

Multiple paternity, reproductive skew and correlates of male reproductive success in a wild population of the Trinidadian guppy

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Abstract – In most species, males have a higher reproductive potential than females, leading to skewed reproductive success, particularly in mating systems where pre- or postcopulatory sexual selection reinforces inequality in male mating success. We investigated multiple paternity, reproductive skew and correlates of male reproductive success in a wild population of the Trinidadian guppy (*Poecilia reticulata*). We used nine microsatellite loci to assess the frequency of multiple paternity, number of sires per brood and reproductive skew. Across broods, the frequency of multiple paternity was high with 94% of broods having multiple sires (range: 1–5), resulting in a reproductive skew of 0.14. Variation in male reproductive success was high (range: 0–14 offspring per male), suggesting that there is considerable opportunity for sexual selection. Next, we examined correlations between male reproductive success and sexual coloration, sperm velocity and gonopodium length. Relative area of orange, black, iridescent and total coloration, and sperm velocity were not correlated with reproductive success. However, gonopodium length explained 14% of the variation in reproductive success, suggesting that gonopodium length is likely a sexually selected trait. We discuss these findings in the light of other studies that genetically dissect joint-sex parentage and examine correlates of male reproductive success in wild populations.

Key words: reproductive success; parentage analysis; mating system; sperm; ornamentation; sexual selection; mating success

Introduction

When males and females differ in the total number of offspring they can produce, it can result in intense competition for mating opportunities among members of the sex with a higher reproductive potential (reviewed in Clutton-Brock & Vincent 1991; Shuster 2009). In most species, females are more limited than males in the number of offspring they can produce because of greater initial investment in each gamete and subsequent parental investment, whereas males have the potential to sire numerous progeny but are limited primarily by access to females (Bateman 1948; Trivers 1972; Clutton-Brock & Vincent 1991). Offspring production by males is likely to be more severely skewed than that of females in mating

systems where pre- or postcopulatory sexual selection reinforces inequality in male mating success. Precopulatory sexual selection can lead to exaggerated mate traits, such as weapons for direct competition with rival males or ornaments that increase their attractiveness to females (reviewed in Andersson 1994). Postcopulatory sexual selection acts on male traits that increase the likelihood of fertilisation success when females mate with multiple males, and can result in the elaboration of male genitalia (Arnqvist & Rowe 2005; Hosken & Stockley 2004) or increased investment in sperm quality (Birkhead & Moller 1998; Birkhead et al. 2009).

In the present study, we investigate multiple paternity, reproductive skew and correlates of male reproductive success in a wild population of the

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guppy (*Poecilia reticulata*). The guppy can be found throughout numerous freshwater habitats in Trinidad and is a small, live-bearing fish with internal fertilisation and a non-resource-based, promiscuous mating system (reviewed in Magurran 2005). A number of male traits have been examined with regard to female choice in guppies, the most common finding being a preference for males with a larger area of orange carotenoid coloration (Houde 1987; Brooks & Caithness 1995a; Endler & Houde 1995). For example, females have been shown to 'trade up' and mate with a second male that possesses more area of orange coloration, and is presumably of higher genetic quality (Evans et al. 2004), than a previous mate (Pitcher et al. 2003). Other findings regarding female mate choice in guppies include a preference for the area of black melanin coloration (Brooks & Caithness 1995b), area of iridescent coloration (Kodric-Brown 1985, 1993) or larger body size (Reynolds & Gross 1992; Magellan et al. 2005). However, there is variation in female preference for these traits across populations (Endler & Houde 1995) and among individuals (Brooks & Endler 2001).

Male guppies attempt to initiate copulation by employing a form of courtship known as the sigmoid display (Magurran 2005). Alternatively, males will also attempt a form of sneaky mating, called the gonopodial thrust, in which the male approaches a female and forcefully attempts to insert his gonopodium, the modified anal fin used for sperm transfer, into her gonopore (Constanz 1989). Females typically attempt to avoid gonopodial thrusts by swimming away, and thrusts appear to result in successful insemination on occasion, although determining the frequency of successful gonopodial thrusts in the wild is problematic. However, a study of eight wild populations of Trinidadian guppies found that nearly half of the females examined contained sperm presumed to be from unsolicited copulations (Evans et al. 2003a,b; also see Matthews & Magurran 2000). Finally, male guppies with longer gonopodia achieve higher contact success (the number of thrusts that resulted in contact between the males' and females' genitalia) than those with shorter gonopodia (Evans et al. 2011).

Frequent unsolicited copulations and the potential benefits of polyandry (Evans & Magurran 2000; Pitcher et al. 2003; Ojanguren et al. 2005) are likely responsible for the high levels of multiple paternity observed in wild populations of guppies (Kelly et al. 1999; Neff et al. 2008), which results in a high risk of sperm competition (also see Elgee et al. 2010). Experimental studies that involved artificial insemination of a female, which removes any precopulatory cues, with sperm from two competing males

suggest that sperm competition success in guppies is mediated, in large part, by sperm velocity. Evans et al. (2003a,b) used artificial insemination to demonstrate that sperm competition success is correlated with area of orange coloration, which in turn is correlated with sperm velocity (see Locatello et al. 2006; Pitcher et al. 2007; but see Skinner & Watt 2007). A more recent artificial insemination experiment has confirmed a positive relationship between competitive fertilisation success and sperm velocity (C. Boschetto, C. Gasparini & A. Pilastro, unpublished results).

Despite the long history of the guppy as a model system in the study of sexual selection (reviewed in Magurran 2005), no study to date has examined what morphological traits correlate with male reproductive success in a wild population (but see Johnson et al. 2010 for a study examining multiple paternity and relatedness). Correlates of reproductive success have only been examined in a laboratory setting. For example, Houde (1988) allowed guppies to freely mate in aquaria and examined male reproductive success by scoring paternity of sons using Y-linked colour patterns, revealing no relationship between the amount or type of ornamental coloration and reproductive success. Becher & Magurran (2004) also allowed guppies to mate freely in aquaria, then collected and genotyped all the resulting offspring using four polymorphic microsatellite loci for parentage analysis and related male reproductive success to a variety of morphological and behavioural traits. They found that smaller males sired significantly more offspring, but no other male trait was related to reproductive success, including the amount of orange and black coloration, number of courtship displays and sneaky mating attempts, gonopodium length and total sperm stores (Becher & Magurran 2004).

Although there have been many studies on female choice and male mating success in guppies, our study is distinctive in that it examines male and female reproductive success under natural conditions. As such, this study offers important perspectives on prior findings, many of which are potential artefacts of laboratory study conditions. We used nine microsatellite loci to assess the frequency of multiple paternity, number of sires per brood and reproductive skew in a wild guppy population. Next, we examined correlations between male reproductive success and a variety of traits, including sexual coloration, sperm velocity and gonopodium length, to identify particular characters that are under sexual selection in our study population of guppies. We discuss our results in terms of potential selective pressures on male and female guppies and also compare our results with those of other studies that examine reproductive skew in other wild populations.

Methods

Study system

We isolated a section of the Tunapuna stream (N10°42'38.22" W061°21'20.36") by setting up two barriers in the stream approximately 35 m apart. Tunapuna is a low-predation [i.e., predation is restricted to juvenile guppies by a gape-limited fish, *Rivulus hartii* (Magurran 2005)] guppy population located in Trinidad's Northern Mountain Range. The isolated study area contained 10 pools that varied in depth (approximately 25 cm–90 cm), separated by small riffles. The barriers were permeable to water and did not significantly disrupt stream flow but prevented migration (or emigration) of adult guppies from (or to) the study area. The barriers were constructed by stretching netting across the stream and securing it in place using rocks. The barriers were left in place for 65 days, which is enough time for females that had mated prior to the study to give birth and mate with available males within the study area (Magurran 2005). The study area was inspected periodically to ensure that the barriers remained intact. After 65 days, we seined the study area, taking care to collect all adult guppies between the barriers, ensuring that no adult individuals remained. All females were immediately euthanised and preserved in ethanol to collect offspring. Males were brought to the laboratory and assessed for sexual maturity and traits related to reproductive success.

Male trait assessment

Field-collected males were first assessed for sexual maturation; males were considered to be adult (reproductively mature) if the hood was extended beyond the tip of the gonopodium (Evans et al. 2003a,b). Sperm bundles were extracted to assess sperm velocity, and a digital photograph (Nikon Coolpix 950; Nikon Inc., Melville, NY, USA) was taken of the left side of each adult male. From these photographs, we used ImageJ software (available at <http://rsbweb.nih.gov/ij/>) to measure total body length (from the tip of the mouth to the base of the caudal fin, along the central axis), gonopodium length (from the base to the distal tip, following Kelly et al. (2000)), total body area (excluding all fins except the caudal fin) and total area of orange, black and iridescent coloration (see Pitcher et al. 2007) (Table 1). Because the amount of orange and black coloration was related to body area (orange: $R^2 = 0.48$, d.f. = 80, $P < 0.001$; black: $R^2 = 0.10$, d.f. = 80, $P = 0.004$), we used the relative amount of each colour (percentage of body covered in that colour, hereafter relative orange coloration and relative black coloration) in all analyses. Analyses conducted

Table 1. Summary of traits of male guppies (*Poecilia reticulata*), including mean, standard deviation (SD) and range (minimum to maximum) of relative orange coloration, relative black coloration, relative iridescent coloration, relative total coloration, gonopodium length and sperm velocity. Sperm velocity measures include average path velocity (VAP = average velocity on the smoothed cell path), straight line velocity (VSL = average velocity on a straight line between the start and end points of the track) and curvilinear velocity (VCL = average velocity on the actual point-to-point track followed by the cell).

Male trait	Mean	SD	Range
Orange coloration (%)	7.20	3.1	1.1–16.3
Black coloration (%)	10.1	3.9	1.4–21.7
Iridescent coloration (%)	3.3	1.8	0.6–7.9
Total coloration (%)	20.5	4.9	7.8–32.2
Body length (mm)	17.9	1.01	16.1–20.4
Gonopodium length (mm)	4.08	0.33	3.39–5.24
Sperm velocity ($\mu\text{m}\cdot\text{s}^{-1}$)			
VAP	47.4	5.3	38.3–64.7
VSL	26.5	4.8	13.1–40.8
VCL	106.1	11.5	69.9–131.4

on residuals of colour area on body area produced qualitatively similar results (data not shown). In addition to absolute gonopodium length, the residuals of the regression of \log_{10} gonopodium length on \log_{10} body length ($y = 0.58x - 0.13$, $R^2 = 0.17$, d.f. = 80, $P < 0.001$), hereafter relative gonopodium length, were used in analyses (see Kelly et al. 2000).

Sperm was extracted following the study of Matthews et al. (1997). A fixed number of sperm (25 bundles, to control for sperm density) were drawn up in a pipette and added to 250 μl Courtland's saline, which contained bovine serum albumin at 1% v/v (hereafter saline solution) (Pitcher et al. 2007; Evans 2009). The resulting saline solution was drawn repeatedly into the pipette to break the sperm bundles and activate the sperm for velocity analysis. Video recordings for sperm velocity analyses were made using a CCD B/W video camera module at 50 Hz vertical frequency, mounted on a digital compound microscope (magnification 400 \times , Olympus BX60, Olympus Inc., Center Valley, PA, USA). We used an 8- μl sample of semen in saline solution on a haemocytometer, covered with a cover slip. To maximise the time until the sperm stuck to the glass, the glass slide and cover slip were precoated by immersion in 1% bovine serum albumin followed by a rinse in distilled water (see Billard et al. 1995). Video recordings were analysed using the HTM-CEROS sperm-tracking packing (CEROS version 12; Hamilton Thorne research, Beverly, MA, USA), an objective tool for studying sperm motility in fish (see Kime et al. 2001; Rurangwa et al. 2004; Pitcher et al. 2009). The variables we assessed for each male's sperm were as follows: average path velocity (VAP = average velocity on the smoothed cell path), straight line velocity (VSL = average velocity on a straight line between the start and end points of the track) and

curvilinear velocity (VCL = average velocity on the actual point-to-point track followed by the cell) at 5-s postactivation (i.e., breaking of the bundle) (Table 1). Because the variables describing sperm velocity (VAP, VSL and VCL) were highly correlated, we performed a principle component analysis on these variables, which yielded one PC axis (hereafter referred to as sperm velocity) that explained 69.4% of the variation.

Microsatellite genotyping

All females ($n = 67$) that were considered sexually mature (i.e., larger than a sexually mature male) were dissected. Only broods consisting of embryos that were visible and large enough for DNA extraction were genotyped ($n = 32$ females). DNA was extracted from individual whole embryos [mean \pm SD: 7.19 ± 2.15 offspring per brood (range 4–12)] and from muscle tissue of the caudal peduncle of all females that contained embryos as well as all adult males ($n = 81$) by salt precipitation using the Wizard Genomic DNA Purification Kit (Promega Corp, Madison, WI, USA). Samples were screened using standard techniques (see Neff et al. 2008 for details) with up to nine microsatellite markers (chosen for their ability to detect multiple paternity based on a prescreening of tissue samples from our focal population), including Pr39, Pr92 (Becher et al. 2002), Pre9, Pre15 (Paterson et al. 2005), AGAT-11 (Olendorf et al. 2004), 9-1 (van Oosterhout et al. 2006) and PP_GATG_F4, PP_GATA_H2 and PP_GATA_5 (Nater et al. 2008) (see Table 2).

Reproductive skew

Because we were not able to assign paternity to all of the offspring, we used Colony (version 1.2; Wang 2004) to estimate the number of sires per brood and reproductive skew for each brood. This program uses a maximum likelihood method to estimate full-sib relationships within half-sib broods (i.e., number of

sires per brood, see Neff et al. 2008). We assumed a genotyping error rate of 0.02. Skew was estimated by first calculating the effective number of sires from $1/\sum(rs_i/\text{brood size})^2$, where rs_i is the number of offspring assigned to sire i and the summation is over all sires contributing to a brood. Skew was then expressed as $1 - (\text{effective number of sires}/\text{actual number of sires})$ (see Neff et al. 2008). Linear regressions were used to assess the relationships between female body length and brood size, female body length and number of sires, and brood size and reproductive skew.

Correlates of male reproductive success

Paternity was assigned to the 81 candidate sires using Cervus ver. 3.0 software (available at <http://www.fieldgenetics.com>), which uses a likelihood-based method to assign paternity (Marshall et al. 1998; Kalinowski et al. 2007). We assumed a typing error rate of 0.01. To account for sperm storage by females (Evans et al. 2003a,b) and possible male mortality during the study period, we assumed that 90% of all potential sires were included among the 81 males sampled (see Tatarenkov et al. 2008). Of the 233 offspring genotyped, Cervus was able to assign paternity to 197 individuals with between 80% and 100% certainty. From this, we obtained a total number of offspring for each male, which we divided by 197 to obtain an estimate of relative reproductive success (RRS) for each male. To determine how equally mates and reproductive success were shared among males and among females, we created frequency distributions of the number of offspring and number of mates for each sex. Sample sizes for offspring number per sex differ ($n = 233$ for females, $n = 197$ for males) because for females, we could assign all offspring with absolute confidence and for males, we could assign paternity to 85% of the offspring. Male number of mates represents the number of females with which each male successfully produced offspring. Female number of mates represents the number of sires contributing to a single brood. We then compared these distributions between males and females using Kolmogorov–Smirnov tests.

Relative reproductive success was then related to the male traits described earlier (relative orange coloration, relative black coloration, iridescent coloration, total coloration, body length, absolute and relative gonopodium length and sperm velocity) using linear regressions. Because it is possible that males with relatively less coloration engage in a greater number of sneak copulations, which presumably would select for larger gonopodia, we also examined whether these males have relatively longer gonopodia compared to relatively more colourful males. The

Table 2. List of microsatellite loci used in paternity analysis of guppy (*Poecilia reticulata*) offspring, references for the loci, number of alleles, observed heterozygosity (H_o) and expected heterozygosity (H_E).

Locus	Reference	Alleles	H_o	H_E
Pr92	Becher et al.2002	3	0.525	0.578
Pr39	Becher et al.2002	5	0.757	0.711
Pre15	Paterson et al.2005	6	0.769	0.770
Pre9	Paterson et al.2005	6	0.702	0.678
PP_F4	Nater et al.2008	7	0.792	0.747
PP_H2	Nater et al.2008	5	0.780	0.772
PP_5	Nater et al.2008	5	0.654	0.665
AGAT-11	Olendorf et al.2004	9	0.746	0.785
9-1	van Oosterhout et al.2006	4	0.575	0.594

distribution of RRS was highly skewed (Kolmogorov–Smirnov test for normality, $Z = 2.0$, $P = 0.001$) as is typical of this kind of data and could not be normalised through any transformation. For this reason, we estimated statistical significance for the linear regressions relating RRS to male traits, using a randomisation test (Manly 1997) executed in PopTools, a Microsoft Excel add-in (available at <http://www.cse.csiro.au/poptools>). Briefly, the x and y variables were shuffled (with replacement) to create randomised x and y pairings, from which a new regression coefficient was calculated. This was repeated 10,000 times, creating a distribution of regression coefficients and their associated t (the constant being excluded from the model). Regression coefficients that fell outside the 95% CI of this distribution were considered statistically significant. Exact P values were estimated by calculating the proportion of randomised data sets that had r square values greater than or equal to the r^2 value calculated from the actual data.

Results

Reproductive skew

Nearly all broods (94%) were multiply sired, with a mean (\pm SD) of 3.03 ± 1.21 (range 1–5) sires per brood and a mean reproductive skew of 0.15 ± 0.11 (range: 0–0.48). There was variation in female body length (mean \pm SD: $24.9 \text{ mm} \pm 1.8$; range: 21.6–28.76), and bigger females had larger broods ($R^2 = 0.20$, d.f. = 31, $P = 0.01$). There was no significant relationship between female body length and number of sires ($R^2 = 0.001$, d.f. = 31, $P = 0.84$) or between brood size and reproductive skew ($R^2 = 0.07$, d.f. = 31, $P = 0.15$).

The frequency distribution of number of offspring ($Z = 4.0$, $P < 0.001$; Fig. 1) and the number of mates ($Z = 2.4$, $P < 0.001$; Fig. 2) differed significantly between males and females. For females, both offspring number and mate number did not deviate significantly from normality (offspring: $Z = 1.1$, $P = 0.20$; mates: $Z = 1.3$, $P = 0.07$, Figs 1 and 2), whereas for males, they both deviated significantly from normality (offspring: $Z = 2.0$, $P = 0.001$; mates: $Z = 2.3$, $P < 0.001$) and were instead highly skewed (Figs 1 and 2).

Individual reproductive success was compiled as the total number of offspring assigned to each adult in the population. For females, this number ranged from 4 to a maximum of 12 with a mean of 7.3. For males, the number of offspring ranged from 0 to 14, with a mean of 2.4. Therefore, there was higher variation in reproductive success for males than for females. Male reproductive success was positively related to the number of mates ($R^2 = 0.72$, $P < 0.001$; Fig. 3a), but

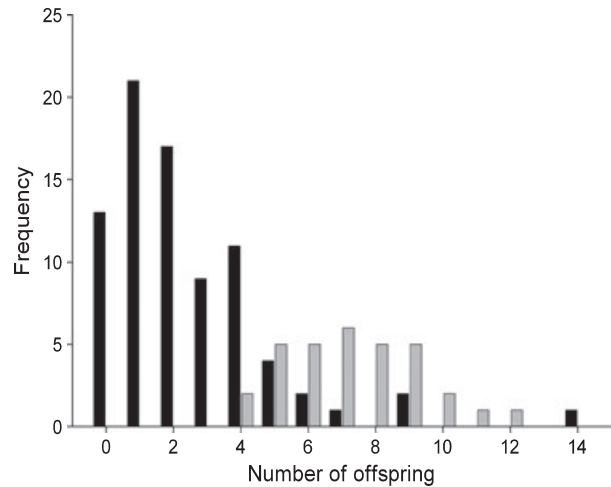


Fig. 1. Total number of offspring of male (black bars, $n = 197$ offspring) and female (grey bars, $n = 233$ offspring) guppies (*Poecilia reticulata*) from an isolated, wild population in Trinidad. Differences in offspring number between males and females are attributable to the fact that all offspring could be assigned to maternal identities, while only a subset (85%) of the offspring could be assigned paternal identities.

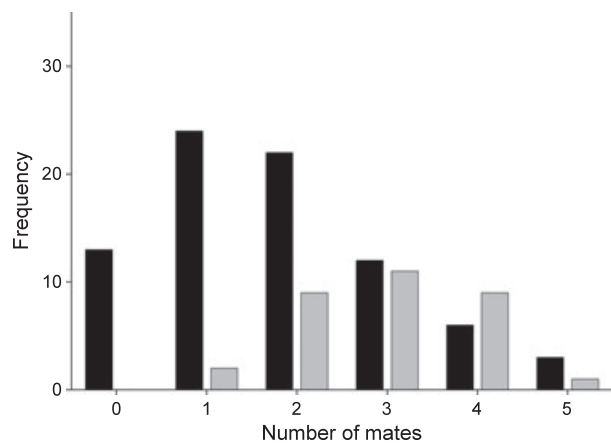


Fig. 2. Total number of mates of male (black bars) and female (grey bars) guppies (*Poecilia reticulata*) from an isolated, wild population in Trinidad. Male number of mates represents the number of females with which each male successfully produced offspring, based on paternity analysis. Female number of mates represents the number of sires contributing to a single brood.

there was no such relationship for females ($R^2 = 0.08$, $P = 0.11$; Fig. 3b).

Correlates of male reproductive success

Linear regressions revealed no relationship between male RRS and relative orange coloration ($R^2 = 0.01$, $P = 0.62$, $n = 81$), relative black coloration ($R^2 = 0.007$, $P = 0.66$, $n = 81$), iridescent coloration ($R^2 = 0.046$, $P = 0.60$, $n = 81$), total coloration ($R^2 = 0.01$, $P = 0.51$, $n = 81$), body length ($R^2 = 0.012$,

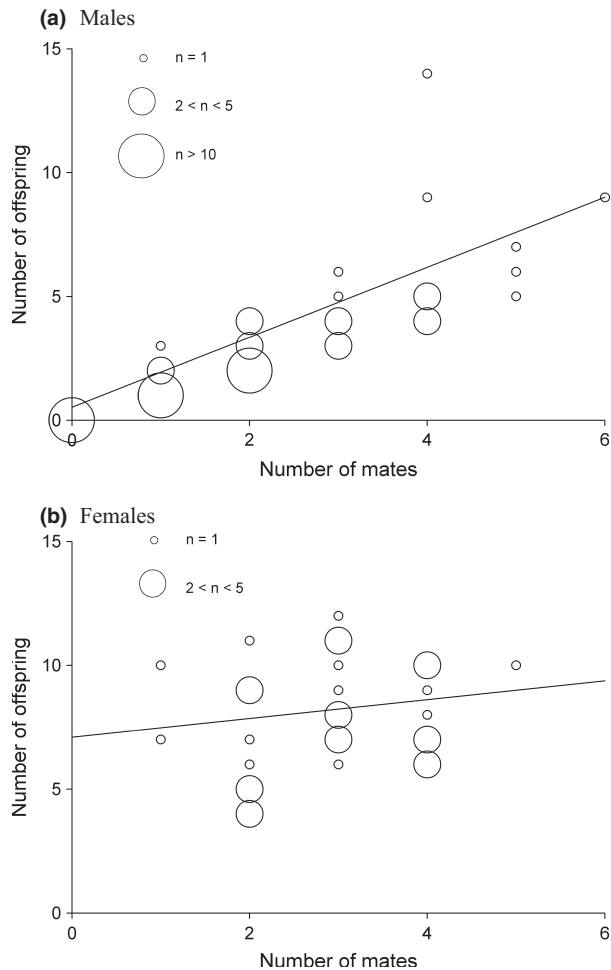


Fig. 3. The relationship between number of mates and number of offspring for (a) male ($n = 81$) and (b) female ($n = 32$) guppies (*Poecilia reticulata*). Symbol size is proportional to the sample size at overlapping data points.

$P = 0.32$, $n = 81$) or sperm velocity ($R^2 = 0.03$, $P = 0.23$, $n = 81$). However, there was a significant positive relationship between male RRS and relative gonopodium length ($R^2 = 0.14$, $P = 0.002$, $n = 81$; Fig. 4). The relationship between male RRS and gonopodium length remained significant even when not controlling for body size ($R^2 = 0.14$, $P = 0.001$, $n = 81$). Neither measure of gonopodium length was significantly correlated with any of the coloration metrics (all $P > 0.15$).

Discussion

Our study is unique because we examined not only patterns of multiple paternity and reproductive skew in a wild population of Trinidadian guppies but also phenotypic correlates of reproductive success. Consistent with previous studies, we found that nearly all of the females produced broods of offspring sired by multiple males. We also found that the distribution of

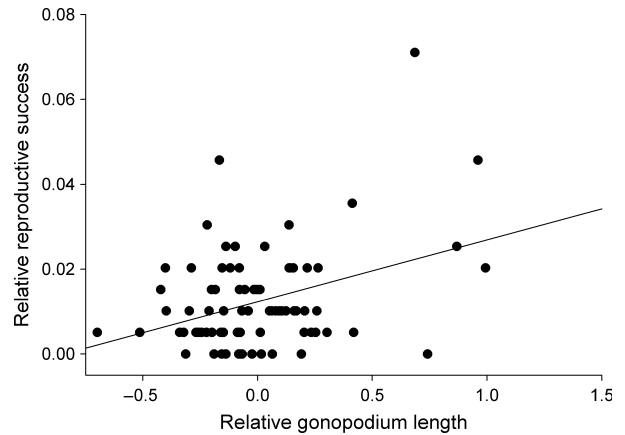


Fig. 4. The relationship between male relative reproductive success (proportion of offspring produced by each male) and relative gonopodium length (residuals from the regression of gonopodium length on body length) in male guppies (*Poecilia reticulata*).

the number of mates and offspring was significantly different between males and females; females exhibited relatively even success compared to the large differences in success observed among males. Finally, among the male sexually related traits we examined, we found that gonopodium length explained a significant amount of variation in reproductive success, while more traditional measures of male attractiveness, including colour patterns, did not explain patterns of reproductive success in our wild study population.

The mean reproductive skew in our guppy population was 0.15, 94% of the broods were multiply sired, and the mean number of sires per brood was 3.0. These are similar to results found by Neff et al. (2008), who found that the mean reproductive skew was 0.14 and the proportion of broods that were multiply sired was 100% in this same population, although they found a higher mean number of sires per brood (3.6), possibly due to a higher mean brood size in their study. In contrast, Kelly et al. (1999) found a proportion of multiply sired broods in the same population of only 35%. (They did not examine reproductive skew and the number of sires contributing to broods.) The discrepancy between our results and Kelly et al.'s (1999) is most likely due to the fact that we used more microsatellite loci and therefore had much greater power to detect multiple paternity (see Neff & Pitcher 2002).

The frequency distribution of the number of mates was significantly different between males and females. For females, the number of mates was normally distributed, an indication that reproductive success was relatively evenly distributed among females. For males, on the other hand, the number of mates was highly skewed, indicating that most males were able to secure very few mating partners and sire few offspring. These findings are in contrast to those of

Becher & Magurran's (2004) laboratory study of multiple paternity and reproductive skew in guppies that found that there was no significant difference in the distribution of number of mates between sexes. This difference is likely a result of the fact that the Becher and Magurran's laboratory-based study took place over 3 months and each female had on average 2.6 broods compared to our wild study where each female only had one brood.

Because a small number of males appear to have sired the majority of offspring in our study, there appears to be considerable opportunity for sexual selection. Reproductive success of female guppies in our population is relatively even and largely related to body size, whereas male reproductive success appears to be highly skewed. Compared to two other studies of wild fish populations, the Atlantic salmon (*Salmo salar*) (Garant et al. 2001) and the Green swordtail (*Xiphophorus helleri*) (Tatarenkov et al. 2008), male guppies in our study population qualitatively appear to be intermediate in terms of their variation in reproductive success. The relatively high variation in reproductive success in male guppies begs the question as to which male traits are correlated with reproductive success in our wild study population.

We found that male relative area of orange coloration, relative area of black coloration, area of iridescent coloration, total coloration and body length did not predict male reproductive success. It is possible that an analysis of coloration based on qualities such as chroma or brightness would yield different results; however, previous laboratory-based research on female choice in guppies has primarily focused on the area of coloured spots. Studies thus far have indicated that female choice, particularly for male colour patterns, is an important determinant of male mating success, and many studies have demonstrated a strong preference for area of orange coloration (Kodric-Brown 1985; Houde 1987; Endler & Houde 1995; Brooks & Endler 2001; Pitcher et al. 2003). Studies examining the area of black and iridescent spots are more contradictory, with some studies showing a female preference for these colours and others showing an aversion or indifference (Kodric-Brown 1985; Brooks & Caithness 1995a,b; Endler & Houde 1995). Evidence regarding preference for body size is similarly contradictory (Reynolds & Gross 1992; Endler & Houde 1995). These contradictions may be due to differences in experimental conditions among studies but also to variation in female preferences among populations (Endler & Houde 1995) and among individual females (Brooks & Endler 2001). In spite of the considerable body of work on female choice in guppies, very few studies have examined the extent to which male traits preferred by females translate into reproductive success. Becher & Magurran (2004)

conducted paternity analysis on offspring born under controlled laboratory conditions, with all mating taking place in aquaria, and found that smaller males sired more offspring than larger males, but the relative area of orange, black and iridescent coloration was not significantly related to reproductive success (also see Houde 1988). It is possible that small male body size led to higher reproductive success in the Becher & Magurran's (2004) study because they examined a high-predation population (in contrast to our low-predation population), where gonopodial thrusting is likely to be more frequent in nature. However, because female choice and male sexual behaviour studies have not been performed on our study population, it is not possible to determine whether our results are attributable to a lack of female preference for these male traits or minimal female control over paternity owing to the prevalence of sneaky mating.

Multiple mating is the norm in this species, so sperm competition is likely to affect male reproductive success; however, we found that sperm velocity did not predict male reproductive success. Recent studies using artificial insemination, with one focal female and two males contributing sperm, have demonstrated that sperm velocity is a predictor of paternity success in guppies (C. Boschetto, C. Gasparini & A. Pilastro, unpublished results), confirming previous artificial insemination experiments by Evans et al. (2003a,b), which found that male paternity success was correlated with the amount of orange coloration, which in turn has been associated with sperm velocity (Locatello et al. 2006; Pitcher et al. 2007; but see Skinner & Watt 2007). Our results indicate that sperm velocity may not be as important a predictor of paternity success in the wild when other male traits are taken into account or that sperm competition is not a primary determinant of fertilisation success in our study population. It is also possible that the artificial insemination experiments described earlier do not replicate normal sperm competition mechanisms that occur in the wild, where more than two males are often contributing sperm to a female's reproductive tract (see Zeh & Zeh 1994).

We found that males with relatively longer gonopodia had higher reproductive success than males with relatively shorter gonopodia, a finding that is most likely due to an association between gonopodium length and success at gonopodial thrusts. Males with relatively longer gonopodia were more successful at making contact with female reproductive tracts than males with relatively shorter gonopodia (Evans et al. 2011), and Reynolds et al. (1993) demonstrated a positive correlation between gonopodium length and frequency of thrusts. There is also evidence that the shape of the gonopodium, including the angle of the hook and the length of the apical tip, contributes

to insemination success (Cheng 2004; Evans et al. 2011). The positive relationship between male RRS and gonopodium length did not change when we used absolute length instead of relative length, suggesting that there is a reproductive advantage of longer gonopodia *per se*, not relative investment in gonopodium growth. It would be useful to conduct a more detailed analysis of gonopodium shape in relation to reproductive success in a wild population of guppies. It is also possible that the relationship between gonopodium length and male reproductive success is the result of female choice. Brooks & Caithness (1995b) found some evidence of female preference for gonopodium length in guppies, and Langerhans et al. (2005) demonstrated female preference for gonopodium length in two other Poeciliid species, *Gambusia hubbsi* and *G. affinis*. Finally, we found no significant relationships between sexual coloration metrics and gonopodium length, suggesting that males with less coloration do not compensate by investing more in gonopodium length in our focal study population.

The relationship between gonopodium length and male reproductive success suggests that gonopodium length is a sexually selected trait. Sexual selection on male genitalia is common and is likely to account for the diversity in male genitalia, even among closely related species (Eberhard 1985, 2010). In guppies, the association between gonopodium length and gonopodial thrusts suggests that the mechanism of sexual selection is likely to be sexual conflict over mating decisions. A positive relationship was found between male gonopodium morphometrics and the shape of the female oviduct across ten wild guppy populations (Evans et al. 2011), suggesting a role for sexually antagonistic selection. In addition, differences in predation pressure have likely influenced the evolution of gonopodium length in guppies because males are more likely to use sneak copulations when females are under predation risk (Houde 1997) and males in high-predation populations sneak copulate more often than males in low-predation populations (Magurran & Seghers 1994). Male guppies from populations that have evolved with high predation intensity have been shown to have relatively longer gonopodium than males from low-predation populations (Kelly et al. 2000; also see Evans et al. 2011), suggesting that in addition to sexual selection, natural selection also plays a role in determining gonopodium size. Because our focal guppy population was a low-predation population, sexual selection may be a more important driver of gonopodium size than natural selection. Future studies that examine whether certain aspects of female oviduct morphology are able to help thwart thrusting attempts by males and whether there is sufficient additive genetic variance underlying the

expression of these traits (in both high- and low-predation populations) to permit response to selection are needed.

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