

Male phenotype and sperm number in the guppy (*Poecilia reticulata*)

Trevor E. Pitcher and Jonathan P. Evans

Abstract: The idea that female mate choice might be adaptive is relatively easy to understand in species with resource-based mating systems in which females gain access to a territory, food, or other forms of parental care from the males with whom they mate. In contrast, the evolution of female mate choice in species exhibiting resource-free mating systems remains controversial. One such species in which males contribute nothing but sperm during mating is the guppy (*Poecilia reticulata*). Here, we examined whether female guppies can obtain information on male fertility (i.e., direct fertility benefits) via cues used during mate choice. Specifically, we examined whether male guppy colour patterns, body size, and mating behaviour signal their functional fertility, that is, their ability to supply a large number of sperm at copulation. We found significant correlations between male phenotype parameters and the number of sperm in male guppies originating from two wild Trinidadian populations. There were, however, significant interpopulation differences with respect to which traits were good predictors of sperm load. In the low-predation Paria River population, larger males and males with relatively more carotenoid colouration had significantly larger sperm loads, but mating behaviour (i.e., sigmoids) and melanin colouration were not good predictors of sperm load. In the high-predation Tacarigua River population, larger males, males that displayed more, and males with less yellow colouration had significantly more sperm, but other colour pattern components (area of orange and black colouration) were not good predictors of sperm load. Overall, our results suggest that there is the potential for direct fertility benefits through mate choice in the promiscuous, non-resource-based mating system of the guppy.

Résumé : L'idée que le choix d'un partenaire chez les femelles puisse être adaptatif est facile à comprendre dans le cas d'espèces dont le système reproducteur est basé sur les ressources et chez lesquelles la femelle gagne du mâle avec lequel elle s'accouple l'accès à un territoire, à de la nourriture ou à d'autres formes de soins parentaux. En revanche, l'évolution du choix d'un partenaire chez les femelles d'espèces dont le système reproducteur n'est pas basé sur les ressources demeure sujet à controverse. Le Guppy (*Poecilia reticulata*) est un exemple d'une telle espèce et les mâles n'ont que leur sperme à donner au cours de l'accouplement. Nous avons tenté de savoir si les femelles peuvent obtenir de l'information sur la fertilité du mâle (i.e., bénéfices directs de la fertilité) par certains indices lors de leur choix d'un partenaire. Plus précisément, nous avons examiné si la coloration des mâles, leur taille et leur comportement à l'accouplement peuvent signaler leur fertilité fonctionnelle, c'est-à-dire leur capacité de produire un grand nombre de spermatozoïdes durant l'accouplement. Nous avons trouvé des corrélations significatives entre les paramètres du phénotype mâle et le nombre de spermatozoïdes chez des guppys mâles provenant de deux populations sauvages de Trinidad. Il y a cependant des différences significatives entre les populations quant aux caractères qui sont de bons indices de la quantité de spermatozoïdes. Chez la population de la rivière Paria, où la prédation est faible, les mâles plus gros et les mâles à coloration à caroténoïdes plus importante ont plus de spermatozoïdes, mais leur comportement à l'accouplement (i.e., sigmoïdes) et leur coloration de mélanine sont de mauvais indices de la fertilité. Chez la population de la rivière Tacarigua, où la prédation est forte, les mâles plus gros et les mâles au comportement de parade élaboré et les mâles avec moins de coloration jaune ont plus de spermatozoïdes, mais les autres composantes de la coloration (zone de coloration orangée et noire) ne sont pas de bons indices de l'importance du nombre de spermatozoïdes. Dans l'ensemble, nos résultats indiquent qu'il y a, chez les espèces à système d'accouplement par promiscuité, non basé sur les ressources, possibilité de tirer des bénéfices directs de la fertilité lors du choix d'un partenaire.

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Introduction

Studies have revealed that females of nearly all taxa frequently engage in mate choice (reviewed in Bateson 1983; Andersson 1994). Two major types of potential benefit arising

from mate choice have been identified. First, females may obtain direct benefits from males such as food, territories, male parental care, or high quality ejaculates that assure fertilization of all eggs (reviewed in Andersson 1994; Birkhead and Møller 1998). Second, females may secure indirect (i.e.,

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T.E. Pitcher.¹ Department of Zoology, University of Toronto, 25 Harbord Street, Toronto, ON M5S 3G5, Canada.

J.P. Evans.² Environmental and Evolutionary Biology, University of St. Andrews, St. Andrews, Fife, KY16 9TS, U.K.

¹Corresponding author (e-mail: tpitcher@zoo.utoronto.ca).

²Present address: Department of Biology, University of Padova, via Ugo Bassi 58/B, I-35131, Padova, Italy.

genetic) benefits for their offspring, such as increased survival and reproductive success (von Schantz et al. 1989; Houtman 1992; Norris 1993; Petrie 1994; Welch et al. 1998; Møller and Alatalo 1999; Drickamer et al. 2000; Jennions and Petrie 2000; Sandvik et al. 2000). Some of the most compelling evidence for female mate choice has been provided by studies of the guppy, *Poecilia reticulata* (reviewed in Houde 1997). Guppies are small live-bearing fish with internal fertilization in which females are fertile for a relatively short period each reproductive cycle. When females are sexually receptive they show preferences for males on the basis of body size, colour patterns, and courtship rate (e.g., Houde 1987; Kodric-Brown 1989; Reynolds and Gross 1992; Endler and Houde 1995; reviewed in Houde 1997), although the extent to which each of these components of mate choice is used by females can depend on the predation regime from which they originate (Endler 1995; Endler and Houde 1995; Godin 1995; Houde 1997).

Because guppies are characterized by a non-resource-based mating system in which males apparently provide nothing but sperm, previous studies have focused on the evolution of female mate choice through indirect benefits. Reynolds and Gross (1992) provided some of the first evidence that mate choice enables female guppies to obtain heritable benefits for their offspring (i.e., good genes). They found that females from the Quare River prefer larger males and that offspring sired by larger males had higher growth rates than offspring sired by smaller males. With respect to colour patterns, Nicoletto (1991) found that male swimming performance was an increasing function of the relative area of orange on the body, which suggests that display is an indicator of male condition. It is not known, however, whether female guppies also garner direct benefits by mating with larger males or males with more elaborate phenotypes. Because male guppies provide females with nothing but sperm when mating, it has been assumed that females are unlikely to obtain direct benefits through mate choice. However, it has been postulated that direct fertility benefits may be obtained by females if the phenotypic traits used by females during mate choice are associated with those that enhance a male's functional fertility (Sheldon 1994; Engen and Folstad 1999).

Ensuring an optimal sperm supply is considered a direct, not indirect, benefit of mate choice because sperm shortage can reduce female fertility (Jennions and Petrie 2000). Evidence is accumulating that within-species variation in sperm production is common (Sheldon 1994; Matthews et al. 1997; Birkhead and Møller 1998; Engen and Folstad 1999). Males can either run out of sperm or become functionally infertile by failing to produce enough sperm to successfully fertilize a female's entire complement of eggs (Nakatsuru and Kramer 1982; Petersen et al. 1992; Shapiro et al. 1994; Engen and Folstad 1999). Sheldon (1994) suggested that if functional fertility covaries with the male phenotype, females are just as likely to obtain direct as indirect benefits by choosing to copulate with more vigorous or more attractive males. It is not yet clear whether and (or) how females assess male sperm stores and as such benefit directly by mating with males with more sperm. Few studies to date have provided evidence for the phenotype-linked fertility hypothesis (Sheldon 1994). Birkhead and Fletcher (1995) and Birkhead et al.

(1997) found no evidence for the predicted positive relationship between sperm load and phenotypic characters preferred by females in the zebra finch (*Taeniopygia guttata*) or sedge warbler (*Acrocephalus schoenobaenus*), respectively. In contrast, evidence for the phenotype-linked fertility hypothesis has been documented in fish. Evidence from guppies and cod (*Gadus morhua*) suggests that females may be able to detect subtle differences in male fertility on the basis of behavioural cues used during mate choice (Matthews et al. 1997; Engen and Folstad 1999).

Here, we use the guppy to provide a further test of the phenotype-linked fertility hypothesis (Sheldon 1994). Specifically, we test the hypothesis that sperm load of male guppies is an increasing function of the male phenotype. The traits chosen for this study have been shown to be preferred by female guppies (relative cover of orange, yellow, and black; total colour area; body size; and sigmoid-display rate (Houde 1987; Houde and Torio 1992; Reynolds and Gross 1992; Nicoletto 1993; Endler and Houde 1995; Houde 1997)). We examined these traits in males originating from two populations that differ in the level of predation because the cues used by females during mate choice vary with predation intensity (Endler 1995; Endler and Houde 1995). Typically, males in high-predation locales tend to be less colourful than those in low-predation locales (Endler 1995; Houde 1997).

Materials and methods

Guppies used in this experiment were descendants of wild-caught fish from the Paria and lower Tacarigua rivers, Trinidad. The Paria River (henceforth referred to as Paria) is a low-predation locale where guppies coexist with the gape-limited cyprinodontid *Rivulus hartii*, which feeds on juvenile guppies (Houde 1997). The lower Tacarigua River (henceforth referred to as Tacarigua) is a high-predation locale where guppies coexist with a variety of predators, including the pike cichlid, *Crenicichla alta*. All males examined (Tacarigua: $n = 50$; Paria: $n = 48$) were 3–6 months old and sexually mature according to the gonopodium (intromittent organ) development criterion (see Houde 1997). Temperature was maintained between 24 and 25°C and illumination kept on a 12-h light:dark cycle.

Male mating behaviour

We examined male courtship behaviour (termed sigmoid display) in Tacarigua and Paria males. A sigmoid display is characterized by the male orientating itself in front of a female and vibrating its body in a S-shaped posture (Houde 1997). Sigmoid displays were only recorded if they were directed at a particular female. In each of the 10 Tacarigua behavioural trials, 5 males were placed in a 59 × 29 × 35 cm deep observation tank containing 5 fully mature non-virgin females that were all unfamiliar to the test males. Individual males were sketched and subsequently recognized by their unique colour patterns. The fish were allowed to settle overnight before observations commenced. The number of sigmoid displays performed by males was recorded over a 15-min period. The sigmoid display rate of one randomly chosen male in each of the 10 tanks was used in the correlation analysis to avoid pseudoreplication.

In each of the 24 Paria behavioural trials, 1 male was placed in a 59 × 29 × 35 cm observation tank containing 1 fully mature virgin female that was unfamiliar to the test male. The fish were allowed to settle overnight before observations commenced and the number of sigmoid displays performed by males in 20 min of observation was recorded. The methods of collecting behavioural data differ for the two populations because these observations were initially made in isolation of one another (i.e., prior to collaboration). Other

methods regarding sperm counts and colour pattern analysis, the main focus of this study, were equivalent throughout (see below).

Male morphology and colour patterns

Males were isolated for 2 days following behavioural trials, at which time males of both populations were photographed and examined for sperm load (see below). Tacarigua males were anaesthetized in a water bath containing 0.4 g/L Benzocaine (ethyl *p*-amino benzoate) and photographed using a mounted camera (Nikon FM 35-mm camera with a 100-mm macro lens and two electronic flash guns) loaded with Kodak Ektachrome 64 slide film. Of the 50 Tacarigua males photographed, only 43 slides were of high enough quality to be assessed for colour patterns. Paria males were anaesthetized in a water bath containing 0.15 g/L MS-222 and photographed with a digital camera (Nikon CoolPix 950). For scale and colour identification, a section of ruler and a selection of colours (paint chips) were included in each photograph, respectively. Slides were digitized and all colours were quantified (by T.E.P.) using an image analysis software package (NIH image). The total surface area of each fish was measured to calculate the relative area covered by the two main colour types: carotenoids (which include orange and yellow) and black spots/lines (melanin) (see Kodric-Brown 1989; Houde 1997). Because of subtle interpopulation differences in female mate choice preferences, we examined total, composite (i.e., both carotenoid colours), and individual (orange, yellow) colour measures (see Endler and Houde 1995). To control for the potentially confounding effects of body size, we used relative area of colouration in all of our analyses.

Measuring the number of sperm

Following photography, each anaesthetized male was placed on a petri dish under a dissecting microscope. The gonopodium was swung forward and slight pressure was applied to the side of the abdomen, just anterior to the gonopodium, to release the spermatozeugmata (i.e., sperm bundles) (see Matthews et al. 1997). This procedure was repeated until all sperm bundles were removed. The sperm bundles were then drawn up a pipette and added to 100 μ L of 0.9% saline solution. To distribute sperm evenly, samples were repeatedly drawn up and expelled from the pipette. Sperm counts were calculated by counting sperm cells in an "improved Neubauer chamber" haemocytometer under a magnification of 400 \times . The number of sperm in each sample was determined by multiplying the mean of five counts by the sample's dilution factor and initial volume. Sperm counts were expressed as the total number of sperm per stripped ejaculate.

Because of the nature of the data, non-parametric statistics were primarily used. Given that several correlations were performed, a sequential Bonferroni correction was applied to decrease the probability of committing a Type I error by chance alone (Rice 1989; Zar 1996; Peres-Neto 1999). The correction ($\alpha = 0.05$) was applied to the body colour versus the number of sperm correlations. No significant relationships were rendered nonsignificant due to the correction factor. The guppies used in this study were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care and the United Kingdom Animal Welfare Legislation.

Results

Body size and the number of sperm

Similar to previous studies (Matthews et al. 1997; Pilastro and Bisazza 1999), we found a significant positive correlation between body size (standard length) and the number of sperm per stripped ejaculate in males from both populations (Spearman's rank correlation; Tacarigua: $r_S = 0.46$, $p < 0.001$, $n = 43$; Paria: $r_S = 0.32$, $p = 0.03$, $n = 48$; Fig. 1a).

Mating behaviour and the number of sperm

Males that performed high numbers of sigmoid displays had larger sperm reserves in the Tacarigua population ($r_S = 0.84$, $p = 0.002$, $n = 10$). Body size also correlated with the number of sigmoid displays in the Tacarigua population ($r_S = 0.55$, $p = 0.01$, $n = 10$); however, the relationship between the number of sigmoid displays and the number of sperm remained significant when body size is controlled for (partial correlation, $r_{\text{partial}} = 0.83$, $p = 0.01$). In the Paria population, sigmoid display rate was not significantly correlated with either sperm load ($r_S = -0.34$, $p = 0.1$, $n = 24$) or body size ($r_S = -0.16$, $p = 0.45$, $n = 24$).

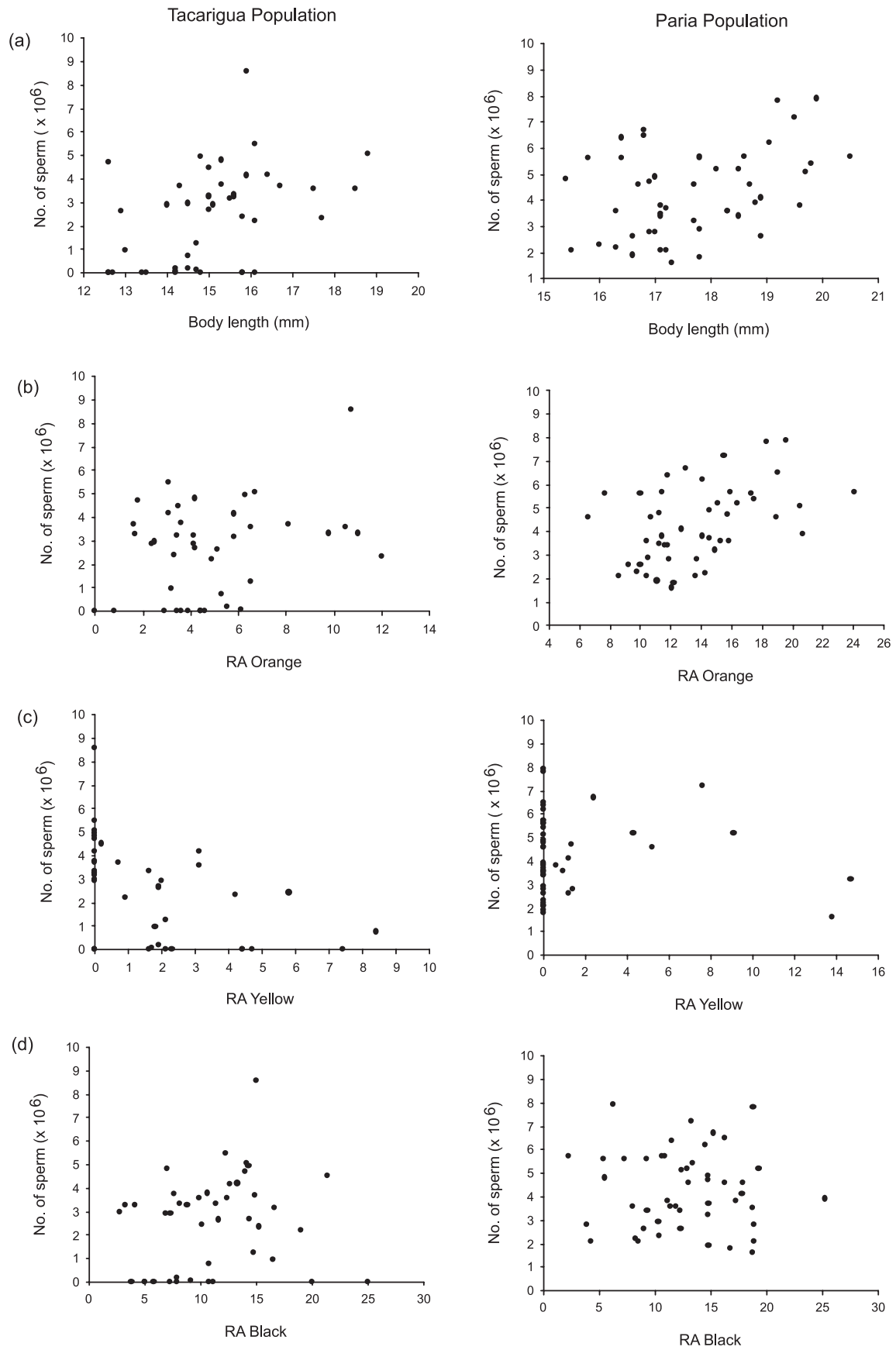
Colour pattern and the number of sperm

More colourful males had significantly more sperm than their less colourful counterparts in the Paria population but not in the Tacarigua population (see Fig. 1). The relative area of carotenoid colouration (a composite measure of orange and yellow) was positively correlated with the number of sperm per ejaculate in Paria males ($r_S = 0.38$, $p = 0.009$, $n = 48$) but not in Tacarigua males ($r_S = 0.18$, $p = 0.19$, $n = 43$). When the colours were examined individually (see Endler and Houde 1995), the relative area of orange was positively correlated with the number of sperm per ejaculate in Paria males but not in Tacarigua males (Paria: $r_S = 0.47$, $n = 48$, $p = 0.001$; Tacarigua: $r_S = 0.17$, $p = 0.27$, $n = 43$; Fig. 1b), and the relative area of yellow was not significantly related to sperm reserves in the Paria population but was significantly and negatively related to the number of sperm in the Tacarigua population (Paria: $r_S = 0.19$, $p = 0.19$, $n = 48$; Tacarigua: $r_S = -0.55$, $p < 0.001$, $n = 43$; Fig. 1c). The relative area of black (melanin) on the body surface of males was not related to sperm reserves in either the Tacarigua population ($r_S = 0.21$, $p = 0.18$, $n = 43$; Fig. 1d) or the Paria population ($r_S = -0.03$, $p = 0.83$, $n = 48$; Fig. 1d). Finally, the relative amount of the body covered by colour (orange, yellow, and black, which is a composite measure) was not significantly related with the sperm reserves in either population (Tacarigua: $r_S = 0.17$, $p = 0.29$, $n = 43$; Paria: $r_S = 0.22$, $p = 0.14$, $n = 48$).

Discussion

Our data lend general support to Sheldon's (1994) phenotype-linked fertility insurance hypothesis which suggests that female mate choice may offer direct benefits because the traits (e.g., colour, courtship display, and body size) that females find attractive covary with a male's sperm load. Our results indicate that for both populations, the number of sperm per stripped ejaculate is correlated with body size and certain components of body colouration (see Fig. 1). This has significant implications for female mate choice in guppies. Our results suggest that female guppies may not only garner indirect benefits by mating with larger, more vigorous, or more attractive males (Reynolds and Gross 1992; Nicoletto 1991), but they may also garner direct benefits (i.e., more sperm per mating). Recent work has demonstrated that there is a positive correlation between natural ejaculate size (the number of sperm delivered at copulation) and the size of stripped ejaculates (Pilastro and Bisazza 1999). Thus, females choosing to mate with larger or more colourful males could benefit by receiving more sperm. Although

Fig. 1. The relationship between the number of sperm per stripped ejaculate and male body size (standard length) (a), relative areas (RAs) of orange (b), yellow (c), and black (d) body coverage in the Tacarigua and Paria populations. See text for details.



it is uncertain how often reduced fertility in female guppies is attributable to sperm limitation, evidence from recent work suggests that brood size is a function of the number of males a female secures (Evans and Magurran 2000). Since males can become sperm limited after a single copulation (Pilastro and Bisazza 1999), it is possible that sperm limitation does influence the tendency of female guppies to mate with other males (see Evans and Magurran 2000). Females who choose to mate with “sperm-rich” males may be able to reduce the need to secure future partners and therefore reduce the costs associated with multiple matings (Sheldon 1993; Magurran and Seghers 1994; Jennions and Petrie 2000).

Because of the geographic variation in female guppy preferences for male traits and differences in the costs and benefits of different male mating tactics in low- and high-predation populations (Endler 1995; Endler and Houde 1995; Godin 1995; Houde 1997), it is not surprising that the potential cues for sperm load differ between the two populations examined. Females in high-predation locales tend to encounter males that are typically less colourful than males in low-predation locales (reviewed in Houde 1997). We found evidence of a positive correlation between the relative amount of carotenoid colouration and the number of sperm stripped in the low-predation Paria population but not in the high-predation Tacarigua population (see Fig. 1). Interestingly, relative orange area is one of the most important determinants of female mate choice in this low-predation population (Houde 1987; Endler and Houde 1995). In contrast, in high-predation populations male body size appears to be an important mate-choice cue (Reynolds and Gross 1992). We found evidence of a positive correlation between body size and sperm load and a negative correlation between the relative amount of yellow colouration and the number of sperm in the Tacarigua population. These findings suggest that in this high-predation population, male body size is a good indicator of sperm load, while, contrary to Sheldon’s (1994) hypothesis, there is a possible trade-off between the size of colour ornaments and the number of sperm.

We also found evidence of a positive correlation between male sigmoid display rate and the number of sperm stripped in one population but not in the other (see Matthews et al. 1997; Pilastro and Bisazza 1999). The high-predation Tacarigua population exhibited a significant positive correlation between courtship display intensity and sperm load. This relationship between sperm load and courtship display is consistent with earlier work (Matthews et al. 1997) and suggests that females in the Tacarigua population have the potential to assess male fertility status on the basis of male courtship behaviour. One possible reason why our study did not uncover a relationship between male behaviour and the number of sperm in the low-predation Paria population is that we used a different experimental protocol to examine male behaviour in the two populations (see also Pilastro and Bisazza 1999). Although we failed to detect a correlation between sperm load and sigmoid display rate in the Paria population, the correlations between colour components and sperm load differed between the two populations when we used identical methodologies.

In conclusion, females choosing mates on the basis of body size, behaviour, and (or) colouration may not only be seeking indirect benefits, but also mates that maximize their

fertility (see Grey 1997a, 1997b). Direct benefits of mate choice should therefore be more carefully considered by those studying potential indirect benefits of mate choice in guppies and warrants further study.

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