

Sire attractiveness influences offspring performance in guppies

Jonathan P. Evans^{1*}, Jennifer L. Kelley¹, Angelo Bisazza², Elisabetta Finazzo³ and Andrea Pilastro³

¹School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW, 2052, Australia

²Dipartimento di Psicologia Generale, Università di Padova, via Venezia 8, I-35131 Padova, Italy

³Dipartimento di Biologia, Università di Padova, via U. Bassi 58/B, I-35131 Padova, Italy

According to the good-genes hypothesis, females choose among males to ensure the inheritance of superior paternal genes by their offspring. Despite increasing support for this prediction, in some cases differential (non-genetic) maternal effects may obscure or amplify the relationship between paternal attractiveness and offspring quality. Artificial insemination controls such effects because it uncouples mate choice from copulation, therefore denying females the opportunity of assessing male attractiveness. We adopted this technique in the live-bearing fish *Poecilia reticulata* and examined whether paternal coloration was associated with the behavioural performance of newborn offspring. Sexually receptive virgin females were inseminated with sperm taken individually from donor males that exhibited high variation in the area of orange pigmentation, a trait known to influence female choice in the study population. Our analysis of offspring performance focused on the anti-predator behaviour of newborn fish, including schooling by sibling pairs, the response (swimming speed) of these fishes to a simulated avian predator, and the time taken for a naive investigator to capture the offspring. Although we found no significant effect of sire coloration on either schooling or swimming speed, our analysis revealed a significant positive association between sire coloration and the ability of newborn offspring to evade capture. This finding supports the view that at least one aspect of anti-predator behaviour in newborn offspring is influenced by sire genotype, which in turn is revealed by the expression of secondary sexual traits.

Keywords: sexual selection; differential allocation; genetic benefits; sperm competition; guppy; indirect benefits

1. INTRODUCTION

A major controversy in sexual selection is whether female mate choice evolves as a consequence of an underlying preference for genetically superior males (see, for example, Rowe & Houle 1996; Kirkpatrick & Barton 1997; Møller & Alatalo 1999; Kokko *et al.* 2003). Preferences for males of high genetic quality and the inheritance of viability genes by the progeny of choosy females form the basis of 'good-genes' models of sexual selection (Andersson 1994). According to these models, male ornaments reliably indicate viability because only males of high genetic quality are able to develop and maintain elaborate traits (Zahavi 1975). Consequently, females are expected to obtain indirect (genetic) benefits when mating with attractive males, resulting in the production of high-quality offspring. In support of these predictions, several studies have reported a positive correlation between paternal attractiveness and offspring fitness components (e.g. Reynolds & Gross 1992; Norris 1993; Møller 1994; Petrie 1994; Iyengar & Eisner 1999).

The possibility that females differentially invest resources at reproduction according to the attractiveness of their current mate has important implications for good-genes explanations of mate choice. For example, the differential allocation hypothesis (Burley 1986) predicts that females

should preferentially allocate resources to breeding events when mating with attractive males, and that energy invested in the current mating is traded against that available for future matings (reviewed by Sheldon 2000). Indeed, several recent studies have revealed that females tailor their investment in eggs or offspring according to male attractiveness (Gil *et al.* 1999; Cunningham & Russell 2000; Saino *et al.* 2002). Failure to control differential maternal effects when testing the good-genes hypothesis may therefore result in non-genetic relationships between sire attractiveness and offspring fitness. Artificial insemination provides a potential method for controlling such effects because it uncouples mate choice from copulation, thus preventing the direct assessment of male attractiveness by females. For example, *in vitro* fertilization techniques for external fertilizers (see Welch *et al.* 1998; Barber *et al.* 2001; Sheldon *et al.* 2003) and *in vivo* artificial insemination for internal fertilizers (Parker 2003) have facilitated powerful tests of the good-genes hypothesis in recent studies. Here, we also use artificial insemination to test whether early offspring performance in juvenile guppies (*Poecilia reticulata*) is related to paternal attractiveness.

(a) *The study species*

The guppy is a model system for studying the evolution of male ornaments and female mating preferences (Houde 1997). Like other poeciliids, guppies are live-bearers with

* Author and address for correspondence (jon.evans@unsw.edu.au).

internal fertilization (Constantz 1989). Several studies indicate that females base their mating preferences on the proportion of the male's body covered with carotenoid (orange) pigmentation (Brooks & Endler 2001*b*; and references in Houde 1997; but see Houde & Endler 1990; Reynolds & Gross 1992; Endler & Houde 1995 for exceptions). The area of orange pigmentation in these colour patterns, and consequently male attractiveness, is highly heritable and due in part to Y-linked genes that influence the presence of particular spots (Winge 1927; Houde 1992; Brooks 2000; Brooks & Endler 2001*a*). However, X-linked and autosomal genes also contribute substantial additive genetic variation to the area of orange (Brooks & Endler 2001*a*). Importantly, in the context of its value as a potential indicator of male quality, orange area provides information about condition (van Oosterhout *et al.* 2003) and therefore may function as an indicator of male genetic quality (Reinhold 2002).

Multiple mating by females, which is common in guppies (Constantz 1984; Kelly *et al.* 1999; Evans & Magurran 2000; Pitcher *et al.* 2003), extends the opportunity for sexual selection beyond copulation (Jennions & Petrie 2000; Fedorka & Mousseau 2002). Indeed, studies indicate that male phenotype covaries with sperm load (Matthews *et al.* 1997; Pilastro & Bisazza 1999; Pitcher & Evans 2001) and that males with preferred phenotypes are likely to be favoured during post-copulatory sexual selection (Pilastro & Bisazza 1999; Evans & Magurran 2001; Pilastro *et al.* 2002; Evans *et al.* 2003; see also Pitcher *et al.* 2003). Evans and Magurran (2000) found that offspring from multiply mated female guppies exhibited enhanced schooling abilities and better developed anti-predator escape responses than their singly sired counterparts. They speculated, though could not confirm, that the differences between the progeny of singly and multiply mated female guppies arose because genetically superior males were favoured during sperm competition (e.g. Hosken *et al.* 2003). However, other explanations for their findings include the possibility that females influenced offspring fitness via non-genetic differential maternal effects (e.g. Kozielska *et al.* 2003), or that the benefits of polyandry were mediated by male-female genetic compatibility. Guppies are ovoviparous and therefore the scope for differential investment via nutrient donations from mother to developing offspring is likely to be limited (Thibault & Schultz 1978). Nevertheless, ovoviparous females may potentially influence offspring fitness by investing differentially in ova before fertilization (Heath & Blouw 1998), selectively fertilizing particular eggs (Johnston *et al.* 1979), or by prolonging uterine retention of embryos (see, for example, Shine 2002; Shine & Olsson 2003).

We use artificial insemination to control these potential maternal effects and examine the influence of sire coloration on the behavioural performance of newborn offspring. Our analyses of offspring performance focused on schooling behaviour, the response by offspring to a simulated predation threat and the ability of offspring to evade capture. These behavioural traits are likely to provide good measures of an individual's ability to evade predators (Magurran 1990; Fuiman & Magurran 1994) and are therefore potentially important (early) components of fitness, especially in populations characterized by high levels of predation.

2. MATERIAL AND METHODS

(a) *Study population and its maintenance*

The guppies used in this experiment were second-third generation descendants of wild-caught fish from the Tacarigua River in Trinidad (national grid reference PS 787 804). This is a high-predation site where guppies coexist with several predator species (Magurran & Seghers 1994), including the pike cichlid *Crenicichla alta*, which preys on all size classes of guppy (Mattingly & Butler 1994). Female guppies store sperm for several months (Constantz 1984); we therefore used virgin females for the artificial insemination trials (see § 2c) to avoid the possibility that fertilizations were due to prior males. Females were reared in single-sex tanks and males were reared in mixed-sex aquaria (*ca.* 1:1 sex ratio) until required. Water temperature was maintained between 25 °C and 27 °C and illumination was set on a 12 L:12 D cycle (Philips TLD 36W fluorescent lamps). All fishes were fed a mixed diet of brine shrimp nauplii and commercially prepared flake food.

(b) *Selection of males and measurement of colour patterns*

We have previously demonstrated that females from the study population prefer males with larger total combined areas of orange, yellow and red coloration, irrespective of variation in body size (Evans *et al.* 2004). To estimate male attractiveness we therefore calculated the total area of these colour spots (hereafter collectively termed 'orange') in the colour patterns. Males were aged between five and six months and exhibited a range of colour phenotypes. We confirmed that each male was fully sexually mature, as evident by the maturational state of the gonopodium's apical hood (see Houde 1997 for details), before the extraction of sperm for artificial insemination. To measure orange pigmentation (done immediately after artificial insemination), individual test males were anaesthetized in a water bath containing MS222 and photographed using a digital camera. Image analysis software (UTHSCSA Image Tool: <http://www.ddsdx.uthscsa.edu/dig/download.html>) was used to measure male standard length and measure the total area of the body covered with orange spots (for details see Pitcher & Evans 2001).

(c) *Artificial insemination*

Artificial insemination (see Clark 1950; Grove 1980; Lodi 1981; Evans *et al.* 2003) was used to inseminate sexually naive, six-month-old virgin female guppies. Females were approximately matched for size among replicates (mean standard length \pm s.d. = 24.4 \pm 1.70). Sperm were manually stripped from individual males following Matthews *et al.* (1997). In guppies, sperm are packaged in bundles (spermatozeugmata), each containing approximately 27 000 individual sperm cells (authors' own estimates, and see Billard (1969)). Previous work on this population has confirmed that among-male variation in the number of sperm cells per bundle does not exceed that within males, and that the number of sperm per bundle does not correlate with either body size or the extent of body coloration (Evans *et al.* 2003). Thus, it was possible to control the number of sperm used for each insemination by counting the number of sperm bundles, which we based on the number of sperm inseminated during natural copulations in this population (*ca.* 0.5×10^6 cells = 18–20 bundles) (see Pilastro *et al.* 2004). For each insemination, a virgin female was anaesthetized in a water bath containing a mild dose of MS222 and placed in a polystyrene 'cradle' with her genital pore exposed. A machine-pulled plastic micropipette was used to inseminate 18 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 fresh water, gravel, aquatic weed and an airstone), where they remained isolated until they produced their first broods. Each male was used only once (final sample sizes given in § 2e). There was no significant correlation between male colour phenotype (proportion and absolute area of orange pigmentation) and female size (p -values greater than 0.26).

(d) Offspring performance measures

The schooling and escape responses by juvenile guppies were assessed *ca.* 24 h after their birth. To eliminate the possibility of observer bias during the offspring behaviour trials, and subsequently during the video playback analyses (see below), all broods were tested 'blind' of experimental treatment (each brood was given a unique code by an investigator who was not involved in the offspring performance trials). Our basic protocol was to measure the duration that pairs of offspring spent schooling over two consecutive 5 min periods, intersected by a simulated predation threat in the form of an avian predator. This allowed us to measure schooling by pairs of offspring before and after a simulated threat (e.g. Evans *et al.* 2002) and assess the response by offspring to that threat. Additionally, 'capture time'—the time taken to catch both offspring sequentially with a hand net (for details see Evans & Magurran 2000)—was used to assess the escape abilities of the schooling fish (see Birkhead *et al.* (1998) for a similar protocol in birds). Our preliminary investigations performed before this experiment confirmed that the simulated bird flight elicited a strong anti-predator response in the newborn fish (see also Seghers 1974), characterized by sudden bursts of 'darts' and erratic swimming as the bird model 'flew' over the schooling arena. This response usually persisted for *ca.* 2–3 s before the fish settled again.

The schooling arena was constructed from a circular disc of white plastic sheeting (35 cm in diameter) marked with 2 cm gridlines. The walls of the arena (6.5 cm high) were attached to the circular base with silicon sealant, which was placed inside a circular plastic tray (44 cm in diameter) for support. Illumination was provided by two 60 W bulbs positioned 40 cm above the arena. Before each schooling trial, the test arena was filled with conditioned water (depth of 2.5 cm) and labelled with a code that subsequently identified the brood and the individuals being tested. At the start of each trial, a pair of juveniles was caught using a hand-net and transferred via a small tub to the test arena where they were allowed to acclimate for 10 min before the schooling trial commenced.

Following the settlement period the schooling behaviour of both fish was video recorded until 5 min had elapsed, at which point the observer pulled the model bird over the arena using a system of pulleys operated from behind a blind. The model bird (10.5 cm in length, wingspan of 12.5 cm, constructed of black plastic) was suspended on a length of monofilament line 26 cm above the arena. The line was held taut by two 43 cm long vertical poles clamped to the desk. A second length of monofilament line that was tied to the front of the bird allowed the observer to control the movement of the bird from behind the blind. Two plastic platforms were constructed at either end of the arena so that the fish were unable to see the model bird when it was not in use. Following the simulated bird flight a further 5 min of schooling behaviour was recorded before the offspring were captured with a net (see previous paragraph) and photographed using a digital camera (each photograph included a section of ruler for calibration). Offspring body size (standard length) was measured to within 0.1 mm from these photographs.

The water in the arena was changed between consecutive trials (both within and between broods) to control for the possibility that chemical cues released by frightened fishes affected the behaviour of fishes taking part in the subsequent trials (Wisenden *et al.* 1995; Mirza & Chivers 2002). For each family, schooling was expressed as a mean value for all sibling pairs (where broods contained uneven numbers of offspring, one individual was therefore not tested in the schooling trials).

The schooling and responses by offspring to the simulated predation threat were analysed from the videos using ZOOM PLAYER software (available online at: http://www.inmatrix.com/files/zoomplayer_download.html). Our criterion for 'schooling' was that the two fish should be within 4 cm of each other and swimming and turning in synchrony. In practice, fish were either schooling tightly (less than 2 cm apart) or were widely separated in the arena (more than 10 cm apart). In each trial, the percentage of time that the two fish spent schooling was estimated before and after the simulated bird flight. The response by each offspring to the bird flight was measured from video footage taken during the trials. Specifically, we viewed the section of video during which the bird moved over the arena frame by frame (at 4 frames s^{-1}) and measured the distance travelled by each offspring during this time (using PESCI2 software, developed at the University of Padova, Italy). After estimating the total distance travelled by each individual offspring (using the gridlines on the arena) and the number of frames it took for the bird to pass (at 0.25 s frame $^{-1}$), it was possible to calculate the mean speed (centimetres per second) travelled by each pair of babies during the simulated bird flight.

(e) Statistical analyses

For logistical reasons the artificial inseminations were performed in two blocks: winter 2003 (January and February) and summer 2003 (July and August). The timing of insemination had an effect on brood development times (mean \pm s.d., winter: 38.8 ± 1.96 days, summer: 30.7 ± 2.14 ; $t_{50} = 2.77$, $p = 0.008$), offspring size (mm) (mean \pm s.d., winter: 3.45 ± 0.20 ; summer: 3.30 ± 0.13 ; $t_{48} = 3.19$, $p = 0.003$) and capture times (s) (mean \pm s.d., winter: 9.74 ± 3.56 ; summer: 7.45 ± 2.93 ; $t_{43} = 2.31$, $p = 0.025$). We suspect that these seasonal effects were caused by minor temperature differences between the winter and summer trials (although differences did not exceed 2 °C; temperatures always remained between 25 °C and 27 °C), which may have influenced aspects of brood development (see Dzikowski *et al.* 2001). We noted that the duration of brood development was weakly positively associated with mean offspring size ($r = 0.20$, $n = 50$, $p = 0.16$) and more strongly with median capture times ($r = 0.34$, $n = 45$, $p = 0.02$), suggesting a functional relationship between gestation time and these traits. By contrast, the mean number of offspring produced by females was unaffected by the timing of insemination ($t_{50} = 0.30$, $p = 0.77$). To control for possible seasonal effects on offspring traits we entered 'insemination group' as a factor in a multivariate analysis of variance (MANOVA), in which offspring traits (schooling behaviour, capture times, offspring body size and swimming speed) were entered simultaneously as dependent variables, and sire coloration (proportion of orange area, following arcsine square-root transformation) was entered as a covariate. We also tested whether sire body size (SL) was significantly associated with any of the analysed offspring traits, by entering sire SL as a covariate in the MANOVA. We tested for significant effects of the interactions between predictors on offspring traits. Because none of the interactions was significant, they were not included in the final models.

Offspring size was measured for all individual juveniles and the mean value for each brood was used in the MANOVA. The mean standard length (SL) of offspring was negatively correlated with brood size across broods (Pearson's correlation, $r = -0.41$, $n = 50$, $p = 0.003$); we therefore used the residuals of offspring SL (mm) on brood size as an index of offspring size (linear regression: $Y = -0.015X + 3.516$). However, the results from our analyses remained unchanged when we entered mean SL rather than the residuals of SL on brood size in the model. Our final analyses included $n = 52$ families (each sired by a different donor male), from which we obtained size data from $n = 50$ broods (two photographs were unusable). Schooling was measured for pairs of juveniles and therefore where females produced a single offspring ($n = 4$) we were unable to measure this behavioural trait. Sample sizes therefore sometimes vary between analyses. In some cases capture time and swimming speed data exhibited high variation within broods (s.d. greater than the mean); we therefore used the median value for these traits for each brood. Schooling and colour pattern data were proportional and therefore arcsine square-root transformed before statistical analyses. All probabilities are two-tailed and the analyses were performed on SPSS v. 11.5.

3. RESULTS

Schooling times did not differ significantly before and after the simulated bird flight (paired t -test, $t_{45} = 0.75$, $p = 0.46$). When analysing schooling data we therefore used the mean value per brood for the entire 10 min schooling trials. The overall effect of male coloration on offspring traits was significant after taking seasonal effects into account (MANOVA: proportion of orange, $p = 0.019$; insemination group $p = 0.003$; table 1). Tests of the between-subject effects indicated that sire coloration was significantly and positively correlated with capture time ($F_{1,44} = 14.25$, $p < 0.001$; figure 1). None of the other offspring traits was correlated with orange area in these tests (residual SL: $F_{1,44} = 0.34$, $p = 0.57$; schooling: $F_{1,44} = 0.93$, $p = 0.34$; swimming speed: $F_{1,44} = 0.07$, $p = 0.79$). When sire SL was also included in the model as a covariate, similar results were obtained: the area of orange coloration in males was significantly and positively associated with capture time ($F_{1,44} = 10.38$, $p = 0.002$), whereas none of the other offspring traits was correlated with orange area (residual SL: $F_{1,44} = 0.35$, $p = 0.56$; schooling: $F_{1,44} = 0.15$, $p = 0.70$; swimming speed: $F_{1,44} = 0.13$, $p = 0.72$). By contrast, sire SL was not significantly associated with any of the offspring traits (all $F_{1,44} < 1.63$, all $p > 0.21$).

We tested whether offspring capture time was influenced by other paternal or maternal traits using a stepwise multiple regression, in which capture time was the dependent variable and male coloration (proportion of orange area, following arcsine square-root transformation), male SL, female SL, brood development time and brood size were the independent variables. Initially, we analysed data from the two insemination groups separately to account for differences in capture times between the two groups (see § 2e). The final models selected the proportion of orange area as a predictor in both groups (winter group: $F_{1,23} = 4.46$, $r = 0.41$, $p = 0.046$; summer group: $F_{1,19} = 12.37$, $r = 0.64$, $p = 0.002$; figure 1). Pooling the data from the two groups gave similar results, with the proportion of orange and brood development time significantly

predicting capture time (final model: $F_{1,44} = 6.78$, $r^2 = 0.24$, $p = 0.003$; proportion of orange: $t = 2.51$, $r_{\text{partial}} = 0.34$, $p = 0.016$; brood development time: $t = 2.60$, $r_{\text{partial}} = 0.35$, $p = 0.013$). None of the other male or female traits was selected in these models. Furthermore, we found that sire body size, which has been shown to exhibit significant father-son heritability (Reynolds & Gross 1992; Brooks & Endler 2001a) was not significantly correlated with the mean size of newborn offspring ($r = -0.15$, $n = 50$, $p = 0.31$ and $r = -0.14$, $n = 50$, $p = 0.32$, respectively, for mean SL and their residuals).

Additionally, our results suggest that schooling, although not associated with male coloration in our study, is likely to influence the susceptibility of juveniles to predation. We detected a significant positive correlation between mean schooling times for pairs of fish and swimming speed during the simulated bird flight ($r = 0.35$, $n = 46$, $p = 0.018$). This finding indicates that offspring pairs that schooled more exhibited enhanced escape responses (see also Evans & Magurran 2000). Schooling and swimming speed were also significantly positively correlated with residual and absolute measures of offspring size (e.g. for absolute SL $r = 0.40$, $n = 46$, $p = 0.006$; $r = 0.48$, $n = 46$, $p = 0.001$, respectively). However, offspring size was not significantly correlated with capture times ($r = 0.13$, $n = 45$, $p = 0.41$). This was also confirmed by a MANOVA in which schooling time, swimming speed during the simulated bird flight and capture time were the dependent variables, insemination group the factor, and male coloration and residual offspring size the covariates. Male coloration remained significantly and positively associated with capture time ($F_{1,44} = 13.62$, $p = 0.001$), whereas none of the other offspring traits was correlated with male coloration (schooling time: $F_{1,44} = 0.64$, $p = 0.43$; swimming speed: $F_{1,44} = 0.26$, $p = 0.61$). By contrast, residual offspring SL was significantly associated with schooling time ($F_{1,44} = 5.25$, $p = 0.027$) and swimming speed ($F_{1,44} = 6.25$, $p = 0.017$), but not with capture time ($F_{1,44} = 0.12$, $p = 0.74$). Similar results were obtained when actual, rather than residual offspring SL was used as a covariate (data not shown).

4. DISCUSSION

Our findings indicate that variation in one measure of offspring performance can be predicted by sire attractiveness, when controlling for the potentially confounding effect of differential maternal investment through the use of artificial insemination. Recent studies that have adopted artificial fertilization techniques have provided some of the most compelling evidence to date for the good-genes hypothesis (Welch *et al.* 1998; Barber *et al.* 2001; Parker 2003; Sheldon *et al.* 2003). Among these, Sheldon *et al.* (2003) reported that under certain predation conditions sire coloration (and therefore presumably male attractiveness) influenced the susceptibility of juvenile moorfrogs to predation by water beetles. Our results for guppies similarly suggest that paternal genes influence the susceptibility of juveniles to capture (see also Sandvik *et al.* 2000).

We found that paternal phenotype did not predict variation in either swimming speed (in relation to the simulated threat) or schooling behaviour, both of which are likely to influence the susceptibility of newborn offspring to predators (Fuiman & Magurran 1994). However, both of

Table 1. Results of a MANOVA in which four offspring fitness traits (see § 2) are the dependent variables, insemination group is the factor and sire attractiveness (proportion of the body covered by orange) is the covariate. (The multivariate test indicates the overall significance, whereas the between-subjects effects indicate the effect of the two independent variables on each offspring trait (n.s. when $p > 0.05$; d.f., degrees of freedom).)

(a) multivariate test						
effect	<i>F</i>	hypothesis d.f.	error d.f.	<i>p</i>		
intercept	94.829	4	39	0.000		
proportion of orange	3.358	4	39	0.019		
insemination group	4.903	4	39	0.003		
(b) between-subjects effects						
source	dependent variable	type III sum of squares	d.f.	mean square	<i>F</i>	<i>p</i>
corrected model	offspring SL	0.20	2	0.10	3.84	0.029
	schooling	0.01	2	0.01	0.49	n.s.
	speed	105.97	2	52.98	2.08	n.s.
intercept	capture time	176.58	2	88.29	10.63	< 0.001
	offspring SL	0.00	1	0.00	0.09	n.s.
	schooling	4.06	1	4.06	304.01	< 0.001
proportion of orange	speed	600.75	1	600.75	23.58	< 0.001
	capture time	55.31	1	55.31	6.66	0.013
	offspring SL	0.01	1	0.01	0.34	n.s.
insemination group	schooling	0.01	1	0.01	0.93	n.s.
	speed	1.88	1	1.88	0.07	n.s.
	capture time	118.31	1	118.31	14.25	< 0.001
error	offspring SL	0.15	1	0.15	5.77	0.021
	schooling	0.00	1	0.00	0.01	n.s.
	speed	102.65	1	102.65	4.03	n.s.
total	capture time	112.33	1	112.33	13.53	0.001
	offspring SL	1.11	42	0.03	—	—
	schooling	0.56	42	0.01	—	—
corrected total	speed	1069.96	42	25.48	—	—
	capture time	348.70	42	8.30	—	—
	offspring SL	1.31	45	—	—	—
corrected total	schooling	38.98	45	—	—	—
	speed	8377.61	45	—	—	—
	capture time	3948.75	45	—	—	—
corrected total	offspring SL	1.31	44	—	—	—
	schooling	0.57	44	—	—	—
	speed	1175.93	44	—	—	—
corrected total	capture time	525.28	44	—	—	—

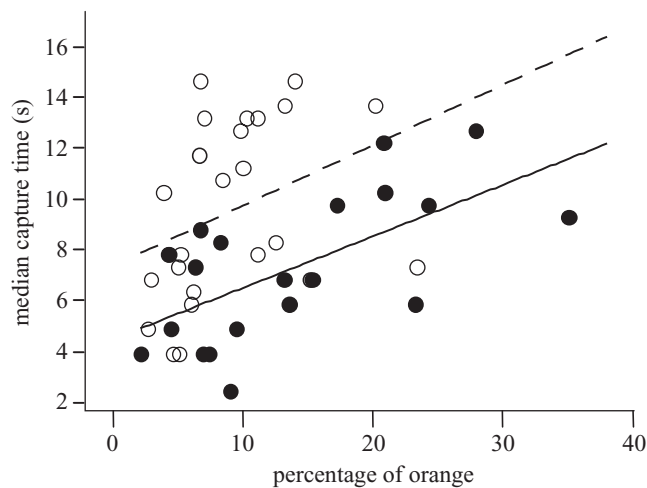


Figure 1. The time to capture newborn offspring as a function of sire coloration (percentage of body area covered by orange, red and yellow spots). Filled circles represent values from winter inseminations (solid line: least-square regression line); open circles are for summer inseminations (dotted line: least-square regression line; see text for details).

these variables were positively correlated with offspring size, which is more likely to be influenced by maternal than paternal effects in newborn offspring (see Heath & Blouw 1998 and references therein). Indeed, paternal phenotype did not significantly predict offspring size, and therefore the lack of association between sire coloration and the correlates of offspring size is not surprising. Our results do, however, differ from those presented in an earlier study, which revealed a significant positive association between offspring schooling and capture times (Evans & Magurran 2000). However, Evans and Magurran observed the behaviour of offspring following natural (solicited) copulations and therefore could not rule out the possibility that adaptive differential maternal effects (in addition to possible sire effects) influenced offspring performance.

An increasing number of studies have investigated the influence of sire attractiveness on components of offspring fitness in guppies. For example, in a laboratory study Brooks (2000) reported a negative genetic correlation between sire attractiveness and the survival of male offspring, possibly owing to the genetic 'hitch-hiking' of deleterious genes with attractiveness genes on the Y sex chromosome. Reynolds & Gross (1992), on the other hand, reported a positive association between sire attractiveness and the success (growth rates and fecundity) of both male and female offspring. Reynolds and Gross found that females from their study population did not use variation in the area of orange pigmentation as a mate-choice cue, but instead based their mating preferences on male body size (which in turn was weakly correlated with courtship display rates and more strongly with the area of iridescent coloration). Hence, the positive relationship between sire body size (which showed significant heritability) and both growth rate and fecundity was easily explicable in their study (Reynolds & Gross 1992). Similarly, our findings indicate that sire ornamentation can have a positive influence on one aspect of offspring performance, although this trait may in turn show positive or negative correlations with other fitness-related components

(van Noordwijk & de Jong 1986; Hunt *et al.* 2004), including survival later in life (e.g. Brooks 2000).

Most studies identifying orange pigmentation as a target of female choice (Endler & Houde 1995; Houde 1997; Brooks & Endler 2001*b*) have been performed on upstream populations (but see Godin & Briggs 1996; Houde & Hankes 1997), where levels of predation are relatively low (Houde 1997). Thus, the extent to which the 'benefit' of evading capture revealed by our study on high-predation fish occurs (and indeed is relevant) to fish from low-predation populations remains to be tested. However, predation on juvenile fish is likely to be a cause of mortality in both low- and high-predation environments. Indeed, most so-called 'low-predation' streams in Trinidad are characterized by the presence of the killifish *Rivulus hartii* (Magurran *et al.* 1995), to which juvenile guppies are especially vulnerable (Seghers 1973). Thus, although in upstream populations the mean level of predation experienced by fish over their lifetimes may be relatively low, the predation they do experience is likely to be skewed towards early life-history stages. Hence, by focusing on behavioural traits in newborn offspring, we suggest that our findings may be generally applicable among populations that differ in overall predation intensity. Moreover, traits that are likely to be correlated with escape behaviour, for example sustained swimming ability, which has been shown to be correlated with sexually selected traits (Nicoletto 1991, 1993), are likely to be advantageous in all environments and at all life-history stages.

Our study was designed to test whether sire coloration correlates with offspring performance, and thus whether mate choice can result in indirect fitness benefits for choosing females. The use of artificial fertilization controlled the potentially confounding effect of differential maternal investment based on the females' perception of male attractiveness. In principle, however, cues other than the appearance of males, such as ejaculate compounds, may stimulate females to differentially invest in offspring. If females can discriminate among males on this basis, we can envisage two potential forms of differential investment available to female guppies. First, they may manipulate brood development times so that embryos from preferred males are allowed to develop for longer. Such adaptive maternal effects have been shown to influence the locomotor skills of offspring in other viviparous species (Shine & Olsson 2003). However, we found no significant correlation between brood development time and male coloration ($r = -0.07$, $n = 52$, $p = 0.62$). Second, females may use the sperm from preferred males to fertilize larger, or more eggs. Again, correlations between sire coloration and both the mean size and number of offspring were not significant in our study (offspring size: $r = -0.05$, $n = 50$, $p = 0.73$; brood size: $r = -0.19$, $n = 52$, $p = 0.18$). Similarly, sire SL was not significantly correlated with brood size ($r = 0.05$, $n = 52$, $p = 0.75$). Thus, although we cannot rule out the possibility of ejaculate-induced maternal investment (see also Parker 2003), our results are more consistent with the idea that variation in offspring capture times is explained by genes correlated with sire attractiveness.

The authors thank R. Brooks for discussion and comments, T. Pitcher and an anonymous referee for comments on the

manuscript, and Clelia Gasparini for her help with the experiments. This work was supported by fellowships to J.P.E. from the European Union and subsequently the Australian Research Council, and grants to A.P. and A.B. from the Italian Research Ministry (Cofin2001 and Cofin2003) and Institutional Research Grants from the University of Padova (ex60% 2000-3). The research conformed with the relevant Italian laws governing the care of animals in research (D.L. 116/27-01-92, C.M.S. 8/22-04-94) and was authorized by the Istituto Superiore di Sanita (National Health Institute).

REFERENCES

- Andersson, M. 1994 *Sexual selection*. Princeton University Press.
- Barber, I., Arnott, S. A., Braithwaite, V. A., Andrew, J. & Huntingford, F. A. 2001 Indirect fitness consequences of mate choice in sticklebacks: offspring of brighter males grow slowly but resist parasitic infections. *Proc. R. Soc. Lond. B* **268**, 71–76. (doi:10.1098/rspb.2000.1331)
- Billard, R. 1969 La sperm spermatogenese de *Poecilia reticulata*. *Ann. Biol. Anim. Biochem. Biophys.* **9**, 251–271.
- Birkhead, T. R., Fletcher, F. & Pellatt, E. J. 1998 Sexual selection in the zebra finch *Taeniopygia guttata*: condition, sex traits and immune capacity. *Behav. Ecol. Sociobiol.* **44**, 179–191.
- Brooks, R. 2000 Negative genetic correlation between sexual attractiveness and survival. *Nature* **406**, 67–70.
- Brooks, R. & Endler, J. A. 2001a Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution* **55**, 1002–1015.
- Brooks, R. & Endler, J. A. 2001b Female guppies agree to differ: phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution* **55**, 1644–1655.
- Burley, N. 1986 Sexual selection for aesthetic traits in species with biparental care. *Am. Nat.* **127**, 415–445.
- Clark, E. 1950 A method for artificial insemination in viviparous fishes. *Science* **112**, 722–723.
- Constantz, G. D. 1984 Sperm competition in poeciliid fishes. In *Sperm competition and the evolution of animal mating systems* (ed. R. L. Smith), pp. 465–485. Orlando, FL: Academic Press.
- Constantz, G. D. 1989 Reproductive biology of poeciliid fishes. In *Ecology and evolution of livebearing fishes* (ed. G. K. Meffe & F. F. Snelson Jr), pp. 33–50. Englewood Cliffs, NJ: Prentice-Hall.
- Cunningham, E. J. A. & Russell, A. F. 2000 Egg investment is influenced by male attractiveness in the mallard. *Nature* **404**, 74–77.
- Dzikowski, R., Hulata, G., Karplus, I. & Harpaz, S. 2001 Effect of temperature and dietary L-carnitine supplementation on reproductive performance of female guppy (*Poecilia reticulata*). *Aquaculture* **199**, 323–332.
- Endler, J. A. & Houde, A. E. 1995 Geographical variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* **49**, 456–468.
- Evans, J. P. & Magurran, A. E. 2000 Multiple benefits of multiple mating in guppies. *Proc. Natl Acad. Sci. USA* **97**, 10 074–10 076.
- Evans, J. P. & Magurran, A. E. 2001 Patterns of sperm precedence and predictors of paternity in the Trinidadian guppy. *Proc. R. Soc. Lond. B* **268**, 719–724. (doi:10.1098/rspb.2000.1577)
- Evans, J. P., Kelley, J. L., Ramnarine, I. W. & Pilastro, A. 2002 Female behaviour mediates male courtship under predation risk in the guppy. *Behav. Ecol. Sociobiol.* **52**, 496–502.
- Evans, J. P., Zane, L., Francescato, S. & Pilastro, A. 2003 Directional postcopulatory sexual selection revealed by artificial insemination. *Nature* **421**, 360–363.
- Evans, J. P., Bisazza, A. & Pilastro, A. 2004 Female mating preferences for colourful males in a high predation population of guppies. *J. Fish Biol.* (In the press.)
- Fedorka, K. M. & Mousseau, T. A. 2002 Material and genetic benefits of female multiple mating and polyandry. *Anim. Behav.* **64**, 361–367.
- Fuiman, L. A. & Magurran, A. E. 1994 Development of predator defences in fishes. *Rev. Fish. Biol. Fish.* **4**, 145–183.
- Gil, D., Graves, J., Hazon, N. & Wells, A. 1999 Male attractiveness and differential testosterone investment in zebra finch eggs. *Science* **286**, 126–128.
- Godin, J. G. J. & Briggs, S. E. 1996 Female mate choice under predation risk in the guppy. *Anim. Behav.* **51**, 117–130.
- Grove, B. D. 1980 An analysis of intraovarian sperm interactions in the guppy, *Poecilia reticulata*. In *Faculty of graduate studies*. Vancouver: University of British Columbia.
- Heath, D. D. & Blouw, D. M. 1998 Are maternal effects in fish adaptive or merely physiological side effects? In *Maternal effects as adaptations* (ed. T. A. Mousseau & C. W. Fox), pp. 178–201. Oxford University Press.
- Hosken, D. J., Garner, T. W. J., Tregenza, T., Wedell, N. & Ward, P. I. 2003 Superior sperm competitors sire higher-quality young. *Proc. R. Soc. Lond. B* **270**, 1933–1938. (doi:10.1098/rspb.2003.2443)
- 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.
- Houde, A. E. 1997 *Sex, color, and mate choice in guppies*. Princeton University Press.
- Houde, A. E. & Endler, J. A. 1990 Correlated evolution of female mating preferences and male color patterns in the guppy, *Poecilia reticulata*. *Science* **248**, 1405–1408.
- Houde, A. E. & Hankes, M. A. 1997 Evolutionary mismatch of mating preferences and male colour patterns in guppies. *Anim. Behav.* **53**, 343–351.
- Hunt, J., Bussière, L. F., Jennions, M. D. & Brooks, R. 2004 What is genetic quality? *Trends Ecol. Evol.* **19**, 329–333.
- Iyengar, V. K. & Eisner, T. 1999 Female choice increases offspring fitness in an arctiid moth (*Utetheisa ornatrix*). *Proc. Natl. Acad. Sci. USA* **96**, 15 013–15 016.
- Jennions, M. D. & Petrie, M. 2000 Why do females mate multiply? A review of the genetic benefits. *Biol. Rev.* **75**, 21–64.
- Johnston, N. P., Warner, D. H. & Arscott, G. H. 1979 Effect of egg position at insemination on selective fertilization of turkeys and chickens. *J. Poultry Sci.* **58**, 1071.
- Kelly, C. D., Godin, J.-G. J. & Wright, J. M. 1999 Geographic variation in multiple paternity within natural populations of the guppy (*Poecilia reticulata*). *Proc. R. Soc. Lond. B* **266**, 2403–2408. (doi:10.1098/rspb.1999.0308)
- Kirkpatrick, M. & Barton, N. H. 1997 The strength of indirect selection on female mating preferences. *Proc. Natl Acad. Sci. USA* **94**, 1282–1286.
- Kokko, H., Brooks, R., Jennions, M. D. & Morley, J. 2003 The evolution of mate choice and mating biases. *Proc. R. Soc. Lond. B* **270**, 653–664. (doi:10.1098/rspb.2002.2235)
- Kozielska, M., Krzeminska, A. & Radwan, J. 2003 Good genes and the maternal effects of polyandry on offspring reproductive success in the bulb mite. *Proc. R. Soc. Lond. B* **271**, 165–170. (doi:10.1098/rspb.2003.2585)
- Lodi, E. 1981 Competition between pall and normal bearing spermatozoa of *Poecilia reticulata* (Pisces: Poeciliidae). *Copeia* **1981**, 624–629.
- Magurran, A. E. 1990 The adaptive significance of schooling as an anti-predator defence in fish. *Ann. Zool. Fenn.* **27**, 51–66.
- Magurran, A. E. & Seghers, B. H. 1994 Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. *Proc. R. Soc. Lond. B* **255**, 31–36.

- Magurran, A. E., Seghers, B. H., Shaw, P. W. & Carvalho, G. R. 1995 The behavioural diversity and evolution of guppy, *Poecilia reticulata*, populations in Trinidad. *Adv. Stud. Anim. Behav.* **24**, 155–202.
- Matthews, I. M., Evans, J. P. & Magurran, A. E. 1997 Male display rate reveals ejaculate characteristics in the Trinidadian guppy, *Poecilia reticulata*. *Proc. R. Soc. Lond. B* **264**, 695–700. (doi:10.1098/rspb.1997.0099)
- Mattingly, H. T. & Butler, M. J. 1994 Laboratory predation on the Trinidadian guppy: implications for the size-selective predation hypothesis and guppy life history evolution. *Oikos* **69**, 54–64.
- Mirza, R. S. & Chivers, D. P. 2002 Behavioural responses to conspecific disturbance chemicals enhance survival of juvenile brook charr, *Salvelinus fontinalis*, during encounters with predators. *Behaviour* **139**, 1099–1109.
- Møller, A. P. 1994 Male ornament size as a reliable cue to enhanced offspring viability in the swallow. *Proc. Natl Acad. Sci. USA* **91**, 6929–6932.
- Møller, A. P. & Alatalo, R. V. 1999 Good-genes effects in sexual selection. *Proc. R. Soc. Lond. B* **266**, 85–91. (doi:10.1098/rspb.1999.0607)
- Nicoletto, P. F. 1991 The relationship between male ornamentation and swimming performance in the guppy, *Poecilia reticulata*. *Behav. Ecol. Sociobiol.* **28**, 365–370.
- Nicoletto, P. F. 1993 Female sexual response to condition-dependent ornaments in the guppy, *Poecilia reticulata*. *Anim. Behav.* **46**, 441–450.
- Norris, K. 1993 Heritable variation in a plumage indicator of viability in male great tits *Parus major*. *Nature* **362**, 537–539.
- Parker, T. H. 2003 Genetic benefits of mate choice separated from differential maternal investment in red junglefowl (*Gallus gallus*). *Evolution* **57**, 2157–2165.
- Petrie, M. 1994 Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature* **371**, 598–599.
- Pilastro, A. & Bisazza, A. 1999 Insemination efficiency of two alternative male mating tactics in the guppy (*Poecilia reticulata*). *Proc. R. Soc. Lond. B* **266**, 1887–1891. (doi:10.1098/rspb.1999.0862)
- Pilastro, A., Evans, J. P., Sartorelli, S. & Bisazza, A. 2002 Male phenotype predicts insemination success in guppies. *Proc. R. Soc. Lond. B* **269**, 1325–1330. (doi:10.1098/rspb.2002.2017)
- Pilastro, A., Simonato, M., Bisazza, A. & Evans, J. P. 2004 Cryptic female preferences for colorful males in guppies. *Evolution* **58**, 665–669.
- Pitcher, T. E. & Evans, J. P. 2001 Male phenotype and sperm number in the guppy (*Poecilia reticulata*). *Can. J. Zool.* **79**, 1891–1896.
- Pitcher, T. E., Neff, B. D., Rodd, F. H. & Rowe, L. 2003 Multiple mating and sequential mate choice in guppies: females trade up. *Proc. R. Soc. Lond. B* **270**, 1623–1629. (doi:10.1098/rspb.2002.2280)
- Reinhold, K. 2002 Modelling the evolution of female choice strategies under inbreeding conditions. *Genetica* **116**, 189–195.
- Reynolds, J. D. & Gross, M. R. 1992 Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. *Proc. R. Soc. Lond. B* **264**, 57–62.
- Rowe, L. & Houle, D. 1996 The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* **263**, 1415–1421.
- Saino, N., Ferrari, R. P., Martinelli, R., Romano, M., Rubolini, D. & Møller, A. P. 2002 Early maternal effects mediated by immunity depend on the sexual ornamentation of the male partner. *Proc. R. Soc. Lond. B* **269**, 1005–1009. (doi:10.1098/rspb.2002.1992)
- Sandvik, M., Rosenqvist, G. & Berglund, A. 2000 Male and female mate choice affects offspring quality in a sex-role-reversed pipefish. *Proc. R. Soc. Lond. B* **267**, 2151–2155. (doi:10.1098/rspb.2000.1262)
- Seghers, B. H. 1973 An analysis of geographic variation in the antipredator adaptations of the guppy, *Poecilia reticulata*. PhD thesis, University of British Columbia.
- Seghers, B. H. 1974 Geographic variation in the responses of guppies (*Poecilia reticulata*) to aerial predators. *Oecologia* **14**, 93–98.
- Sheldon, B. C. 2000 Differential allocation: tests, mechanisms and implications. *Trends Ecol. Evol.* **15**, 397–402.
- Sheldon, B. C., Arponen, H., Laurila, A., Crochet, P.-A. & Merilä, J. 2003 Sire coloration influences offspring survival under predation risk in the moorfrog. *J. Evol. Biol.* **16**, 1288–1295.
- Shine, R. 2002 Eggs in autumn: responses to declining incubation temperatures by the eggs of montane lizards. *Biol. J. Linn. Soc.* **76**, 71–77.
- Shine, R. & Olsson, M. 2003 When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizard (Scincidae). *J. Evol. Biol.* **16**, 823–832.
- Thibault, R. E. & Schultz, R. J. 1978 Reproductive adaptations among viviparous fishes (Cyprinodontiformes: Poeciliidae). *Evolution* **32**, 320–333.
- van Noordwijk, A. J. & de Jong, G. 1986 Acquisition of allocation of resources: their influence on variation in life history tactics. *Am. Nat.* **128**, 137–142.
- van Oosterhout, C., Trigg, R. E., Carvalho, G. R., Magurran, A. E., Hauser, L. & Shaw, P. W. 2003 Inbreeding depression and genetic load of sexually selected traits: how the guppy lost its spots. *J. Evol. Biol.* **16**, 273–281.
- Welch, A. M., Semlitsch, R. D. & Gerhardt, H. C. 1998 Call duration as an indicator of genetic quality in male gray tree frogs. *Science* **280**, 1928–1930.
- Winge, O. 1927 The location of eighteen genes in *Lebistes reticulatus*. *J. Genet.* **18**, 1–43.
- Wisenden, B. D., Chivers, D. P. & Smith, R. J. F. 1995 Early warning in the predation sequence—a disturbance pheromone in Iowa darters (*Etheostoma exile*). *J. Chem. Ecol.* **21**, 1469–1480.
- Zahavi, A. 1975 Mate selection: a selection for a handicap. *J. Theor. Biol.* **53**, 205–214.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.