

Colorful male guppies do not provide females with fecundity benefits

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The phenotype-linked fertility hypothesis (PLFH) predicts that males with elaborated sexual ornaments signal their high fertilizing efficiency to females and that female preferences for ornamented males are driven by direct fecundity benefits. Although some studies have demonstrated that attractive males produce more or higher quality sperm, there is limited experimental evidence that females derive fecundity benefits by mating with attractive males. Some of the best indirect evidence for the PLFH comes from work on guppies (*Poecilia reticulata*), an internally fertilizing species of freshwater fish in which phenotypically attractive males produce larger and relatively higher quality ejaculates than their less attractive counterparts. We used artificial insemination to impregnate female guppies using known numbers of sperm from a range of males with different phenotypes and related female fecundity (brood success, time from insemination to parturition, and brood size) to sperm numbers and male phenotype (body size and the relative area of color spots). We found no evidence that male phenotype or experimentally adjusted “ejaculate” size influenced any of our measures of female fecundity. These results highlight the importance of experimentally investigating potential fecundity benefits associated with female mating preferences before concluding that the maintenance of these preferences is driven by the pursuit of such benefits. *Key words:* artificial insemination, direct benefits, fecundity, female choice, male sexual secondary characters, sperm number. [*Behav Ecol* 19:374–381 (2008)]

Extravagant male secondary sexual characters (ornaments) evolve in many species because females preferentially mate with males exhibiting the most exaggerated traits (Darwin 1874; Andersson 1994). By mating with the most ornamented males, females are expected to obtain either direct or indirect benefits, for example, because such traits signal the male’s ability to provide resources that *directly* influence the choosing female’s fitness or because they signal a male’s genetic quality which will benefit females *indirectly* via the enhanced (genetic) quality of their offspring (Andersson 1994). In species with resource-free mating systems (where males contribute only sperm at reproduction), female preferences for ornamented males are thought to be driven by genetic benefits inherited through the father, such as “good genes” for offspring survival and/or future reproduction (e.g., Andersson 1994; Møller and Alatalo 1999; Kokko et al. 2006).

The phenotype-linked fertility hypothesis (PLFH) offers an alternative explanation for female preferences for ornamented males by proposing that females will benefit from mating with males with elaborated sexual ornaments if these males also have high fertilizing efficiency (Sheldon 1994). According to this hypothesis, the coevolution of male ornaments and female preferences for such ornaments is driven by the pursuit of fertility benefits by females. To support this hypothesis, it is therefore necessary to demonstrate first that there is a phenotypic correlation between male ornaments and fertility (due either to an underlying genetic correlation between the 2 traits or because they are both dependent on condition) and second that females exhibit enhanced fecundity by mating with highly ornamented males.

The PLFH has received considerable experimental attention, most of which has concentrated on the association between proxy measures of fertilizing efficiency (testis size, sperm production, ejaculate size, and sperm quality) and male attractiveness (ornamentation or courtship behavior) (reviewed in Birkhead and Pizzari 2002; Pizzari et al. 2002). Several physiological mechanisms have been suggested to explain the positive covariance between ornament expression and fertilizing efficiency. For example, Hillgarth et al. (1997) and Folstad and Skarstein (1997) proposed a hypothesis that links androgen-dependent male secondary characters with sperm quality. According to this hypothesis, male ornaments are correlated with levels of gonadal hormones (androgens). The immunosuppressive effect of these androgens, although potentially dangerous to the males because they increase their susceptibility to parasite attacks, also reduces the autoimmune response to antigenic sperm, resulting in covariance between ejaculate quality and male sexual secondary characters. More recently, Blount et al. (2001) suggested an alternative mechanism for species in which males exhibit antioxidant-dependent ornamental displays (e.g., carotenoid-based ornaments). This hypothesis suggests that both sperm quality (fertility; integrity of DNA) and the substrates responsible for male ornamentation may be vulnerable to free radical attack, which can be mitigated by antioxidants (e.g., contained in carotenoid-rich food sources). A positive correlation between ornamentation and sperm quality could arise if antioxidants are in limited supply and the showiest males are most likely to produce high-quality ejaculates (e.g., Peters et al. 2004).

As Birkhead and Pizzari (2002) have noted, although we often assume positive correlations between fitness-related traits, there is no a priori reason why this should always be true. Indeed, life-history traits are often traded off (Stearns 1992), and males may not be able to increase their investment in postcopulatory traits without reducing investment in those involved in precopulatory sexual selection, assuming that the resources allocated to reproduction are constant (Parker

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1998). Indeed, studies aimed at testing for phenotypic correlations between a male's ornament and his fertilizing efficiency have yielded contrasting results. For example, in the greenfinch (*Carduelis chloris*) there is a positive correlation between plumage brightness and testis size (Merila and Sheldon 1999), and in male mallards (*Anas platyrhynchos*) carotenoid-based ornaments positively covary with ejaculate quality (Peters et al. 2004). Likewise, in red deer, male attractiveness (relative antler size) is positively correlated with relative testes size and sperm quality (Malo, Roldan, et al. 2005). By contrast, other studies have reported no relationship between male phenotype and ejaculate traits (Birkhead and Fletcher 1995; Birkhead and Petrie 1995), and some studies on fishes have reported negative associations between sperm quality and male secondary characters, including Arctic charr (*Savelinus alpinus*) (Liljedal et al. 1999, but see Masvaer et al. 2004), salmon (Vladic and Jarvi 2001; Vladic et al. 2002), and corksing wrasse (*Symphodus melops*) (Uglen et al. 2001). Ambiguous results in tests of the PLFH are not unexpected, especially in studies done on animals taken from natural populations where confounding factors that may influence fertilizing efficiency (e.g., male condition, age, recent copulation history, differential maternal effects) are difficult to control. Even when the association between male phenotype and fertilizing efficiency is tested under controlled, experimental conditions, results can be contradictory. For example, in a detailed and well-controlled study on the domestic fowl, the expression of one male ornament (the comb), but not that of another (the spur), predicted testicular mass, but neither ornament significantly covaried with sperm velocity, another important component of male fertilizing efficiency (Pizzari et al. 2004).

All these previous studies have estimated phenotypic correlations between sperm traits and male ornaments. However, as emphasized above, a positive correlation between fertilizing efficiency and male ornaments is not sufficient to demonstrate that female preferences for ornamented males are driven by the pursuit of fecundity benefits (or alternatively that females mating with males with lower expression of their ornaments suffer a fecundity cost). To support the PLFH, it is therefore necessary to demonstrate that females obtain fecundity benefits by mating with showy males (Wagner and Harper 2003; Malo, Garde, et al. 2005). The guppy (*Poecilia reticulata*), a live-bearing freshwater fish with internal fertilization and a nonresource-based mating system, represents an ideal species with which to test the PLFH hypothesis. Females exhibit sexual preferences for males with large color spots, which are displayed during elaborate "sigmoid" courtship displays (Houde 1997). Several studies have revealed that phenotypically attractive males produce larger and higher quality ejaculates. For example, male sigmoid display rate, and in some populations the size of the males' body color spots, is positively associated with sperm production (as revealed by manually stripping sperm) (Matthews et al. 1997; Pitcher and Evans 2001, but see Skinner and Watt 2007). Furthermore, colorful male guppies transfer higher numbers of sperm to females than their less colorful counterparts (Pilastro et al. 2002), although females also influence sperm transfer independently according to their perception of male color phenotype (Pilastro et al. 2004). Nevertheless, when sperm numbers from competing males are held experimentally constant, relatively attractive males have higher parentage success than their less conspicuous counterparts (Evans, Zane, et al. 2003), which is likely to be due to the strong positive association between sperm quality (sperm motility, viability, and swimming speeds) and male attractiveness (Locatello et al. 2006; Pitcher et al. 2007). This preference by female guppies for highly ornamented males that produce larger and higher quality ejaculates has been offered as some of the strongest

support for the PLFH (Matthews et al. 1997; Birkhead and Pizzari 2002). However, evidence that female guppies obtain direct fecundity benefits by mating with colorful males is still lacking. Observations from natural copulations cannot be used to test this prediction because of the potentially confounding influence of differential maternal effects (e.g., Reyer et al. 1999) and variable ejaculate size (Pilastro and Bisazza 1999; Pilastro et al. 2002). In this study, we use artificial insemination to control these potentially confounding effects and test the potential for females to obtain fecundity benefits by mating with colorful males.

METHODS

The study population and its maintenance

The guppies used in this experiment were descendents of wild-caught fish from the Tacarigua River in Trinidad (national grid reference PS 787 804). The Tacarigua population is ideal for this study for several reasons. First, females exhibit sexual preferences for males with relatively large areas of orange spots (Evans, Bisazza, et al. 2004). Second, males that have relatively high levels of orange spots in their color patterns produce faster and more viable sperm (Locatello et al. 2006), consequently enjoying higher fertilization success when the number of competing sperm from rival males is regulated through artificial insemination (Evans, Zane, et al. 2003). Finally, a recent study has confirmed that in this population relatively colorful males have higher sperm replenishment rates (and therefore potentially sperm production) than their less ornamented counterparts (Gasparini C, Pilastro A, in preparation). A previous study (Pitcher and Evans 2001) failed to find such a positive correlation between sperm reserves and the relative area of orange spots in this population. However, in the study of Pitcher and Evans (2001), males were not isolated from females before sperm collection and their estimates of sperm reserves may therefore have been influenced by the females' previous mating history. When males are stripped to remove any stored sperm and subsequently prevented from mating, the number of sperm produced in the ensuing 7 days is positively correlated with the relative area of orange spots ($r = 0.24$, $P < 0.001$, $n = 110$, Gasparini C, Pilastro A, in preparation).

Because female guppies store sperm for several months (Constantz 1989), we used virgin females to avoid the possibility that fertilizations were due to prior males. Females were reared in single-sex groups, and males were reared in mix-sex aquaria (ca. 1:1 sex ratio) until required. Water temperature was maintained between 25 and 27 °C, and illumination was set on a 12:12 h light:dark cycle (Philips TLD 36-W fluorescent lamps). All fish were fed a mixed diet of brine shrimp nauplii and commercially prepared flake food.

Selection of males and measurement of color patterns

Males were aged between 4 and 6 months and exhibited a range of color phenotypes. We confirmed that each male was fully sexually mature, as evident by the maturational state of the gonopodium's apical hood (for details see Houde 1997). To measure male color patterns (done immediately after artificial insemination), individual test males were anesthetized in a water bath containing Tricaine Methanesulfonate (MS222) and photographed using a digital camera (Nikon Coolpix 4300). UTHSCSA Image Tool (University of Texas Health Science Center, San Antonio, TX; <http://ddsdx.uthscsa.edu/dig/download.html>) was then used to estimate the body area of each male (including caudal fin but excluding dorsal fin) as well as the surface area of orange, yellow, and red spots (hereafter orange). Black spots (encompassing fuzzy

black lines) and iridescent spots (combined measures of blue, green, purple, and silver) were also included in the analysis because in some populations these colors are known to influence female mating preferences (e.g., Brooks 1996; Kodric-Brown and Nicoletto 1996). In the population used for this study, however, only the area of the orange spots is positively correlated with sperm competition success and sperm quality (Evans, Zane, et al. 2003; Locatello et al. 2006). Color measures were made on the left side of each male's body. To control for the effects of body size, the relative area of each color pigment was used in the analyses. The standard length (distance from the snout to the tip of the caudal peduncle = SL) of each female was measured with a ruler to the nearest 1 mm.

Artificial insemination

Artificial insemination (see Clark 1950; Lodi 1981; Evans, Zane, et al. 2003; Evans, Kelley, et al. 2004) was used to inseminate sexually naive virgin female guppies with known numbers of sperm from different males. This technique makes it possible to control for the number of sperm inseminated (Pilastro et al. 2002, 2004). Following the methods in Evans, Kelley, et al. (2004), sperm were manually stripped from individual males that were previously isolated from females for 3–5 days. Sperm are packaged in bundles (spermatozeugmata), which in the focal population contain approximately 27 000 individual sperm cells (Evans, Kelley, et al. 2004). Previous work has confirmed that the number of sperm per bundle does not correlate with either body size or the extent of body coloration (Evans, Zane, et al. 2003). It was therefore straightforward to control the number of sperm used for each insemination by counting the number of sperm bundles. The number of sperm bundles used for the inseminations corresponded to the natural variation in ejaculate size observed in this population during "sneaky" (forced) copulations (mean ejaculate size = $52.1 \pm 174.4 \times 10^3$ sperm, corresponding to 2 bundles, $n = 18$; Pilastro A, Gasparini C, Boschetto C, Evans JP, unpublished observations) and courtship (solicited) copulations ($0.91 \pm 0.83 \times 10^6$ cells, $n = 27$, corresponding to ca. 33 bundles, Pilastro et al. 2004). For each insemination, a virgin female was anesthetized in a water bath containing a mild dose of MS222 and placed in a polystyrene cradle with her genital pore exposed. A Drummond 3- μ L micropipette was used to inseminate the sperm bundles from each stripped ejaculate (suspended in 10 μ L of 0.9% NaCl) into the female's gonoduct (penetration depth, ca. 2 mm). Immediately after insemination, females were revived in a 5-L plastic container (containing conditioned freshwater, gravel, aquatic vegetation, and an airstone) where they remained isolated until they produced their first broods.

In a previous study that tested the association between sire phenotype and offspring performance, Evans, Kelley, et al. (2004) found no evidence that male phenotype was associated with subsequent brood sizes or the timing to parturition. Here we extend this analysis to examine the combined effects of sire phenotype and experimentally manipulated ejaculate size on the success of artificial inseminations (i.e., whether the female produce a brood or not), hereafter termed brood success (with binomial distribution). To this end, we performed 2 groups of artificial inseminations, 1 in 2002 ($n = 30$) and 1 in 2006 ($n = 70$), comprising $n = 100$ replicate fertilizations (mean SL of the females, 21.56 ± 1.93 standard deviation [SD], range = 18–26 mm, $n = 100$; mean SL of males, 18.35 ± 1.26 SD, range = 15.7–22.1 mm; mean relative area of color spots was $11.72 \pm 6.14\%$ SD for orange, $3.67 \pm 1.62\%$ SD for melanistic spots and $5.07 \pm 2.63\%$ SD for iridescent colors, $n = 99$; for 1 male phenotype measures were missing). Artificial inseminations were carried out by 2 operators (author 2, $n = 70$, and author

4, $n = 30$). The 2 artificial insemination (AI) groups were temporally separated due to the logistic constraints of housing all females individually for sufficient time to produce a brood (up to 2 months). Brood success did not differ between operators (author 3 = 75.7%, author 4 = 83.3%, Fisher's Exact test, $P = 0.45$). During the artificial inseminations, we experimentally manipulated ejaculate sizes by using 2 ($n = 30$, among which author 4 = 10 and author 2 = 20), 15 ($n = 35$, author 4 = 10 and author 3 = 25), and 30 ($n = 35$, author 4 = 10 and author 2 = 25) sperm bundles. This enabled us to also examine the effect of ejaculate size on female fecundity. To increase the power to detect an effect of male phenotype on brood success, this dataset was then enlarged (incorporating 29 females from study of Evans, Kelley, et al. [2004] and a further 89 females from previously unpublished data) to comprise all artificial inseminations performed by the same 2 operators in 4 temporal groups during the period 2003–2006, in which different numbers of sperm bundles were used (total $n = 218$, author 3 = 159 and author 4 = 59). The phenotypic traits of the males included in the additional sample did not differ significantly from those of the initial dataset (male SL: additional [$n = 118$] 18.10 ± 1.18 mm, restricted [$n = 99$] 18.35 ± 1.26 mm; orange: additional $10.74 \pm 5.51\%$, restricted $11.72 \pm 6.14\%$; melanistic: additional $3.13 \pm 1.64\%$, restricted $3.67 \pm 1.62\%$; iridescent: additional $4.27 \pm 3.45\%$, restricted $5.07 \pm 2.63\%$; all P 's > 0.05, except for size of melanistic spots, $F_{1,216} = 5.86$, $P < 0.02$). Variances were homogeneous between the 2 datasets (all P 's > 0.05).

In total (including reduced and enlarged datasets), artificial inseminations were done with 2 ($n = 30$), 5 ($n = 20$), 15 ($n = 35$), 20 ($n = 68$), and 30 ($n = 65$) sperm bundles. Brood success did not differ among operators (author 2 = 77.4%, author 4 = 83.1%, Fisher's Exact test, $P = 0.46$) or between the 2 datasets (restricted dataset = 78.0%, additional dataset = 79.7%, Fisher's Exact test, $P = 0.87$, $n = 218$).

Statistical methods

Time to parturition (the number of days between insemination and the production of offspring) and brood size differed among insemination groups (see also Evans, Kelley, et al. 2004 and below), and therefore, insemination group was entered as a factor in the following analyses. The association between male phenotype and brood success (0 = no brood produced, 1 = brood produced) was tested using a logistic regression, where brood success was the dependent binomial variable and number of bundles, female SL, and male phenotype (SL, relative area of color spots) were the independent variables. To reduce multicollinearity in the logistic multiple regression analyses (see below), we grouped ejaculate size into 2 groups: small ejaculates (2–5 bundles) and large ejaculates size (15–30 bundles). Reducing this explanatory variable to just 2 levels better enabled the regression model to reach convergence and accurately estimate the model deviance. Nevertheless, we confirmed in separate analyses that when all 5 levels of sperm bundles (2, 5, 15, 20, and 30) were used, the results were qualitatively similar (although in these instances the model did not always fully reach convergence). Furthermore, we used centered variables (where the mean has been subtracted from each datum), a transformation which often reduces multicollinearity (Cohen and Cohen 1983). To test for the effect of male phenotype on brood success, we first entered all the independent variables (male ornaments, female size, AI group, and ejaculate size). We noted the deviance and whether any variable was a significant predictor of brood success. We then used a backward stepwise elimination procedure to exclude interactions between ejaculate size and male traits from the model. This procedure excluded all nonsignificant terms,

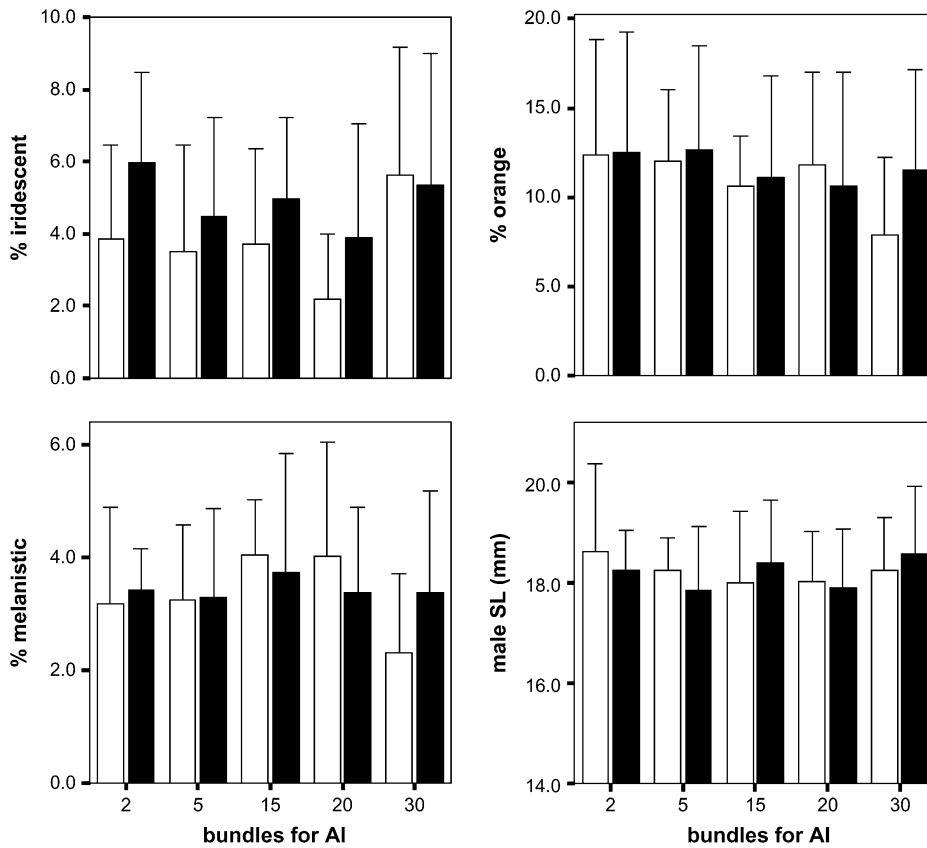


Figure 1
Phenotypic traits (mean \pm SD) of the males according to brood success (open bars = no brood produced; filled bars = brood produced) and to the number of sperm bundles used for artificial insemination.

controlling step by step whether the elimination of each term caused a significant reduction of the model's fit (using the goodness-of-fit procedure GOODFIT [SPSS ver. 15.0] based on chi square of Hosmer and Lemeshow; Hosmer and Lemeshow 2000). The final model included the original predictors and any significant interaction left in the model after the backward elimination.

To test for an effect of male phenotype on brood size and time to parturition, we used a general linear model, where the time to parturition (in days) and brood size (number of offspring produced) were the dependent variables, insemination group was a random factor, and number of bundles, female SL, and male phenotype (SL, relative area of color spots) were entered as covariates. Data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene's test of equality of error variances). Time to parturition (in days) did not show homogeneity of variance across time blocks ($P = 0.025$) even with log transformation. Analysis of covariance is generally robust to heteroscedasticity, in particular when the differences in variance between the dependent variables are small and the covariate means and variances are similar between the samples (Larholt and Sampson 1995; see Figure 1 for a comparison of means and variances of covariates in this study). Furthermore, when, as in the case of our dataset, there is an inverse relationship between sample size and variance (brood size, $r = -0.33$; gestation $r = -0.49$), alpha is inflated (Glass et al. 1972). This decreases the likelihood of accepting the null hypothesis when it is false and is therefore conservative with respect to the conclusions of the present study (see Results). Proportions (relative area of color spots) were arcsine square root transformed. All probabilities are 2 tailed. If not otherwise stated, means \pm 1 SD are given. Statistical analyses were done using SPSS ver. 15.

RESULTS

Among the females that were artificially inseminated, 78.0% delivered a brood in the restricted dataset that did not include data from study of Evans, Kelley, et al. (2004) (mean time to parturition = 34.4 ± 7.18 , range = 25–54, $n = 78$) and 78.9% in the total dataset (mean time to parturition = 34.1 ± 8.56 , range = 18–68, $n = 172$). Male phenotype and the number of bundles used for artificial inseminations did not affect brood success, both in the full model and in the restricted model after stepwise elimination of nonsignificant interactions (Table 1). The effect of male color pattern on brood success was not significant even at low sperm numbers, as evident by nonsignificant interactions between color measures and the number of bundles inseminated (Table 1). Including the identity of the operator as a factor in the model did not change the results substantially.

Mean brood size was 8.66 ± 5.95 (range = 1–28, $n = 172$). After excluding cases where the timing to parturition exceeded 50 days (gestation is usually 3–4 weeks, Houde 1997, and therefore offspring produced >50 days could be second broods), mean time to parturition was 33.2 ± 6.55 (range = 18–50) and mean brood size was 8.70 ± 5.99 (range = 1–28, $n = 165$). In the analysis of the effect of male phenotype on the time to parturition and brood size, we excluded females that produced offspring more than 50 days after artificial insemination because these were likely to be second broods (Houde 1997). However, including these data did not change the results substantially. The models included time to parturition and brood size as dependent variables, AI group as a factor, and female and male phenotypic traits and ejaculate size as covariates. Although we found a significant difference in brood size and time to parturition among AI groups, none of the male

Table 1

Results of the logistic regression model testing the influence of female size (SL), male phenotype (SL and area of color spots relative to body area), and number of sperm inseminated on brood success, which was the dependent variable with binomial distribution (0 = no brood produced and 1 = brood produced)

Goodness-of-fit test

Model	Restricted dataset ($n = 99$)			Total dataset ($n = 215$)		
	Chi square	df	P	Chi square	df	P
Saturated	3.559	8	0.895	8.435	8	0.392
Final	3.030	8	0.932	8.969	8	0.345

Variable	Restricted dataset ($n = 99$)				Extended dataset ($n = 215$)			
	b	SE	Wald	P	b	SE	Wald	P
Constant	0.072	0.993	0.005	0.943	0.409	0.755	0.294	0.588
AI group			0.191	0.662			4.410	0.492
Female SL	0.815	0.596	1.870	0.171	0.395	0.464	0.727	0.394
Male SL	0.098	0.157	0.393	0.531	-0.036	0.102	0.123	0.726
Ejaculate size	0.076	0.227	0.110	0.740	-0.063	0.152	0.173	0.678
Orange	-0.113	0.173	0.427	0.513	0.055	0.036	2.347	0.126
Melanistic	0.095	0.187	0.261	0.609	0.117	0.121	0.942	0.332
Iridescent	0.147	0.105	1.950	0.163	0.104	0.067	2.401	0.121
Ejaculate size \times orange	0.171	0.116	2.178	0.140				
AI group \times orange	-0.203	0.117	2.987	0.084				

The analysis was conducted on the restricted dataset ($n = 99$) and the extended dataset ($n = 215$). Predictors were forced into the model, whereas the interactions were selected using a backward stepwise elimination based on the likelihood ratio test. P values for each independent variable are shown for the final model after backward elimination. df, Degrees of freedom; SE, standard error.

phenotypic traits was significantly associated with time to parturition or brood size (Table 2, Figure 2). Similar results were obtained with the reduced dataset ($n = 78$).

DISCUSSION

Our analysis revealed that male attractiveness was not associated with increased female fecundity (brood size, the duration of brood development, and brood success) in the guppy population used for this study. We did not find an effect of sperm

numbers on brood success even at the lowest sperm concentration. It has been argued that colorful males are more likely to provide fecundity benefits to females because males exhibiting the most colorful phenotypes produce better-quality ejaculates (Locatello et al. 2006; Pitcher et al. 2007; but see Skinner and Watt 2007), produce higher numbers of sperm (Pitcher and Evans 2001), and deliver larger ejaculates to females during experimentally controlled matings (Pilastro et al. 2002). Hence, colorful males are less likely to be sperm depleted (having on average larger reserves, although this will depend on the population and on the balance between their mating rate and sperm production) than less colorful males and will deliver a larger proportion of their sperm reserves to females. Female fecundity may in principle be limited because males do not provide them with high enough numbers of sperm or sperm of sufficient quality (or a combination of both). In the following paragraphs, we address both these points and argue on several counts that such benefits are unlikely to apply in guppies.

First, we demonstrated that female fecundity is not limited by ejaculate size, even when extremely low numbers of sperm were used during artificial insemination. This suggests that, on average, male sperm reserves and ejaculate size (Pitcher and Evans 2001; Pilastro et al. 2004) are substantially larger than the minimum number of sperm needed to guarantee a female's brood success (and hence fecundity) and that females are unlikely to be sperm limited in nature. Second, during their sexually receptive phase, females typically mate cooperatively with more than one male (e.g., Evans and Magurran 2000; Pitcher et al. 2003) and the likelihood that all males are sperm depleted seems unlikely. Furthermore, outside these periods of sexual receptivity, females typically receive about 1 forced copulatory attempt per minute (Magurran and Seghers 1994) and these mating attempts often result in sperm transfer (Pilastro

Table 2

General linear models testing the effect of male and female phenotype, the number of sperm inseminated (covariates), and the experimental group (factor) on brood size and the timing from insemination to parturition (after log transformation, $n = 171$)

	Time to parturition			Brood size	
	Degrees of freedom	F	P	F	P
Model	1	4.303	<0.001	2.320	0.012
Intercept	1	82.290	<0.001	0.338	0.562
Female SL	1	1.458	0.229	3.185	0.076
Male SL	1	0.013	0.910	0.476	0.491
Orange area	1	0.360	0.549	0.547	0.461
Melanistic area	1	0.159	0.691	0.003	0.957
Iridescent area	1	0.205	0.651	0.000	0.990
No. of bundles	1	0.073	0.788	0.028	0.868
AI group	5	6.157	<0.001	4.145	0.001
Error	152				

Interactions, which were not significant, were removed from the final models. Similar results were obtained using the restricted dataset ($n = 78$).

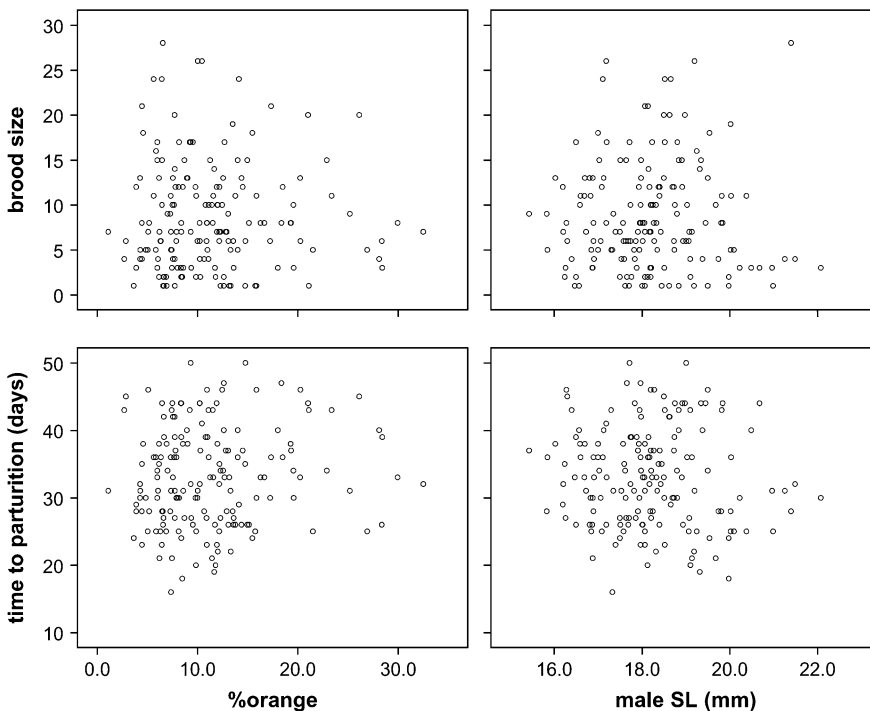


Figure 2

The duration in days for females to produce offspring (time to parturition) and brood size in relation to the proportion of male's orange (%) and body size (SL). None of the male phenotypic traits were correlated with brood size and time to parturition (male SL: brood size, $r = 0.002$, $P = 0.99$; time to parturition, $r = -0.08$, $P = 0.33$; orange: brood size, $r = 0.001$, $P = 0.99$; time to parturition, $r = 0.10$, $P = 0.19$; melanistic: brood size, $r = -0.14$, $P = 0.07$; time to parturition, $r = 0.10$, $P = 0.20$; iridescent: brood size, $r = 0.04$, $P = 0.61$; time to parturition, $r = -0.02$, $P = 0.84$; $n = 165$).

and Bisazza 1999; Pilastro et al. 2002). Field surveys have revealed that up to 45% of unreceptive females have sperm in their gonoducts resulting from forced copulations (Matthews and Magurran 2000; Evans, Pilastro, et al. 2003; but see Russell et al. 2006). Third, females can store sperm for several months and can produce numerous consecutive broods after a single copulation (Constantz 1989). Finally, during cooperative copulations, females actively limit the number of sperm transferred from males that they perceive to be less attractive (Pilastro et al. 2004), despite the fact that males often attempt to remate after the first copulation (Pilastro and Bisazza 1999). This would not be expected if females were sperm limited or if females select males on the basis of their sperm production. Nevertheless, our analysis was limited to the first brood cycle, and it could be argued that differences in sperm quality among males may affect brood success over several clutches. However, because female guppies are highly promiscuous (Kelly et al. 1999; Pitcher et al. 2003, see above), it seems unlikely that long-term sperm fertility would play anything other than a minor role, although such effects cannot be completely eliminated on the basis of the results presented here.

Colorful male guppies produce relatively high-quality ejaculates (Locatello et al. 2006; Pitcher et al. 2007; but see Skinner and Watt 2007), which is likely to account for their disproportionately high success in sperm competition (Evans, Zane, et al. 2003). In principle, therefore, it is possible that female fecundity is limited by sperm quality (e.g., sperm viability and sperm swimming speed). However, we did not find statistically significant interaction between male phenotype and ejaculate size on brood success, suggesting that mating with colorful males does not increase female fecundity, even when male sperm reserves are limited. It would be worth investigating whether male courtship rate, a sexually selected trait which is positively correlated with sperm reserves in this guppy population (Matthews et al. 1997; Pitcher and Evans 2001), is correlated with ejaculate characteristics and hence with female fecundity. The lack of association between male phenotype and brood size revealed by our study further suggests that "intrauterine" survival rate of the offspring is not influenced by male pheno-

type, confirming previous indirect evidence (Evans, Zane, et al. 2003). Thus, embryo viability does not seem to be an important factor underlying the observed benefits of female choice (see below) and, possibly, polyandrous behavior (e.g., Garcia-Gonzalez and Simmons 2005) in guppies.

In conclusion, despite the positive covariance found in some guppy populations between male attractiveness and sperm production (Matthews et al. 1997; Pitcher and Evans 2001) and between male attractiveness and sperm quality/competitiveness (Evans, Zane, et al. 2003; Locatello et al. 2006; Pitcher et al. 2007), the results of our experiment indicate that female fecundity is unlikely to be influenced by female preferences for highly ornamented males. Thus, even if fecundity benefits may have initially reinforced the coevolution of female choice and male ornaments in this species, it seems unlikely that these benefits actually maintain such preferences. Our sample size was large, and the lack of correlation between male attractiveness and female fecundity suggests that, at least in this captive population, any fecundity benefits derived from mating with attractive males are likely to be small. Other benefits, such as the reduced probability of contracting parasites or disease (e.g., Kennedy et al. 1987; Grether et al. 2004), increased offspring performance (e.g., Reynolds and Gross 1992; Evans, Kelley et al. 2004), and enhanced reproductive performance of male offspring (e.g., Houde 1992; Brooks 2000), are more likely to account for the maintenance of female preference for showy males in guppies. Although a positive correlation between male attractiveness and ejaculate quality has often been interpreted as evidence supporting the PLFH, the results of the present study highlight the importance of experimentally investigating fecundity benefits associated to female preference for attractive males (see Wagner and Harper 2003; Malo, Garde, et al. 2005) to find firm support for this theory.

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