What do orange spots reveal about male (and female) guppies? A test using correlated responses to selection

Magdalena Herdegen-Radwan,[1,2] (iD) Silvia Cattelan,[3] (iD) Jakub Buda,[4] (iD) Jarosław Raubic,[5] and Jacek Radwan[6] (iD)

[1]Department of Behavioural Ecology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poznan 61-614, Poland

[2]E-mail: magdalena.herdegen@amu.edu.pl

[3]Department of Biology, University of Padova, Padova 35121, Italy

[4]Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poznan 61-614, Poland

[5]Population Ecology Group, Faculty of Biology, Adam Mickiewicz University in Poznan, Poznan 61-614, Poland

[6]Evolutionary Biology Group, Faculty of Biology, Adam Mickiewicz University in Poznan, Poznan 61-614, Poland

Female preferences for male ornamental traits can arise from indirect benefits, such as increased attractiveness or better viability of progeny, but empirical evidence for such benefits is inconsistent. Artificial selection offers a powerful way to investigate indirect effects of male ornaments. Here, we selected for the area of orange spots on male guppies, a trait subject to female preferences in our population, in replicated up- and down-selected lines. We found a significant direct response to selection, and a correlated response in female preferences, with females from down-selected lines showing less interest in more orange males. Nevertheless, up-selected males sired more offspring in direct competition with low-selected males, irrespective of female origin. We did not find a significantly correlated response to selection among any other fitness correlates we measured. Our results imply that female preferences for orange spots can lead to increased reproductive success of their sons, with no effect on general viability of progeny. Furthermore, although we demonstrate that female preferences may evolve via linkage disequilibrium with the preferred trait, the potential for runaway selection by positive feedback may be constrained by the lack of corresponding linkage with male reproductive competitiveness.

KEY WORDS: Ornaments, *Poecilia reticulata*, preferences, sexual selection.

Sexual selection arises from reproductive competition, typically among males, and favors traits useful in intrasexual combat or in attracting the opposite sex (Darwin 1871; Andersson 1994; Andersson and Simmons 2006). The reasons why traits like elaborate ornaments or bright coloration attract females remain controversial, particularly in species in which males do not provide material benefits to females (Kokko et al. 2003; Andersson and Simmons 2006; Prokop et al. 2012; Radwan et al. 2016; Achorn and Rosenthal 2020). Fisher (1930) argued that, given additive genetic variance (V_A) in both female preference

for male trait and in the trait itself, a self-reinforcing runaway process will ensue, leading to elaboration of the trait. The process arises because progeny of females expressing the preferences will inherit both the preference and the preferred trait. Males inheriting the trait will achieve above-average mating success, spreading genes for both the trait and preference and thus leading to further reproductive advantage of the trait. Furthermore, costs of elaborated sexual traits are expected to make their expression condition dependent (Andersson 1986; Cotton et al. 2004). Because condition, in turn, is likely to integrate genetic variance

for organismal efficiency, reflecting, for example, mutation load, sexually selected traits were hypothesized to signal "genetic quality" of their bearers and bring genetic benefits to choosy females in terms of improved offspring fitness (Zahavi 1975; Andersson 1986; Rowe and Houle 1996; Houle and Kondrashov 2002; Tomkins et al. 2004). Good genes and Fisherian mechanisms are not mutually exclusive, as the latter is an inevitable component of the former (Kirkpatrick and Ryan 1991). Indeed, Fisherian versus good genes dichotomy was argued to be redundant as genes that enhance offspring fitness via sons' mating success can also be considered "good genes" (Kokko et al. 2002; Kokko et al. 2003, but see Fuller et al. 2005). Nevertheless, it remains critically important to understand how sexual ornaments are correlated with fitness-related life-history traits (Kokko and Brooks 2003; Kokko et al. 2003; Candolin and Heuschele 2008; Whitlock and Agrawal 2009; Radwan et al. 2016; Achorn and Rosenthal 2020). For example, if sexual ornaments are significantly genetically correlated with general health and vigor, we can expect their evolution to enhance the ability of populations to adapt to novel environments (Lorch et al. 2003) or help avoid extinction (Martinez-Ruiz and Knell 2017), but if male reproductive benefit is the only component of fitness associated with exaggerated sexual trait, associated cost may in fact increase the risk of extinction (Kokko and Brooks 2003; Martins et al. 2018). A recent meta-analysis of experimental evolution studies manipulating the magnitude of sexual selection found its generally positive effect on traits indirectly related to fitness, but not on fitness components measured directly (Cally et al. 2019). An earlier meta-analysis, more directly relevant to the value of exaggerated sexual traits as indicators of genetic quality, found mixed evidence too: male traits associated with attractiveness were positively associated with "performance traits" indirectly associated with fitness, but not with life-history traits directly associated with fitness (Prokop et al. 2012). Thus, the question whether exaggerated sexual traits reveal general health and vigor, and consequently how they affect the ability of populations to adapt and/or avoid extinction, remains open.

Selection experiments are powerful tools for testing genetic correlations because correlated responses to selection accumulated across several generations are easier to detect (Boake 1985; Conner 2003; Kawecki et al. 2012). Furthermore, selection experiments are well suited to reflect fundamental associations between traits resulting from pleiotropy (or tight physical linkage), rather than transient linkage disequilibria arising, for example, from drift, selection, or nonrandom mating. Such transient disequilibria should break down within a few generations in the absence of forces maintaining them in a sampled population, or, if they arise in selection lines by drift, the disequilibria should become inconsistent between the lines (Roff 1997). However, selection experiments have rarely been used to investigate correlated responses of fitness components to selection for elaborated sexually selected traits, and when they have been, it was mostly with respect to male weapons (Harano et al. 2010; Okada et al. 2011; Plesnar-Bielak et al. 2014) rather than traits subject to female preferences (but see Jia et al. 2000).

Additionally, in the context of sexual selection, selection experiments provide an elegant means to demonstrate the operation of the Fisherian process. This process does not assume a pleiotropic effect (although it does not exclude it), but a linkage disequilibrium arising from nonrandom mating between males carrying a trait under selection and females showing preferences for this trait. Assuming genetic variance for female preferences, artificial selection for or against a sexually selected trait can be expected to lead to correlated response in female preferences if females are allowed to exert mate choice. This is because males of selected phenotypes should carry genes for preferences passed from their mothers. However, widely cited studies that aimed to demonstrate the Fisherian mechanism using this design in fact provide an ambiguous support. In guppies (*Poecilia reticulata*), a correlated response to selection observed in the first two generations of selection disappeared in the third generation (Houde 1994), and in another study the response was significant selection-wide only in the less conservative of the two tests reported (Brooks and Couldridge 1999). In another widely cited example, the stalk-eyed fly *Cyrtodiopsis dalmanni*, the number of replicate selection lines was too low to enable confident conclusions (only one down selected line was used, and compared with a pool of two up selected lines, Wilkinson and Reillo 1994), and in *Drosophila mercatorum* no response was observed (Ikeda and Maruo 1980). Some inconsistencies between these studies might stem from confounding effects of inbreeding (e.g., as a result of different effective population sizes between directions of selection) or genetic drift.

Moreover, if choosy females are to benefit by passing their genes via reproductively successful sons, male attractiveness should be positively correlated with male reproductive success, which may not be the case, for example, if there is a trade-off between pre- and post-insemination sexual selection (Tomášek et al. 2017). There is a wealth of data on phenotypic correlations between male mating success and post-insemination competitiveness, which a meta-analysis reported to be positive on average, but not to be significantly different from zero (Mautz et al. 2013). Negative genetic correlations, which are indicative of trade-offs but often not visible in phenotypic data owing to positive environmental correlations (Tuni et al. 2018), have indeed been reported between courtship song quality and sperm viability in the field cricket *Teleogryllus oceanicus* (Simmons et al. 2010) and between iridescent coloration and sperm quality in guppies (Evans 2010), but, in a different population of the

latter species, positive genetic correlation was reported between sperm quality and orange coloration (Cattelan et al. 2018). Thus, it remains unclear whether increased sexual attractiveness typically translates into increased reproductive success.

Here, we evaluated correlated responses to selection for carotenoid coloration in the Trinidadian guppy. Guppies are characterized by short generation time (about 3–4 months), which makes them an excellent vertebrate for selection or experimental evolution studies (e.g., Endler 1980, Houde 1994, Kotrschal et al. 2014, Cole and Endler 2018). Furthermore, guppies are an established model system for studying the evolution of male ornaments and female mating preferences (Endler 1980; Houde 1997; Magurran 2005; Hughes et al. 2013). Males in this species are ornamented with highly variable color patterns, of which carotenoid spots (mostly orange, but also red and yellow) are reported to attract females in several populations (Endler 1980; Kodric-Brown 1985; Houde 1997; Brooks and Endler 2001b; Evans et al. 2004a; Magurran 2005), although there is some variation between guppy populations in this respect (Houde and Endler 1990; Endler and Houde 1995). Here, we selected male guppies bidirectionally, for high and low carotenoid spots area, for six generations. We then quantified correlated responses in female preferences, as well as in a range of behavioral, reproductive, and life-history traits. This allowed us to test (i) if selection for a male ornament resulted in a correlated response in female preferences, as proposed by Fisher (1930), and (ii) if selection caused a significant correlated increase in male reproductive competitiveness. To dissect the latter, we also investigated (iii) correlated responses in behavioral and sperm traits presumed to affect male mating and fertilization success. Finally, to assess the potential of female preferences for orange males to affect population fitness, we quantified (iv) correlated responses in the values of life-history traits directly related to population dynamics: juvenile survival, time to maturation, female gestation time, and fecundity.

Methods

STUDY POPULATION

Experimental fish came from a laboratory population established in 2010, and were descendants of wild-caught guppies collected in 2002 from Tacarigua river in Trinidad and reared in the laboratory of Andrea Pilastro (University of Padova, Italy). Our stock population was kept in 100-L tanks in standard conditions: water temperature of $25 \pm 1°C$, an alternating light/dark regime every 12 h, density of about 1 fish per 1 L, and feeding twice per day (once with live brine shrimp nauplii [*Artemia* spp.] and once with generic fish flakes). Selection lines at all stages were kept under identical conditions, except for the aquaria dimensions and fish density described below.

SELECTION DESIGN

We applied selection to a male ornamental trait, the total area of carotenoid (orange, red, and yellow) spots relative to body area (see next subsection for measurement details). Selection was carried out in both directions (treatments henceforth), that is, we selected for small and large carotenoid area (henceforth "LOW" and "HIGH" treatment, respectively). We applied selection to groups of fish to allow a potential Fisherian process to operate: females were free to choose their mates, and if V_A in female preference occurred, males in the next generation would be able to inherit preference genes from their mothers.

For each treatment, four independent lines were created, each consisting of 25 females randomly selected from the stock population and 25 males chosen based on their carotenoid coloration (details on selection procedure below). The 50 fish per selection line were kept in a 50-L aquarium and allowed to freely mate for about 4 months. Aquaria were daily checked for newborn offspring, which were immediately transferred to a separate aquarium, assigned to their line of origin. This was done to avoid cannibalism by the adults, a common phenomenon among captive guppies (e.g., Loekle et al. 1982). As soon as sex could be assessed (from change in the anal fin shape of males or the occurrence of the female dark speckling in the anal area), male and female offspring were further split into separate aquaria, so that no matings among them took place before the next round of selection (sex can be identified before males are capable of inseminating females). As soon as approximately 200 offspring of either sex per line reached maturity, male coloration was measured and the next generation set up from 25 males per line having the biggest (HIGH lines) or smallest (LOW lines) orange area plus 25 females randomly sampled among female offspring from the respective line. We conducted the selection for five generations (F1–F5), and each new generation was set up by applying the same procedure as for F1. The last (F6) generation born (not selected itself) was subject to measurements of correlated responses to selection.

MALE SELECTION PROCEDURE

The area of carotenoid spots on male body, as well as male body size, were measured from digital photographs. Each male was briefly anaesthetized using a water solution of tricaine methanesulfonate (MS-222; 0.15 g/L) and photographed from his left side. Body area (size) and the area of all carotenoid spots except those situated on the fins (which are difficult to measure and may thus increase the measurement error) were measured manually using ImageJ software (http://rsbweb.nih.gov/ij/). As orange is the most frequently occurring color among carotenoid spots, in the remaining text we will use the terms "(total) orange" (measurement units are pixels) and "relative orange" to refer to total and relative (to body size) area of all carotenoid spots measured

from a male picture, respectively. In the first round of selection, we measured the relative orange in 300 males from stock culture. Males were then ranked by their relative orange and a hundred of those from the right and left tail of the trait distribution were assigned to HIGH and LOW treatment, respectively. From each of those groups, 25 males were randomly assigned to each line and placed in an aquarium with 25 randomly selected females. In the following five generations, 25 males from the respective tail of the distribution were taken from among all male offspring born in a given line and generation.

MEASURING CORRELATED RESPONSE TO SELECTION

In F6, in addition to measuring male orange area, we investigated correlated responses in a set of traits including female preferences, male reproductive competitiveness, traits associated with male reproductive success (sperm quality and competitiveness, boldness, and courtship display), and life-history traits directly related to fitness (juvenile survival, male time to maturity, female fecundity, and gestation time), and body sizes of both sexes. Each gravid female from the fifth generation of selection was individually housed in a 3-L tank with partitioned breeding chamber. After birth, the offspring of each female (i.e., family) were reared separately. As soon as sex could be determined, brothers were separated from sisters so that each female's progeny was housed in unisex groups, visually isolated from each other. After sexual maturity, that is, when the hood on the gonopodium has grown even or beyond the gonopodial tip (Houde 1997), each male's orange was measured as described above. For logistical reasons, time to maturity was recorded for only a part of males (about 30%, never more than one male per family), with representation for all lines. As we wanted to keep track of male identities throughout the experiments, we housed separately one or two (if available) males from each family after completing all measurements of male coloration. Other traits were measured as described below.

Female preferences

Precopulatory sexual preference of F6 females from selection lines was assessed in a dichotomous preference test (e.g., Godin and Dugatkin 1996, Evans et al. 2004a), where each experimental female was given a choice between one F6 male from her own treatment (but never from her own line) and one from the opposite. The males were 6–8 months old at the time of the test. Only one male and one female per family were used in this experiment. Each male pair was presented to one HIGH and one LOW female, in consecutive trials, so that the smallest independent test unit consisted of a group of four fish: one HIGH and one LOW male both assessed by one HIGH and one LOW female. The minimum difference in the relative area of orange spots within each male pair presented to a female was 0.02 (mean = 0.054, SD =

0.023). At the beginning of a trial, the focal female was placed in a transparent cylinder in the middle of the main compartment of the test aquarium (40 × 20 cm, filled up to 10 cm with water), so that she could observe but not explore the new tank. Each of the males was put in a separate smaller compartment, where they were kept visually separated from the female and from each other (Fig. S1). The position of males in the two compartments, as well as the order of testing pairs of females and order of females within pairs, was randomized with respect to treatment. Fish were left for 10 min to acclimatize, after which the opaque division between male and female compartments was raised and the female was allowed to observe, but not approach, the males for further 10 min. The males remained visually separated from each other throughout the test to avoid the confounding effect of male-male interactions. Finally, the cylinder was lifted and the female was allowed to swim freely for 10 min. Throughout these final 10 min, the behavior of the female was recorded by a camera (Microsoft LifeCam Studio Q2F-00018) above the aquarium. Time spent by the female in each of the preference zones, that is, marked fields within 5 cm from individual male compartments, was measured, excluding the time when she was turned with her back to the male compartment. Female presence in this zone was interpreted as preference for that male (e.g., Breden and Hornaday 1994, Evans et al. 2004a). The female was returned to her home aquarium immediately after the test. Recordings were analyzed blindly with respect to treatment.

Male reproductive competitiveness

After a group of four fish performed the preference tests, they were given 2 days of rest. The pair of males was then housed with one of the females in a common aquarium and allowed to interact freely for 2 days. Thereafter, the female was placed in a breeding chamber where she stayed until parturition, whereas the males were given another 2 days of rest to ensure sperm replenishment (Bozynski and Liley 2003), after which the mating procedure was repeated with the female from the opposite treatment. Newborn offspring resulting from these matings were euthanized with an excess of MS-222 and their entire body was preserved for DNA analysis. Tail fin-clips were taken from all experimental adult fish. Tissues were kept in ethanol at −20°C until DNA isolation. Males were photographed following the procedure described above (see MALE SELECTION PROCEDURE section), and the length of their gonopodia (sperm transfer apparatus) was measured from the images.

Sperm competitiveness in artificial insemination experiment

Fifty-three virgin females from the stock population were artificially inseminated with the sperm of F6 males 8–10 months old (from the set of males subject to sperm assays). Each

female was inseminated with an equal number of sperm bundles (spermatozeugmata) from one HIGH and one LOW selected male, following the procedure described in Evans et al. (2004b). Previous work on the same population recommended to use the number of bundles as a proxy for number of sperm and has shown that the among-male variation in the number of sperm per bundle does not exceed that within males (Evans et al. 2003), while Cattelan et al. (2018) found no differences in the number of sperm per bundle among lines selected for the number of sperm. Males from the selected lines were paired based on their age. For each pair of males, a virgin female was anaesthetized, placed under a stereomicroscope and, using a Drummond micropipette, artificially inseminated with 20 bundles from each male (40 bundles in total) freshly collected from males in 2 µL of saline solution. After insemination, each female was kept separately in a breeding chamber until parturition. A tissue sample was collected from each male and female by fin clipping and stored in ethanol at −20°C. Newborn fish were euthanized with an excess of MS-222 and their entire body was preserved as for the adults' fins until required for DNA analysis.

Sperm collection and count

Sperm parameters were analyzed in 83 F6 males from each treatment (8–10 months old), with at least 14 fish from each line. Only one male per family was measured. Sperm were collected and counted following an established protocol (Cattelan et al. 2018). The number of sperm produced correlates with the number of sperm transferred during copulation in the guppy (Pilastro and Bisazza 1999), whereas the latter has been shown to correlate with male reproductive success in this species (Boschetto et al. 2011). Seven days before sperm collection, each male was individually isolated to ensure the full replenishment of sperm reserves (Bozynski and Liley 2003). Males were anesthetized in a water bath of MS-222 (0.15 g/L) and placed on a slide under a stereomicroscope (OLYMPUS SZ61). A gentle abdominal pressure on the male was applied to release the sperm in 800 µL of saline solution (NaCl 0.9%). Sperm bundles were photographed against a black background and counted using ImageJ. We then transformed the number of sperm bundles into the actual number of sperm following the method described in Cattelan et al. (2018), that is, the total number of sperm was approximated by multiplying the number of bundles by the mean number of sperm per bundle observed in this population (about 22K; see supporting information methods in Cattelan et al. 2018). A picture of each male was taken after sperm collection, as described above (see MALE SELECTION PROCEDURE section).

Sperm velocity

Sperm velocity was estimated after sperm collection following an established protocol (Gasparini et al. 2013). Bundles were collected using a Drummond micropipette and activated with 3 µL of 150 mM KCl solution in 2 mg/L bovine serum albumin on a 12-cell multiset slide (MP Biomedicals) coated with a 1% polyvinyl alcohol to prevent sperm from sticking to the glass slide. Sperm swimming velocity was measured using a computer-assisted sperm analyzer, CASA (CEROS, Hamilton-Thorne). Sperm velocity assays were performed twice for each male using three bundles per sample from the same ejaculate, and the mean (based on at least 100 sperm cells per male) was used in the final analysis. We measured three standard metrics: the average path velocity (VAP), which estimates the average velocity of sperm cells over a smoothed cell path; the straight line velocity (VSL), the average velocity on a straight line between the start and end point of the track; and curvilinear velocity (VCL), the actual velocity along the trajectory (see Gasparini et al. 2013 for setting parameters). These measures are known to be positively correlated with fertilization success in this guppy laboratory population (Boschetto et al. 2011).

Sperm viability

After sperm collection and sperm velocity assays, we assessed the viability of sperm. Sperm viability was measured using a live/dead viability Kit (Halotech). The kit consists of a membrane-permeant nucleic acid stain (acridine orange) that labels live sperm green, and a membrane-impermeant stain (propidium iodide) that labels dead or damaged sperm red. From each male, we collected 50 bundles in 40 µL of saline solution and then vortexed the solution for 90 s. Six microliters of the solution containing sperm were placed on a microscopic slide and 1 µL of acridine orange and 1 µL of propidium iodide were gently added. After covering the final solution with a coverslip, fluorescent images of samples were taken at ×40 magnification using a fluorescent microscope (Axiovert 25 clf inverted microscope, Zeiss). From digital images, we counted the number of live and dead spermatozoa on a minimum of 200 sperm cells per male. Only cells totally dyed in green were considered viable.

Male courtship and boldness

Male courtship behavior was recorded in the presence of a female from the stock population. The focal male was put into one compartment of a 3-L tank, whereas a virgin mature female was placed in the other one, the compartments being divided by a transparent partition. After 3 minutes of acclimation, the partition was removed and the fish were allowed to freely interact for the subsequent 5 min. Behavior was recorded by a video camera placed above the tank. We measured the number of courtship behaviors named sigmoid displays (the male presents his side to the female, takes an s-shaped posture, and quivers). Water in the test tank was changed after each test.

We also assayed boldness of the same males. To do this, we performed an emergence test as described in Herdegen (2019b). In short, a male was placed in a closed opaque box (10 × 10 cm) within an unfamiliar aquarium (40 × 20 cm) and left to acclimate for 5 min. Thereafter, the door in one of the walls of the box was opened and the time taken by the male to swim out from the box was measured. Males who took less time to come out were considered bolder.

MOLECULAR ANALYSES

DNA was extracted from tail fins or fry by a magnetic beads-based method, using Mag-Jet Genomic DNA Kit (Thermo Fisher Scientific) according to the manufacturer's protocol. Individuals were screened for variation at seven previously described microsatellite loci: AGAT11 (Olendorf et al. 2004), Pret77 (Watanabe et al. 2003), G75 and G389 (Shen et al. 2007), TTA (Taylor et al. 1999), KonD15 (Seckinger et al. 2002), and TACA33 (H. Alexander, unpubl., based on *Xiphophorus* sequence GeneBank No. AY258896). The first three and the other four markers were amplified in two multiplex polymerase chain reactions. Each 2-µL reaction mix contained 1.1 µL of PCR Master Mix (Qiagen), 0.04−0.33 µM of each primer (one of which in each pair was fluorescently labeled), and 4−20 ng of genomic DNA (previously dried), overlain with 5 µL of mineral oil to prevent evaporation. The reaction conditions were as follows: a 15-min denaturation step at 95°C, followed by 36 cycles of 30 s at 94°C, 1 min at 52°C, and 1 min at 72°C, then 10 min of final extension at 72°C. PCR products were mixed with a GeneScan LIZ600 size standard and electrophoresed on an ABI 3130xl Genetic Analyzer. Genotyping was performed using the ABI software GeneMapper 4.0.

Parentage analysis

Paternity was assigned using COLONY 2.0 (Wang and Santure 2009). In all analyses, to increase assignment accuracy, offspring were a priori assigned to their mothers as part of the COLONY input (maternity was always known). One long run of simulations was performed using full-likelihood method with high likelihood precision, polygamy was allowed for both sexes, and no sibship prior was set. A male was considered to be the father of an individual if the associated probability of assignment of the putative offspring was above 0.8.

STATISTICAL ANALYSES

Data were analyzed with linear and generalized linear models/mixed models, with parameters estimated using maximum likelihood method. Differences in intercept were allowed for all random effects (random intercept). Where appropriate (see detailed model descriptions below), we allowed the effect of treatment (or orange coloration) to vary between lines (i.e., random

slopes were modeled). We report models with estimates of random effect even if the estimates approached zero, but models with these terms removed gave the same conclusions. Model assumptions were checked by examining diagnostic plots and, in case of Gaussian distributions, by Shapiro test for normality of residuals. All analyses were performed in R (R Core Team 2016), using packages lme4 (Bates et al. 2015) and glmmTMB (for fitting models with the cbind function, Brooks et al. 2017). All continuous variables were scaled. *P*-values for general linear models were based on *t*-tests using Satterthwaite's method calculated using lmerTest package (Kuznetsova et al. 2017), and for generalized linear mixed models (GLMMs) based on type III Wald test. The models were visualized based on partial residuals using visreg 2.7.0.1 (Breheny and Burchett 2017).

Microsatellite data were checked for conforming to assumptions for molecular markers. All loci were checked for deviations from Hardy-Weinberg equilibrium and tested for linkage disequilibrium in GENEPOP 4.3 (Rousset 2008), using F6 data. To control for error rate from multiple testing, the Holm-Bonferroni correction (Holm 1979) was applied to the results of both analyses. The frequencies of null alleles were estimated using the algorithm of Dempster et al. (1977) implemented in FreeNA (Chapuis and Estoup 2007).

Selection experiment

The effect of artificial selection on the ornamental trait, that is, total orange area corrected for body size used as a covariate, was tested in F6 with a linear mixed model, with treatment as fixed effect, whereas line and family (linking brothers) were random effects. Realized heritability of relative orange area was estimated separately for each line as a ratio of the cumulative response to selection to cumulative selection differential from all six generations of selection. The ratio was then multiplied by two, as only one sex was selected. We compared heritabilities between HIGH and LOW lines using a simple *t*-test, as the distribution was not significantly different from normal by Shapiro-Wilks test and variances did not differ among treatments. To assess any potential confounding effects of inbreeding accumulation via drift, which could vary between treatments, for example, due to differences in the degree of male reproductive skew, we estimated the level of genetic diversity within lines, as well as fixation index (F_{ST}) measuring genetic differentiation among lines. Observed heterozygosity was calculated for each line at F6 using FSTAT version 2.9.3.2 (Goudet 2001), and a linear model was used to test for any differences in this measure of genetic diversity among HIGH and LOW lines. Also for the last generation of selection, allelic richness was estimated in FSTAT, whereas global, as well as pairwise (for pairs of lines), F_{ST} indices were estimated in FreeNA (Chapuis and Estoup 2007).

Preference experiment

To test the effect of treatment on female mating preferences, we ran a linear mixed model. One data point was removed from the models based on the percentiles method (outside the 99th percentile upper bound). The response variable was calculated as the proportion of time spent by a female in the preference zone of HIGH male (i.e., the time was divided by the total time she spent in both preference zones). Difference in relative orange between HIGH and LOW males within a pair ("difference in relative orange" henceforth), and female treatment were fixed effects in the model, whereas female selection line and pair (linking females tested with the same pair of males) were random effects, where the effect of the difference in orange was allowed to vary among female lines (i.e., random slopes were modeled). The model also tested the interaction between difference in relative orange and female treatment.

Male reproductive competitiveness

Because mixed paternity was found in only 21.9% of cases in competitive trials, we analyzed paternity success (which could take values 0 or 1 for either of the two competing males) as our response variable. Apart from male and female treatment, male body size and gonopodium length were included as fixed effects, together with the interaction between male and female treatment. Male and female line and ID were fitted as random effects with the effect of male treatment allowed to vary among female lines (random slope). To examine if any effect of male treatment could be accounted for by a corresponding change in male coloration, we examined two additional models, either by replacing male treatment with orange area or adding orange area as a covariate to the analysis above.

Sperm characteristics

The effect of treatment on sperm number was tested with a linear mixed model with body size included as a covariate, to account for its possible effect on sperm production, and sperm number was log-transformed to conform to normality of distribution. In the GLMM model testing the effect of treatment on sperm viability, the response variable was expressed as the number of alive and dead sperm using the cbind function (Brooks et al. 2017) and the model was fitted with a binomial distribution and logit link function. In both models, selection line and family were included as random effects.

The effect of treatment on sperm velocity was tested with two linear mixed models, one where VLC was the response variable, and a second where it was the first principal component (PC1) resulting from the principal components analysis performed on all three parameters of sperm velocity (VCL, VSL, and VAP). PC1 explained over 93% of variance among samples. Both models included selection line and family as random effects.

Similar to the competitiveness models, to examine if any effect of male treatment could be accounted for by a corresponding change in male coloration, all four models for sperm traits were rerun either with male treatment replaced with orange area or the latter added as a covariate to the analyses above.

Artificial insemination experiment

The effect of treatment on the proportion of offspring sired by HIGH males (expressed as the number of offspring of HIGH and LOW males using cbind function) was tested with an intercept only generalized linear model, fitted with a binomial distribution and logit link function. In the binomial model, the intercept estimates the log odds ratio, and thus the associated significance level indicates whether the proportion of offspring sired by HIGH and LOW males is significantly different from 0.5. A second model tested the effect of coloration, by fitting the difference in relative orange.

Life-history and behavioral traits

The effect of treatment on female body size was tested with a linear mixed model, with line included as random effect. In separate linear mixed models, female fecundity (obtained from male reproductive competitiveness experiment) and gestation time were modeled in response to treatment, with female body size fitted as a fixed factor and line as a random effect.

Differences in juvenile mortality among treatments were tested with a generalized linear model with a two-column binomial response variable (survived, dead, linked by cbind function). Correlated responses in the time to maturity and male body size at maturity in F6 males were tested using linear mixed models, with line included as random effect. Both response variables were log-transformed to conform to the assumption of normality.

The effect of treatment on F6 male boldness, one of the personality traits, was tested with a linear mixed model, with the response variable log-transformed to better conform to normality assumption. The effect of treatment on courtship behavior, that is, number of sigmoids performed in presence of a female, was tested with a GLMM fitted with Poisson distribution. In both models, treatment was included as a fixed effect, and line and family as random effects.

Results

DIRECT RESPONSE TO SELECTION

The effect of artificial selection on male relative orange is shown in Figure 1, and means ($\pm$SE) are given in Table S1, panel A. In generation F6, males selected for high relative orange had significantly larger total orange than their down-selected counterparts (Fig. 1 and Table 1), controlling for body size (which was significantly positively correlated with total orange
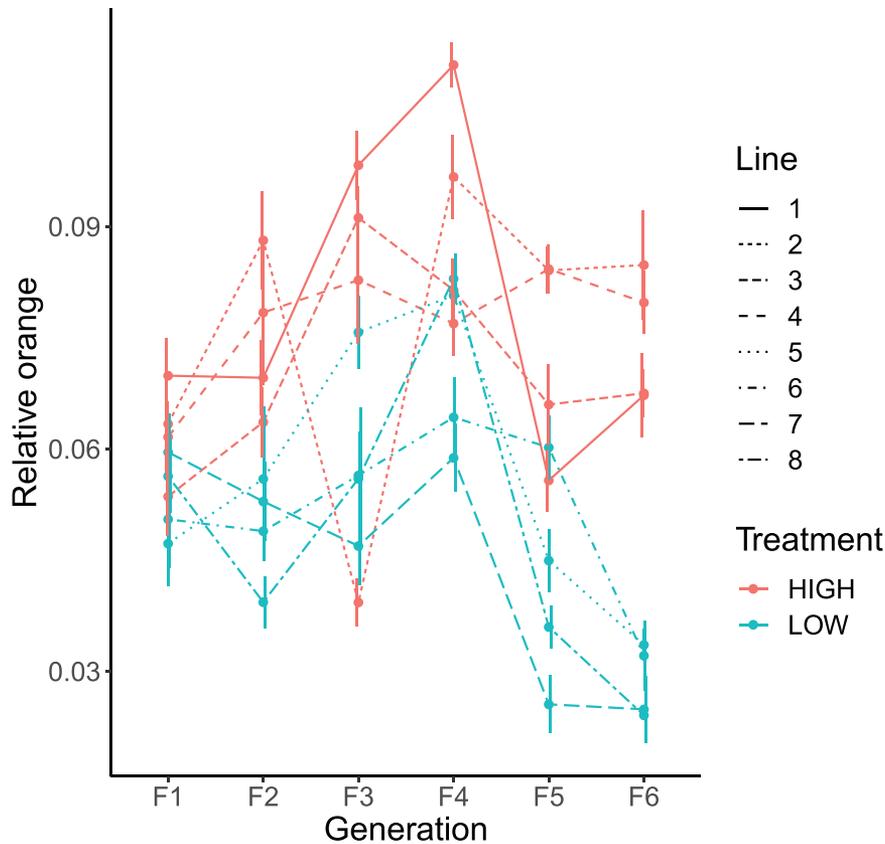
Figure 1. Selection effect. The graph presents changes in the mean values (±SE) of relative orange area (proportion of body covered) of males over six generations of selection for increased (HIGH) or decreased (LOW) orange area.

Table 1. Linear mixed model testing the effect of selection on male orange coloration, controlling for body size. Significant terms are in bold.

	Term	Estimate	SE	*df*	*t*	*P*-value
Fixed effects	(Intercept)	15,289.58	1774.31	6.5	8.62	**<0.001**
	Male treatment (HIGH)	26,917.40	2559.01	7.1	10.52	**<0.001**
	Body size (scaled)	1659.44	745.26	266.53	2.23	**0.027**
		Variance		SD		
Random effects	Male line:(Intercept)	58,539		2419		
	Male family:(Intercept)	529,950		7280		
Residual		99,736		9987		

; Table 1)). Realized heritability of the selected trait was on average 0.04 (SE = 0.079) and 0.58 (SE = 0.11) for HIGH and LOW treatment, respectively (see Table S2 for per line heritabilities), with the difference between treatments in heritability estimates being significant ($t_{1,6} = -3.95$, $P = 0.007$). No significant differences were found in observed heterozygosity among fish from HIGH and LOW lines ($t = 0.232$, $P = 0.824$; Table S2).

MICROSATELLITE METRICS
After correcting for multiple comparisons, significant deviations from Hardy-Weinberg equilibrium were detected in seven out of 56 (12.5%) tests, which was probably due to the presence of null alleles that we detected at frequencies ranging from 0.00 to 0.16. Linkage disequilibrium was detected in 13 out of 168 (7.7%) tests. This likely results from drift, strongly operating in small populations, rather than from actual physical linkage, as different

Table 2. Linear mixed model testing female preferences. Proportion of time spent by female in a HIGH male preference zone is the response variable. Significant terms are in bold.

	Term	Estimate	SE	df	t	P-value
Fixed effects	(Intercept)	0.51	0.03	12.7	16.04	**<0.001**
	Female treatment (LOW)	−0.10	0.04	9.9	−2.28	**0.046**
	Difference in relative orange	0.07	0.03	8.1	2.19	0.059
	Female treatment (LOW) × difference in orange	−0.06	0.04	6.6	−1.41	0.204
		Variance		SD		
Random effects	Pair:(Intercept)	0.0042		0.0650		
	Female line:(Intercept)	0.0009		0.0306		
	difference in orange	0.0011		0.0330		
Residual		0.0231		0.1520		

Table 3. Generalized linear mixed model testing the effect of male and female treatment on male reproductive success. Paternity success (0/1) is the response variable. Significant terms are in bold.

	Term	Estimate	SE	z	P-value
Fixed effects	Intercept	10.27	4.03	2.55	**0.011**
	Female treatment (LOW)	−0.94	3.76	−0.25	0.802
	Male treatment (LOW)	−17.70	6.59	−2.69	**0.007**
	Gonopodium length (scaled)	0.55	1.85	0.30	0.766
	Body size (scaled)	−0.27	1.40	−0.19	0.845
	Female treatment (LOW) × male treatment (LOW)	−4.36	10.14	−0.43	0.667
		Variance		SD	
Random effects	Male ID:(Intercept	398.20		19.95	
	Female ID:(Intercept)	0.2483		0.4983	
	Male line:(Intercept)	0.0012		0.0354	
	Female line:(Intercept)	0.46380		0.68103	
	Male treatment (LOW)	2.13		1.46	

pairs of loci were linked in different lines. We thus did not exclude any loci from further analyses. Allelic richness assessed for each treatment and juxtaposed with allelic richness in the stock population and in a natural population in Trinidad (same set of markers) is reported in Table S3. We found that microsatellite heterozygosity did not differ significantly between selection treatments ($t = 0.232$, $P = 0.824$). Global F_{ST} in the selection experiment in F6 was 0.077 (pairwise F_{ST} values among lines are in Table S4).

## CORRELATED RESPONSE IN FEMALE PREFERENCES
Female preferences
There was a significant effect of female treatment on female preferences, with females from LOW lines spending significantly less time in proximity of HIGH males compared to HIGH females (Table 2). The magnitude of the difference in male coloration had a marginally nonsignificant effect, and the interaction was not significant (Fig. 2 and Table 2).

CORRELATED RESPONSE IN MALE REPRODUCTIVE COMPETITIVENESS
Male reproductive competitiveness
Eighty-one percent of HIGH males from the experiment sired any offspring, whereas only 42% of LOW males did. Among HIGH females, 10 broods were sired by HIGH males, two by LOW males, and in five both males from both treatments shared paternity; for LOW females, those numbers were nine, four, and two, respectively. Males from HIGH lines had higher probability of siring offspring than LOW males (Table 3). Female treatment did not affect male reproductive success, nor was there a significant interaction between male and female treatment (Table 3). Orange was not a significant predictor in alternative models in which orange was either added as a covariate or replaced
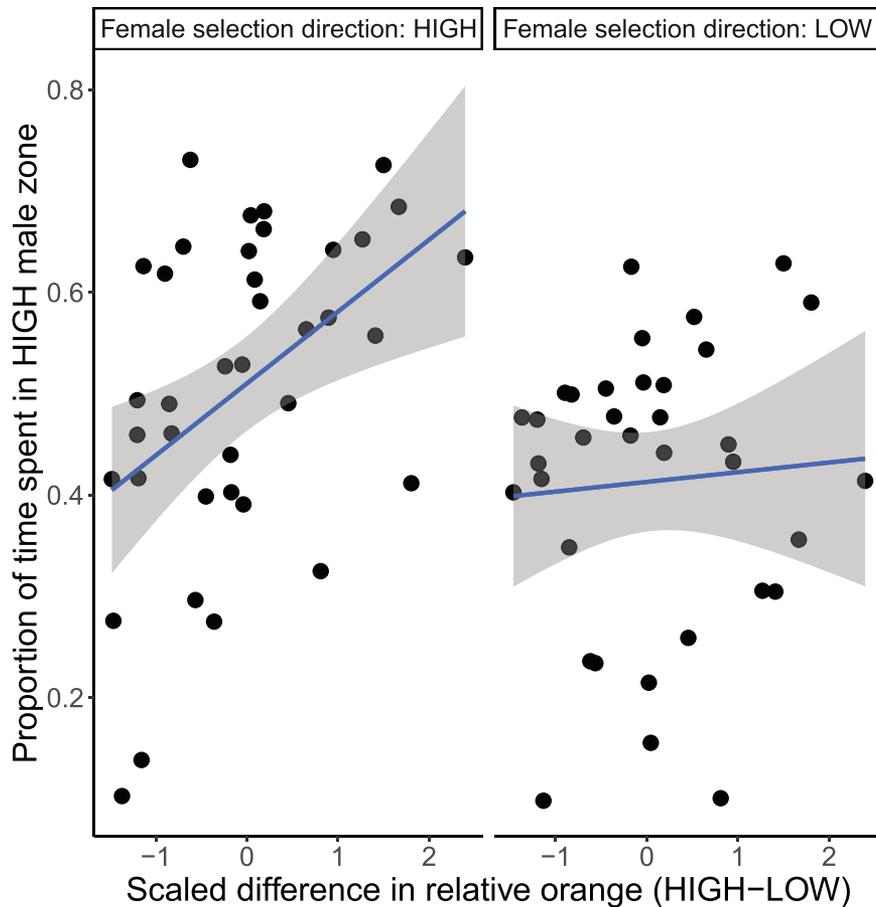
Figure 2. Partial residuals plot from the model reported in Table 2, examining the proportion of time spent by female in a HIGH male preference zone as a function of the scaled difference in male orange and female treatment.

treatment (Tables S5 and S6), suggesting that the effect of treatment was not due to female preferences for orange males.

Sperm characteristics and competitiveness

Descriptive statistics for sperm characteristics are given in Table S1, panel A. Males from LOW lines produced significantly more sperm than their HIGH counterparts (Table 4, panel A and Fig. S2). This effect was not mediated by body size, which did not affect sperm number. HIGH and LOW treatment males did not differ in sperm velocity, both assessed based on VCL and on the first principal component of the PCA of the three different velocity parameters (Table 4, panels B and C). Treatment did not affect sperm viability (Table 4D). Relative orange did not affect any of the sperm characteristics in alternative models with treatment plus orange and orange alone (Tables S7 and S8, respectively).

From the 53 artificially inseminated females, 45 gave birth to offspring. Neither treatment ($z = -0.07$, $P = 0.945$) nor the difference in relative orange ($z = -0.72$, $P = 0.474$; Table 5 and Fig. 3) did influence paternity share in the artificial insemination experiment.

Male courtship and boldness

Males from opposite treatments did not differ significantly in the number of sigmoid displays toward females from the stock population ($z = 0.559$, $P = 0.576$; Table S1, panel A). There was also no significant difference in the level of boldness between HIGH and LOW selected males ($z = -1.255$, $P = 0.255$; Table S1, panel A).

CORRELATED RESPONSES LIFE-HISTORY TRAITS

Descriptive statistics for female traits are given in Table S1, panel B. Fecundity of females from opposite treatments did not differ significantly ($t = 0.217$, $P = 0.831$), nor did the body size ($t = 0.854$, P $= 0.416$) or gestation time, although the latter tended to be shorter for females from HIGH lines ($t = -1.916$, $P = 0.062$). Irrespective of the treatment, bigger females had both more offspring ($t = 5.433$, $P < 0.001$) and longer gestation time ($t = 3.159$, $P = 0.003$).

No significant juvenile mortality differences were found between HIGH and LOW selection lines ($z = 1.593$, $P = 0.111$; Table S9). There was also no significant difference in time to maturity between males from HIGH and LOW lines ($t = 1.189$,

Table 4. Mixed models testing the effect of treatment on sperm qualities: (A) number; (B) velocity (VCL); (C) PC1 from principal components analysis on sperm velocity related measures; (D) viability. Significant terms are in bold.

		Term	Estimate	SE	*df*	Test value[1]	*P*-value
	Fixed effects	(Intercept)	20.43	0.07	86.9	278.58	**<0.001**
A. Sperm number							
		Male treatment (HIGH)	−0.31	0.10	86.2	−2.95	**0.004**
		Male body size (scaled)	0.04	0.48	132.7	0.85	0.396
	Random effects		Variance		St. Dev.		
		Male line:(Intercept)	0.0000		0.0000		
		Male family:(Intercept)	0.0960		0.3098		
	Residual		0.2324		0.4821		
	Fixed effects	(Intercept)	148.13	2.54	165	58.30	**<0.001**
A. Sperm velocity (VCL)							
		Male treatment (HIGH)	1.28	3.60	165	0.35	0.724
	Random effects		Variance		St. Dev.		
		Male line:(Intercept)	0.00000		0.00026		
		Male family:(Intercept)	0.00000		0.00000		
	Residual		535.80		23.15		
	Fixed effects	(Intercept)	−0.19	0.18	161	−1.06	0.288
A. Sperm velocity (PCA)							
		Male treatment (HIGH)	0.36	0.26	161	1.37	0.173
	Random effects		Variance		St. Dev.		
		Male line:(Intercept)	0.00000		0.00000		
		Male family:(Intercept)	0.00000		0.00000		
	Residual		2.72		1.65		
	Fixed effects	(Intercept)	−3.77	0.14	–	−26.32	**<0.001**
A. Sperm viability							
		Male treatment (HIGH)	0.33	0.20	–	−1.64	0.099
	Random effects		Variance		St. Dev.		
		Male line:(Intercept)	0.00000		0.00000		
		Male family:(Intercept)	1.05		1.02		

[1] *t*-test in A, B, and C, and *z*-test in D.

Table 5. Generalized linear mixed model testing reproductive success of experimental males whose sperm was used to artificially inseminate stock population females.

Term	Estimate	SE	*z*	*P*-value
(Intercept)	−0.02	0.14	−0.11	0.910
Difference in relative orange	−0.11	1.16	−0.72	0.474

$P = 0.267$; Table S1, panel A), nor in body size at maturity ($t = 0.677$, $P = 0.519$).

Discussion

Our selection experiment was successful in modifying body area covered by carotenoid spots, a sexually selected trait in the guppy. This confirmed the general pattern of significant heritability of traits subject to sexual selection (Prokop et al. 2012; Prokuda and Roff 2014) and allowed us to investigate correlated responses to selection for female preferences, male reproductive competitiveness and its determinants, as well as in several fitness-related life-history traits. We found that selection resulted in a significant correlated response in female preferences, with females from LOW lines being significantly less attracted to HIGH males compared
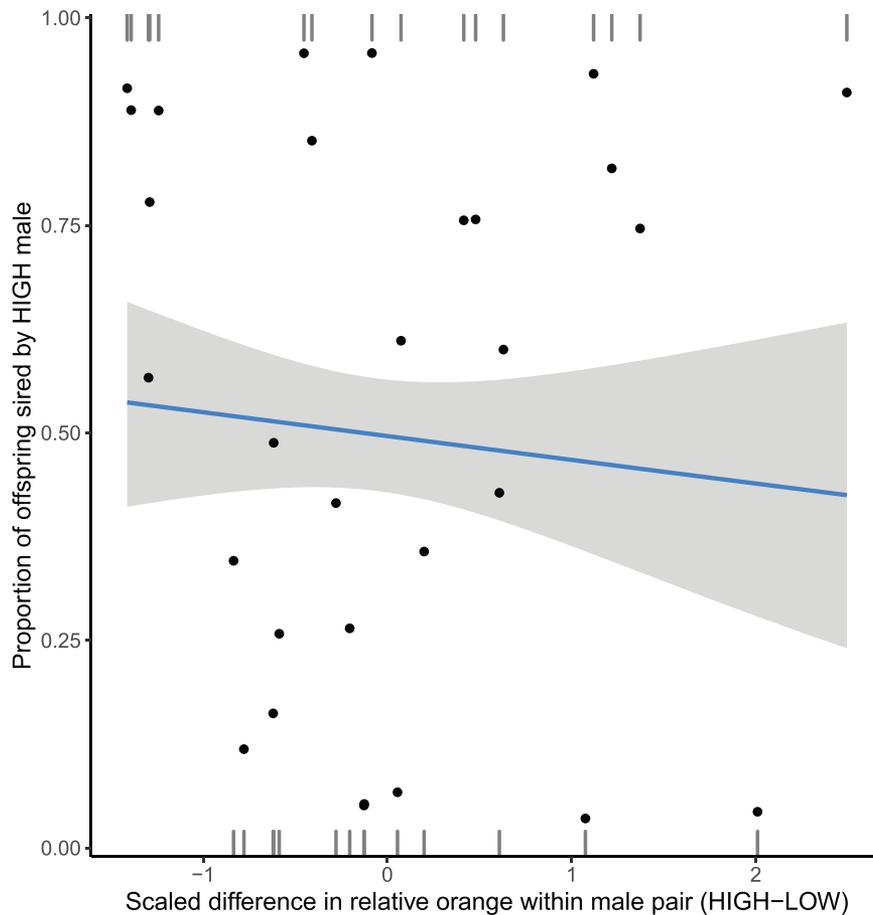
Figure 3. Partial residuals plot from the model reported in Table 5, examining the proportion of offspring sired by male from HIGH treatment within a brood produced following artificial insemination, with scaled difference in orange as covariate.

to females from HIGH lines. Despite this correlated response in preferences, males from HIGH lines achieved reproductive success in competitive trials more often than LOW males did, irrespective of the female treatment of origin. This reproductive advantage could not be explained by any of the potential determinants of male reproductive success we measured. Finally, we have not found a significant response to selection in any fitness component other than male reproductive competitiveness. Below we discuss these findings in detail, and consider their implications for sexual selection theory.

DIRECT RESPONSE TO SELECTION

Our results confirm significant heritability of relative orange spot area reported for other guppy populations (Houde 1992; Breden and Hornaday 1994; Brooks 2001; Hughes et al. 2005). Importantly, unlike earlier studies, we investigated if our result could be affected by differential inbreeding rates between treatments. Such differences could arise if, for example, selection treatments differed in the number of males contributing to the next generation, for example, as a result of selecting descendants of males carrying rare variants strongly affecting the trait under selection,

or by among-treatment differences in variance in male reproductive success. Differential inbreeding could potentially confound both the area of orange (van Oosterhout et al. 2003; Mariette et al. 2006) as well as correlated responses in life-history traits (Charlesworth and Willis 2009). We found that genome-wide heterozygosity did not differ significantly between selection treatments, indicating that differential inbreeding had little to no effect on our results. Furthermore, fixation indexes (F_{ST}) among replicate lines were small, indicating that genetic drift did not play a major role in shaping genetic variance of our selection lines.

Our estimates of heritability of relative orange area were significantly different for HIGH and LOW lines (averages from per-line estimates from Table S2 were 0.04 and 0.58, respectively), suggesting stronger response to selection in the latter treatment. Such asymmetric response may reflect the fact that our stock population evolved under the lack of predation in the laboratory for about 40 generations since the population was founded. Given female preferences for orange males, frequencies of alleles contributing to large carotenoid area might have increased in the absence of natural selection against orange coloration (i.e., predation), limiting further response in this direction (Roff et al.

1997). However, the apparent asymmetry should be interpreted with caution, as we have not maintained control (unselected) lines, and therefore we cannot exclude common environmental factors affecting expression of carotenoid spots. Indeed, such factors could have caused a drop in carotenoid spot area observed in the last two generations across all lines (Fig. 1). Although such common environmental effects could have affected our parametric estimates of heritability, they do not change our conclusion of significant divergence between the lines, which demonstrates significant heritability of relative orange in our population. Our estimates of heritability are comparable to the average of 0.18 reported earlier by Breden and Hornaday (1994), but are lower than those reported by Houde (1994), Brooks and Endler (2001a), and Hughes et al. (2005) (on average 0.97, 0.96, and 1, respectively). Heritability is a population parameter, and populations may differ in the amount of additive genetic variance due to the history of selection and genetic drift. Our stock population has earlier been shown to host significant additive genetic variance in other traits (Gasparini et al. 2013; Cattelan et al. 2018), and allelic richness for microsatellites in our population is actually higher than the average for several natural Trinidad populations studied using the same set of markers (Herdegen-Radwan et al. 2021; see Table S3 for comparison). This reflects the fact that the Lower Tacarigua population is large, and our stock population was founded by a large number of individuals (400 adult fish at approximately 1:1 sex ratio; as most females were gravid, the effective population size was probably even larger; Andrea Pilastro, pers. commun.). Thus, lower heritability of relative orange area is unlikely to be due to low overall genetic variance in our population compared to earlier studies. Instead, it may reflect the history of selection in the wild. Interestingly, the population studied by Breden and Hornaday (1994) evolved under high predation, similarly to Lower Tacarigua population used here, whereas high heritability estimates come from low predation sites (Houde 1994; Hughes et al. 2005). High predation should impose selection against orange coloration (Endler 1983), which might decrease genetic variance in this trait, either via strong directional selection, if females from high predation sites prefer drab males in comparison to females from low predation sites (Breden and Stoner 1987), or due to stabilizing selection (Roff 1997), if females show preferences for more orange males, as in our population (Evans et al. 2004a). Data on more populations are needed to formally test whether low predation on the guppy does indeed coincide with high heritability of carotenoid coloration, and if so, for what reason. In any case, our results are in line with results of the research syntheses reporting significant heritability of traits contributing to male attractiveness across species (Prokop et al. 2012; Prokuda and Roff 2014) and confirming that sexual selection does not typically lead to depletion of genetic variance in

sexually selected traits. In addition to genic capture mechanisms (Rowe and Houle 1996), this could also be due to balancing selection resulting from fitness trade-offs associated with elaboration of sexual ornaments (Connallon and Clark 2014; Radwan et al. 2016; Zajitschek and Connallon 2018).

CORRELATED RESPONSE IN FEMALE PREFERENCES

We found that selection on relative orange in males resulted in a correlated response in female preferences, confirming that genetic variation for female preferences, necessary to observe Fisherian process in action, is present in our population. Thus, by selecting males for relative orange, and allowing females to exercise mate choice, we indirectly passed to the next-generation genes that affect female preferences for the artificially selected phenotypes. Our results thus help resolve ambiguous results of earlier studies (see *Introduction* and Table S10 for the summary of the results of those earlier selection experiments) that provided only partial support for the evolution of linkage disequilibrium between selected male traits and female preferences (Houde 1994; Brooks and Couldridge 1999). Our study provides unambiguous results likely due to having a larger number of generations of selection (6) compared to earlier studies (3–4), and possibly, higher level of standing genetic variation for female preferences, as the founding population was large and maintained as such. Our results contrast with those obtained by Breden and Hornaday (1994), who have not recorded a correlated response in female preferences to selection on male orange area. However, these authors have not allowed for free mating within their selection lines, which implies that linkage disequilibrium between female preference and orange could not build up, and if such linkage existed in the source population, it would have broken down under enforced random mating, unless it resulted from pleiotropy. Given the absence of pleiotropy implied by the results of Breden and Hornaday (1994), our results indicate that the linkage disequilibrium between preferences and preferred trait can build up via selection, as postulated by Fisher (1930). This further implies that these traits may coevolve in different directions across populations (Schwartz and Hendry 2007), for example, in response to differences in additional selection pressures acting on either trait, potentially leading to speciation (Lande 1981).

CORRELATED RESPONSE IN MALE REPRODUCTIVE COMPETITIVENESS

Fisherian benefits arise if sons of females expressing preferences for male sexual ornaments achieve higher than average reproductive success by being more attractive to females. Because females from HIGH and LOW lines diverged in their preferences for carotenoid coloration, we expected this to be reflected in reproductive competitiveness of males, specifically, HIGH males were predicted to win reproductive competition with LOW males

over HIGH females, and the reverse ranking was predicted for LOW females. In contrast to this prediction, we found that HIGH males sired higher proportion of offspring across female treatments. One explanation for this inconsistency is that the choice test, which does not allow males to exhibit a typical behavior of following females and positioning themselves in close proximity in front of their snout to display (Liley 1966), could represent an inaccurate measure of female mating preferences. However, time spent in proximity of males significantly predicted male mating success in guppies in an earlier study (Brooks and Endler 2001a). Alternatively, more orange males may be able to achieve higher reproductive success with LOW females by means other than attracting them to approach, for example, by inducing females to use their sperm preferentially (Pilastro et al. 2004; Firman et al. 2017).

Our finding that HIGH males had higher probability of reproductive success, despite that the effect of orange area was not significant, implies that traits other than carotenoid coloration contributed to their reproductive competitiveness. Yet, none of the candidate traits we measured provided a clear indication of the underlying mechanism. HIGH and LOW males did not differ significantly in another trait subject to female preferences in guppies, that is, courtship display (Kodric-Brown and Nicoletto 2001), or in boldness, which can also affect male attractiveness (Godin and Dugatkin 1996) and reproductive success (Herdegen-Radwan 2019a). Likewise, our data do not indicate that HIGH males should outcompete LOW male guppies during sperm competition. Success in sperm competition is predicted by the number of sperm transferred during insemination (Boschetto et al. 2011), but we found that LOW males produced a significantly higher number of sperm. The proportion of live sperm spermatozoa or sperm velocity did not differ between HIGH and LOW males, and when females were inseminated with an equal number of sperm bundles from males from opposite treatments, fertilization success did not differ between treatments (Fig. 3). Previous studies conducted in the same population showed that colorful males transfer more sperm during copulation than less ornamented males (Pilastro et al. 2002) due to cryptic choice of females accepting more sperm from more attractive males (Pilastro et al. 2004). Under this scenario, it could be expected that LOW treatment females would accept more sperm from LOW males, being more attractive to them. In contrast to this expectation, HIGH males outcompeted LOW males in terms of paternity share. We do not have data about number of sperm inseminated by males, but it is likely that HIGH males won competition for insemination with LOW males for reasons other than sperm competitiveness. Although guppy males rarely engage in aggressive combat (Farr 1975), they adjust their mating tactic in the presence of rivals, switching between courtship and sneaky copulations (Liley 1966). It has been reported that dull guppy males more often abandoned their courtship toward a female when the rival was more colorful (Yoshikawa et al. 2016). It has also been shown that during copulations following courtship display, guppy males transfer more sperm than during enforced copulations (Pilastro and Bisazza 1999). Although we did not observe differences in the frequency of courtship between HIGH and LOW males, we tested fish individually, so we cannot exclude this possibility. In contrast, in reproductive trials, LOW males were confronted with more colorful HIGH males, so they could have switched to sneaky copulations.

The lower number of sperm produced by HIGH males in our study contrasts with the results of Cattelan et al. (2018) from the same source population. The authors selected for sperm number and found that it resulted in a correlated increase in orange coloration. As both experiments used the same base populations, this inconsistency is puzzling. The two studies, however, used different designs: unlike our mass selection, Cattelan et al. (2018) minimized sexual selection within selection lines by using family-level selection. It is possible that, in our study, lines selected for low relative orange evolved increased investment in sperm because postcopulatory selection was more intense in these lines than in HIGH lines. This intriguing possibility remains to be tested in future studies. In any case, lower sperm number did not prevent HIGH males from winning reproductive competition with LOW males, indicating that other factors, such as insemination tactics discussed above, may be more important in contributing to male reproductive success.

Overall, our results highlight that a male advantage due to female preferences may often be overridden by other components of reproductive competition between males. Conflict between female preferences and the outcome of male-male competition have been reported in some other systems (Moore and Moore 1999; Okada et al. 2014) and will affect strength, or even direction of selection on sexually selected traits (Hunt et al. 2009).

CORRELATED RESPONSES IN LIFE-HISTORY TRAITS

Unlike earlier selection experiments, we also measured correlated responses in several life-history traits, to test whether mating advantage of males carrying elaborated sexual ornaments can be expected to improve population fitness (Zahavi 1975; Rowe and Houle 1996; Candolin and Heuschele 2008; Radwan et al. 2016). However, none of the traits, including juvenile survival, male time to maturity, female fecundity, and female gestation time, significantly responded to selection on relative orange.

Previous work investigating genetic correlations between orange coloration and fitness components using other designs than selection experiments is scant. Brooks (2000) found a negative genetic correlation between offspring survival and fathers' attractiveness, itself predicted by orange area, but there was no significant effect on daughters' brood size. Evans et al. (2004b) found a

positive correlation between offspring capture avoidance (but not swimming speed) and fathers orange area. Our results are consistent with those of Brooks (2000) in that we observed a tendency for higher juvenile mortality in lines selected for increased relative orange area, but this result was not significant ($P = 0.11$). Overall, the results reported so far, including ours, suggest that carotenoid coloration is typically not associated with fitness-related life-history traits, and in cases where it is, relationships are both positive and negative. This implies that female preferences for carotenoid coloration are unlikely to improve population fitness in guppies. This conclusion is in line with the results of a meta-analysis by Prokop et al. (2012), who have not found a significant association between traits contributing to fathers' attractiveness and fitness components in the progeny across 55 species.

WHAT DO ORANGE SPOTS REVEAL ABOUT MALE GUPPIES?

One possible reason why male reproductive competitiveness stood out among life-history traits in showing significant, correlated response to selection on carotenoid coloration is that it reflects male genetic quality better than the other measured traits. Much of the genetic variation in fitness-related traits is attributed to the load of deleterious mutations, with traits differing in their "mutational target," that is, the number of genes affecting them (Rowe and Houle 1996; Houle 1998). Because male reproductive competitiveness is a complex trait likely affected by the overall health and vigor, cognitive abilities, and condition-dependent sexually selected traits, which include both traits enhancing mating and fertilization success (Cattelan et al. 2020), it is likely that it indeed represents a larger mutational target than the other traits we measured. Thus, if selection for large relative orange area helped to purge deleterious mutations in HIGH lines more effectively than in LOW lines, then the difference in mutation load between treatments could be captured by male reproductive competitiveness more than by other traits. A similar case, however, could be made for female fecundity, which did not show a correlated response to selection. In this case, the positive effect of reduced mutation load could be obscured by intersexual ontogenetic conflict (Rice and Chippindale 2001), resulting in a negative genetic correlation between male and female fitness. Indeed, Hall et al. (2004) found that female guppies originating from lines selected for male attractiveness were less fecund than females from lines where males selected against attractiveness. Nevertheless, other considerations argue against the mutational target explanation. First, Herdegen and Radwan (2015) found no effect of induced mutations on guppy carotenoid coloration. Second, at least two traits we measured here (time to maturity and the rate of sigmoid display) have been shown to be sensitive to deleterious mutations (Herdegen and Radwan 2015), and yet they did not show a correlated response to selection on relative orange, pointing against the possibility that the selection helped purge deleterious mutations from HIGH lines. Alternatively, genes affecting male reproductive competitiveness may be located in male-specific genomic regions on the Y chromosome, known to affect male coloration (Lindholm and Breden 2002; Tripathi et al. 2009). Indeed, a pseudoautosomal region of guppy sex chromosome has been implicated in determining sex-specific reproductive success (Wright et al. 2018). Overall, our results indicate that female preferences for more orange male guppies may benefit females by producing sons with high reproductive competitiveness, but the competitiveness is not solely due to attractiveness of the carotenoid spots.

Conclusions

Our results, by demonstrating significant correlated response in female preferences to selection on male ornament, support the potential for an indirect selection model proposed by Fisher (1930) to drive self-reinforcing selection for preference and preferred trait known as "runaway process". However, we also found that change of female preferences due to this indirect selection did not translate into corresponding differences in male reproductive success, implying that female preferences may not be enough to change the direction of selection on male ornaments. This result highlights that components of male reproductive competitiveness uncorrelated to sexual attractiveness may interfere with Fisherian runaway process (Hunt et al. 2009), and possibly sometimes prevent female preferences from causing reproductive isolation between populations (Kirkpatrick and Ravigné 2002; Schwartz and Hendry 2007; Schwartz et al. 2010). However, higher reproductive competitiveness of males selected for increased carotenoid area in competition with males from lines selected in the opposite direction shows that female guppies mating with more orange males obtain indirect genetic benefits via their competitive sons. The nature of reproductive advantage of males selected for increased carotenoid area will require further investigation, as it was not explained by the correlated traits we measured, including sperm competitiveness. The absence of significant correlated improvement in other fitness components, or in energetically costly sexual display known to reveal mutation load (Herdegen and Radwan 2015), suggests that the advantage is not based on carotenoid area reflecting genome-wide genetic quality, and implies that female preferences for orange coloration in guppies are unlikely to improve population fitness.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

LITERATURE CITED

Achorn, A. M., and G. G. Rosenthal. 2020. It's not about him: mismeasuring 'good genes' in sexual selection. Trends Ecol. Evol. 35:206–219.

Andersson, M. 1986. Evolution of condition dependent sex ornaments and mating preferences: sexual selection based on viability differences. Evolution 40:804–816.

Andersson, M., and L. W. Simmons. 2006. Sexual selection and mate choice. Trends Ecol. Evol. 21:296–302.

Andersson, M. B. 1994. Sexual selection. Princeton Univ. Press, Princeton, NJ.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1–48.

Boake, C. R. B. 1985. Genetic consequences of mate choice: a quantitative genetic method for testing sexual selection theory. Science 227:1061–1063.

Boschetto, C., C. Gasparini, and A. Pilastro. 2011. Sperm number and velocity affect sperm competition success in the guppy (*Poecilia reticulata*). Behav. Ecol. Sociobiol. 65:813–821.

Bozynski, C. C., and N. R. Liley. 2003. The effect of female presence on spermiation, and of male sexual activity on 'ready' sperm in the male guppy. Anim. Behav. 65:53–58.

Breden, F., and G. Stoner. 1987. Male predation risk determines female preference in the Trinidad guppy. Nature 329:831–833.

Breden, F., and K. Hornaday. 1994. Test of indirect models of selection in the Trinidad guppy. Heredity 73:291–297.

Breheny, P., and W. Burchett. 2017. Visualizing regression models using visreg. R J. 9:56–71.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Machler, and B. M. Bolker. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J. 9:378–400.

Brooks, R. 2000. Negative genetic correlation between male sexual attractiveness and survival. Nature 406:67–70.

Brooks, R., and J. A. Endler. 2001a. Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). Evolution 55:1002–1015.

———. 2001b. Female guppies agree to differ: phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. Evolution 55:1644–1655.

Brooks, R., and V. Couldridge. 1999. Multiple sexual ornaments coevolve with multiple mating preferences. Am. Nat. 154:37–45.

Cally, J. G., D. Stuart-Fox, and L. Holman. 2019. Meta-analytic evidence that sexual selection improves population fitness. Nat. Commun. 10:2017.

Candolin, U., and J. Heuschele. 2008. Is sexual selection beneficial during adaptation to environmental change? Trends Ecol. Evol. 23:446–452.

Cattelan, S., A. Di Nisio, and A. Pilastro. 2018. Stabilizing selection on sperm number revealed by artificial selection and experimental evolution. Evolution 72:698–706.

Cattelan, S., J. P. Evans, F. Garcia-Gonzalez, E. Morbiato, and A. Pilastro. 2020. Dietary stress increases the total opportunity for sexual selection and modifies selection on condition-dependent traits. Ecol. Lett. 23:447–456.

Chapuis, M. P., and A. Estoup. 2007. Microsatellite null alleles and estimation of population differentiation. Mol. Biol. Evol. 24:621–631.

Charlesworth, D., and J. H. Willis. 2009. The genetics of inbreeding depression. Nat. Rev. Genet. 10:783–796.

Cole, G., and J. A. Endler. 2018. Change in male colouration associated with artificial selection on foraging colour preference J. Evol. Biol. 31:1227–1238.

Connallon, T., and A. G. Clark. 2014. Balancing selection in species with separate sexes: insights from Fisher's geometric model. Genetics 197:991–1006.

Conner, J. K. 2003. Artificial selection: a powerful tool for ecologists. Ecology 84:1650–1660.

Cotton, S., K. Fowler, and A. Pomiankowski. 2004. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? Proc. R. Soc. Lond. B Biol. Sci. 271:771–783.

Darwin, C. 1871. The descent of man and selection in relation to sex. John Murray, Lond.

Dempster, A. P., N. M. Laird, and D. B. Rubin. 1977. Maximum likelihood from incomplete data via the EM algorithm. J. R. Stat. Soc. Ser. B Stat. Methodol. 39:1–22.

Endler, J. A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34:76–91.

———. 1983. Natural and sexual selection on color patterns in poeciliid fishes. Environ. Biol. Fishes 9:173–190.

Endler, J. A., and A. E. Houde. 1995. Geographic variation in female preferences for male traits in Poecilia reticulata. Evolution 49:456–468.

Evans, J. P. 2010. Quantitative genetic evidence that males trade attractiveness for ejaculate quality in guppies. Proc. R. Soc. B Biol. Sci. 277:3195–3201.

Evans, J. P., L. Zane, S. Francescato, and A. Pilastro. 2003. Directional postcopulatory sexual selection revealed by artificial insemination. Nature 421:360–363.

Evans, J. P., A. Bisazza, and A. Pilastro. 2004a. Female mating preferences for colourful males in a population of guppies subject to high predation. J. Fish Biol. 65:1154–1159.

Evans, J. P., J. L. Kelley, A. Bisazza, E. Finazzo, and A. Pilastro. 2004b. Sire attractiveness influences offspring performance in guppies. Proc. R. Soc. Lond. B Biol. Sci. 271:2035–2042.

Farr, J. A. 1975. Role of predation in evolution of social behavior of natura populations of guppy, *Poecilia reticulata* (Pisces: Poeciliidae). Evolution 29:151–158.

Firman, R. C., C. Gasparini, M. K. Manier, and T. Pizzari. 2017. Postmating female control: 20 years of cryptic female choice. Trends Ecol. Evol. 32:368–382.

Fisher, R. A. 1930. The genetical theory of natural selection. Oxford Univ. Press, Oxford, U.K.

Fuller, R. C., D. Houle, and J. Travis. 2005. Sensory bias as an explanation for the evolution of mate preferences. Am. Nat. 166:437–446.

Gasparini, C., A. Devigili, R. Dosselli, and A. Pilastro. 2013. Pattern of inbreeding depression, condition dependence, and additive genetic variance in Trinidadian guppy ejaculate traits. Ecol. Evol. 3:4940–4953.

Godin, J. G. J., and L. A. Dugatkin. 1996. Female mating preference for bold males in the guppy, *Poecilia reticulata*. Proc. Natl. Acad. Sci. USA 93:10262–10267.

Goudet, J. 2001. FSTAT, version 2.9.3, A program to estimate and test gene diversities and fixation indices. Available via http://www.unil.ch/izea/softwares/fstat.html.

Hall, M., A. K. Lindholm, and R. Brooks. 2004. Direct selection on male attractiveness and female preference fails to produce a response. BMC Evol. Biol. 4:1.

Harano, T., K. Okada, S. Nakayama, T. Miyatake, and D. J. Hosken. 2010. Intralocus sexual conflict unresolved by sex-limited trait expression. Curr. Biol. 20:2036–2039.

Herdegen-Radwan, M. 2019a. Bolder guppies do not have more mating partners, yet sire more offspring. BMC Evol. Biol. 19:211.

———. 2019b. Does inbreeding affect personality traits? Ecol. Evol. 00:1–9.

Herdegen-Radwan, M., K. P. Phillips, W. Babik, R. S. Mohammed, and J. Radwan. 2021. Balancing selection versus allele and supertype turnover in MHC class II genes in guppies. Heredity 126:548–560.

Herdegen, M., and J. Radwan. 2015. Effect of induced mutations on sexually selected traits in the guppy, *Poecilia reticulata*. Anim. Behav. 110:105–111.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6:65–70.

Houde, A. E. 1992. Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces: Poeciliidae) (guppies). Heredity 69:229–235.

———. 1994. Effect of artificial selection on male colour patterns on mating preference of female guppies. Proc. R. Soc. Lond. B Biol. Sci. 256:125–130.

———. 1997. Sex, color and mate choice in guppies. Princeton Univ. Press, Princeton, NJ.

Houde, A. E., and J. A. Endler. 1990. Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. Science 248:1405–1408.

Houle, D. 1998. How should we explain variation in the genetic variance of traits? Genetica 103:241–253.

Houle, D., and A. S. Kondrashov. 2002. Coevolution of costly mate choice and condition-dependent display of good genes. Proc. R. Soc. Lond. B Biol. Sci. 269:97–104.

Hughes, K. A., F. H. Rodd, and D. N. Reznick. 2005. Genetic and environmental effects on secondary sex traits in guppies (*Poecilia reticulata*). J. Evol. Biol. 18:35–45.

Hughes, K. A., A. E. Houde, A. C. Price, and F. H. Rodd. 2013. Mating advantage for rare males in wild guppy populations. Nature 503:108–110.

Hunt, J., C. J. Breuker, J. A. Sadowski, and A. J. Moore. 2009. Male-male competition, female mate choice and their interaction: determining total sexual selection. J. Evol. Biol. 22:13–26.

Ikeda, H., and O. Maruo. 1980. The genetic basis of courtship sounds in *Drosophila mercatorum*. Jpn J. Genet. 55:459–459.

Jia, F. Y., M. D. Greenfield, and R. D. Collins. 2000. Genetic variance of sexually selected traits in waxmoths: maintenance by genotype x environment interaction. Evolution 54:953–967.

Kawecki, T. J., R. E. Lenski, D. Ebert, B. Hollis, I. Olivieri, and M. C. Whitlock. 2012. Experimental evolution. Trends Ecol. Evol. 27:547–560.

Kirkpatrick, M., and M. J. Ryan. 1991. The evolution of mating preferences and the paradox of the lek. Nature 350:33–38.

Kirkpatrick, M., and V. Ravigné. 2002. Speciation by natural and sexual selection: models and experiments. Am. Nat. 159:S22–S35.

Kodric-Brown, A., and P. F. Nicoletto. 2001. Female choice in the guppy (*Poecilia reticulata*): the interaction between male color and display. Behav. Ecol. Sociobiol. 50:346–351.

Kodric-Brown, A. B. 1985. Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). Behav. Ecol. Sociobiol. 17:199–205.

Kokko, H., and R. Brooks. 2003. Sexy to die for? Sexual selection and the risk of extinction. Ann. Zool. Fenn. 40:207–219.

Kokko, H., R. Brooks, J. M. McNamara, and A. I. Houston. 2002. The sexual selection continuum. Proc. R. Soc. Lond. B Biol. Sci. 269:1331–1340.

Kokko, H., R. Brooks, M. D. Jennions, and J. Morley. 2003. The evolution of mate choice and mating biases. Proc. R. Soc. Lond. B Biol. Sci. 270:653–664.

Kotrschal, A., E. Lievens, J. Dahlbom, A. Bundsen, S. Semenova, M. Sundvik, A. Maklakov, S. Winberg, P. Panula, and N. Kolm. 2014. Artificial selection on relative brain size reveals a positive genetic correlation between brain size and proactive personality in the guppy. Evolution 68:1139–1149.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest package: tests in linear mixed effects models. J. Stat. Softw. 82:1–26.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. Proc. Natl. Acad. Sci. USA 78:3721–3725.

Liley, N. R. 1966. Ethological isolating mechanisms in four sympatric species of poeciliid fishes. Behaviour 13:1–19.

Lindholm, A., and F. Breden. 2002. Sex chromosomes and sexual selection in poeciliid fishes. Am. Nat. 160:S214–S224.

Loekle, D. M., D. M. Madison, and J. J. Christian. 1982. Time dependency and kin recognition of cannibalistic bahevior among Poeciliid fishes. Behav. Neural Biol. 35:315–318.

Lorch, P. D., S. Proulx, L. Rowe, and T. Day. 2003. Condition-dependent sexual selection can accelerate adaptation. Evol. Ecol. Res. 5:867–881.

Magurran, A. E. 2005. Evolutionary ecology: the Trinidadian guppy. Oxford Univ. Press, Oxford, U.K.

Mariette, M., J. L. Kelley, R. Brooks, and J. P. Evans. 2006. The effects of inbreeding on male courtship behaviour and coloration in guppies. Ethology 112:807–814.

Martinez-Ruiz, C., and R. J. Knell. 2017. Sexual selection can both increase and decrease extinction probability: reconciling demographic and evolutionary factors. J. Anim. Ecol. 86:117–127.

Martins, M. J. F., T. M. Puckett, R. Lockwood, J. P. Swaddle, and G. Hunt. 2018. High male sexual investment as a driver of extinction in fossil ostracods. Nature 556:366–369.

Mautz, B. S., A. P. Møller, and M. D. Jennions. 2013. Do male secondary sexual characters signal ejaculate quality? A meta-analysis. Biol. Rev. Camb. Philos. Soc. 88:669–682.

Moore, A. J., and P. J. Moore. 1999. Balancing sexual selection through opposing mate choice and male competition. Proc. R. Soc. Lond. B Biol. Sci. 266:711–716.

Okada, K., M. Katsuki, Y. Okada, and T. Miyatake. 2011. Immature performance linked with exaggeration of a sexually selected trait in an armed beetle. J. Evol. Biol. 24:1737–1743.

Okada, K., M. Katsuki, M. D. Sharma, C. M. House, and D. J. Hosken. 2014. Sexual conflict over mating in Gnatocerus cornutus? Females prefer lovers not fighters. Proc. R. Soc. B Biol. Sci. 281:20140281.

Olendorf, R., B. Reudi, and K. A. Hughes. 2004. Primers for 12 polymorphic microsatellite DNA loci from the guppy (*Poecilia reticulata*). Mol. Ecol. Notes 4:668–671.

Pilastro, A., and A. Bisazza. 1999. Insemination efficiency of two alternative male mating tactics in the guppy (*Poecilia reticulata*). Proc. R. Soc. Lond. B Biol. Sci. 266:1887–1891.

Pilastro, A., J. P. Evans, S. Sartorelli, and A. Bisazza. 2002. Male phenotype predicts insemination success in guppies. Proc. R. Soc. Lond. B Biol. Sci. 269:1325–1330.

Pilastro, A., M. Simonato, A. Bisazza, and J. P. Evans. 2004. Cryptic female preference for colorful males in guppies. Evolution 58:665–669.

Plesnar-Bielak, A., A. M. Skrzynecka, K. Miler, and J. Radwan. 2014. Selection for alternative male reproductive tactics alters intralocus sexual conflict. Evolution 68:2137–2144.

Prokop, Z. M., L. Michalczyk, S. M. Drobniak, M. Herdegen, and J. Radwan. 2012. Meta-analysis suggests choosy females get sexy sons more than "good genes. Evolution 66:2665–2673.

Prokuda, A. Y., and D. A. Roff. 2014. The quantitative genetics of sexually selected traits, preferred traits and preference: a review and analysis of the data. J. Evol. Biol. 27:2283–2296.

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Radwan, J., L. Engqvist, and K. Reinhold. 2016. A paradox of genetic variance in epigamic traits: beyond "good genes" view of sexual selection. Evol. Biol. 43:267–275.

Rice, W. R., and A. K. Chippindale. 2001. Intersexual ontogenetic conflict. J. Evol. Biol. 14:685–693.

Roff, D. A. 1997. Evolutionary quantitative genetics. Chapman and Hall, New York.

Roff, D. A., G. Stirling, and D. J. Fairbairn. 1997. The evolution of threshold traits: a quantitative genetic analysis of the physiological and life-history correlates of wing dimorphism in the sand cricket. Evolution 51:1910–1919.

Rousset, F. 2008. GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol. Ecol. Resour. 8:103–106.

Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. Proc. R. Soc. Lond. B Biol. Sci. 263:1415–1421.

Schwartz, A. K., and A. P. Hendry. 2007. A test for the parallel co-evolution of male colour and female preference in Trinidadian guppies (*Poecilia reticulata*). Evol. Ecol. Res. 9:71–90.

Schwartz, A. K., D. J. Weese, P. Bentzen, M. T. Kinnison, and A. P. Hendry. 2010. Both geography and ecology contribute to mating isolation in guppies. PLoS ONE 5:e15659.

Seckinger, J., H. Brinkmann, and A. Meyer. 2002. Microsatellites in the genus *Xiphophorus*, developed in *Xiphophorus montezumae*. Mol. Ecol. Notes 2:4–6.

Shen, X., Y. Guanpin, and L. Meijie. 2007. Development of 51 genomic microsatellite DNA markers of guppy (*Poecilia reticulata*) and their application in closely related species. Mol. Ecol. Notes 7:302–306.

Simmons, L. W., R. M. Tinghitella, and M. Zuk. 2010. Quantitative genetic variation in courtship song and its covariation with immune function and sperm quality in the field cricket *Teleogryllus oceanicus*. Behav. Ecol. 21:1330–1336.

Taylor, J. S., J. S. P. Sanny, and F. Breden. 1999. Microsatellite allele size homoplasy in the guppy (*Poecilia reticulata*). J. Mol. Evol. 48:245–247.

Tomášek, O., J. Albrechtová, M. Němcová, P. Opatová, and T. Albrecht. 2017. Trade-off between carotenoid-based sexual ornamentation and sperm resistance to oxidative challenge. Proc. R. Soc. B Biol. Sci. 284:20162444.

Tomkins, J. L., J. Radwan, J. S. Kotiaho, and T. Tregenza. 2004. Genic capture and resolving the lek paradox. Trends Ecol. Evol. 19:323–328.

Tripathi, N. H. M., E. Willing, C. Lanz, D. Weigel, and C. Dreyer. 2009. Genetic linkage map of the guppy, *Poecilia reticulata*, and quantitative trait loci analysis of male size and colour variation. Proc. R. Soc. Lond. B Biol. Sci. 276:2195–2208.

Tuni, C., C. S. Han, and N. J. Dingemanse. 2018. Multiple biological mechanisms result in correlations between pre- and post-mating traits that differ among versus within individuals and genotypes. Proc. R. Soc. B Biol. Sci. 285:20180951.

van Oosterhout, C., R. E. Trigg, G. R. Carvalho, A. E. Magurran, L. Hauser, and P. W. Shaw. 2003. Inbreeding depression and genetic load of sexually selected traits: how the guppy lost its spots. J. Evol. Biol. 16:273–281.

Wang, J., and A. W. Santure. 2009. Parentage and sibship inference from multilocus genotype data under polygamy. Genetics 181:1579–1594.

Watanabe, T., M. Yoshida, M. Nakajima, and N. Taniguchi. 2003. Isolation and characterization of 43 microsatellite DNA markers for guppy (*Poecilia reticulata*). Mol. Ecol. Notes 3:487–490.

Whitlock, M. C., and A. F. Agrawal. 2009. Purging the genome with sexual selection: reducing mutation load through selection on males. Evolution 63:569–582.

Wilkinson, G. S., and P. R. Reillo. 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. Proc. R. Soc. Lond. B Biol. Sci. 255:1–6.

Wright, A. E., M. Fumagalli, C. R. Cooney, N. I. Bloch, F. G. Vieira, S. D. Buechel, N. Kolm, and J. E. Mank. 2018. Male-biased gene expression resolves sexual conflict through the evolution of sex-specific genetic architecture. Evol. Lett. 2:52–61.

Yoshikawa, T., Y. Ohkubo, K. Karino, and E. Hasegawa. 2016. Male guppies change courtship behaviour in response to their own quality relative to that of a rival male. Anim. Behav. 118:33–37.

Zahavi, A. 1975. Mate selection - a selection for a handicap. J. Theor. Biol. 53:205–214.

Zajitschek, F., and T. Connallon. 2018. Antagonistic pleiotropy in species with separate sexes, and the maintenance of genetic variation in life-history traits and fitness. Evolution 72:1306–1316.

Associate Editor: T. Flatt
Handling Editor: T. Chapman

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Preference test arena consisting of a: female (F) compartment with a cylinder in the middle (dashed circle) and preference zones (P1 and P2), and male compartments (M1 and M2), visually separated from each other.

Fig. S2 Histograms of the sperm number distribution, plotted separately for male treatments.

Tab. S1 Descriptive statistics for F6 male (A) and female (B) traits, with respect to treatment.

Tab. S2 Average observed heterozygosities (Ho) in the last generation of selection (F6) per line, and heritability (h^2) per line, followed by mean (SE) for each treatment.

Tab. S3 Per locus allelic richness (AR) in the whole generation F6, as well as per treatment, for comparison juxtaposed with AR data from a different experiment on the same stock population used in the present study (Herdegen-Radwan 2019a), and AR in a set of wild guppy populations from Trinidad (Herdegen-Radwan et al. 2021).

Tab. S4 Pairwise F_{ST} values among selection lines in generation F6, estimated with the method of Weir (1996) implemented in FreeNA. ENA correction applied.

Tab. S5 Generalized linear mixed model testing the effect of male and female treatment on male reproductive success, with orange as covariate. Paternity success (0/1) is the response variable. Significant terms are in bold.

Tab. S6 Generalized linear mixed model testing the effect of female treatment and orange on male reproductive success. Paternity success (0/1) is the response variable.

Tab. S7 Mixed models testing the joint effect of treatment and orange on sperm qualities: (A) number; (B) velocity (VCL); (C) PC1 from principal components analysis on sperm velocity-related measures; (D) viability. Significant terms are in bold.

Tab. S8 Mixed models testing the effect of orange on sperm qualities: (A) number; (B) velocity (VCL); (C) PC1 from principal components analysis on sperm velocity-related measures; (D) viability. Significant terms are in bold.

Tab. S9 Per line percentage of F6 juveniles that did not survive to adulthood.

Tab. S10 Summary of the results of earlier studies looking at correlated responses to selection on male color traits in guppies. Correlated responses: YES = significant, NS = nonsignificant, FP = female preferences. Source population: LP = low predation site, HP = high predation site. *One line in each combination: orange up black up, orange up black down, orange down black up, orange down black down; **became marginally significant after replicates were pooled within orange up and orange down treatments.