Male phenotypic diversity experienced during ontogeny mediates female mate choice in Trinidadian guppies

Abbreviated title: Male diversity and female mate choice in guppies

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Abstract

Early social experience can be important in shaping female mate choice. Previous work has shown that females adjust their decisions based on the distribution of male sexual trait values encountered during development. However, other phenotypic features could be important in the formation of mate preferences if, for example, they provide additional information about the males available. Here, we examined how the level of overall phenotypic variance (independent of trait values) experienced during ontogeny, mediated female choice in guppies, *Poecilia reticulata*. Developing females were reared with males either all different in colouration or all similar in colouration or with adult females representing high variance, low variance and no experience of male variance respectively. We found that females were more sexually responsive when reared with females only than in either of the male treatments. When reared with males, responsiveness was greater in the low-variance compared to the high variance treatment. Moreover, females had stronger sexual preferences following rearing in the high variance compared to the low variance condition. In turn, males switched mating tactics, increasing the rate of coerced copulation attempts when facing choosier females, possibly to balance the loss in mating opportunities. Taken together, these results demonstrate the adaptive plasticity of female mating decisions and the dynamic selection pressures they might impose on the evolution of male sexual traits, potentially contributing to the maintenance of the extreme polymorphism found in male colour patterns.

Keywords: Mate choice, early social environment, ontogeny, adaptive plasticity, colour pattern polymorphism, *Poecilia reticulata*

Introduction

Research on female mating preferences has historically focused on the role of average preferences on the evolution of elaborated male traits and species recognition (Andersson and Simmons, 2006; Andersson, 1994). However, a growing body of evidence suggests there is substantial phenotypic variation among and within females in mate choice behaviour and this can have significant ecological and evolutionary implications (Ah-King and Gowaty, 2016; Brooks, 2002; Brooks and Endler, 2001; Jennions and Petrie, 1997; Widemo and Saether, 1999). This variability has been linked to genetic differences (Bakker and Pomiankowski, 1995; Brooks, 2002; Jennions and Petrie, 1997; Widemo and Saether, 1999), environmental factors (e.g. perceived predation risk (Johnson and Basolo, 2003; Kim et al., 2009), signalling environment (Endler, 1991; Gordon and Uetz, 2011)), intrinsic factors (e.g. female age and condition (Coleman et al., 2004; Cotton et al., 2006), cost of sampling males (Milinski and Bakker, 1992)), social experience (Jirotkul, 1999; Mery et al., 2009; Rutledge et al., 2010; Witte and Nöbel, 2011) and distinct developmental trajectories (Bailey and Zuk, 2008; Macario et al., 2017). The conditions experienced early in life strongly influence the development of an individual's morphology, behaviour and cognition (Buchanan et al., 2013; Stamps, 2016; West-Eberhard, 2003), however, some gaps remain in the understanding of the effects of the social context experienced during ontogeny on mate choice.

Developmental plasticity enables juveniles to evaluate environmental conditions and adapt their behaviour accordingly to maximize fitness once adult, especially in variable environments (Kasumovic and Brooks, 2011; West-Eberhard, 2003). In species with parental care, young individuals may use visual (Kendrick et al., 1998), acoustic (Riebel, 2003, 2009) and olfactory (Penn and Potts, 1998) cues displayed by their parents to adjust mate preferences, allowing for the

recognition of conspecifics as prospective partners. However, mate preferences can also be formed during ontogeny in group living species with no parental care referred to as "oblique imprinting" (Hebets and Sullivan-Beckers, 2010). This type of mate choice imprinting in which juveniles learn characteristics of nonparental adults encountered during development has received considerably less attention than "parental imprinting" and its potential evolutionary consequences have not been fully explored.

Previous studies on oblique imprinting have investigated how the manipulation of secondary sexual traits known to be good predictors of mating success affected the outcome of mate choice. For example, Macario et al. (2017) found that, in the Trinidadian guppy (*Poecilia reticulata*), both the values of male coloration experienced as juveniles and the duration of exposure influenced females' preferences and choosiness. Walling et al. (2008) varied the size of the sword – an important criterion of mate choice in the genus *Xiphophorus* - to which growing females were exposed and demonstrated that the preference for long swords could be reversed if female experience was restricted to short-sworded males. A similar effect has been observed in the wolf spider *Schizocosa ocreata* where females shifted their preferences towards males displaying the sexual trait they experienced as juveniles. Hence female *S. ocreata* previously exposed to only large-tufted males or only small-tufted males chose, as adults, large-tufted and small-tufted males respectively (Stoffer and Uetz, 2016). In the related species *S. uetzi*, females preferred to associate with the phenotype of males – either brown or black painted legs - encountered while maturing (Hebets, 2003). Moreover, it has been shown that female *S. ocreata* exposed to males with a mixture of tuft sizes (Stoffer and Uetz, 2016) and female guppies exposed to males with low- and high- orange colouration (Rosenqvist and Houde, 1997) both increased the strength of their mating

97 preferences. Thus, previous work has considered how ontogenetic exposure to sexual traits that
98 vary quantitatively (e.g. short or long sword, small or large tufts) or qualitatively (e.g. brown or
99 black legs) influenced mate choice. It has also highlighted the importance of perceiving variation
100 in a sexual trait for the development of mate preferences. However, there are still some aspects of
101 the social environment that, to our knowledge, have not been investigated and could potentially
102 drive variation in individual preferences. Sexual displays are often complex, consisting of many
103 different signal components and in species where males display such complex traits, female mate
104 choice is based on these multiples cues (Candolin, 2003). For instance, in guppies, a species in
105 which males display multicomponent colour signals, females favour groups of colours rather than
106 individual colours independently and the preferences for different colour combinations vary
107 depending on the environment (Cole and Endler, 2015). Unlike previous studies which focused on
108 the influence of variation in a single sexually selected trait, we aimed here to analyse how being
109 exposed during development to different levels of variation in overall phenotypes affects the
110 process of mating decision.

111 We examined how the frequency distribution of whole male phenotypes (independently of
112 the value or salience of any specific secondary sexual trait), experienced during development
113 shaped female mate choice in adult life. Using the Trinidadian guppy, we tested the hypothesis
114 that females adjust their responsiveness to males' sexual solicitation and preference functions (how
115 potential mates are ranked based on inherent characteristics) following exposure to males with
116 similar colour patterns (low between-male variance) or males with entirely different colour
117 patterns (high between-male variance). The extreme polymorphism found in their coloration
118 (Houde, 1997) and a strong environmental component accounting for phenotypic variation in

female mate choice (Brooks, 2002; Brooks and Endler, 2001) provide an excellent model system to address the developmental trajectory of female preferences and its potential evolutionary implications, such as the maintenance of variation in male ornamentation and population divergence. We predicted that females would be choosier, that is less responsive, after exposure to high variance in male phenotype because they would learn as juveniles that their social environment provided relative diversity in males, improving the benefits of sampling multiple males prior to making mating decisions. Similarly, we predicted that females would increase the strength of their preference functions as it paid off to invest in prospecting for the best possible mate. Furthermore, to more fully understand the implications of female choice behaviour, we investigated the possibility that males shift their reproductive tactics – courtship display versus sneak matings (e.g. forced copulation attempts) – in response to changes in female sexual behaviours. Guppy males exhibit a high level of plasticity in their mating behaviour driven by multiple factors such as the operational sex ratio (Jirotkul, 1999), male trait distribution (Jirotkul, 2000), female reproductive status (Guevara-Fiore et al., 2010) and social experience (Guevara-Fiore, 2012; Price and Rodd, 2006). We predicted that males would shift from courting females to sneaky attempts as females become choosier and variance in male reproductive success increases.

Methods

Study organisms

Guppies are small livebearing freshwater fish native to the coastal streams of the north-eastern part of South America. We used descendants of individuals collected in the lower part of the Aripo River on the island of Trinidad (N 10°39’03’’; W 61°13’40’’). Neonates were selected from housing tanks at day 5 post-birth and placed into treatment groups. All fish housed in the laboratory

were maintained on a 12h light:dark cycle at 24-25°C and were fed twice daily: in the mornings with commercial flake and in the afternoons with brine shrimp (*Artemia nauplii*). Plastic plants were placed into the tanks to physically enrich the environment and provide shelter for the fish.

Rearing setup

Neonates were evenly distributed into one of three types of rearing groups composed of adult fish representing two levels of phenotypic variance and a control group (high and low between-male variance and a female-only condition, respectively, N=6 stimuli fish/group, Fig. 1) and reared for 84 days (all females were sexually mature at this point: A.M., personal observation). The "high variance" treatment was composed of males that differed distinctively in their colour patterns. The "low variance" treatment was achieved by assembling similar males for which nearly all the coloured patches coincided in both colour classes and topographic position although could slightly vary in their shape. The "no male" treatment was composed of mature non-virgin females with no secondary body colouration. Due to the Y-linked inheritance of a large part of the colour pattern alleles (Haskins and Haskins, 1951; Houde, 1992; Winge, 1927; Yamamoto, 1975), the "low variance" condition was represented by collections of half or full brothers unlike "high variance" males that were unrelated or very distantly related. The experiment consisted of four replicates of each treatment. The rearing tanks were divided into two compartments of unequal size (30x30x18cm + 15x30x18cm). The developing fry were housed in the large compartment and through a perforated, transparent Perspex sheet were exposed to groups of 6 stimulus fish representing the three different conditions. The partition allowed olfactory and visual cues to pass from one compartment to the other. Males were removed from rearing groups before reaching sexual maturity (i.e. before the gonopodial hood extended beyond the tip of the fin (Reznick,

164 1990)). The number of focal females varied across treatments and across replicates depending on
165 brood size and brood sex ratio (see Table 1 in electronic supplementary method).
166
167 *Behavioural trials*
168 Following the 84-day rearing period, we assessed mate choice in a 42 litre open arena
169 (60x35x20cm). In each trial an equal number of stimulus males and experimentally reared females
170 were placed in the arena (see electronic supplementary methods for details on number of males
171 and females used in each trial) as an even sex ratio reduces male-male competition which in turn
172 facilitates the full expression of female preferences. Stimulus males for these trials were randomly
173 drawn from different stock tanks and chosen by eye to differ in colour patterns and size. Females
174 were from the same rearing treatment and had not been exposed during development to the colour
175 patterns of the stimuli males used in the behavioural trials. In total 4 replicate groups for each
176 treatment were observed. As females show little discrimination prior to their first mating, focal
177 females were mated with an unfamiliar male (i.e. neither represented in the rearing treatments nor
178 in the behavioural trials) the day before testing to ensure the full expression of their preferences
179 (Daniel and Rodd, 2016; Endler and Houde, 1995; Houde, 1997). On the day of the trial, females
180 were released in the test tank two hours before observation to acclimatize them to the novel
181 environment.
182 We recorded sexual behaviour following standard methods (Endler and Houde, 1995;
183 Houde, 1997; Houde and Endler, 1990). During a trial we carried out five minutes of focal
184 sampling on each male during each of 6 sessions across a single day: 3 sessions in the morning
185 and 3 in the afternoon. The order in which males were sampled in a session was randomized. Two
186 male behaviours were recorded: the number of courtship displays and the number of sneak mating

187 attempts. The courtship display, or "sigmoid" display (Houde, 1997), takes the form of a male
188 quivering stiffly in front of a female while he bends his body into an S-shape potentially ending
189 with a consented insemination. Alternatively, a male can adopt a sneaker strategy, attempting
190 copulation without female cooperation by thrusting his gonopodium at the female's gonopore
191 (Houde, 1997). Males that performed less than five displays (seven males in total) throughout the
192 6 observation sessions were excluded from the analysis. Male displays were registered only if they
193 were directed toward a particular female, if other males did not interrupt them and if they started
194 after the male became the focal male. Moreover, during focal observations we recorded the sexual
195 responses of females to the courtship display of the male being observed. The relative
196 attractiveness of a given male was evaluated as the proportion of his displays that elicited at least
197 a "glide" response from the females in the group (coined the "fraction response" D; see electronic
198 supplementary methods for details on a female sexual response and on the measurement of D).
199 Individual females within an experimental group were not distinguishable so D represents an
200 aggregate measure of overall female responsiveness for that particular male. The fraction response
201 is a reliable predictor of male mating success in guppies (Houde, 1987, 1988). The degree of
202 preference for a sexual trait was calculated as the regression of D on that trait for all males used in
203 a given experimental group (see below and electronic supplementary methods). At the end of the
204 day, females were placed back in their housing tanks and males were kept in the observational
205 arena.
206
207 *Male trait analysis*
208 Male colour patterns were photographed with a digital camera (Nikon coolpix 8800) in a custom-
209 made box filled with a small volume of water where fish were free to swim. All the pictures were

taken under the same light conditions when the fish was parallel to the glass face of the box and the images analyzed with UTHSCSA ImageTool (http://compdent.uthscsa.edu/dig/itdesc.html). Colour patches were grouped into the following colour classes: black, orange (including red), yellow, iridescent (including silver/white, blue, violet, and bronze-green) and total colour area. Colour classes were measured as relative total area (relative to the body + caudal fin). Total body length (from the tip of the snout to the tip of the longer lobe of the caudal fin) was recorded using a digital caliper. A measure of diversity of the colour pattern was also calculated for each male. This male trait, which is rarely examined in the study of female preference, was computed with Simpson's Reciprocal Diversity Index. The values span from 1 to X with X being the number of categories being used (with 5 colour classes, the highest possible value, X=5, when each of the 5 colours have equal areas). The lower the value the less diversity and vice versa (see electronic supplementary method for more detailed explanations).

Female preference analysis

We divided female sexual behaviour into two measurable components (Brooks and Endler, 2001; Jennions and Petrie, 1997; Widemo and Saether, 1999). *Choosiness* is the effort an individual is prepared to invest in mate assessment and is represented in this study by females' responsiveness D. The *preference function* is the ranking order of the male sexual signal, measured as the relationship between female responses D and the male trait they are evaluating (see method in electronic supplementary).

Statistical analyses were performed in R version 3.3.2 (The R Foundation for Statistical Computing, Vienna, Austria). To determine the effects of the rearing treatments on female responsiveness (D), we used a generalized linear mixed model (GLMMs, 'glmer' function of the

233 'lme4' R package) fit by maximum likelihood (Laplace approximation) with a binomial error
234 distribution and logit link. A model selection procedure, using the 'LMERConvenienceFunctions'
235 R package that performed backward selection of fixed effects and forward fitting of the random
236 effect kept in the final model Treatment, Orange, Yellow and Total Colour as fixed effects. To
237 establish whether the total number of male courtship displays varied across treatments we used a
238 generalized linear mixed model (GLMMs, 'glmmadmb' function of the 'glmmADMB' R package)
239 with a negative binomial error distribution and logarithmic link to account for overdispersion. The
240 influence of the rearing conditions on total number of gonopodium thrusts was examined
241 performing a generalized linear mixed model ('glmmadmb' function of the 'glmmADMB' R
242 package) with a Poisson error distribution and logarithmic link. To determine the most adequate
243 model, we used Likelihood Ratio Tests via the 'drop1' R function. Based on Akaike Information
244 Criterion (AIC) criterion, we kept in our final model the fixed effects Treatment, Black and Total
245 Colour. Experimental group was included as a random term in all models to account for the non-
246 independence of the behaviours of males and females within the same trial. To adjust for multiple
247 testing and decide which differences were significant across treatment groups, we corrected *p*-
248 values following a Holm procedure.
249 For each sexual trait, the overall degree of female preference was analysed among
250 treatments. To do so, preference slopes of these traits were compared using a Kruskal-Wallis
251 procedure performed with the "qn.test" function developed in the "kSamples" R package. Post-
252 hoc analyses were carried out applying the Nemenyi test found in the "DescTools" R package.
253 Finally, we tested for a correlation between female preference slopes and the level of variance
254 experienced as juveniles using a spearman rank correlation.
255

Results

Responsiveness

The females from the no-male group (reared with females) were significantly more responsive than those from both the low- and high-variance groups and females from the low-variance condition were more responsive than females from the high-variance group (χ^2= 25.8, *df*=2, p<0.0001, Fig. 2). Female responsiveness was thus greatest when the level of phenotypic variance experienced during development was lowest in the case of females that had experienced males, or non-existent in the case of females' naïve to male variance. There was no significant difference in the intensity (i.e. display rate) of male courtship towards females reared in the different treatments (χ^2= 0.73, *df*=2, p=0.69) so it is unlikely that variation in female responsiveness among treatments was due to differences in male display behaviour.

Linear preference functions

Female preferences were estimated as the linear regression slope coefficients of female response (D) on the male trait being evaluated. We estimated the female preference function for each male trait (orange, yellow, black, iridescent, total colour area, total body length and diversity of colour pattern) separately within each trial. Our four replicates yielded four slope coefficients per treatment per male trait. Females that had experienced different phenotypes during development showed stronger preferences for yellow (p=0.02), black (p=0.08) and total colour area (p=0.01) than females exposed to similar males (Table 1). Moreover, females with no male experience preferred smaller males relative to females reared with similar males (p=0.03). In contrast, there were no significant differences with respect to preference for body size between females from the high variance treatment and the two other experimental groups (Table 1, Fig.3). We also found a

positive correlation between the strength of Yellow (p=0.04), Total Colour (p=0.08) and Total Body Length (p=0.06) preferences and the level of phenotypic variability experienced as juveniles (Table 1). Finally, we analysed whether the observed differences between treatments resulted from variation in the overall phenotypic variance *per se* or because of variation in a particular sexual trait experienced during rearing. To do so, we compared the average values of sexual traits experienced in the low and high variance treatments and for which females displayed a preference during the behavioural trials. Independent t-tests did not reveal any differences between the two experimental conditions ruling out the possibility that the observed effects resulted from exposure to variation in the value of a particular sexual trait (yellow: t_{46}=1.35, p=0.18; black: t_{46}=0.95, p=0.35; total colour: t_{46}=0.29, p=0.78; total body length: t_{46}=1.85, p=0.07).

Male alternative reproductive tactic

When males were in contact with females from the "high variance" group, they attempted more sneak copulations than when in contact with females reared in the two other conditions (χ^2= 16.8, *df*=2, p=0.0002; high-variance vs. no-male group: p=0.07, and high- vs. low-variance group<0.001, Fig. 4). Moreover, males attempted fewer gonopodium thrusts towards females reared in the "low variance" than in the "no male" condition (p<0.001). There is a possibility that less attractive males (males not bearing preferred traits) would switch from courtship displays to gonopodium thrusts in order to offset an initial reduced mating success. Although we found a significant effect of Black and Total Colour covariates, these did not drive the differences observed among treatments as signified by the lack of interaction.

Discussion

Our findings demonstrate for the first time that variation in female mate preferences can arise through early social experience with different degrees of phenotypic variability found in males and independently of variation in any particular sexual trait. Females differed in both aspects of mate preference under scrutiny (responsiveness and preference functions) after prior exposure to two different levels of male phenotypic variance and to a control group of females (i.e. naïve to male phenotypic variation). We present evidence that female guppies were less responsive when reared with males than when reared in the absence of males. Following exposure to different levels of variance in male phenotypes, females were more responsive if males were less variable in their phenotypes. Moreover, the strength of preference for male traits such as yellow body colouration or total colour area increased for females having experienced a higher level of phenotypic variance. In response, males shifted their reproductive tactics, augmenting the rate of forced copulation in the presence of those females.

Phenotypic variance and responsiveness

Females were more responsive to males' solicitations as the overall level of phenotypic variance experienced during development decreased. Females from the no-male group (no experience of male variance) were more responsive than females from both the "low-" and "high-variance" male treatments possibly emphasizing that males are a limited resource in the local environment. This may work to augment the willingness of females to respond positively (e.g. be more responsive) and thus engage more in sexual behaviours. These findings support a previous study (Bailey and Zuk, 2008), which showed that female field crickets *Teleogryllus oceanicus* reared in silent conditions (comparable to our "no male" treatment) are more responsive to playbacks than females

reared with male song (comparable to our "low" and "high" variance treatments). Secondly, we found that after being exposed to similar males ("low variance"), females responded more to male displays than females exposed to different males ("high variance"). Zajitschek & Brooks (2008) and Hampton et al. (2009) investigated how sexually mature females accommodated their preferences to different levels of colour pattern rarity (common or redundant vs. unique vs. novel colour pattern) encountered within their social environment and showed that females found unique and novel phenotypes equally more attractive relative to common phenotypes. In our experiment, although adult females were tested only with novel phenotypes, the effects of early social experience were not overridden by the immediate experience of surrounding males.

Phenotypic variance and preference functions

There were no differences between treatments in the direction of preferences for the different traits under investigation (no difference in the sign of the median preference slopes). Females were applying the same ranking criteria independent of their early experience and male attractiveness did not vary as a function of the amount of phenotypic variance that females observed during development. In contrast, there were clear-cut differences in the strength of preferences (magnitude of the slopes) between treatments. Overall, females tended to increase their degree of preferences for sexual cues as the level of phenotypic variance experienced as juveniles increased. Females from the "high variance" condition had stronger preferences for greater amount of yellow, black and total colour relative to females from the "low variance" condition. In other words, the level of phenotypic variance displayed by males during female maturation influenced female choosiness (measured here as the magnitude of preference slopes). Even if rather uncommon, females may base their choice on male size in some guppy populations (Endler and Houde, 1995;

347 Magellan et al., 2005; Reynolds and Gross, 1992), favoring larger males (but see Endler & Houde
348 (1995) for Paria river). Here, we report results showing that females were indifferent to male size
349 when reared in contact with males but preferred smaller males when reared without male contact.
350 It is not clear why this preference arose and further examination in the acquisition of preference
351 for male size in guppies is needed.
352
353 *Implications for sexual selection*
354 Our results suggest that phenotypic variation found in the social environment during development
355 is unlikely to alter the direction of sexual selection, as females from the three different treatments
356 tended to use the same sexual cues. It may, however, change the strength of sexual selection since
357 choosiness increased when developing females had experienced more variation in male
358 phenotypes.
359 Developmental plasticity in the degree of choosiness can generate dynamic fluctuations in
360 the selection exerted on male traits following this scenario: in a population where phenotype
361 diversity is relatively high, females increase their choosiness, leading to more variance in mating
362 success between males. After a while the diversity in sexual phenotypes is eroded by the strong
363 directional selection imposed by females that are now experiencing less diversity during ontogeny.
364 Less variance in male phenotypes drives females to be less choosy, decreasing the threshold at
365 which they accept males, which in turn relaxes sexual selection on male traits, allowing for more
366 or different sexual phenotypes to spread in the population. The importance of this feedback loop
367 between plasticity in female preferences, environmental variation and selection can explain the
368 maintenance of the polymorphism found in male colour patterns alongside other mechanisms such
369 as frequency-dependent selection or antagonistic pleiotropy for fitness-related traits. From a

female perspective, plasticity in choosiness as a function of male phenotypic variance could represent an adaptive strategy diminishing the cost associated with the process of mate choice. Indeed, widening the range of accepted stimuli (e.g. decreasing choosiness) when the variability in male phenotypes is low allows females to spend time and energy on other activities than searching and assessing potential partners. The effect of plasticity in female preferences was balanced, to some extent, by plasticity in the reproductive tactics adopted by males. In response to females being choosier, males attempted more unsolicited copulations in the form of gonopodium thrusts, which are less costly than courtship rituals. High relative rates of sneak copulation diminish the importance of mate choice as a determinant of male mating success (Kelly et al., 1999; Magurran, 2001), potentially decreasing the strength of sexual selection which in turn can undermine population divergence (Evans et al., 2003; Matthews and Magurran, 2000). Our finding supports previous work showing that the relative importance of sneak attempt versus courtship display within population depends on environmental factors (Endler, 1987; Farr, 1976; Gamble et al., 2003; Godin, 1995; Jirotkul, 1999), morphological characteristics (Karino and Kobayashi, 2005) and prior sexual experience with females (Balaban-Feld and Valone, 2018).

More surprisingly, our results suggest that males surrounded by females from the "low variance" treatments attempted significantly less gonopodium thrusts than males with females from the two other treatments. These differences cannot be explained by differences in male size (Becher and Magurran, 2004; Houde, 1997) or male attractiveness, as there were no correlations between different colour classes used as sexual cues and the rate of thrusts. Females from the "low variance" treatment were exposed during rearing to similar males closely related to each other. We cannot rule out that being exposed to half- or full-siblings affected female behaviours (for other

392 reasons than their similar phenotypes and not observable in our measures) that would in turn affect
393 the rate at which males performed their gonopodium thrusts.
394
395 To conclude, we have demonstrated that the frequency distribution of males' phenotype
396 experienced before reaching maturity (independent of the values of sexual signals) affected female
397 mate choice in guppies; females were choosier when reared with more diverse male phenotypes.
398 Our work adds to the growing literature on phenotypic plasticity in mate choice highlighting the
399 importance of focusing on the physiological, social and ecological conditions of the choosing
400 subject rather than only on the traits of potential mates to understand reproductive decision-making
401 (Ah-King and Gowaty, 2016). This plasticity can have substantial ecological and evolutionary
402 implications such as variation in the relative strength of sexual selection. Most traditional sexual
403 selection models assume that mate choice is directional, fixed within a species and static within
404 individuals over time, missing important properties of the mate choice process. A recent review
405 (Ah-King and Gowaty, 2016) pointed towards the need for elaborating state-dependent models
406 such as the one developed by Gowaty and Hubbell (2009) to study the flexible nature of mate
407 choice. This model, in which our results fit, has the advantage of capturing the variation induced
408 by chooser intrinsic features and ecological and social situations while categorizing the large
409 number of variables associated with within-individual plasticity into few unifying parameters.
410

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References

Ah-King M, Gowaty PA, 2016. A conceptual review of mate choice: stochastic demography, within-sex phenotypic plasticity, and individual flexibility. Ecol Evol 6:4607-4642. doi: 10.1002/ece3.2197.

Andersson M, Simmons LW, 2006. Sexual selection and mate choice. Trends in Ecology & Evolution 21:296-302. doi: DOI 10.1016/j.tree.2006.03.015.

Andersson MB, 1994. Sexual Selection. Princeton: Princeton University Press.

Bailey NW, Zuk M, 2008. Acoustic experience shapes female mate choice in field crickets. Proceedings of the Royal Society B: Biological Sciences 275:2645-2650. doi: 10.1098/rspb.2008.0859.

Bakker TCM, Pomiankowski A, 1995. The genetic basis of female mate preferences. Journal Of Evolutionary Biology 8:129-171. doi: 10.1046/j.1420-9101.1995.8020129.x.

Balaban-Feld J, Valone TJ, 2018. Changes in courtship behaviour following rejection: The influence of female phenotype in Drosophila melanogaster. Ethology 124:149-154. doi: 10.1111/eth.12715.

Becher SA, Magurran AE, 2004. Multiple mating and reproductive skew in Trinidadian guppies. Proc R Soc Lond Ser B-Biol Sci 271:1009-1014. doi: 10.1098/rspb.2004.2701.

448 Brooks R, 2002. Variation in female mate choice within guppy populations: population
449 divergence, multiple ornaments and the maintenance of polymorphism. Genetica 116:343-
450 358.

451 Brooks R, Endler JA, 2001. Female guppies agree to differ: Phenotypic and genetic variation in
452 mate-choice behavior and the consequences for sexual selection. Evolution 55:1644-1655.

453 Buchanan KL, Grindstaff JL, Pravosudov VV, 2013. Condition dependence, developmental
454 plasticity, and cognition: implications for ecology and evolution. Trends in Ecology &
455 Evolution 28:290-296. doi: 10.1016/j.tree.2013.02.004.

456 Candolin U, 2003. The use of multiple cues in mate choice. Biol Rev Cambridge Philosophic Soc
457 78:575-595. doi: 10.1017/s1464793103006158.

458 Cole GL, Endler JA, 2015. Variable Environmental Effects on a Multicomponent Sexually
459 Selected Trait. Am Nat 185:452-468. doi: 10.1086/680022.

460 Coleman SW, Patricelli GL, Borgia G, 2004. Variable female preferences drive complex male
461 displays. Nature 428:742-745. doi: 10.1038/nature02419.

462 Cotton S, Small J, Pomiankowski A, 2006. Sexual selection and condition-dependent mate
463 preferences. Curr Biol 16:R755-R765.

464 Daniel MJ, Rodd FH, 2016. Female guppies can recognize kin but only avoid incest when
465 previously mated. Behavioral Ecology 27:55-61. doi: 10.1093/beheco/arv122.

466 Endler JA, 1987. Predation, Light-Intensity and Courtship Behavior in Poecilia-Reticulata (Pisces,
467 Poeciliidae). Animal Behaviour 35:1376-1385.

468 Endler JA, 1991. Variation in the Appearance of Guppy Color Patterns to Guppies and Their
469 Predators under Different Visual Conditions. Vision Research 31:587-608.

470 Endler JA, Houde AE, 1995. Geographic-Variation in Female Preferences for Male Traits in
471 Poecilia-Reticulata. Evolution 49:456-468.

472 Evans JP, Pilastro A, Ramnarine IW, 2003. Sperm transfer through forced matings and its
473 evolutionary implications in natural guppy (Poecilia reticulata) populations. Biological
474 Journal of the Linnean Society 78:605-612. doi: 10.1046/j.0024-4066.2002.00193.x.

475 Farr JA, 1976. Social facilitation of male sexual behaviour, intra-sexual competition and sexual
476 selection in guppy, *Poecilia reticulata* (Pisces - poeciliidae). Evolution 30:707-717. doi:
477 10.2307/2407811.

478 Gamble S, Lindholm AK, Endler JA, Brooks R, 2003. Environmental variation and the
479 maintenance of polymorphism: the effect of ambient light spectrum on mating behaviour
480 and sexual selection in guppies. Ecology Letters 6:463-472.

Godin JGJ, 1995. Predation risk and alternative mating tactics in male Trinidadian Guppies (*Poecilia reticulata*). Oecologia 103:224-229.

Gordon SD, Uetz GW, 2011. Multimodal communication of wolf spiders on different substrates: evidence for behavioural plasticity. Animal Behaviour 81:367-375. doi: 10.1016/j.anbehav.2010.11.003.

Gowaty PA, Hubbell SP, 2009. Reproductive decisions under ecological constraints: It's about time. Proc Natl Acad Sci U S A 106:10017-10024. doi: 10.1073/pnas.0901130106.

Guevara-Fiore P, 2012. Early social experience significantly affects sexual behaviour in male guppies. Animal Behaviour 84:191-195. doi: 10.1016/j.anbehav.2012.04.031.

Guevara-Fiore P, Stapley J, Watt PJ, 2010. Mating effort and female receptivity: how do male guppies decide when to invest in sex? Behav Ecol Sociobiol 64:1665-1672. doi: 10.1007/s00265-010-0980-6.

Hampton KJ, Hughes KA, Houde AE, 2009. The Allure of the Distinctive: Reduced Sexual Responsiveness of Female Guppies to 'Redundant' Male Colour Patterns. Ethology 115:475-481. doi: 10.1111/j.1439-0310.2009.01634.x.

Haskins CP, Haskins EF, 1951. The inheritance of certain color patterns in wild populations of *Lebistes reticulatus* in Trinidad. Evolution 5:216-225.

Hebets EA, 2003. Subadult experience influences adult mate choice in an arthropod: Exposed female wolf spiders prefer males of a familiar phenotype. Proc Natl Acad Sci U S A 100:13390-13395. doi: 10.1073/pnas.2333262100.

Hebets EA, Sullivan-Beckers L, 2010. Mate choice and learning. In: Breed MD, Moore J, editors. Encyclopedia of Animal Behavior Amsterdam: Elsevier Science. p. 389-393.

Houde AE, 1987. Mate Choice Based Upon Naturally-Occurring Color-Pattern Variation in a Guppy Population. Evolution 41:1-10.

Houde AE, 1988. The Effects of Female Choice and Male Male Competition on the Mating Success of Male Guppies. Animal Behaviour 36:888-896.

Houde AE, 1992. Sex-Linked Heritability of a Sexually Selected Character in a Natural-Population of Poecilia-Reticulata (Pisces, Poeciliidae) (Guppies). Heredity 69:229-235.

Houde AE, 1997. Sex, color and mate choice in Guppies. Princeton: Princeton University Press.

Houde AE, Endler JA, 1990. Correlated evolution of female mating preferences and male color patterns in the Guppy (Poecilia reticulata) Science 248:1405-1408.

512 Jennions MD, Petrie M, 1997. Variation in mate choice and mating preferences: A review of causes
513 and consequences. Biological Reviews Of The Cambridge Philosophical Society 72:283-
514 327.

515 Jirotkul M, 1999. Operational sex ratio influences female preference and male-male competition
516 in guppies. Animal Behaviour 58:287-294.

517 Jirotkul M, 2000. Male trait distribution determined alternative mating tactics in guppies. Journal
518 of Fish Biology 56:1427-1434.

519 Johnson JB, Basolo AL, 2003. Predator exposure alters female mate choice in the green swordtail.
520 Behav Ecol 14:619-625. doi: 10.1093/beheco/arg046.

521 Karino K, Kobayashi M, 2005. Male alternative mating behaviour depending on tail length of the
522 guppy, Poecilia reticulata. Behaviour 142:191-202. doi: 10.1163/1568539053627695.

523 Kasumovic MM, Brooks RC, 2011. It's all who you know: the evolution of socially cued
524 anticipatory plasticity as a mating strategy. Quarterly Review of Biology 86:181-197. doi:
525 10.1086/661119.

526 Kelly CD, Godin JGJ, Wright JM, 1999. Geographical variation in multiple paternity within
527 natural populations of the guppy (Poecilia reticulata). Proc R Soc Lond Ser B-Biol Sci
528 266:2403-2408.

529 Kendrick KM, Hinton MR, Atkins K, Haupt MA, Skinner JD, 1998. Mothers determine sexual
530 preferences. Nature 395:229-230.

531 Kim TW, Christy JH, Dennenmoser S, Choe JC, 2009. The strength of a female mate preference
532 increases with predation risk. Proceedings of the Royal Society B: Biological Sciences
533 276:775-780. doi: 10.1098/rspb.2008.1070.

534 Macario A, Croft DP, Endler JA, Darden SK, 2017. Early social experience shapes female mate
535 choice in guppies. Behavioral Ecology 28:833-843. doi: 10.1093/beheco/arx043.

536 Magellan K, Pettersson LB, Magurran AE, 2005. Quantifying male attractiveness and mating
537 behaviour through phenotypic size manipulation in the trinidadian guppy, Poecilia
538 reticulata. Behav Ecol Sociobiol 58:366-374. doi: 10.1007/s00265-005-0950-6.

539 Magurran AE, 2001. Sexual conflict and evolution in Trinidadian guppies. Genetica 112:463-474.
540 doi: 10.1023/a:1013339822246.

541 Matthews IM, Magurran AE, 2000. Evidence for sperm transfer during sneaky mating in wild
542 Trinidadian guppies. Journal of Fish Biology 56:1381-1386. doi: 10.1111/j.1095-
543 8649.2000.tb02150.x.

544 Mery F, Varela SAM, Danchin t, Blanchet S, Parejo D, Coolen I, Wagner RH, 2009. Public Versus
545 Personal Information for Mate Copying in an Invertebrate. Current biology : CB 19:730-
546 734.

547 Milinski M, Bakker TCM, 1992. Costs Influence Sequential Mate Choice in Sticklebacks,
548 Gasterosteus aculeatus. Proceedings: Biological Sciences 250:229.

549 Penn D, Potts W, 1998. MHC-disassortative mating preferences reversed by cross-fostering. Proc
550 R Soc Lond Ser B-Biol Sci 265:1299-1306.

551 Price AC, Rodd FH, 2006. The effect of social environment on male-male competition in guppies
552 (Poecilia reticulata). Ethology 112:22-32. doi: 10.1111/j.1439-0310.2006.01142.x.

553 Reynolds JD, Gross MR, 1992. Female mate preference enhances offspring growth and
554 reproduction in a fish, *Poecilia reticulata*. Proc R Soc Lond Ser B-Biol Sci 250:57-62.

555 Reznick DN, 1990. Plasticity in age and size at maturity in male guppies (*Poecilia reticulata)* - an
556 experimental evaluation of alternative models of development. Journal Of Evolutionary
557 Biology 3:185-203. doi: 10.1046/j.1420-9101.1990.3030185.x.

558 Riebel K, 2003. The "mute" sex revisited: Vocal production and perception learning in female
559 songbirds. Advances in the Study of Behavior, Vol 33 33:49-86. doi: 10.1016/s0065-
560 3454(03)33002-5.

561 Riebel K, 2009. Song and Female Mate Choice in Zebra Finches: A Review. In: Naguib M,
562 Zuberbuhler K, Clayton NS, Janik VM, editors. Advances in the Study of Behavior, Vol
563 40 San Diego: Elsevier Academic Press Inc. p. 197-238.

564 Rosenqvist G, Houde A, 1997. Prior exposure to male phenotypes influences mate choice in the
565 guppy, Poecilia reticulata. Behav Ecol 8:194-198. doi: 10.1093/beheco/8.2.194.

566 Rutledge JM, Miller A, Uetz GW, 2010. Exposure to multiple sensory cues as a juvenile affects
567 adult female mate preferences in wolf spiders. Animal Behaviour.

568 Stamps JA, 2016. Individual differences in behavioural plasticities. Biol Rev 91:534-567. doi:
569 10.1111/brv.12186.

570 Stoffer B, Uetz GW, 2016. Social experience affects female mate preferences for a visual trait in
571 a wolf spider. Behavioral Ecology 27:252-261. doi: 10.1093/beheco/arv143.

572 Walling CA, Royle NJ, Lindström J, Metcalfe NB, 2008. Experience-induced preference for short-
573 sworded males in the green swordtail, Xiphophorus helleri. Animal Behaviour 76:271-276.

574 West-Eberhard MJ, 2003. Developmental Plasticity and Evolution. New York: Oxford University
575 Press.

576 Widemo F, Saether SA, 1999. Beauty is in the eye of the beholder: causes and consequences of
577 variation in mating preferences. Trends in Ecology & Evolution 14:26-31.

578 Winge Ø, 1927. The location of eighteen genes in *Lebistes reticulatus* Journal of Genetic 96:1-43.

579 Witte K, Nöbel S, 2011. Learning and mate choice. In: Brown C, Laland KN, Krause J, editors.
580 Fish Cognition and Behavior: Wiley-Blackwell.

581 Yamamoto TO, 1975. The medaka, *Oryzias latipes*, and the guppy, *Lebistes reticularis*. In: King
582 RC, editor. Handbook of Genetics New York: Plenum Press. p. 133-149.

583 Zajitschek SRK, Brooks RC, 2008. Distinguishing the Effects of Familiarity, Relatedness, and
584 Color Pattern Rarity on Attractiveness and Measuring Their Effects on Sexual Selection in
585 Guppies (Poecilia reticulata). Am Nat 172:843-854. doi: 10.1086/593001.

Figure legends:

Figure 1: Example of one replicate made of a control group and two different levels of male phenotypic variance to which fry were exposed to. The scale among the different pictures in the figure varies.

Figure 2: Females responsiveness in the 3 different treatments. Bars represent estimated marginal means +/- 1SE. ***$p<0.0001$, **$p<0.01$, *≤ 0.05.

Figure 3: Degree of female preference for different male sexual traits after exposure to *no male* (■), *low variance* (▲) and *high variance* (●) treatments. Each data point represents the degree of preference in one observation session.

Figure 4: Gonopodium thrusts attempted by males to females reared in the 3 different treatments. Bars represent estimated marginal means +/- 1SE. ***$p<0.001$, *<0.1.

Table 1: Median of the preference slope for the three treatments. Each row represents a trait that could affect the linear preference function of females. K-W is the Kruskall-Wallis statistic testing for differences in degree of preference between treatments; multiple comparisons between treatments; r_s is the spearman rank correlation coefficient between preference slope and the treatments ordered from "no male" to "high variance"; n is the total number of observation session.

	Median of preference slope			Preference Differences		Multiple comparisons			Preference and Treatment	
						No male - Low variance	No male - High variance	Low variance - High variance		
	No male	Low variance	High variance	K-W (df)	*P* –value **	Test statistic + adjusted significance	Test statistic + adjusted significance	Test statistic + adjusted significance	r_s	n
Orange area	1.94	1.14	2.61	3.04 (2)	0.23	-	-	-	0.12	12
Yellow area	2.10	0.38	5.05	8.0 (2)	0.005*	2.0 p=0.71	-5.0 p=0.12	-7.0 **p=0.017**	0.59 ‡	12
Black area	1.88	0.26	3.12	4.77 (2)	0.09	3.5 p=0.36	-2.0 p=0.71	-5.5 **p=0.08**	0.24	12
Iridescent area	0.24	-0.24	-0.18	0.15 (2)	0.94	-	-	-	-0.06	12
Total colour area	1.50	0.55	3.09	8.77 (2)	0.001*	3.0 p=0.47	-4.5 p=0.18	-7.5 **p=0.009**	0.53 †	12
Total body length	-0.15	-0.01	-0.02	6.96 (2)	0.02	-6.5 **p=0.029**	-4.75 p=0.15	1.75 p=0.77	0.56 †	12
Simpson's Reciprocal Index	0.09	0.06	0.14	3.85 (2)	0.15	-	-	-	0.3	12

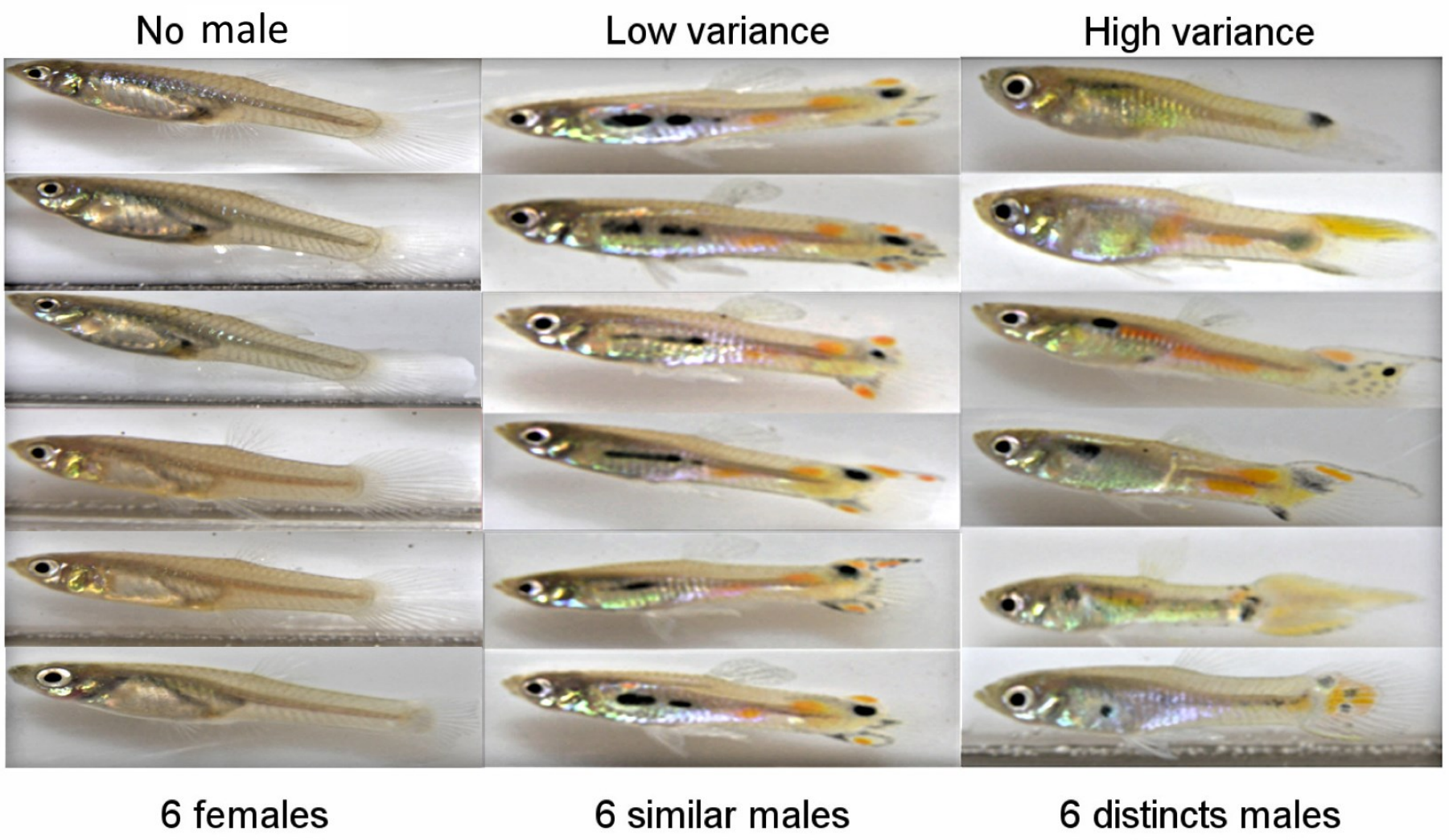
* *The significance level is based on the exact distribution of the test statistic providing an exact *p*-value

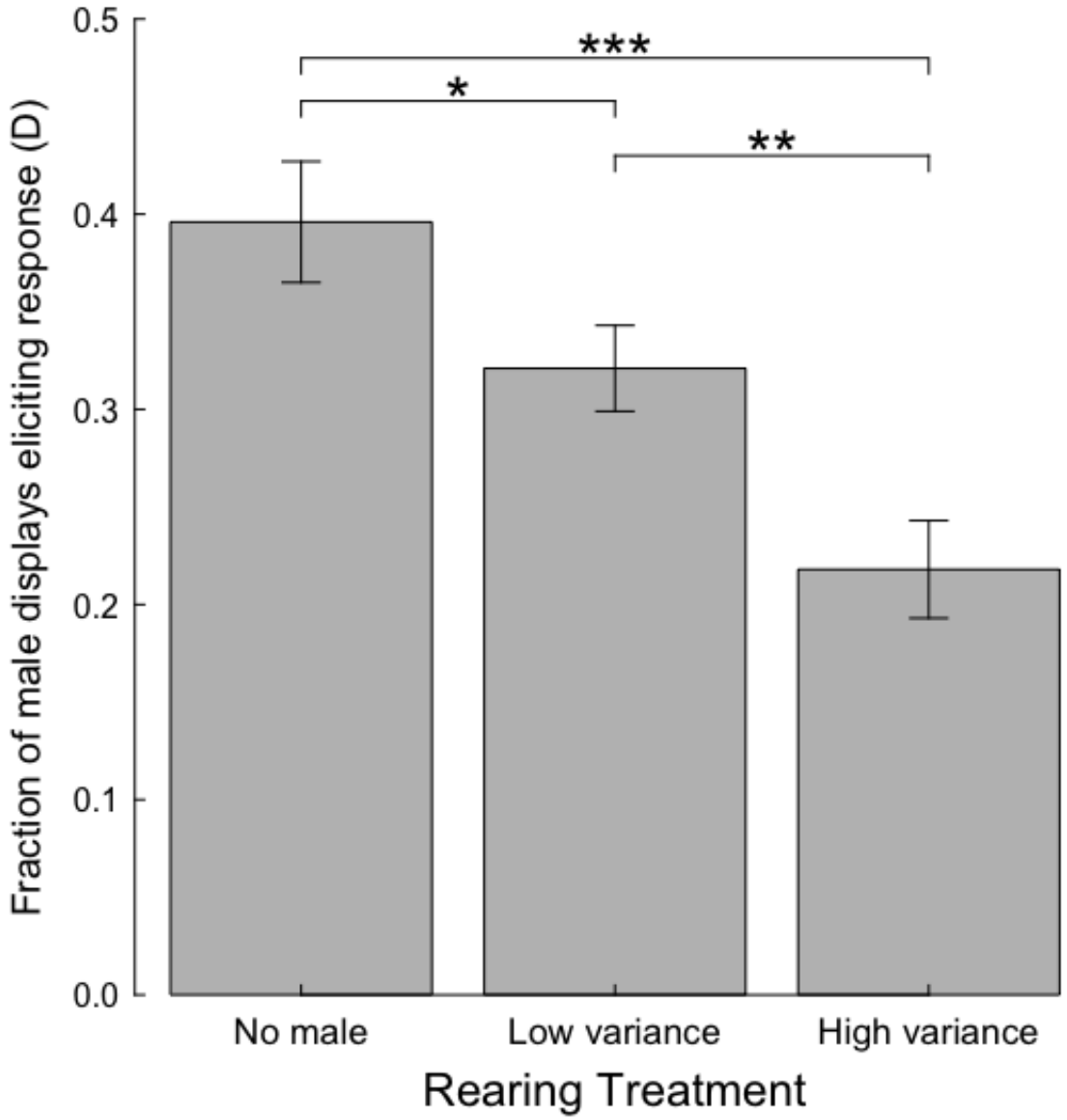
* Significant after Bonferroni correction for number of tests in column

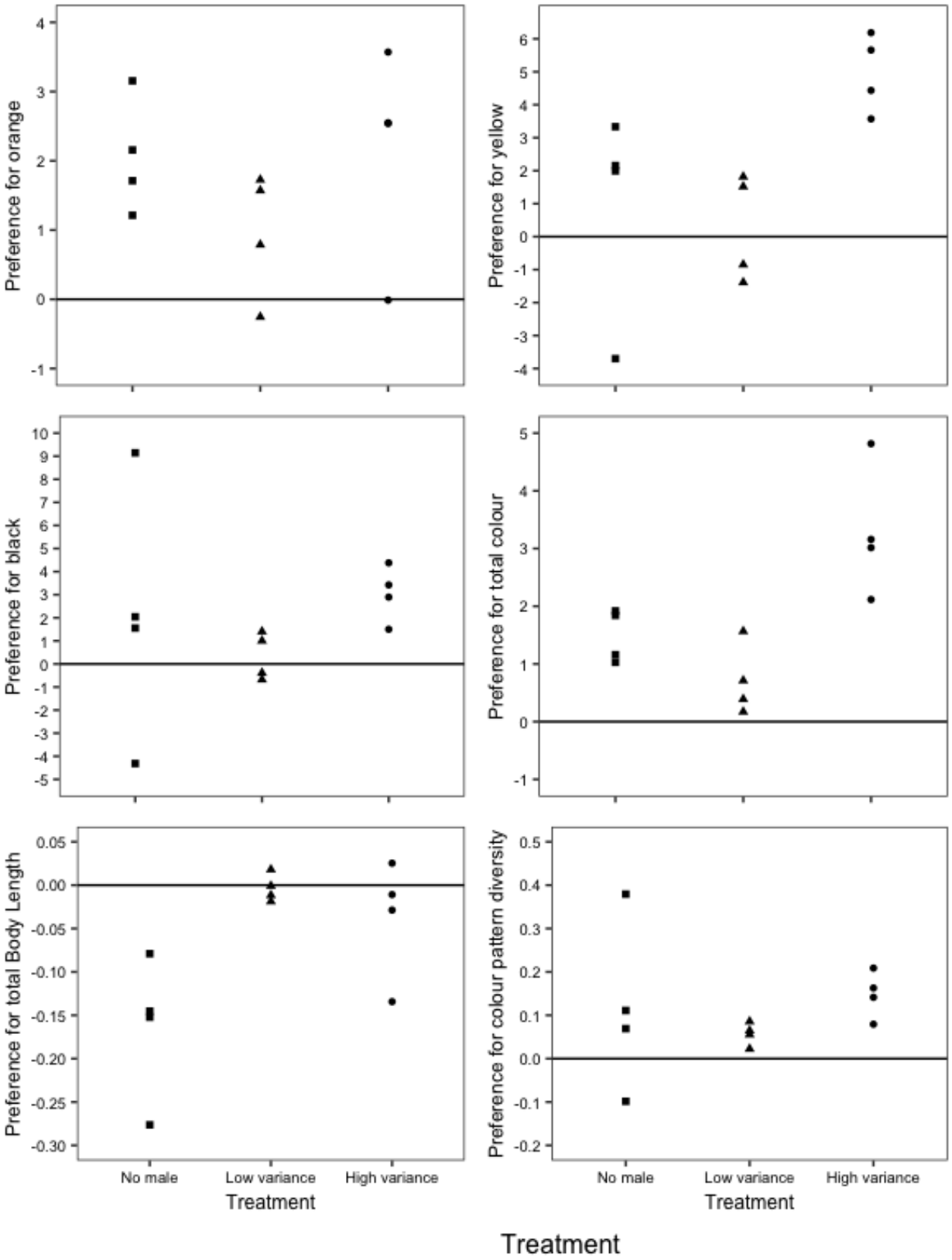
- No pairwise comparisons when no overall significant differences across treatments

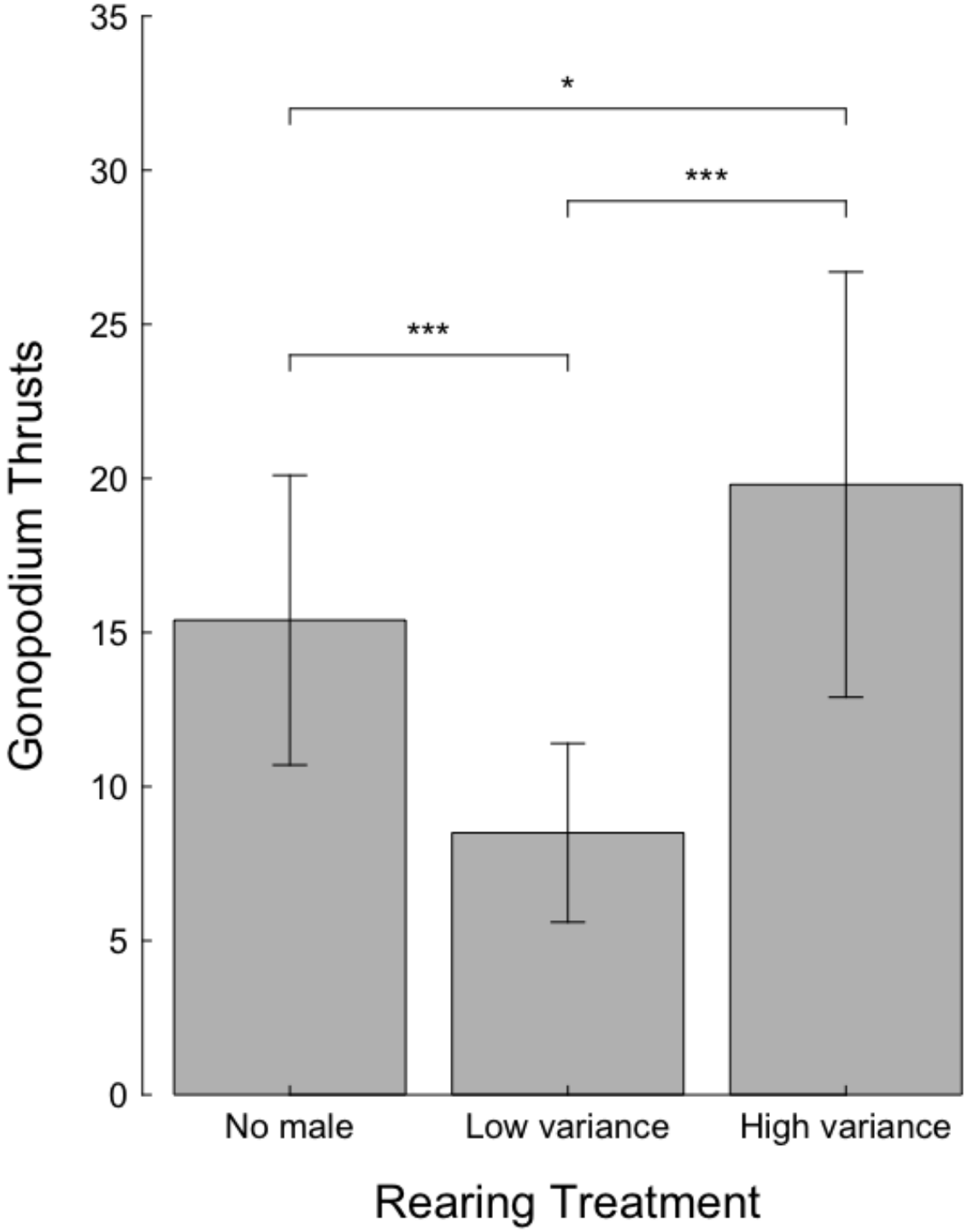
† $p < 0.1$

‡ $p < 0.05$


No male
Low variance
High variance
6 females
6 similar males
6 distincts males


0.5
0.4
0.3
0.2
0.1
0.0
Fraction of male displays eliciting response (D)
***
*
**
No male
Low variance
High variance
Rearing Treatment


Preference for orange
Preference for yellow
Preference for black
Preference for total colour
Preference for total Body Length
Preference for colour pattern diversity
No male
Low variance
High variance
Treatment
Treatment


35
30
25
20
15
10
5
0
Gonopodium Thrusts
*
***
***
No male
Low variance
High variance
Rearing Treatment