowed the fish to freely interact for 5 min. Using JWatcher, v 1.0 [33], we recorded number of male courtship displays and the number of displays to which females responded positively by orienting to the male, approaching him, and/or performing a "glide" response [30] (see Supplementary Table 1 for ethogram). Within the timeframe of behavioural trials, copulations were too sparse for statistical analyses. Positive responses are more common and predict eventual mating success [30], making them an effective measure of female mating interest. We analysed female mating interest in two ways. To control for variation in male courtship effort, we measured female mating interest as the proportion of courtship displays to which the female responded positively. We also analysed the number of positive responses (without controlling for male courtship displays). While we report the results for the former measure of female mating interest, using the latter metric gave similar results (see Supplementary Results). To ensure consistency, all females were exposed to males by the same person (MJD) and mating trials were all scored by the same person (LK). The experiment was

blind, as LK was not aware of the female's treatment at the time of observation. Additional details on our behavioural observations are in Supplementary Methods.

Statistical analyses

All analyses were performed in R, v 3.5.1 [34]. We fit generalized linear mixed models, using the package lme4, 1.1-18-1 [35], to female mating interest using a binomial distribution and Laplace approximation for estimating the marginal likelihood. We included the argument "weights", which is used with binomial data to account for between-sample variation in the number of trials (in our case, variation in the number of male courtship displays per trial). Treatment, the Iso-Y line of the test male, and their interaction were modelled as fixed effects. Random effects included the time and day of testing. We used likelihood ratio tests to assess the significance of fixed effects. The interaction between treatment and line was not significant (see results), and was not included in the final model. The effect of treatment was significant (see results); we therefore performed planned contrasts to test our *a priori* hypotheses about differences between certain treatment levels. Because we found significant evidence of responsiveness decline when comparing the 0 (naïve) and 15 treatments, we additionally performed post-hoc tests comparing the 0 treatment with the 3, 6, 9, and 12 treatments to determine how many exposures were required to elicit a significant effect on female mating interest. We avoided inflation of type 1 error rate for this set of 4 post-hoc tests by applying the Bonferroni-Holm correction for multiple comparisons [36].

We also asked whether there were differences in amounts of male courtship between lines and treatments that could have confounded any effects of these factors on female mating interest. We

fit generalized linear mixed models to courtship count data, following the same procedures above, but used the Poisson distribution. The interaction between treatment and line was not significant (see results) and was therefore excluded from the final model.

Results

During the mating interest trials, males performed an average of 10.077 ± 0.223 courtships displays (mean ± SE). Females responded positively to an average of 26.9 ± 1.0 % of displays (mean ± SE). Number of male courtship displays was not significantly influenced by treatment (χ^2_{10} = 7.498, P = 0.678), line (β = 0.008 ± 0.042, χ^2_{10} = 0.036, P = 0.849), or their interaction (χ^2_{10} = 13.291, P = 0.208). Thus, any effects of treatment and/or line on female mating interest cannot be attributed to differences in male courtship behaviour.

Female mating interest was significantly influenced by treatment (χ^2_{10} = 72.329, P < 0.001) and line (β = 0.441 ± 0.524, χ^2_1 = 5.812, P = 0.016), with females showing more interest in males from line 9. All estimates and test statistics for this analysis are reported in Supplementary table 2. However, the interaction between treatment and line was not significant (χ^2_{10}= 12.562, P = 0.249). We therefore found no evidence that habituation differed between lines.

As a test of responsiveness decline (criterion 1), we asked whether exposure to a series of males with a given colour pattern reduced female interest in males with that same colour pattern, relative to the mating interest of naïve females. Naïve females responded positively to male courtship nearly twice as often as females exposed to 15 stimulus males (see Figure 3 for all treatment comparisons on the untransformed scale; β = -0.720 ± 0.556, χ^2_1 = 4.227, P < 0.001).

Therefore, repeated exposure to several males with a particular colour pattern reduces females' mating interest in males with that same pattern. Post-hoc comparisons revealed that the mating interest of naïve females was not significantly different from that of females exposed to 3 stimulus males (β = 0.568 ± 0.553, χ_1^2 = 1.306, P = 0.192), but did differ significantly from that of females exposed to 6, (β = 0.697 ± 0.556, χ_1^2 = 3.721, P < 0.001), 9 (β = 0.719 ± 0.555, χ_1^2 = 4.227, P < 0.001), and 12 (β = 0.757 ± 0.559, χ_1^2 = 4.814, P < 0.001) stimulus males. This indicates that exposure to 6 males, over a period of 12 minutes, was sufficient to reduce mating interest for similar colour patterns.

To test for spontaneous recovery (criterion 2), we compared the mating interest of females in the "15" treatment with that of females also exposed to 15 stimulus males, but that were isolated from males for 30 min between exposure and testing. The mating interest of females that were temporarily isolated was 43% higher than that of females that were tested immediately (Figure 3; β = 0.347 ± 0.556, χ_1^2 = 2.831, P = 0.005). Therefore, isolation from males allows spontaneous recovery of mating interest.

We tested dishabituation (criterion 3) by comparing mating interest of females in the "15" treatment with females also exposed to 15 stimulus males, but that were additionally exposed to a 16th male that had a colour pattern different from that of the stimulus and test males. The mating interest of females shown the dissimilar male was 45% higher (Figure 3; β = 0.349 ± 0.556, χ_1^2 = -2.787, P = 0.005). This indicates that exposure to a novel colour pattern results in females dishabituating to the familiar colour pattern.

To test stimulus specificity (criterion 4), we compared the mating interest of females in the "15" treatment with females also exposed to 15 stimulus males, but that were tested with a male that had a different colour pattern than those males. Females tested with a male bearing a novel colour pattern showed 50% more mating interest than those tested with males bearing a familiar pattern (Figure 3; β = 0.356 ± 0.557, χ_1^2 = -2.584, P = 0.010). In addition, the mating interest of females in the stimulus specificity treatment against naïve females. The mating interest of females in the stimulus specificity treatment was not significantly different from that of naïve females (Figure 3; β = -0.352 ± 0.212, χ_1^2 = 1.659, P = 0.097). Therefore, the decline in female mating interest caused by exposure to males does not extend to males with novel colour patterns.

To assess long-term habituation, we first tested for long-term responsiveness decline that persists without spontaneous recovery (criterion 5). As predicted, the mating interest of females exposed to 15 stimulus males per day over 4 days, and then isolated for 24 h prior to testing, was not greater than that of females exposed to 15 stimulus males and immediately tested (β = -0.625 ± 0.565, χ_1^2 = 1.951, P = 0.051), and the trend was for females in the long-term responsiveness decline treatment to show lower mating interest than females in the 15 treatment. Therefore, we found no evidence of spontaneous recovery for females given extended exposure to stimulus males, indicating long-term responsiveness decline. We next determined whether this was attributable to habituation *per se* by testing for long-term stimulus specificity (criterion 6). As predicted, females in the long-term stimulus specificity treatment (exposed to 15 stimulus males per day over 4 days, and then tested with a male that had an unfamiliar colour pattern), showed higher mating interest than females in the long-term responsiveness decline treatment (β = 0.284 ± 0.435, χ_1^2 = 0.435, P < 0.001). Because stimulus specificity cannot be explained by fatigue,

sensory adaptation, or other known forms of learning, our results demonstrate that repeated exposure to male colour patterns over an extended period resulted in long-term habituation.

Discussion

Our results demonstrate that visual exposure to males affects the mating interest of female guppies in a manner that fulfills the major criteria of habituation learning. First, exposure to males with a given colour pattern reduced female mating interest in males with that pattern. Second, female mating interest recovered when females were briefly isolated from males. These results indicate responsiveness decline and spontaneous recovery, respectively. We also demonstrated two characteristics that distinguish habituation from alternative explanations such as sensory adaptation, fatigue, or other forms of learning. We observed stimulus specificity: female mating interest in males with novel colour patterns was greater than interest in males with familiar patterns. Furthermore, interest in males with the familiar colour pattern recovered when females were shown a male with an unfamiliar colour pattern, demonstrating dishabituation. Together, these results indicate that female guppies exhibit short-term habituation to the colour patterns of males that they encounter. By reducing female mating interest in males with familiar colour patterns, but not those with unfamiliar patterns, habituation produced a preference for novel phenotypes. These results provide, to our knowledge, the first evidence that habituation can lead to a preference for mates with novel sexual signals. Our findings provide new insight into the proximate mechanisms underpinning this preference.

We were also able to show long-term habituation to male colour patterns. Female mating interest did not spontaneously recover when we applied an extended exposure regime. Therefore,

413 sufficient exposure can cause an enduring reduction in mating interest in males with familiar
414 colour patterns. We demonstrated that this effect is the result of long-term habituation *per se* by
415 showing stimulus specificity: the long-term exposure resulted in females showing less mating
416 interest in males with familiar colour patterns than unfamiliar patterns. These results are
417 consistent with previous experiments in the guppy describing both short- (e.g. [27]) and long-
418 term (e.g. [7, 26]) preference for novel patterns.
419
420 That habituation underpins preference for novel colour patterns helps to explain some of the key
421 findings made in a previous study of this preference. We found a significant reduction in female
422 mating interest in males with the familiar colour pattern after exposure to 6 males, which
423 corresponds to a total of 12 minutes of exposure. Similarly, Graber et al. [27] allowed females to
424 freely interact with several males and found that females shifted their preferences on a timescale
425 of minutes, preferring males with colour patterns different from that of their immediately
426 previous suitor. We suggest that this short-term change in preference can be explained by
427 repeated habituation and dishabituation. Exposure to a courting male likely reduced female
428 interest in males with similar colour patterns through habituation. When courted by a male with a
429 different colour pattern, female interest recovered–likely as a result of dishabituation–causing
430 females to discriminate against whichever males resembled their most recent suitor. However,
431 this dynamic may be transient: Graber et al. [27] found that after 24 h of continuously interacting
432 with these males, females showed less mating interest overall and no longer discriminated
433 between different and same morphs. This result could be explained by an additional
434 characteristic of habituation called habituation of dishabituation [14]: upon repeated application
435 of a dishabituating stimulus (i.e. a novel colour pattern), the amount of dishabituation produced

436 decreases. The results of Graber et al. [27], when contextualized by the findings of the present
437 experiment, suggest that when individuals are allowed to freely interact in social groups,
438 habituation shapes patterns of mate choice in complex ways over multiple timescales.
439
440 Habituation has been observed at the behavioural, physiological, and neural levels, and involves
441 changes in neurons and synapses [13,14]. These changes are believed to result primarily from
442 transient epigenetic markings that reduce synaptic efficacy by lowering expression levels of key
443 receptor genes, and increasing the activation threshold of receptors by inducing conformational
444 changes [37]. The particulars of the processes involved are highly evolutionary conserved, but
445 vary depending on the timeframe of stimulation, type of sensory pathway, and the hierarchical
446 level of signal processing [38]. Nevertheless, given that habituation is among the simplest forms
447 of learning, investigation of the mechanisms of habituation to visual patterns is an exciting
448 avenue for future work to elucidate the molecular, genetic, and neural underpinnings of mate
449 choice plasticity.
450
451 It is likely that habituation plays a pervasive role in mate choice, given our results and the
452 observation that habituation is highly conserved across taxa and contexts [13,14] – including
453 responses to visual stimuli [39]. This raises the question of whether habituation causes
454 preference for novel phenotypes in taxa beyond the handful of species in which such preferences
455 have been documented. Published tests of negative frequency-dependent mate choice have thus
456 far been limited to a fairly small number of taxa, so preference for novel phenotypes may be an
457 underappreciated type of mate choice. Additionally, individuals in many species recognize and
458 discriminate against conspecifics that they have previously encountered as a means of avoiding

459 re-mating and/or inbreeding (e.g. [11,31,40]). Such preference for novel individuals represents a
460 preference for novelty in a broader sense, and might be underlain or reinforced by habituation to
461 the particular phenotypes of familiar individuals. Habituation to sexual signals need not always
462 produce a preference for novel phenotypes. In species in which females exhibit consistent
463 preferences for a given phenotype, habituation would be expected to diminish attraction to the
464 preferred phenotype. Preference for novelty should arise only if there are one or more alternative
465 phenotypes that are attractive enough that they become preferred because of the decline in
466 female interest in the originally preferred phenotype. Therefore, habituation is compatible with
467 consistent mating preferences. Indeed, we observed both habituation and an overall preference
468 for male line 9. The preference for male line 9 may be due to these males having more orange
469 colouration (figure 1; Supplementary Results), as females from our study population prefer
470 males with a large area of orange colouration [30,41]. Habituation is thus most likely to lead to
471 preference for novel phenotypes in species like the guppy in which there are multiple, attractive
472 male phenotypes. We suggest that future work investigate the generality of habituation as a
473 process shaping mate choice, and whether habituation causes widespread mating preferences for
474 novel phenotypes and/or individuals. This is an intriguing possibility because preference for
475 novel sexual signals can promote and maintain genetic variation within populations, potentially
476 helping to explain the paradoxically high levels of genetic diversity in ecologically-relevant
477 traits–including sexual signals–that are widely observed in nature [1,2,42].
478
479 The role of habituation in preference for novel colour patterns also provides insight into the
480 evolutionary origins of this mating preference. Several hypotheses have been proposed for why
481 preferring males with novel patterns might be adaptive. The preference could confer genetic

benefits by promoting inbreeding avoidance [8] and/or polyandry [26]. Additionally, preference for novel phenotypes could evolve and be maintained (though only at intermediate frequencies) through a Fisherian "sexy sons" process [43]. Our results raise an alternative explanation: the sensory bias hypothesis, which posits that mating preferences can arise as a by-product of sensory mechanisms favoured by selection in non-mating contexts [44]. Habituation is believed to be widely favoured by selection because it causes organisms to filter out the repetitive sensory "noise" of their environment and instead focus on processing and responding to novel stimuli, which tend to be most biologically relevant [13,14]. Preference for novel colour patterns might have arisen as a pleiotropic consequence of selection favouring habituation to visual stimuli in non-mating contexts. Sensory bias of a different kind has already been found in the guppy: female mating preference for males with large orange spots has been linked to foraging preference for orange food items [45]. However, the potential role of sensory bias in explaining preference for novelty (and thus, frequency-dependent mating preferences) has not, to our knowledge, been previously explored in any taxa.

Habituation to male sexual signals may also provide an explanation for the evolution of multi-component signals. As females become less responsive to common sexual signal(s), males with novel components in their signal should be released from habituation. This may help to explain the complexity of sexual signals found in many species [46], and in guppy colour patterns in particular.

In summary, we have demonstrated that female guppies habituate to male sexual signals, and that this process results in preference for unfamiliar phenotypes during mate choice. By identifying a

505 psychological process underpinning preference for unfamiliar phenotypes, our results provide
506 insight into the mechanism and evolutionary origins of this ecologically important type of mate
507 choice. Given that habituation is pervasive among animal sensory systems, these findings
508 suggest that preference for novel sexual signals may be common, and plays an underappreciated
509 role in the maintenance of genetic diversity.
510
511 **Data accessibility**
512 All data have been deposited in the Dryad repository at http:// datadryad.org/review?doi=doi:
513 10.5061/dryad.fp030jg
514
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Figure 1. Examples of male colour patterns from the two Iso-Y lines (left: line 9; right: line 10) used in the experiment. Males from the same Iso-Y line have patterns that are similar in terms of the number, colour, position, and size of their colour spots, especially on the body. Colour patterns vary substantially between lines.

Figure 2. Summary of our habituation paradigm. Treatments are listed on the left. The procedure for exposing females to stimulus males is described in the centre (grey) panel. Listed on the right is the novelty status of the colour pattern of the males used to assay female mating interest.

706 Figure 3. Mating interest of females from each treatment (n = 20 per treatment), on the
707 untransformed scale, measured as the proportion of male courtship displays to which the female
708 responded positively. The left, white colour frame indicates treatments used to test for
709 responsiveness decline, in which females were sequentially exposed to a number of similarly-
710 patterned males (either 0 (naïve), 3, 6, 9, 12, or 15) and then tested. The grey colour frame
711 indicates treatments compared against the 15 treatment to test for additional criteria of short-term
712 habituation: spontaneous recovery (SR), dishabituation (Dis), and stimulus specificity (SS). The
713 right, white colour frame indicates treatments compared against the 15 treatment to test for long-
714 term habituation: long-term responsiveness decline (LTRD), and long-term stimulus specificity
715 (LTSS). Error bars denote the mean ± SE.