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Mating Preference for Novel Phenotypes Can Be Explained by General Neophilia in Female Guppies

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ABSTRACT: Understanding how genetic variation is maintained in ecologically important traits is a fundamental question in evolutionary biology. Male Trinidadian guppies (*Poecilia reticulata*) exhibit extreme genetic diversity in color patterns within populations, which is believed to be promoted by a female mating preference for rare or novel patterns. However, the origins of this preference remain unclear. Here, we test the hypothesis that mating preference for novel phenotypes is a by-product of general neophilia that evolved in response to selection in nonmating contexts. We measured among-female variation in preference for eight different, novel stimuli that spanned four ecological contexts: mate choice, exploration, foraging, and social (but nonsexual) interactions. Females exhibited preference for novelty in six out of eight tests. Individual variation in preference for novelty was positively correlated among all eight types of stimuli. Furthermore, factor analysis revealed a single axis of general neophilia that accounts for 61% of individual variation in preference for novel color patterns. The single-factor structure of neophilia suggests that interest in novelty is governed primarily by shared processes that transcend context. Because neophilia likely has a sizable heritable component, our results provide evidence that mating preference for novel phenotypes may be a nonadaptive by-product of natural selection on neophilia.

Keywords: genetic diversity, frequency dependence, sexual selection, novelty seeking, mate choice, sensory bias.

Introduction

Populations of organisms harbor enormous genetic diversity in ecologically important traits. Understanding the processes that maintain this variation is a fundamental challenge in evolutionary biology (Lewontin 1974; Mitchell-Olds 2007; Leffler et al. 2012; Charlesworth 2015). Levels of genetic variation are often greater than expected under mutation-selection-drift balance (Bürger and Lande 1994; Turelli and Barton 2004). One potential explanation for this diversity is negative frequency-dependent (NFDS) selection, in which the fitness of a genotype is greater when it is rare (Ayala and Campbell 1974). By favoring rare genotypes, NFDS can prevent the loss of rare alleles, thereby maintaining high levels of genetic variation without genetic load (Tobari and Kojima 1967; O'Donald and Majerus 1988). Consequently, there has been much interest in elucidating the ecological processes that can generate NFDS.

One such process is a rare male mating advantage, which can be driven by female mating preference for rare or novel phenotypes. Female preference for novel or rare sexual signals has been reported in several species, including in three poeciliid fishes (Lindholm et al. 2004; Royle et al. 2008), fruit flies (Tan et al. 2013), and potentially in humans (Janif et al. 2014), although published tests of this preference remain relatively rare. Mating preference for novelty has been proposed to play a broad role in the maintenance of genetic variation (Daniel et al. 2019); however, the evolutionary origins of this preference remain unclear. Resolving this issue should help to predict the generality of this preference and thus its importance in the maintenance of genetic variation.

Several adaptive explanations for mating preference for novel phenotypes have been suggested. This preference might have evolved because of selection favoring inbreeding avoidance or because of benefits from mating with multiple males, such as enhanced genetic diversity of offspring (Zajitschek and Brooks 2008; Hughes et al. 2013). It is also possible that this preference evolved because of a survival advantage to rare phenotypes, as documented in several taxa (e.g., Reid 1987; Sinervo et al. 2001; Trachtenberg et al. 2003; Olendorf et al. 2006; Fraser et al. 2013), which could kick-start a Fisherian "sexy sons" effect (Kokko

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et al. 2007). However, preference for novel phenotypes is expected to reach only an intermediate frequency in this scenario, because, as the preference increases in frequency, phenotypes that are rare in the parental generation become common (and thus confer lower attractiveness or survival) in the offspring generation (Kokko et al. 2007). Finally, there might be a direct benefit to preference for rare sexual signals. In some species, common phenotypes confer greater predation risk (e.g., Reid 1987; Olendorf et al. 2006), and this heightened risk might extend to females being courted by males with common phenotypes. If preference for novel phenotypes dissuades common males from courtship, the preference could enhance female survival.

An alternative to these adaptive hypotheses is that preference for novel sexual signals may have arisen as a by-product of a general interest in novel stimuli, or general neophilia (Hughes et al. 1999, 2013). According to this hypothesis, mating preference for novelty arises as a nonadaptive by-product of sensory and/or behavioral processes that evolved for reasons unrelated to mating, as in the sensory bias model of sexual selection (Kirkpatrick and Ryan 1991; Fuller et al. 2005). The sensory bias model has typically been used to explain the evolution of fixed or directional preferences for particular traits (e.g., preference for the presence of a given sexual signal or for greater values of a trait). The neophilia hypothesis notably differs in that it seeks to explain a nondirectional preference for novel traits, with implications for the maintenance of genetic variation.

Mating preference for novel phenotypes has been most extensively characterized in the Trinidadian guppy (*Poecilia reticulata*). Male guppies have color patterns that are highly heritable and provide one of the most extreme known examples of morphological polymorphism (Brooks and Endler 2001; Hughes et al. 2013). This variation is believed to be promoted by the strong mating preferences of female guppies for males with color patterns that are rare over those that are common (Zajitschek and Brooks 2008; Hampton et al. 2009; Hughes et al. 2013) and color patterns that are novel over those that are familiar (Farr 1977; Hughes et al. 1999; Eakley and Houde 2004; Graber et al. 2015; Valvo et al. 2019). The adaptive hypotheses that have been tested in guppies appear not to explain preference for novel phenotypes. Evidence that female guppies avoid inbreeding in nature is mixed (Johnson et al. 2010), and the strength of preference for novel sexual signals is consistent among populations that differ in inbreeding risk and predator abundance (Valvo et al. 2019). Furthermore, preference for novel sexual signals is strong and prevalent in this species (Hughes et al. 2013; Valvo et al. 2019), suggesting that it cannot be accounted for solely by indirect benefits. Guppies are therefore an excellent system for investigating general neophilia as a potential explanation for mating preference for novelty.

Several lines of reasoning lend plausibility to the neophilia hypothesis. Attraction to novel nonmating stimuli has been observed in many animal taxa (reviewed in Pisula et al. 2013), including in mammals (e.g., Palanza et al. 2001), birds (e.g., Murphy 1977; Fidler et al. 2007), insects (e.g., Liang et al. 2012), and fish (e.g., Øverli et al. 2006; Burns and Rodd 2008; Stewart et al. 2010). Neophilia has also been reported in a wide range of contexts, such as novel environments (e.g., Øverli et al. 2006; Fidler et al. 2007; Burns and Rodd 2008; Stewart et al. 2010), objects (e.g., Fidler et al. 2007), and food items (e.g., Murphy 1977), although some species show aversion to novelty in these contexts (reviewed in Greggor et al. 2015). Additionally, in guppies, mating preference for novelty is underpinned by habituation, whereby an organism becomes less responsive to repeatedly encountered stimuli (e.g., a common color pattern; Daniel et al. 2019). Habituation is a pervasive feature of animal sensory systems, although the sensory and neural mechanisms responsible for habituation can vary depending on factors such as the type of sensory pathway, the time frame of stimulation, and the hierarchical level of signal processing (Schmid et al. 2015). If the mechanisms responsible for habituation to color patterns overlap with those responsible for habituation to nonsexual stimuli, these mechanisms could constitute a sensory bias for novelty. Furthermore, some forms of novelty seeking, such as exploration, are thought to be favored by selection in environments that reward exploration (e.g., variable resources, colonization opportunities) and selected against when exploration is risky (e.g., intense competition or predation; Greenberg and Mettke-Hofmann 2001; Burns and Rodd 2008). Moreover, variation in novelty-seeking behavior such as exploration often has a genetic component (e.g., van Oers et al. 2004; Forstmeier et al. 2011; reviewed in Bell et al. 2009), including in guppies (Kotrschal et al. 2014; White and Wilson 2019). Thus, there are opportunities for neophilia to evolve in response to selection in nonsexual contexts.

Individual variation in behavioral responses is sometimes found to be correlated across different behavioral or ecological contexts, suggesting major axes of behavioral variation (sometimes called personality factors, or behavioral syndromes; Sih et al. 2004; Réale et al. 2007). For example, individuals that are bolder when interacting with a predator have sometimes been found to be bolder when exploring a novel environment (Sih et al. 2004; Réale et al. 2007). If individual variation in novelty seeking is correlated across contexts, this would imply the existence of an axis of variation in general neophilia and indicate overlap in the factors that govern novelty seeking in different contexts, as is predicted by the neophilia hypothesis. However, we note two relevant gaps in the literature on novelty seeking in nonhuman animals. First, to our knowledge, no previous studies have asked whether mating preference for novel phenotypes is associated with other forms of novelty seeking. While a previous study reported that guppy exploratory behavior is associated with mating preference for unfamiliar individuals (Lucon-Xiccato et al. 2019), the distinction between preference for unfamiliar individuals and preference for novel phenotypes is important; it is preference for novel phenotypes (which may be shared by multiple individuals) that can generate NFDS and thus maintain genetic variation within populations. Second, while responses to novel stimuli have been studied extensively, the vast majority of studies conducted on guppies (e.g., Kniel and Godin 2019) and other taxa (reviewed in Carter 2013) have examined novelty seeking in only a small number (i.e., one or two) of contexts. While such studies have provided valuable information about some types of novelty seeking, they provide little insight into general neophilia because such inferences require measuring responses to multiple—and ideally, many—types of stimuli (Carter 2013; Huang et al. 2018). Thus, investigation into general neophilia remains limited, and its relevance to mating preference for novel phenotypes is untested.

We investigated whether female guppies exhibit general neophilia and whether general neophilia can account for mating preference for novel color patterns. We assayed the interest of female guppies in novel male color patterns and in seven other types of novel stimuli spanning four ecological contexts. These data allowed us to test three predictions of the neophilia hypothesis. First, if female guppies are generally neophilic, then they should exhibit preference for novel stimuli in a majority of contexts, including nonmating contexts. Second, if the processes governing neophilia are shared among contexts, then individual variation in preference for novel color patterns should be correlated with preference for novelty in nonsexual contexts. Third, if these shared processes of general neophilia can explain preference for novel color patterns, then factor analysis should reveal one or more axes of behavioral variation onto which preference for novelty loads in the same direction for multiple contexts, and which explain a substantial amount of variation in preference for novel color patterns. If the neophilia hypothesis is incorrect, then we would predict that guppies show preference for novelty in a minority of contexts, that preference for novelty is uncorrelated across contexts, and that preference for novelty loads in different directions and/or onto different factors. Such results would imply that the processes governing interest in novelty are largely context specific, falsifying the neophilia hypothesis.

Methods

Study System and Husbandry

Guppies are live-bearers with a promiscuous mating system (Houde 1997). Males persistently pursue females and frequently perform courtship displays that advertise their color patterns (Rodd and Sokolowski 1995), while females are choosy (Houde 1997). The guppies in our experiment were laboratory-reared descendants of fish from the "Houde" tributary (Trinidad National Grid System: PS 896 886) of the Paria River in Trinidad.

We reared females that were naive to male color patterns at the start of the experiment. We accomplished this by transferring our experimental fish to juvenile-only tanks within 24 h of their birth (only males develop color patterns at maturity). As they matured (at ~100 days old), we moved the fish into single-sex tanks. Both the offspring-only and female-only tanks were visually isolated from all tanks containing adult males. The fish were held in the single-sex tanks until they were used in the experiment, beginning at 100–120 days old. All tanks were lined with a layer of gravel on the bottom and illuminated by full-spectrum fluorescent bulbs. Fish were kept on a 12 : 12-h light : dark cycle and fed twice daily. They were given TetraMin fish food in the mornings and brine shrimp (nauplii larvae of *Artemia salina*) in the afternoons.

Overview

To test the neophilia hypothesis, we measured the interest in novelty of adult female guppies when exposed to eight different stimulus types. We chose stimulus types that span multiple, ecologically relevant contexts in which novelty seeking is believed to be important for fitness (Boissy 1995; Réale et al. 2007; Smith and Blumstein 2008): exploration, foraging, mate choice, and social (but nonsexual) interactions. These data allowed us to determine whether females exhibit preference for novelty in each of these contexts and to measure the covariance structure of interest in novelty across contexts.

Experimental Design

We placed the experimental females individually into 15-L home tanks (30 × 22 × 25 cm). We allowed the females a 31-day acclimation period in their home tank prior to use in the experiment. We tested each female with all eight stimulus types sequentially, using a balanced Latin square design to determine the order of exposure to the stimulus types. This design accounts for both order effects and first-order carryover effects (Hedayat and Afsarinejad 1975).

Novelty-seeking behavior has often been tested by exposing individuals to a single, novel stimulus and using responses to the stimulus as a measure of interest in novelty. This approach has yielded useful insights but has been criticized because it confounds novelty seeking with other factors that can affect how the organism responds to a stimulus (Carere and Locurto 2011; Carter 2013; Huang et al. 2018). For example, high response toward a novel food item could indicate that an individual has a strong appetite (and is thus more responsiveness to food items in general) or that it has a high general activity level, making it responsive to a wide range of stimuli (regardless of novelty). To control for such confounding effects, for each stimulus type we presented the female with a novel and a familiar stimulus simultaneously. For example, one of our stimulus types was novel objects; we tested interest in novel objects by familiarizing the female to one object (e.g., a rectangular cuboid) and then assaying her responses toward both the familiar object and a different, novel object (e.g., a triangular prism). Importantly, we varied which stimulus was novel (e.g., the rectangular cuboid or the triangular prism) in a balanced manner to disentangle novelty status from the intrinsic properties of each stimulus. For all stimulus types, we observed the female for 15 min after both stimuli were added and live-scored her behaviors using JWatcher version 1.0 (Blumstein and Daniel 2007). We measured interest in novelty as a proportion: the amount of response directed toward the novel stimulus divided by the total amount of response directed toward both stimuli. This approach is informative because it measures the effect of novelty per se, controlling for other factors affecting responsiveness toward a given stimulus type.

For all stimulus types, the experiment was balanced with respect to on which side of the tank the stimulus was located during familiarization. For all stimulus types (except the novel environment, see below), the experiment was also balanced for whether the familiar stimulus was on the same side of the tank during familiarization as during testing. We removed the familiar and novel stimuli (and the compartments they were in) after testing for all stimulus types except the novel environment.

For each stimulus type, we began by familiarizing the female with one of the two stimuli (e.g., rectangular cuboid in the example above), by exposing the female to the stimulus for 7 days. We chose this time frame because previous studies have shown that 7 days of exposure is sufficient for guppies to become familiar with the stimulus for at least two of the stimulus types that we investigated: color patterns (Graber et al. 2015; Daniel et al. 2019) and females (Griffiths and Magurran 1997*a*). Immediately following the familiarization period, we assayed female responses toward the novel and familiar versions of the stimulus. To minimize diel variation in behavior, all assays were performed between 9 and 11 a.m., when guppies are most sexually active (Houde 1997). For all stimulus types except those related to foraging, we fed the females 30 min before testing to discourage foraging during the assay. Below, we describe the specific protocols used for familiarization and testing for each of the eight stimulus types.

Familiarization and Testing Protocols

Mate Choice Context. We observed the responses of females to two stimulus types associated with mate choice. First, we investigated interest in males with novel color patterns, as our hypothesis is explicitly about the relationship between interest in novel color patterns and interest in other types of novel stimuli. We also tested mating interest in novel (i.e., unfamiliar) individuals, because females in many species (reviewed in Pusey and Wolf 1996; Hepper 2005; Penn and Frommen 2010), including guppies (Mariette et al. 2010; Daniel and Rodd 2016), exhibit mating preferences for unfamiliar individuals. Furthermore, mating preference for unfamiliar individuals is thought to be important for inbreeding avoidance and polyandry (Pusey and Wolf 1996; Hepper 2005; Penn and Frommen 2010).

The males used in the experiment were derived from two Iso-Y lines, kindly provided by A.E. Houde. These lines are paternal lineages that differ from one another in the nonrecombining region of the Y chromosome but are otherwise genetically homogeneous. Because guppy color patterns are highly Y linked (Houde 1992; Lindholm and Breden 2002), males from the same Iso-Y line have similar-looking patterns, while males from different Iso-Y lines have dissimilar-looking patterns—particularly in terms of the orange and black spots on the body and caudal peduncle of the fish (Daniel et al. 2019). We familiarized each female to a particular pattern by exposing her to a series of males all from the same Iso-Y line. We did this by placing the male in a compartment (30 × 6 × 12 cm) at one end of the female's tank. Each subsequent day of the familiarization period, we replaced the familiarization male with another male from the same Iso-Y line. This decoupled familiarity with the color pattern from familiarity with any individual male. The containers were made of clear, watertight plastic, allowing the female to see the male but preventing chemosensory communication and mating.

On the eighth day, we tested female interest. We added a second compartment to the opposite end of the tank. We removed the current familiarization male from his compartment, then placed a male with the familiar pattern in one of the containers and a male with a novel pattern in the other. Both of these males were themselves unfamiliar to the female. The region of the tank accessible to the female was delineated crosswise into three zones: an 8-cm-wide zone of association adjacent to each male compartment and an 8-cm-wide neutral zone in the center between the two zones of association (see fig. S1 for diagram of setup used for all stimulus types; figs. S1–S5 are available online). We scored the amount of time that the female spent in each zone and the amount of time that the female spent oriented toward each male. Both association time and orienting are widely used to measure mating preference in guppies (e.g., Bischoff et al. 1985; Kodric-Brown 1989, 1992; Houde and Torio 1992; Brooks and Caithness 1995; Houde 1997; Houde and Hankes 1997; Rosenqvist and Houde 1997; Hibler and Houde 2006; Hampton et al. 2009), and these behaviors predict cooperative copulation (Bischoff et al. 1985; Kodric-Brown 1989).

The second stimulus type related to mate choice that we measured was males that were themselves novel. We familiarized each female to an individual male by placing him in a compartment placed on one side of the female's tank (as described above). The female was exposed to this male for 7 days. On the eighth day, we removed the familiar male and added a new compartment to the opposite end of the tank. We placed the familiar male in one compartment and placed a novel male in the other. Both the familiar and novel male were from the same Iso-Y line. This disentangled familiarity with the individual male from familiarity with the color pattern and also minimized any effects of female mating preferences for certain color pattern features (e.g., preference for extensive orange [e.g., Houde 1997]). Following the same protocols as for novel color patterns, we recorded the female's behaviors toward the two males.

We mated females before the experiment, because female guppies only discriminate among familiar and unfamiliar males once previously mated (Daniel and Rodd 2016). We mated each female by pairing her with a male that had a color pattern different (i.e., from a different Iso-Y line) from the stimulus males. We ensured that females were receptive during testing and controlled for variation in the female reproductive cycle by testing females during the 3-day window of receptivity between birth and becoming gravid again (Houde 1997). We accomplished this by beginning the 7-day familiarization phase 22 days after the female last gave birth (female guppies gestate for 28 days). Offspring were removed from the female's home tank within 24 h after birth.

Exploratory Context. We exposed females to two types of novel stimuli associated with exploration: novel objects and novel environments. We chose these two types because they are widely used in studies of novelty-seeking behavior (e.g., Boissy 1995; Burns 2008; Heyser and Chemero 2012) and because the willingness of individuals to inspect novel objects and environments has implications for predator avoidance and dispersal (Dall et al. 2004; Sih et al. 2004; Réale et al. 2007).

For the novel object assay, we used two small plastic objects: a yellow rectangular cuboid (5 × 3 × 1.25 cm) and a blue triangular prism (3 × 3 × 3 cm). We familiarized the female with one of the two objects by placing it on the gravel near one side of her tank. On the day of testing, we removed the familiar object and then immediately placed both the familiar and the novel objects near opposite ends of the tank. Following Rodd et al. (2002), we delineated a horizontal zone of association around each object by burying a petri dish (12 cm in diameter) under the gravel beneath the object, with only the rim visible. We delimited the upper boundary of this zone at 6 cm above the gravel. We scored both the time that the female spent in zone around each object and the amount of time the female spent oriented toward each object.

For the novel environment assay, we moved the female from her home tank to a 30-L tank (45 × 30 × 25 cm) divided crosswise into three compartments of equal width. We initially placed the female in the center compartment, which was separated from the compartments on either end by opaque blue plastic dividers. After a 15-min acclimation period, we raised one of the dividers, allowing the female to move freely between the center compartment and one end of the tank throughout the familiarization period. After familiarization, we lowered this divider and returned the female to the center compartment, if necessary. We then raised both dividers and observed the female's behavior. We recorded the amount of time that she spent in the center as well as the familiar and novel ends of the tank. We also measured the latency between when the two dividers were raised and when the female first entered each end of the tank. For the novel environment test, our experiment was not balanced with respect to whether the familiar end of the compartment was on the same side of the tank during familiarization and testing (since the side of the tank was itself the stimulus). Immediately following this test, we moved the female back to her home tank.

Foraging Context. To examine interest in novelty in a foraging context, we observed the response of females to two types of novel food items. We used novel fruit and novel insects, as fruit and insects are sources of nutrients, including protein, sugar, and carotenoids, for wild guppies (Williams and Cheesman 1929; Dussault and Kramer 1981; Rodd et al. 2002; Warbanski et al. 2017).

We used two fruits for our novel fruit assay: blueberries and raspberries. While guppies do not encounter these particular fruits in nature, it is meaningful to assay their interest in these fruits given that our goal is study response to novel stimuli. We familiarized the female with one version of fruit by cutting a berry into a piece ~0.5 cm^3 in size, adhering this piece of berry to a microscope slide using a tiny bead of silicon, and placing the slide on top of the gravel near one end of the female's tank. As for the novel object test, we used a petri dish to delineate a zone of association around the piece of fruit. Every 24 h during the familiarization period, we replaced the piece of fruit with a fresh piece of the same type. To test female interest, we replaced the piece of fruit and simultaneously added a piece of the novel fruit near the opposite end of the tank. We scored the amount of time that the female spent associating with each piece of fruit, in addition to the times the female pecked at it.

For novel insect tests, we used adult flour beetles (*Tribolium castaneum*) and tropical house cricket (*Gryllodes sigillatus*) nymphs obtained from Carolina Biological Supply (Burlington, NC). We presented insects to the female by adhering an individual insect to a microscope slide. Insects were always between 3 and 4 mm in length. We followed the same protocols as for the fruits when familiarizing and testing females with the insects.

Social Context. Finally, we exposed females to two types of social (but nonsexual) stimuli. We observed responses to novel conspecific juveniles, since adults and juveniles co-occur in the wild (Houde 1997). We also examined interest in novel adult females. Female guppies tend to shoal with other females (Croft et al. 2003). As in many species (Barber and Wright 2001; Ward and Hart 2003), female guppies preferentially associate with familiar females over unfamiliar females (e.g., Magurran et al. 1994; Griffiths and Magurran 1997*b*; Bhat and Magurran 2006). Familiarity among group members is thought to confer benefits including improved group cohesion (Chivers et al. 1995), social learning (Swaney et al. 2001), and foraging (Lachlan et al. 1998). Given this preference for familiar females, the female stimulus type provides a point of comparison for any stimuli for which females showed a preference for novelty.

We followed the same protocols used for measuring interest in novel males but using juveniles or nonfocal females. We measured only association time (not orientation time) for these social stimuli because females rarely, if ever, orient toward other females or toward juveniles. The juveniles and nonfocal females were derived from the same laboratory population as the focal females. To minimize variation in traits that might affect female association times, all juveniles were between 5 and 8 weeks old, and each pair of juveniles used together were similar in size (<1 mm difference). Nonfocal females were virgins, and each pair of nonfocal females used together differed in standard length from one another, and from the focal female, by <3 mm.

Across the entire experiment, males, juveniles, and nonfocal females were only used with a single focal female, ensuring independence among females. All females were scored by the same observers: L.K. conducted the novel color pattern and novel environment tests, and M.J.D. conducted the other six tests. We excluded females ($n = 9$) that performed no responses toward one or both stimuli in one or more tests, because these females may not have assessed both the novel and familiar stimulus, making their behavior difficult to interpret. Our remaining sample size was $n = 80$ females. It was not possible for observers to be blind to the stimulus types. However, during testing, observers were unaware of how the female had responded during previous tests.

Statistical Analyses

We used association time as our behavioral measure of interest in novelty for two reasons. First, association time was measured for all eight stimulus types. Using the same behavioral measure for all eight stimulus types simplified analyses and allowed for clearer interpretation of the results. Second, for all stimulus types for which we measured two different behavioral responses (for details, see "Supplementary Methods" in the supplemental PDF, available online), the two measures were significantly correlated (all $P < .001$) and had moderate to high coefficients of determination (R^2 of 0.51–0.72; fig. S2; see table S1 for r values; tables S1–S3 are available online), suggesting that they both provided similar information about response to novelty. Hereafter, we use the phrase "interest in novelty" to refer to the time spent associating with the novel stimulus as a proportion of the total time spent associating with either stimuli.

To determine whether the mean level of interest in novelty varied across stimulus types, and to assess correlations in female behavior across stimulus types, we fit general linear mixed models to interest in novelty, using SAS Proc Mixed version 9.4 (SAS Institute 2013), taking repeated measures into account. There were a total of 640 observations (eight trials per female and 80 females) used in each model. Interest in novelty met the assumptions of this analysis (normal and homoscedastic residuals; see fig. S3). Significance of fixed effects were tested using type 3 tests. Fixed effects in initial models included stimulus type, identity of the novel environment test tank, and their interaction; random effects are described below. The effect of test tank was never significant ($P > .4$ for main effect and $P > .9$ for interaction) and was removed from the model. We also asked whether placement of the novel stimulus, relative to the placement of the stimulus during the familiarization phase, affected interest in novelty. To do so, we fit a model that included a fixed effect describing whether the novel stimulus was on the same or different side as the familiar stimulus had been during familiarization and its interaction with stimulus type. Note that we did not fit this model to the data for the novel environment assay, since the novel stimulus was itself a side of the test tank. Neither the interaction nor the main effect of side were significant (both $P > .2$), so we therefore excluded these terms from the final model.

We accounted for repeated measures on females across stimulus types by using female ID as the subject term in specifying a within-subjects random effect. We modeled two different types of repeated measures structures: observations ordered temporally or observations ordered by stimulus type. Models using stimulus type for the order of repeated measures produced substantially lower corrected Akaike information criterion (AICc) values. We therefore used stimulus type to order the repeated effect in the final model. We chose the unstructured covariance model for repeated measures because it produced the lowest AICc value for both the full and reduced models (Littell et al. 2006). We also included another (between-subject) random effect, which specified the female's home tank.

Because stimulus type was significant (see "Results"), we asked whether females showed a preference for novelty for each stimulus type. We used confidence intervals around the estimates for each stimulus type to infer whether females spent a greater proportion of their association time with the novel stimulus than expected by chance under the null expectation of no preference (0.5). Additionally, using the Tukey-Kramer post hoc correction, we performed post hoc tests comparing mean interest in novelty among stimulus types.

We also assessed the magnitude of correlation between each pair of stimulus types, using the correlation estimates from the linear mixed model. We avoided inflation of type 1 error for this set of 28 tests by applying the Bonferroni-Holm correction for multiple comparisons (Holm 1979; McLaughlin and Sainani 2014).

To determine whether shared processes underpin neophilia in multiple contexts, we used factor analysis. Factor analysis attempts to represent the covariances of a set of observed variables using a smaller number of hypothetical latent variables. If shared process(es), such as general neophilia, underpin interest in novelty in multiple contexts, we predicted that multiple stimulus types should load in the same direction onto the same latent variable(s). Our hypothesis does not make predictions about the specific number of meaningful factors, or which exact combination of variables should load onto each factor. Therefore, we performed exploratory factor analysis. The best fit unstructured covariance structure in the mixed model described above indicates that the correlation matrix for stimulus types differs from an identity matrix, justifying the use of factor analysis to explore the correlational structure of the data (Budaev 2010). Furthermore, the Kaiser-Meyer-Olkin test revealed adequate sampling for factor analysis (measure of sampling adequacy [MSA] $= 0.83$). Because we had no reason to assume that factors underpinning response to novelty should be orthogonal, we used promax (oblique) rotation. We compared four approaches that are recommended for determining the number of meaningful factors to interpret (Raîche et al. 2013) using the R package nFactors version 2.3.3 (Raîche and Magis 2010): graphical inspection of the scree plot, parallel analysis, optimal coordinates, and acceleration factor.

Results

To determine whether females exhibited a preference for novelty for each stimulus type, we asked whether confidence intervals around the mixed model effect estimates overlapped with 0.5 (no preference). Confidence intervals revealed a significant preference for novelty for six of the eight stimulus types: color pattern, male, object, environment, fruit, and insect (fig. 1). Females did not discriminate among novel and familiar juveniles and exhibited a preference for familiar females (fig. 1).

Stimulus types differed significantly in the interest in novelty displayed by females ($F_{7,73} = 73.60$, $P < .001$). Post hoc tests indicated that preference did not differ among males, color patterns, objects, and environments (table S2). However, preference for novelty was greater for males than for fruits, insects, females, and juveniles, while preference for novelty was significantly less for females than for all other stimulus types (fig. 1).

Individuals that exhibited high preference for novelty in one context tended to exhibit high preference in all contests (fig. 2). Based on the correlation estimates from the linear mixed model, individual interest in novelty was significantly positively correlated across all pairs of stimulus types ($r = 0.41$–0.81, all $P < .001$; see table S3 and biplots in fig. S4).

We next examined the correlational structure using factor analysis, which does not assume independence of observations and uses the simple pairwise correlations among variables. Pairwise correlations were only minimally different from the model-based correlations estimates (all $P <$ .001; fig. 3*A*). Only the first factor produced by this analysis was meaningful, according to graphical inspection of the scree plot, parallel analysis, optimal coordinates, and acceleration factor approaches (fig. S5). All eight stimulus types had moderate to strong positive loadings onto this factor (all loadings $= 0.62$–0.82; fig. 3*B*). This factor accounted for 58.1% of the overall variance in interest in novelty and 37.8% to 67.2% of the variance in interest in novelty for each stimulus type. We interpret this factor as representing general neophilia, because it captures variation in interest in novelty across all eight stimulus types. Thus, the results of factor analysis support the existence of an axis of behavioral variation that explains variation in novelty

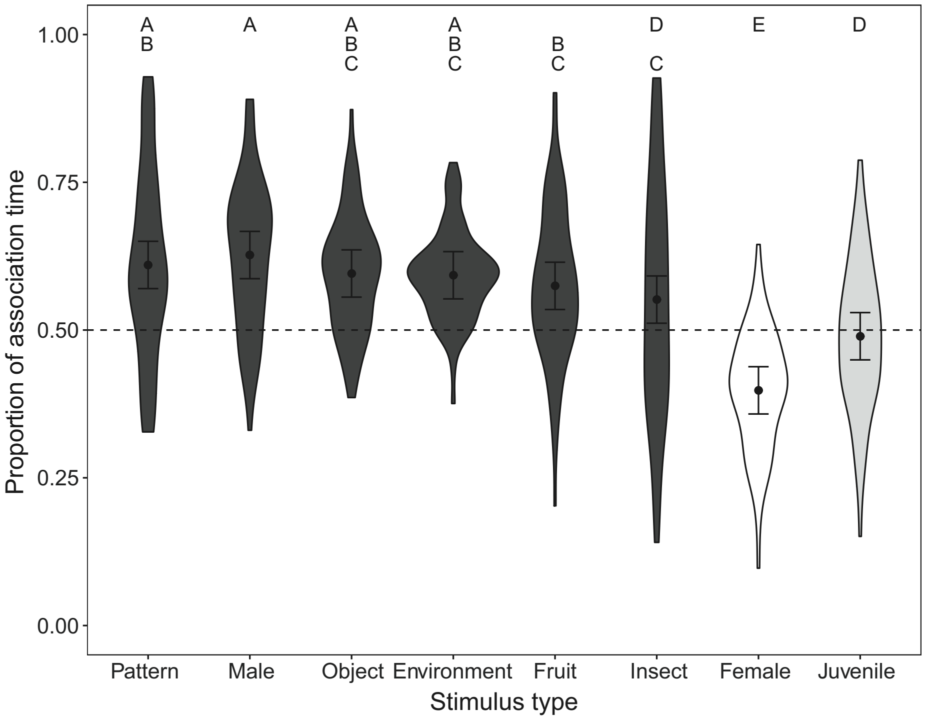

Figure 1: Interest in novelty for each stimulus type. Violins depict the distribution (kernel density) of the data. Black circles represent the mean estimates, flanked by 95% confidence intervals. Dark gray indicates preference for the novel stimulus, white indicates preference for the familiar stimulus, and light gray indicates no preference. Stimulus types labeled with the same letter (A–E) are not significantly different from one another.

seeking across a broad range of contexts. The data supporting this article have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.hqbzkh1cj; Daniel et al. 2020).

Discussion

Females exhibited a preference for novelty for a majority (six out of eight) of stimulus types. Therefore, preference for novelty appears to be broad, but not entirely general, in our study population. Interest in novelty was also moderately to strongly positively correlated among all pairs of stimulus types. These results confirm the prediction that interest in novel color patterns is positively associated with interest in novelty in nonsexual contexts. Moreover, these correlations imply overlap in the sources of variation contributing to and thus the processes governing novelty seeking in different contexts.

Exploratory factor analysis provided more evidence of these shared processes. A single meaningful axis of behavioral variation was identified, onto which interest in novelty in all stimulus types loaded positively. This result supports the existence of a single latent variable that accounts for individual variation in overall novelty seeking, across contexts. We interpret this latent variable as an axis of variation in general neophilia. The loadings of each stimulus type onto this factor were moderate to strong, indicating that differences in general neophilia account for much of the individual variation in novelty seeking within any given context. This result suggests that general neophilia can be measured by assaying interest in novelty for any of the stimulus types that we tested—even those for which females did not show an overall preference for novelty. Behavioral tests in a wide range of contexts can therefore be used to measure neophilia in guppies. General neophilia explained 61% of the variation in interest in novel color patterns, suggesting that this mating preference is governed largely by shared processes rather than specific processes. Therefore, general neophilia appears to play an important role in modulating interest in novelty in a broad range of contexts, including in mating preference for novel color patterns.

Our results are consistent with the neophilia hypothesis, which proposes that mating preference for novel phenotypes is a nonadaptive by-product of natural selection favoring neophilia. If an individual's level of general neophilia has an appreciable genetic component, then natural selection favoring neophilia in one or more contexts

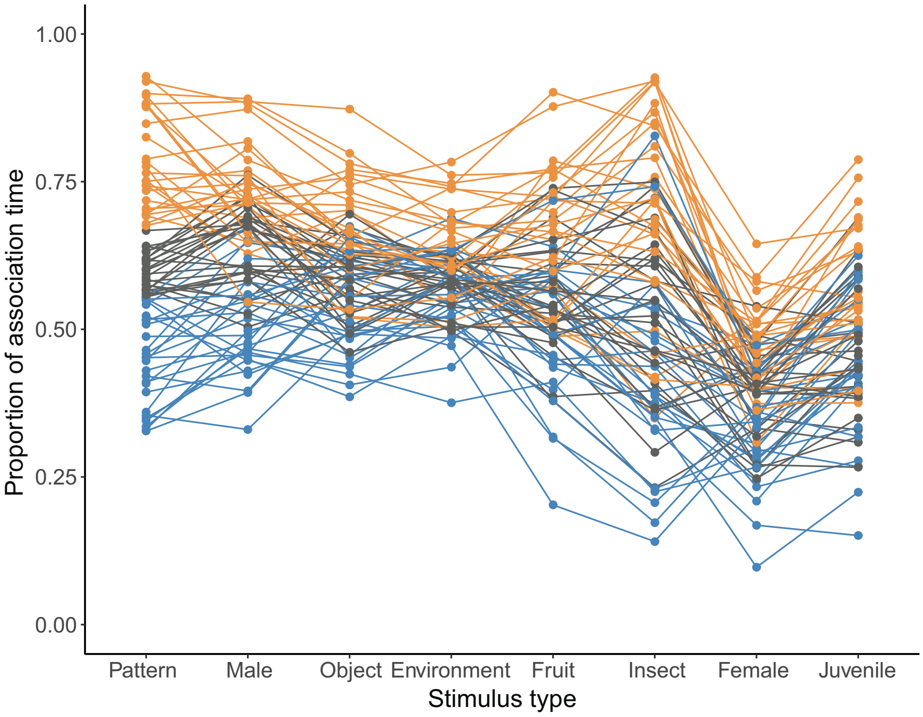

Figure 2: Interest in novelty, for each stimulus type. Each line connects observations of a single female. Color indicates where each female falls in terms of her interest in novel color patterns: blue denotes females that fall below the 0.333 quantile, gray denotes females that fall between the 0.333 and 0.667 quantiles, and orange denotes females that fall above the 0.667 quantile.

could explain the evolution of mating preference for novel color patterns. It is likely that these covariances have a heritable component, since there is substantial genetic variation in response to novelty in many species (e.g., Dingemanse et al. 2004; van Oers et al. 2004; Dochtermann et al. 2015; reviewed in Carere et al. 2010), including guppies (Kotrschal et al. 2014; White and Wilson 2019). The sensory bias model has previously received support in guppies, with female mating preference for orange color spots being linked to preferences for orange food items (Rodd et al. 2002). Our study provides evidence that preference for novel color patterns might similarly be explained by general neophilia, linking sensory bias to a nondirectional preference for novel sexual signals and thus the maintenance of genetic variation. Future work should investigate the extent to which covariance in neophilia across contexts is genetic, for example, through the use of artificial selection and/or quantitative genetics.

Although all the predictions of the neohilia hypothesis were supported in this study, our results do not exclude the possibility that neophilia is favored by selection in the mating context but is neutral in other contexts, such that selection on mating preference could have led to the evolution of general neophilia. Post hoc tests revealed that preference for novelty was not significantly different among the four stimulus types in which preference for novelty was strongest: color patterns, males, objects, and environments. This pattern suggests that general neophilia is the result of selection acting on exploratory behavior and/or mating preference. Opposing selection favoring shoaling with familiar females may explain the neophobic response for that stimulus type. In any case, our results indicate that the processes responsible for neophilia transcend context and that natural or sexual selection could account for mating preference for novel phenotypes.

Previous work has shown that preference for novel color patterns in guppies is underpinned by habituation to familiar color patterns (Daniel et al. 2019). Habituation is a pervasive form of nonassociative learning that can also account for preferences for other kinds of novel stimuli, including food items (Epstein et al. 2009), objects (Leiner and Fendt 2011), and environments (Rodríguez-Prieto et al. 2010). If there is overlap in the mechanisms responsible for habituation to these different kinds of stimuli, individual differences in rate of habituation could underpin variation in general neophilia. The mechanisms of habituation might therefore constitute a sensory bias for novelty. Dopaminergic pathways in the brain may underlie processes of general neophilia, as they are known to regulate aspects of interest in novel stimuli (Dulawa et al. 1999),

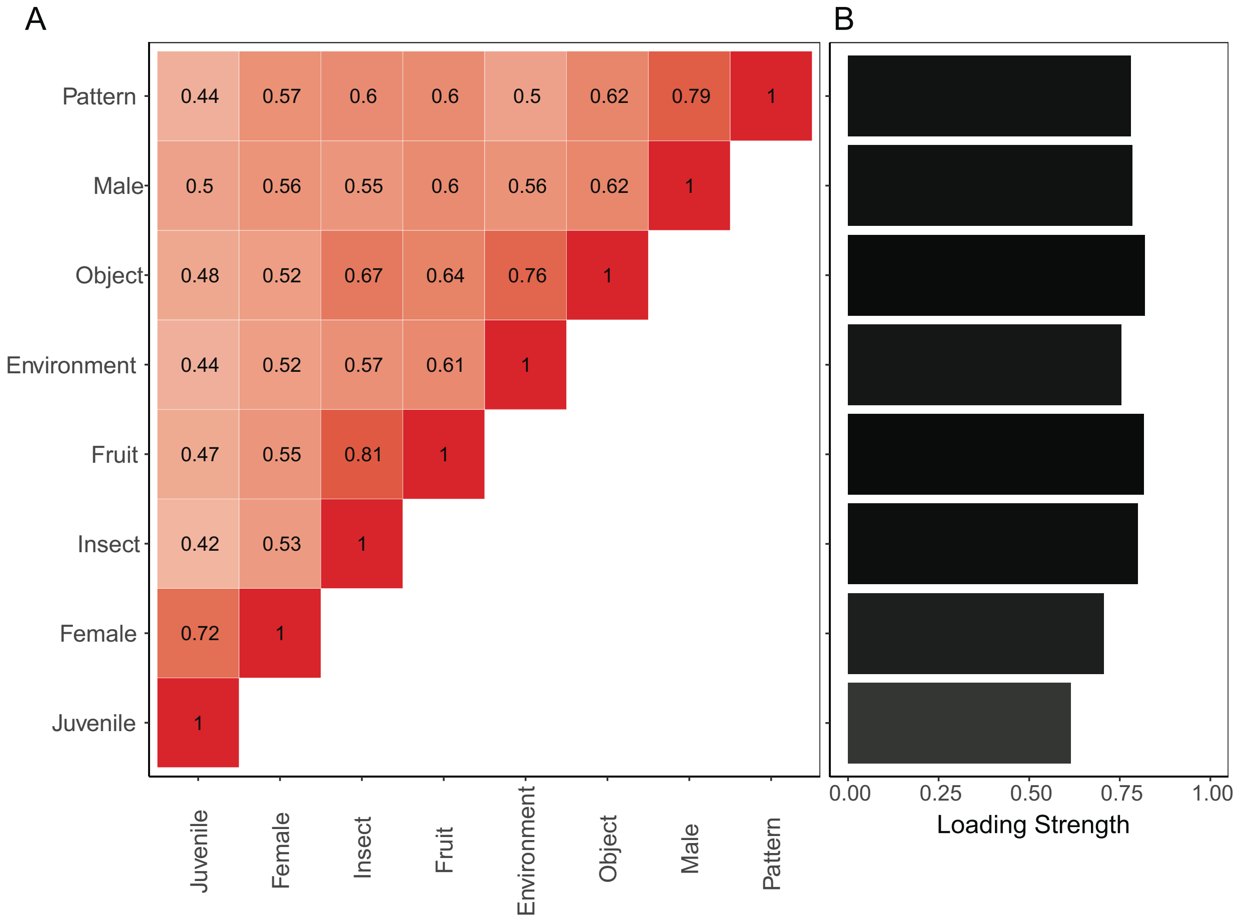

Figure 3: *A*, Pairwise correlations between stimulus types for interest in novelty. Darker red indicates higher correlation coefficients. *B*, Loadings of each stimulus type onto the single-factor (general neophilia) solution.

libido (Zhang et al. 2016), appetite (Salamone and Correa 2009), and habituation (Giles and Rankin 2009).

Our results are consistent with those of a previous study in which exploratory behavior was found to covary with mating preference for unfamiliar individuals (Lucon-Xiccato et al. 2019). Our findings expand on this previous study by showing that preference for novelty exists for and is correlated across a broad range of stimuli, supporting an axis of general neophilia that transcends context. However, other previous studies have provided mixed evidence as to whether responses to novelty are correlated across contexts. Burns (2008) tested different guppy populations than we studied and found that response to novel objects did not covary with response to novel environments, possibly indicating population differences in these covariances. Protocol differences might also explain the discrepancy of results. For example, Burns (2008) measured neophilia as the response to a novel stimulus (which can be affected by behavioral processes such as activity level), whereas we controlled for such factors by measuring response to a novel and a familiar version of each stimulus type presented simultaneously. Indeed, Burns (2008) found that activity level was correlated with response to a novel object. Additionally, Fox et al. (2009) found that exploration in a novel environment does not covary with exploration of a novel object in mountain chickadees. Response to novel environments and novel food items are correlated in some but not all populations of three-spined sticklebacks (Dingemanse et al. 2007) and are correlated for some behavioral measures but not others in Australian desert gobies (Moran et al. 2016). Moreover, a neophilia personality factor (or closely related factors such as openness or exploration) has been supported in several species (e.g., McGuire et al. 1994; Gosling et al. 1999) but appears absent in others (e.g., Neumann et al. 2013). In combination with this literature, our results highlight that the presence and structure of covariances in neophilia across contexts differ among species and populations.

Our results are consistent with those of previous studies reporting that guppies show interest in novel stimuli in specific contexts, including color patterns (Hughes et al. 1999, 2013; Eakley and Houde 2004; Zajitschek and Brooks 2008; Hampton et al. 2009; Graber et al. 2015), individual males (Mariette et al. 2010; Daniel and Rodd 2016), environments (Burns 2008; Burns and Rodd 2008; Burns et al. 2016; Lucon-Xiccato et al. 2019), and food items (Laland and Reader 1999; Kelley et al. 2013). We also found that, as in previous studies, female guppies preferentially shoal with familiar females (Magurran et al. 1994; Griffiths and Magurran 1998; Lachlan et al. 1998). However, past tests of guppy preference for novel objects have produced mixed results. In combination with this literature, our results suggest that age or maturation stage may partially account for behavioral differences, with adult females preferring novel objects (Lucon-Xiccato and Dadda 2016; but see Lucon-Xiccato et al. 2019) and juveniles preferring familiar objects (Petrazzini et al. 2012). These differences in behavior may reflect age-related differences in experience and/or vulnerability to risks such as predation (Reznick et al. 2001). It is likely that selection acts on novelty-seeking behavior in some or all of these nonsexual contexts, lending support to the hypothesis that preference for novel sexual signals is (at least partially) a by-product of natural selection on neophilia.

Perhaps surprisingly, all stimulus types loaded in the same direction on the general neophilia factor, even though females did not exhibit a preference for novelty in all contexts. This pattern suggests that overlap in the processes governing neophilia does not entirely constrain variation in novelty seeking across contexts. Thus, stimulus-specific processes affect overall preference for novelty and contribute to the variation in preference for novelty that is unexplained by the general neophilia factor. One implication of this result relates to potential trade-offs across contexts. Individuals that are highly neophilic in contexts in which novelty seeking is advantageous (e.g., foraging and exploration, in populations with low predation risk) will tend to also be neophilic in contexts in which novelty seeking is costly (e.g., shoaling partner choice). Shared processes of neophilia might thus prevent individuals from simultaneously optimizing responses to novelty in all contexts. However, correlation between response to novelty in different contexts can itself be a target of selection and might have evolved to mitigate this trade-off. That is, selection may have favored a reduction in the amount of overlap in the processes governing neophilia in contexts experiencing opposing selection. This interpretation is consistent with our observation that the two stimulus types that diverged most in terms of overall preference for novelty (females and juveniles) from the stimulus types with the highest level of preference for novelty also had the greatest variation in novelty seeking left unexplained by general neophilia.

In summary, we found that female guppies exhibit a preference for novelty in many ecological contexts (mate choice, foraging, and exploration) but not all (social, but nonsexual, interactions) ecological contexts. Individual variation in preference for novelty is moderately to strongly correlated among contexts, even for contexts in which there is no overall preference for novelty. A general neophilia factor can account for a large share of variation in neophilia, providing insight into the personality structure of guppies as it pertains to novel stimuli. Taken together, these results suggest that the processes governing interest in novel stimuli overlap substantially among contexts. Our results support the hypothesis that selection on novelty seeking in nonsexual contexts can explain female mating preference for novel male color patterns.

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Statement of Authorship

M.J.D. and K.A.H. designed the experiment and analyzed the data. M.J.D. and L.K. collected the data. M.J.D. wrote the manuscript with input from K.A.H.

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