

Effects of polyandry on male phenotypic diversity

M. BARBOSA*, M. DORNELAS†‡ & A. E. MAGURRAN*

*Gatty Marine Laboratory, University of St Andrews, East Sands, Fife, UK

†ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD, Australia

‡CESAM, Department of Biology, Universidade de Aveiro, Campus de Santiago, Aveiro, Portugal

Keywords:

evolution;
phenotypic diversity;
sexual selection;
sexual traits.

Abstract

Polyandry has the potential to affect the distribution of phenotypes and to shape the direction of sexual selection. Here, we explore this potential using Trinidadian guppies as a model system and ask whether polyandry leads to directional and/or diversifying selection of male phenotypic traits. In this study, we compare the phenotypic diversity of offspring from multiply and singly sired broods. To quantify phenotypic diversity, we first combine phenotypic traits using multivariate methods, and then take the dispersion of individuals in multivariate space as our measure of diversity. We show that, when each trait is examined separately, polyandry generates offspring with a higher proportion of bright coloration, indicating directional selection. However, our multivariate approach reveals that this directionality is accompanied by an increase in phenotypic diversity. These results suggest that polyandry (i) selects for the production of sons with the preferred brighter colour phenotypes whereas (ii) enhancing the diversity of male sexual traits. Promoting phenotypic diversity may be advantageous in coping with environmental and reproductive variability by increasing long-term fitness.

Introduction

Multiple mating by females is widespread, occurring even in species previously thought to be monogamous (Birkhead & Møller, 1998). The prevalence of polyandry, in particular in systems where females have no access to resources through mating, has led to a surge in studies that set out to explain its evolution and maintenance based on offspring genetic benefits (Jennions & Petrie, 2000; Simmons, 2005). Curiously, however, despite being recognized as a critical engine of post-mating sexual selection, the role of polyandry in shaping the evolution of sexual traits is only now attracting attention (Birkhead & Pizzari, 2002; Evans & Simmons, 2008). Moreover, empirical studies that consider effects of selection on the production of variable offspring are rare (Tregenza & Wedell, 1998; Crean & Marshall, 2009).

Here, we investigate the effects of polyandry on male phenotypic traits, and specifically ask if polyandry leads to directional and/or diversifying sexual selection.

A consequence of polyandry is that the sperm of two, or more, males compete for fertilization. Post-mating selection mechanisms, such as female cryptic choice (Eberhard, 1996) and/or sperm competition (Parker, 1970; 1998), may therefore bias fertilization towards the sperm of males with certain phenotypic traits. As a consequence of this, there are three possible outcomes of polyandrous mating: (i) males with the “best” and/or preferred phenotype sire the offspring in every brood, (ii) sperm from a single male fertilize the eggs in every brood, but different phenotypes are selected at each different brood and (iii) post-mating selection favours multiple paternity of broods. In reality, these three outcomes span a continuum of possibilities; it is possible, for example, to have a best phenotype being favoured overall, with multiple paternity broods occurring where the preferred phenotype fathers the majority but not all of the offspring. For heritable male phenotypic traits, these different outcomes correspond to different types of

Correspondence: Miguel Barbosa, Gatty Marine Laboratory, University of St Andrews, East Sands, KY16 8LB Fife, UK.
Tel.: +44 (0) 1334 463465; fax: +44 (0) 1334 463443;
e-mail: Miguel.Barbosa2@jcu.edu.au

sexual selection pressures, leading to different phenotypic distributions at the family (brood) and population scales.

The first outcome corresponds to either directional or stabilizing selection. For this outcome to evolve, there must be significant additive genetic quality in the selected phenotype, which is inherited by the offspring (good/sexu sperm hypotheses (Weatherhead & Robertson, 1979; Keller & Reeve, 1995; Kirkpatrick, 1996). One way in which this may arise is if females use the increased variance in the pool of potential mates to become choosy and trade up on the male phenotype that increases females and/or offspring fitness (Halliday, 1983). Under this specific phenotypic outcome, we expect polyandry to promote the prevalence of the selected phenotype among the population of males.

The second possible outcome is an intermediate scenario, where there is directional selection of a particular phenotype at the family level, but with a diversifying result at the population level. Under this second outcome, at each mating event (family level – brood), a particular phenotype is favoured over the others. However, because at each different mating event a different male phenotype is selected, the result is maintenance of multiple phenotypes at the population scale. In contrast to the first proposed outcome, this second outcome is likely to evolve because of nonadditive genetic benefits (Reid, 2007). Evolutionary benefits can arise, for example if post-mating mechanisms select against inbreeding and reduced heterozygosity, which are negatively correlated with fitness (Keller & Walker, 2002; Kussell & Leibler, 2005). One possible consequence of this outcome is the selection of the most different/rarer phenotypes in the population (Zeh & Zeh, 1996; Cornell & Tregenza, 2007; Rubenstein, 2007). Under this second outcome, we expect polyandry to lead to males with specific traits being favoured at the family scale, but the identity of the favoured traits to vary among mating events (population scale).

Finally, the third proposed outcome corresponds to diversifying selection at both the family and population scales (i.e. mixed paternity broods). This idea is known as the phenotypic/genetic diversity hypothesis (Yasui, 1998). Promoting offspring diversity can be advantageous for two reasons. First, it is a risk-spreading strategy: according to the portfolio effect (Tilman *et al.*, 1998), diversifying investment maximizes long-term returns by reducing losses. Popular knowledge warns against putting all one's eggs in the same basket. That is, if the success of each phenotype is variable in time and space, having offspring of variable phenotype is expected to maximize fitness (Marshall *et al.*, 2008). Secondly, having offspring with different phenotypes reduces competition among siblings (Marshall & Keough, 2009), or enhances population productivity by increasing functional diversity (Mattila & Seeley, 2007) thus maximizing total offspring fitness. Under this outcome, we expect

polyandry to promote phenotypic diversity at both the population and family scales.

Here, we use the Trinidadian guppy (*Poecilia reticulata*) (Peters) to examine the effects of polyandry on the selection of male phenotypic traits. We compare sexual traits and phenotypic diversity at the family and population scales, in fathers and sons, and for two mating treatments: monandry vs. polyandry. We combine multiple sexual traits into a single variable by calculating similarities among individuals and measure phenotypic diversity as the dispersion of individuals in multivariate space. We compare phenotypic diversities across generations and mating treatments and discuss our results in the light of the outcomes described earlier.

Guppies live in a resource-free mating system where polyandry is extremely common, and one in which females exhibit strong sexual preference for complex, bright and variable male colour patterns (Endler, 1980; Houde & Endler, 1990). Male guppies are highly polymorphic, displaying three major colour components: carotenoids (orange, red and yellow), melanin (black) pigments and structural colours (blue and iridescent) (Endler, 1980, 1983). Although saturation and brightness of carotenoid pigments are phenotypically plastic (Grether *et al.*, 2001), their size, shape and number are thought to be genetically determined (Endler, 1983; Kodric-Brown, 1989). Lastly, individual colour pattern is highly heritable and Y-linked (Winge, 1927; Haskins *et al.*, 1961).

Previous studies have shown that polyandry enables directional selection on male traits. In a polyandrous context, males with the preferred behavioural and colour traits are favoured (Evans *et al.*, 2003; Pitcher *et al.*, 2003; Pilastro *et al.*, 2004). For example, there is evidence that orange spots are indicative of male quality (van Oosterhout *et al.*, 2003). They are also known to affect offspring performance (Evans *et al.*, 2004a) and to be heritable (Houde, 1992; Brooks & Endler, 2001a). These facts lend support to the hypothesis that polyandry enables directional selection. However, both female choice and sperm competition are highly variable in this species (Brooks & Couldridge, 1999; Brooks & Endler, 2001b). Therefore, multiple male phenotypes may be simultaneously under selection. Post-mating selection is expected to reflect such variability. Overall, although directional selection has featured more prominently in the literature, the three outcomes have some support in this species.

Material and methods

Experimental design

Two hundred and eighty individuals ($n_{\text{females}} = 80$, $n_{\text{males}} = 200$) from a population collected from Trinidad's Lower Tacarigua River were raised in individual tanks from birth until sexual maturation. Mature females and males were then allocated to two communal tanks. At the beginning of the experiment, we characterized the sexual

behaviour of each male. To do this, we quantified the number of sigmoid displays and gonopodial thrusts, over a 10-min period, exhibited by the male when he was placed in a single tank with size-matched females (these females were not included in the treatments). Following this, each male was photographed on both sides to assess the proportion of each main colour components (black, orange/red, green/bronze and blue). After measuring the proportion of each colour component, each male was allocated to an individual tank ($n_{\text{tank males}} = 200$), where it remained until the start of the experiment.

Females (mothers) and males (fathers) were then haphazardly allocated to either a monandrous ($n_{\text{mothers}} = 40$, $n_{\text{fathers}} = 40$) or a polyandrous ($n_{\text{mothers}} = 40$, $n_{\text{fathers}} = 160$) mating treatment. We chose not to focus on female preference, but instead presented females with a random sample of males. Our experimental approach aimed to mimic chance encounters with males, while controlling for potential confounding effects linked to sexual harassment, which are known to strongly influence mating in this species (Magurran, 2001). We adopted a similar experimental design to that used by Tregenza & Wedell (1998), in which the mating frequency remained constant between mating treatments whereas the number of mates varied. Accordingly, in the monandrous treatment, each female was allowed to mate with the same male for four consecutive days, whereas in the polyandrous treatment a new male was introduced to the female each day, for four consecutive days. In both mating treatments, males were introduced to females at 0700 and removed at 1700. The level of disturbance was, therefore, identical for both mating treatments. At the end of the fourth day, all males were removed and females were kept individually in their home tank until broods were produced.

All tanks were checked twice a day (morning/afternoon) for offspring. After birth, each offspring was allocated to an individual tank until its sex could be determined. After reaching sexual maturation, we recorded for all male offspring the frequency of sexual behaviour and the proportion of colour components, as described for fathers (see above for details).

Statistical analysis

We began by making one-way comparisons for each phenotypic trait between monandrous and polyandrous offspring using an analysis of variance (ANOVA). Differences in fecundity between mating treatments were then examined using an unpaired *t*-test whereas differences in sex ratio within each mating treatment were analysed using a binomial test. Finally, differences in the number of sons produced at each mating treatment were examined using a *G*-test.

We estimated phenotypic diversity using a multivariate analysis approach. The proportion of each colour component and frequency of sexual behaviour were used to

calculate phenotypic similarities among individuals. We used Gower distance because of its efficiency dealing with variables of different nature, as is our case (Quinn & Keough, 2002). To avoid any variable dominating the distance measured, variables were standardized by dividing by the range, before computing the similarity matrix, thus ensuring that all variables had the same scale (Gower, 1971; Anderson, 2006). Individuals were then mapped into Euclidean multivariate space by implementing a Principal Coordinate Analysis (PCoA). This allowed us to calculate the position of the centroid (the spatial mean) of each group, and the distance of each individual to its group centroid. Phenotypic diversity was estimated for each treatment, scale or generation as the mean distance to the group centroid in multivariate space (Anderson, 2006).

We compared the distances of each individual to its group centroid to test for differences in phenotypic diversity among groups using three tests: an ANOVA, a permutation test and a bootstrap re-sampling test. The permutation test was run because of the inherent problems of ANOVA with the violation of multivariate normality (Legendre & Legendre, 1983). The permutation test uses the same null hypothesis as the ANOVA, that is, differences in phenotypic dispersion between the two groups of individuals are no more different than expected because of random chance at a level of probability of 5%. In the permutation test, the least-squares residuals of the dispersion matrix were randomly re-shuffled 999 times. This generated a frequency distribution for the *F* statistic under the null hypothesis of no difference in dispersion between phenotypes. Results were considered significant if the observed *F* statistic was greater than 95% of this frequency distribution (for $\alpha = 0.05$). The bootstrap re-sampling test aimed to resolve any bias in the estimate of mean dispersion associated to different numbers of individuals in different groups (in particular the four-fold difference in number of fathers in the monandrous vs. polyandrous mating treatments) (see Arnqvist, 1998, for a similar methodology). In this test, we reduced sample size to the maximum that allowed equal sampling size in each comparison (e.g. comparison between fathers $n_{\text{monandrous}} = 40$, $n_{\text{polyandrous}} = 160$, $N_{\text{bootstrapping}} = 40$). We re-sampled individuals with replacement from each treatment, 1000 times, and calculated Gower distances, and dispersions as described earlier. We then calculated and compared mean dispersions for each bootstrap sample. Differences between mean dispersions were considered significant, if consistent in more than 95% of the bootstrap samples (for example if the mean dispersion of one group was greater than the other in more than 95% of the bootstrap samples).

The phenotypic traits studied here are assumed to be inherited (Houde, 1992; Brooks & Endler, 2001a). Offspring phenotypic diversity must, therefore, be considered in the context of the variability of their fathers. In particular, phenotypic diversity is expected to be identical

between fathers and sons in the monandrous treatment (i.e. there is no gene frequency changes). Conversely, because of the greater gene pool in the polyandrous treatment (four putative fathers), differences in phenotypic diversity between putative fathers and sons can be expected if selection biases paternity. If extreme phenotypes are selected against, we should observe a decrease in phenotypic diversity. On the other hand, if selection favours extreme phenotypes in detriment of intermediate phenotypes, then an increase the phenotypic diversity of sons can be expected. To test for phenotypic shifts between generations we compared the similarities between fathers and sons for each of the two treatments using ANOSIM (analysis of similarity). ANOSIM generates an *R*-value that scales between -1 and +1, with zero indicating that the high and low similarities are perfectly mixed. To test for significance, the ranked similarity within and between groups is compared with the similarity that would be generated by chance. Essentially, the samples are randomly assigned to groups 1000 times and *R* calculated for each permutation. The observed value of *R* is then compared against the random distribution to determine whether it is significantly different from that which could occur at random (Clarke, 1993).

We compared phenotypic diversity both between mating treatments (for each generation) and between fathers and sons (for each mating treatment). These comparisons allowed us to test whether differences in phenotypic diversity between treatments were mere reflections of the differences in sampling pool of father phenotypes in the two treatments. Additionally, for the polyandrous treatment, we compared within family phenotypic diversity between fathers and sons, for all families with more than three sons, using a paired *t*-test. We note that it was not possible to do a similar analysis for the monandrous treatment as there is only one father. This comparison reveals whether there is directional selection at the family scale, and therefore whether population-level differences were driven by post-mating selection-maximizing diversity or by differences among families in phenotype favoured. That is, this comparison allowed discriminate between outcomes two and three described earlier.

All analyses were performed in *R* 2.7.2 (R Development Core Team, 2008). In particular the multivariate analyses of dispersion were run using the *vegan* package (Oksanen *et al.*, 2008) in *R*.

Results

Mean phenotypes

Although females allocated to the polyandrous mating treatment produced more offspring in absolute numbers (monandrous treatment = 121; polyandrous treatment = 155), there were no significant differences in mean fecundity between mating treatments (*t*-test;

$t_{79} = 1.42$, $P = 0.15$). Sex ratios within treatments were not significantly different from 1 : 1 (binomial test (95% CI), monandrous mating treatment, $P = 0.50$ (0.43–0.62); polyandrous mating treatment, $P = 0.56$ (0.40–0.57). Differences in the number of sons produced in each mating treatment were nonsignificant (sons: monandrous = 60, polyandrous = 79, *G*-test = 2.35, $P = 0.12$).

There were no significant differences in any of the phenotypic variables measured between fathers allocated to the monandrous and polyandrous treatments (Table 1, Fig. 1). In contrast, sons produced in the polyandrous treatment were significantly different from sons from the monandrous treatment for all measured phenotypic variables, with the exception of frequency of sexual behaviour (Table 2, Fig. 2). Whereas sons produced in the polyandrous mating treatment had a significantly greater proportion of orange/red, green/bronze and blue colour pigments, sons produced in the monandrous treatment had a significantly higher proportion of black spots (Table 2, Fig. 2). These results are reinforced by the analysis of similarity (ANOSIM), which showed a significant dissimilarity in the dispersion of phenotypes between putative fathers and sons in the polyandrous but not in the monandrous mating treatment (ANOSIM, monandrous treatment, $R = 0.014$, $P = 0.241$; polyandrous treatment, $R = 0.165$, $P = 0.001$).

Phenotypic diversity

Results of the ANOVA, permutation and bootstrapping tests for the analysis of phenotype dispersions at the

Table 1 Summary of analysis of variance (ANOVA) to test for differences in mean traits between fathers allocated to the monandrous or to the polyandrous mating treatment. Means and standard deviations values given for each trait. Values considered significant for $P < 0.05$.

Response variable	d.f.	Sum Sq	<i>F</i> value	Pr (> <i>F</i>)
Sexual behaviour (Mean 3.925; SD 4.883)				
Treatment	1	1.50	0.063	0.800
Residuals	198	4744.3		
Black (Mean 5.837; SD 2.632)				
Treatment	1	13.95	2.022	0.156
Residuals	198	1365.09		
Orange/red (Mean 7.914; SD 2.279)				
Treatment	1	12.41	2.403	0.122
Residuals	198	1022.05		
Green/bronze (Mean 3.910; SD 2.018)				
Treatment	1	11.52	2.854	0.092
Residuals	198	798.89		
Blue (Mean 4.593; SD 2.167)				
Treatment	1	13.96	2.999	0.084
Residuals	198	921.31		

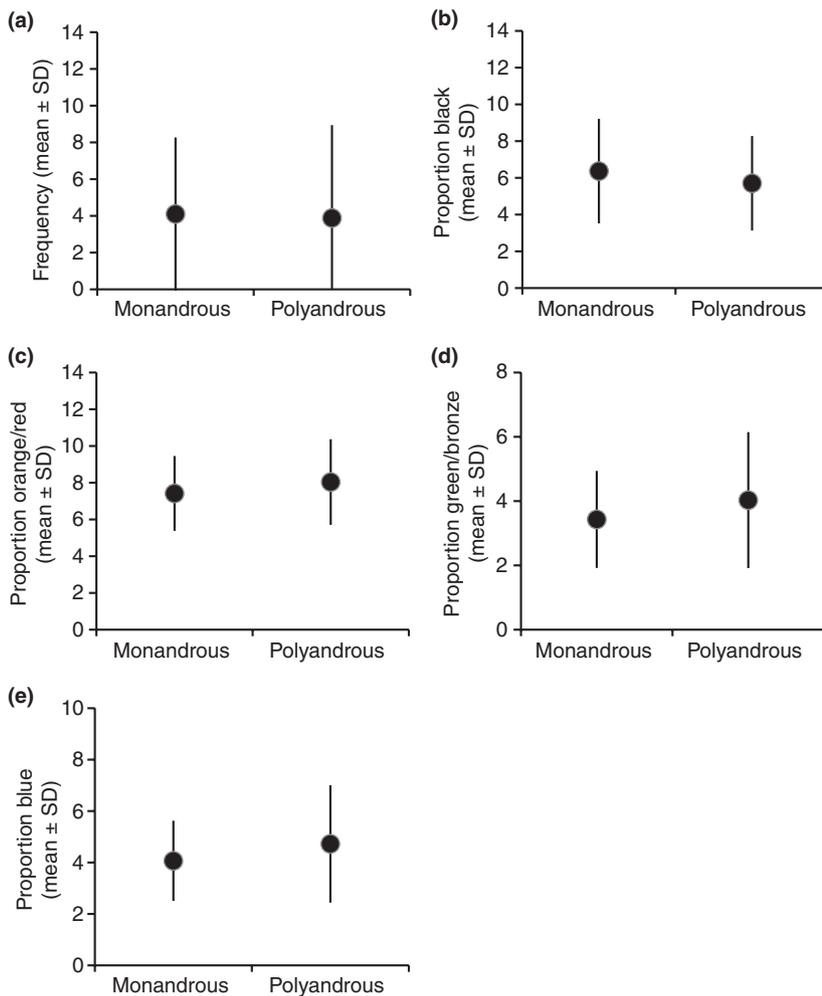


Fig. 1 Differences in mean traits between fathers allocated to the monandrous or to the polyandrous mating treatment. Error bars denote standard deviation. (a) Frequency of sexual behaviour (number of sigmoids and gonopodial thrustings in 10 min); (b) proportion of black; (c) proportion of orange/red; (d) proportion of green/bronze and (e) proportion of blue, colour pigments in both sides of the body.

population level were largely consistent (Table 3). Polyandrous sons were phenotypically more diverse than monandrous sons (Table 3, Figs 3 and S1). This was not a consequence of parent phenotypic diversity because fathers allocated to the monandrous and polyandrous mating treatments had identical phenotypic diversity (Table 3, Figs 3 and S1). In fact, although sons from the polyandrous treatment were phenotypically more diverse than their putative fathers, there was no difference between fathers and sons in the monandrous treatment (Table 3, Figs 3 and S1).

From the initial 40 families allocated to the polyandrous mating treatment ($n_{\text{mothers}} = 40$, $n_{\text{fathers}} = 160$), only 16 produced more than three sons. Differences in the phenotypic dispersion within each family were therefore calculated for these 16 families. A pair-wise paired *t*-test revealed that differences in phenotypic dispersion between sons and putative fathers were nonsignificant (Mean dispersion, \pm SEM; putative fathers = 0.22, \pm 0.006; sons = 0.23, \pm 0.004; *t*-test, $t_{29,06} = 0.26$, $P = 0.80$).

Discussion

Our study focused on the effect of polyandry on the mean and variance of male sexual phenotypes. First, we showed that sons from the polyandrous treatment were more colourful than those from the monandrous treatment. Secondly, this shift was accompanied by an increase in phenotypic diversity. Polyandry can influence the evolution of male phenotypic traits (Andersson & Simmons, 2006). In this study, we found that it can simultaneously act as directional and diversifying selection. Next, we will discuss the implications of the shifts in mean phenotypic traits, and in phenotypic diversity.

Mean phenotypic traits

Polyandry significantly affected the distribution of male traits. All but one trait examined here differed between the monandrous and polyandrous treatments. Sons produced in the polyandrous mating treatment had on average greater areas of orange/red, green/bronze and

Table 2 Summary of analysis of variance (ANOVA) to test for differences in mean traits between sons produced in the monandrous or in the polyandrous mating treatment. Means and standard deviations values given for each trait. Values considered significant for $P < 0.05$. Direction of response for a significant difference indicated.

Response variable	d.f.	Sum Sq	F value	Pr (>F)	Direction of response
Sexual behaviour (Mean 9.581; SD 6.379)					
Treatment	1	79.1	1.957	0.164	
Residuals	137	5536.7			
Black (Mean 6.727; SD 2.842)					
Treatment	1	228.5	35.33	0.001	Monandrous
Residuals	137	886.3			
Orange/red (Mean 7.151; SD 2.679)					
Treatment	1	139.7	22.49	0.001	Polyandrous
Residuals	137	851.2			
Green/bronze (Mean 4.777; SD 2.972)					
Treatment	1	56.07	6.605	0.011	Polyandrous
Residuals	137	1162.8			
Blue (Mean 3.047; SD 1.978)					
Treatment	1	103.4	32.451	0.001	Polyandrous
Residuals	137	436.7			

blue colour pigments, but a smaller area of black spots. Therefore, this first result indicates the existence of post-copulatory mechanisms favouring directional selection of brighter colourations. Male colouration evolves in response to a balance between sexual and natural selection (Endler, 1980; Lande, 1981). In our experiment, natural selection was alleviated (e.g. no predation, abundant food) to focus on the effects of sexual selection. Additionally, female guppies from our study population show strong preferences for males with high levels of bright body pigments (i.e. orange/red, green/bronze and blue) (Evans *et al.*, 2004b). Therefore, our results show that polyandrous females produce sons with colour phenotypes favoured in mate choice.

Polyandry may be beneficial to females through the action of indirect benefits that increase offspring fitness (Jennions & Petrie, 2000). One example of indirect benefits is the production of attractive offspring (Head *et al.*, 2005). In guppies, there is evidence of a direct relationship between the preferred male phenotype and insemination success (Pilastro *et al.*, 2002), and that attractiveness boosts male performance (Evans *et al.*, 2004a; Pilastro *et al.*, 2004). It is therefore plausible to assume that by producing sons with attractive colour patterns, polyandrous females are increasing offspring fitness. Larger offspring with better escape behaviour skills have been suggested as evidence for indirect benefits of polyandry (Evans & Magurran, 2000; Ojanguren *et al.*, 2005). Here, we add the production of offspring with greater areas of bright colouration to this list.

Phenotypes are, however, combinations of all these traits, and trait variables are not completely independent. For example, despite directional selection for bright colouration guppies cannot simultaneously be all orange, all yellow and all blue. Many different combinations of traits can be behind this increase in bright colouration. More importantly, selection affects not only the mean but also the variability of phenotypes. We therefore investigated whether polyandry lead to stabilizing (towards a specific combination of traits) or diversifying (maintaining multiple patterns) selection. Next, we will discuss our results in search of evidence for stabilizing or diversifying selection.

Phenotypic diversity

Sons from the polyandrous treatment were phenotypically more diverse than those from the monandrous treatment. The higher diversity among polyandrous sons was not a mere reflection of the phenotypic diversity of their fathers (i.e. it is not just a sampling effect) (see Arnqvist, 1998, for similar result). In fact, polyandrous sons were phenotypically more diverse than their putative fathers. As expected the same was not observed among fathers and sons in the monandrous mating treatment, as each family had a single possible father. Therefore, we can, with some degree of confidence, reject the hypothesis that polyandry leads to stabilizing selection towards a single male sexual phenotype at the population scale.

The question that arises is whether phenotypic diversity is enhanced because at each mating event fertilization is biased towards different male phenotypes, or as a consequence of brood diversity? Our results showed that within family differences in phenotypic diversity were nonsignificant, suggesting that it is unlikely that stabilizing selection is operating at the family scale. Therefore, within-brood diversity may be contributing towards the maintenance of variability in male sexual traits. This result should, however, be interpreted with caution because of low sample size per brood and the small number of families that had more than three sons.

How can phenotypic diversity increase between generations if sexual characters are genetically controlled? In this case, an increase in diversity can only occur if selection favours extreme, rather than intermediate, phenotypes. Post-mating selection must bias paternity towards phenotypes that are more distant from the centroid. Diversifying selection is in apparent contradiction with some previous work on guppies where paternity was biased towards more orange males (Evans *et al.*, 2003). However, careful examination shows that these two results are not mutually exclusive. We showed here it is possible to have the prevalence of bright colours increase on average and simultaneously increase phenotypic diversity. The former is focused on the mean, whereas the latter is a reflection of the variance. The

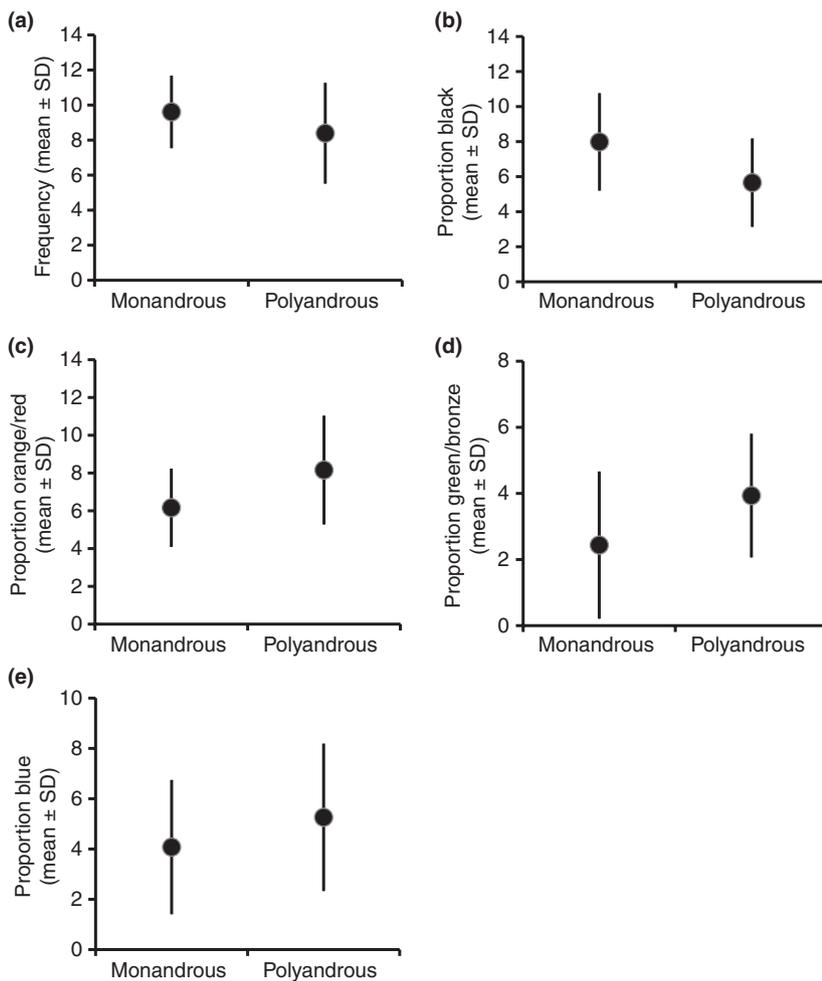


Fig. 2 Differences in mean traits between sons produced in a monandrous and in a polyandrous mating treatment. Error bars denote standard deviation. (a) Frequency of sexual behaviour (number of sigmoids and gonopodial thrustings in 10 min); (b) proportion of black; (c) proportion of orange/red; (d) proportion of green/bronze and (e) proportion of blue, colour pigments in both sides of the body.

shifts in mean phenotype and phenotypic diversity observed suggest an asymmetric bias in paternity translating into the joint action of directional and diversifying selection.

Male sexual traits are those that typically have the highest levels of variability (Pomiankowski & Møller, 1995). The maintenance of this variability through disruptive selection has been shown for numerous different organisms (Sappington & Taylor, 1990; Seehausen & van Alphen, 1999; Barrett, 2002). Our study shows diversifying selection acts to promote variability among male guppies.

The remarkable diversity in colour patterns observed among male guppies has been suggested to co-evolve with differences in female mating preference (Brooks & Coullidge, 1999; Brooks, 2002). Colour perception is highly variable among female guppies (Smith *et al.*, 2002; Hoffmann *et al.*, 2007), and therefore preference based on colour should vary accordingly. Furthermore, there is evidence that broods produced by familiar individuals are significantly smaller than broods produced by unfamiliar ones (Pitcher *et al.*, 2008). Therefore, differences among

females are known to affect sexual selection in this species. However, even in studies where females seem to bias paternity towards a specific male trait, the majority of broods are of mixed paternity (Pitcher *et al.*, 2003).

Implications

Theoretical models of sexual selection traditionally predict that sexual selection drives populations towards a single optimal male phenotype, if species occupy homogeneous environments and both female choice and sperm competition are in unison (Maynard-Smith, 1991). Under these assumptions, polyandry may decrease phenotypic variability because the “best” and/or preferred phenotype is always favoured (i.e. when there is a choice the best phenotype always wins). However, variability in male sexual traits can be maintained under temporally fluctuating selection, provided that generations overlap (Ellner & Hairston, 1994). Temporal fluctuation in both female choice and sperm competition are common in guppies (Brooks & Coullidge, 1999; Brooks & Endler, 2001b), as are overlapping generations. These

Table 3 Analysis of variance (ANOVA), permutation and bootstrapping tests results for the comparison of phenotypic dispersion between and within treatments. The internal line divides comparisons. Above the line, the comparison of phenotypic dispersion between treatments (monandrous vs. polyandrous) for sons and fathers, respectively, are shown. Below the line, we show the results for the comparison of phenotypic dispersion between fathers vs. sons within each mating treatment. Mean distance of each individual to its group centroid is given. SM/SP, sons produced, respectively, in the monandrous/polyandrous mating treatment; FM/FP, fathers allocated, respectively, to the monandrous/polyandrous mating treatment. Direction of response for a significant difference indicated. For the bootstrapping test, differences between mean dispersions were considered significant, if the mean dispersion of one group was greater than the other in more than 95% of the bootstrap samples. Values considered significant for $P < 0.05$.

Comparison	Mean distance to centroid		ANOVA				Permutation	Bootstrapping	
			d.f.	Sum Sq	F-value	Pr (>F)	(n 999)	(n 1000)	P
Sons	Monandrous vs. polyandrous	SM 0.144	Group	1	0.020	6.432	0.0123	0.011	0.013
		SP 0.171	Residuals	137	0.426				
Fathers	Monandrous vs. polyandrous	FM 0.153	Group	1	0.004	2.791	0.096	0.095	0.065
		FP 0.140	Residuals	198	0.340				
Fathers vs. sons	Polyandrous vs. polyandrous	FP 0.129	Group	1	0.030	14.36	0.0002	0.0003	<0.001
Fathers vs. sons	Monandrous vs. monandrous	SP 0.155	Residuals	237	0.499				
		FM 0.152	Group	1	0.005	1.380	0.242	0.228	0.748
		SM 0.148	Residuals	98	0.385				

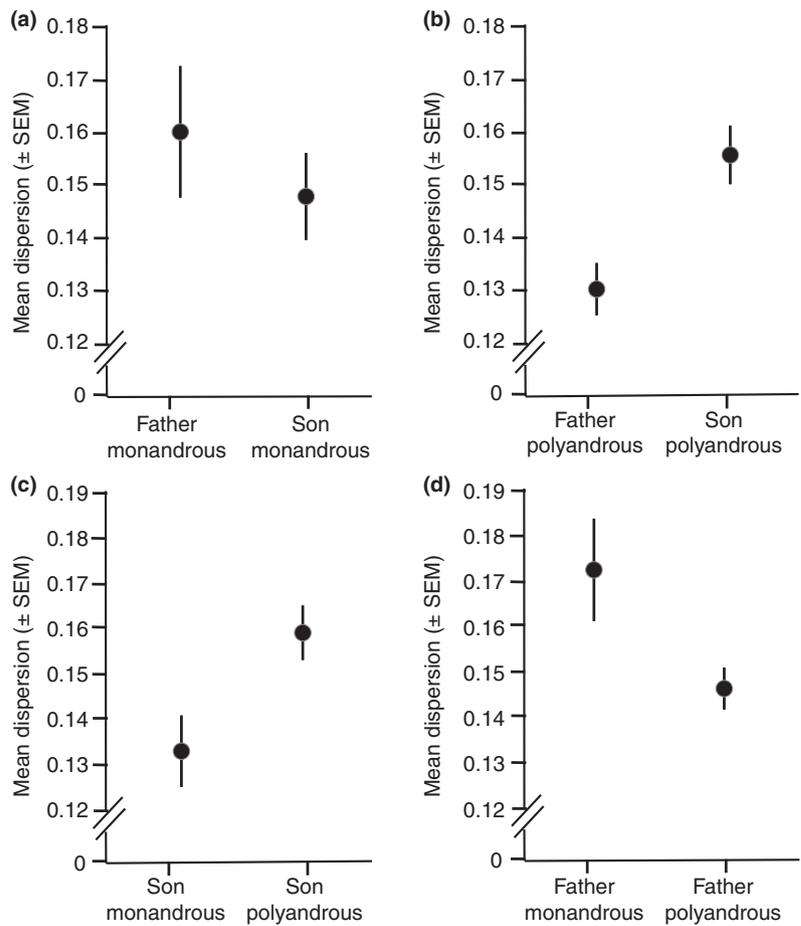


Fig. 3 Comparison of multivariate dispersion in the phenotypes at population level for fathers vs. sons within mating treatments; (a) for monandrous and (b) for polyandrous mating treatment. Also between (c) sons produced in the two mating treatments and (d) fathers allocated to the two mating treatments. Error bars represent standard error of the mean.

conditions contribute to make maximizing offspring diversity selectively advantageous.

Diversifying offspring phenotypes may be adaptive through two mechanisms: (i) by reducing the probability of reproductive failure and (ii) by reducing competition among offspring. Female fitness is the sum of the fitness of all their offspring, and therefore fitness of individuals in each of the two generations is not necessarily correlated (Marshall & Uller, 2007). Even if there is a single optimal phenotype for any set of conditions, female fitness may be maximized by having offspring with a variety of phenotypes if conditions are not constant (Marshall *et al.*, 2008). The benefits of bet hedging are enhanced in highly stochastic systems where changes in environmental and ecological variables occur on small and unpredictable temporal scales (Fox & Rauter, 2003). Although we compared the fitness in the offspring of different treatments, it is reasonable to assume given the unpredictable nature of the environment in which this species lives, that broods with higher phenotypic diversity will have greater fitness.

Apart from a bet-hedging adaptation to changing environments, phenotypic diversity can also boost offspring fitness via the reduction of competition among brothers (Sherman, 1988). For example, male offspring that are phenotypically different from another within the same brood are more likely to mate successfully than brood members that share identical phenotypes. In our particular case, female mate choice is variable, and some females find different and novel male phenotypes more attractive (Hughes *et al.*, 1999; Kelley *et al.*, 1999; Eakley & Houde, 2004). Furthermore, male guppies with uncommon and unfamiliar phenotypic patterns have been found to achieve greater mating success (Farr, 1977). Variability in female mating preference means that a female can potentially increase her fitness by having diverse sons.

In conclusion, we emphasize the following points. First, polyandry results in the production of male offspring with brighter colouration, namely with increased areas of orange/red, green/bronze and blue. This result clearly indicates that polyandry enables directional selection for preferred male sexual patterns. Second, we show that polyandry increases phenotypic diversity in male sexual traits. In combination, these two results provide evidence for sexual selection driving the diversification of bright colouration patterns in male guppies. Furthermore, diversification may be advantageous in coping with environmental and reproductive variability by increasing long-term fitness.

Acknowledgments

This study was supported by a PhD fellowship to MB by Fundação para a Ciência e a Tecnologia (FCT), Portugal. MD thanks the Australian Research Council for funding. We also thank Dr. Andrew Hendry for comments on

early drafts. Additionally, we are grateful to Rhonda Snook and Michael Jennions as well as an anonymous reviewer for providing comments that greatly improved this manuscript. All experiments were carried out at the Gatty Marine Laboratory, University of St Andrews and complied with current UK animal welfare and health and safety regulations.

References

- Anderson, M.J. 2006. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* **62**: 245–253.
- Andersson, M. & Simmons, L.W. 2006. Sexual selection and mate choice. *Trends Ecol. Evol.* **21**: 296–302.
- Arnqvist, G. 1998. Comparative evidence for the evolution of genitalia by sexual selection. *Nature* **393**: 784–786.
- Barrett, S.C.H. 2002. The evolution of plant sexual diversity. *Nature Rev. Genet.* **3**: 274–284.
- Birkhead, T.R. & Møller, A.P. 1998. *Sperm Competition and Sexual Selection*. Academic Press, London.
- Birkhead, T.R. & Pizzari, T. 2002. Postcopulatory sexual selection. *Nature Rev. Genet.* **3**: 262–273.
- Brooks, R. 2002. Variation in female mate choice within guppy populations: population divergence, multiple ornaments and the maintenance of polymorphism. *Genetica* **116**: 343–358.
- Brooks, R. & Coullidge, V. 1999. Multiple sexual ornaments co-evolve with multiple mating preferences. *Am. Nat.* **154**: 37–45.
- 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.
- Clarke, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. *Aust. J. Ecol.* **18**: 117–143.
- Cornell, S.J. & Tregenza, T. 2007. A new theory for the evolution of polyandry as a means of inbreeding avoidance. *Proc. R. Soc. B* **274**: 2873–2879.
- Crean, A.J. & Marshall, D.J. 2009. Coping with environmental uncertainty: dynamic bet hedging as a maternal effect. *Phil. Trans. R. Soc. Lond. B* **364**: 1087–1096.
- Eakley, A.L. & Houde, A.E. 2004. Possible role of female discrimination against 'redundant' males in the evolution of colour pattern polymorphism in guppies. *Biol. Lett.* **271**: S299–S301.
- Eberhard, W.G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press, NJ.
- Ellner, S. & Hairston, N.G. Jr 1994. Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *Am. Nat.* **143**: 403–417.
- Endler, J.A. 1980. Natural selection on colour patterns in *Poecilia reticulata*. *Evolution* **34**: 76–91.
- Endler, J.A. 1983. Natural and Sexual selection on color patterns in poeciliid fishes. *Environ. Biol. Fish* **9**: 173–190.
- Evans, J.P. & Magurran, A.E. 2000. Multiple benefits of multiple mating in guppies. *PNAS* **97**: 10074–10076.
- Evans, J.P. & Simmons, L.W. 2008. The genetic basis of traits regulating sperm competition and polyandry: can selection favour the evolution of good- and sexy sperm? *Genetica* **134**: 5–19.

- 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., Kelley, J.L., Bisazza, A., Finazzo, E. & Pilastro, A. 2004a. Sire attractiveness influences offspring performance in guppies. *Proc. R. Soc. B* **271**: 2035–2042.
- Evans, J.P., Bisazza, A. & Pilastro, A. 2004b. Female mating preferences for colourful males in a population of guppies subject to high predation. *J. Fish Biol.* **65**: 1154–1159.
- Farr, J.A. 1977. Male rarity or novelty, female choice behavior, and sexual selection in the guppy, *Poecilia reticulata*, Peters (Pisces: Poeciliidae). *Evolution* **31**: 162–168.
- Fox, C.W. & Rauter, C.M. 2003. Bet-hedging and the evolution of multiple mating. *Evol. Ecol. Res.* **5**: 273–286.
- Gower, J.C. 1971. A General Coefficient of Similarity and Some of Its Properties. *Biometrics*. **27**: 857–871.
- Grether, G.F., Hudon, J. & Endler, J.A. 2001. Carotenoid scarcity, synthetic pteridine pigments and the evolution of sexual coloration in guppies (*Poecilia reticulata*). *Proc. R. Soc. B* **268**: 1245–1253.
- Halliday, T.R. 1983. The study of mate choice. In: *Mate Choice*, Vol. 1 (P. Bateson, ed), pp. 462. Cambridge Univ. Press, Cambridge.
- Haskins, C.P., Haskins, E.F., McLaughlin, J.J. & Hewitt, R.E. 1961. Polymorphism and population structure in *Lebistes reticulatus*, a population study. In: *Vertebrate Speciation* (W.F. Blair, ed), pp. 320–395. University of Texas Press, Texas.
- Head, M.L., Hunt, J., Jennions, M.D. & Brooks, R. 2005. The indirect benefits of mating with attractive males outweigh the direct costs. *PLoS Biol.* **3**: 289–294.
- Hoffmann, M., Tripathi, N., Henz, S.R., Lindholm, A.K., Weigel, D., Breden, F. & Dreyer, C. 2007. Opsin gene duplication and diversification in the guppy, a model for sexual selection. *Proc. R. Soc. B* **274**: 33–42.
- 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. & Endler, J.A. 1990. Correlated evolution of female mating preferences and male colour patterns in the guppy *Poecilia reticulata*. *Science* **248**: 1405–1408.
- Hughes, K.A., Du, L., Rodd, F.H. & Reznick, D.N. 1999. Familiarity leads to female mate preference for novel males in the guppy *Poecilia reticulata*. *Anim. Behav.* **58**: 907–916.
- Jennions, M.D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biol. Rev.* **75**: 21–64.
- Keller, L. & Reeve, H.K. 1995. Why females mate with multiple males? The sexuality selected sperm hypothesis. *Adv. Stud. Behav.* **24**: 291–315.
- Keller, L.F. & Walker, D.M. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**: 230–241.
- Kelley, J.L., Graves, J.A. & Magurran, A.E. 1999. Familiarity breeds contempt in guppies. *Nature* **401**: 661–662.
- Kirkpatrick, M. 1996. Good genes and direct selection in evolution of mating preferences. *Evolution* **50**: 2125–2140.
- Kodric-Brown, A. 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behav. Ecol. Sociobiol.* **25**: 393–401.
- Kussell, E. & Leibler, S. 2005. Phenotypic diversity, population growth, and information in fluctuating environments. *Science* **309**: 2075–2078.
- Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *PNAS* **78**: 3721–3725.
- Legendre, L. & Legendre, P. 1983. *Numerical Ecology*. Elsevier Science, Amsterdam.
- Magurran, A.E. 2001. Sexual conflict and evolution in Trinidadian guppies. *Genetica* **112**: 463–474.
- Marshall, D.J. & Keough, M.J. 2009. Does Interspecific competition affect offspring provisioning? *Ecology* **90**: 487–495.
- Marshall, D.J. & Uller, T. 2007. When is a maternal effect adaptive? *Oikos* **116**: 1957–1963.
- Marshall, D.J., Bonduriansky, R. & Bussiere, L.F. 2008. Offspring size as a maternal bet-hedging strategy in unpredictable environments. *Ecology* **89**: 2506–2517.
- Mattila, H.R. & Seeley, T.D. 2007. Genetic diversity in honey bee colonies enhances productivity and fitness. *Science* **317**: 362–364.
- Maynard-Smith, J. 1991. Models of female choice. *Trends Ecol. Evol.* **6**: 146–151.
- Ojanguren, A.F., Evans, J.P. & Magurran, A.E. 2005. Multiple mating influences offspring size in guppies. *J. Fish Biol.* **67**: 1184–1188.
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L. & Stevens, M.H.H. 2008. in: *R Package Version*. Community Ecology Package, Vegan.
- 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 loses its spots. *J. Evol. Biol.* **16**: 273–281.
- Parker, G.A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**: 525–567.
- Parker, G.A. 1998. Sperm competition and the evolution of ejaculates: towards a theory base. In: *Sperm Competition and Sexual Selection* (T. Birkhead & A. Møller, eds), pp. 3–54. Academic Press, San Diego.
- Pilastro, A., Evans, J.P., Sartorelli, S. & Bisazza, A. 2002. Male phenotype predicts insemination success in guppies. *Proc. R. Soc. B* **269**: 1325–1330.
- Pilastro, A., Simonato, M., Bisazza, A. & Evans, J.P. 2004. Cryptic female preference for colorful males in guppies. *Evolution* **58**: 665–669.
- 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. B* **270**: 1623–1629.
- Pitcher, T.E., Rodd, H.F. & Rowe, L. 2008. Female choice and the relatedness of mates in the guppy (*Poecilia reticulata*). Mate choice and inbreeding depression. *Genetica* **134**: 137–146.
- Pomiankowski, A. & Møller, A.P. 1995. A resolution of the lek paradox. *Proc. R. Soc. B* **260**: 21–29.
- Quinn, G.P. & Keough, M.J. 2002. *Experimental Design and Data Analysis for Biologists*, 1st edn. Cambridge University Press, Cambridge.
- R Development Core Team. 2008. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
- Reid, J.M. 2007. Secondary sexual ornamentation and non-additive genetic benefits of female mate choice. *Proc. R. Soc. B* **274**: 1395–1402.
- Rubenstein, D.R. 2007. Female extrapair mate choice in a cooperative breeder: trading sex for help and increasing offspring heterozygosity. *Proc. R. Soc. B* **274**: 1895–1903.
- Sappington, T.W. & Taylor, O.R. 1990. Disruptive sexual selection in *Colias eurytheme* butterflies (pheromone variation/aflanes/courtship). *PNAS* **87**: 6132–6134.

- Seehausen, O. & van Alphen, J.J.M. 1999. Can sympatric speciation by disruptive sexual selection explain rapid evolution of cichlid diversity in Lake Victoria? *Ecol. Lett.* **2**: 262–271.
- Sherman, P.W. 1988. Parasites, pathogens, and polyandry in social hymenoptera. *Am. Nat.* **131**: 602–610.
- Simmons, L.W. 2005. The evolution of polyandry: sperm competition, sperm selection, and offspring viability. *Annu. Rev. Ecol. Evol. Syst.* **36**: 125–146.
- Smith, E.J., Partridge, J.C., Parsons, K.N., White, E.M., Cuthill, I.C., Bennett, A.T.D. & Church, S.C. 2002. Ultraviolet vision and mate choice in the guppy (*Poecilia reticulata*). *Behav. Ecol.* **13**: 11–19.
- Tilman, D., Lehman, C.L. & Bristow, C.E. 1998. Diversity-Stability Relationships: statistical inevitability or ecological consequence? *Am. Nat.* **151**: 277–292.
- Tregenza, T. & Wedell, N. 1998. Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evolution* **52**: 1726–1730.
- Weatherhead, P.J. & Robertson, R.J. 1979. Offspring quality and the polygyny threshold – Sexy Son hypothesis. *Am. Nat.* **113**: 201–208.
- Winge, O. 1927. The location of eighteen genes in *Lebistes reticulatus*. *J. Genet.* **18**: 1–43.
- Yasui, Y. 1998. The ‘genetic benefits’ of female multiple mating reconsidered. *Trends Ecol. Evol.* **13**: 246–250.
- Zeh, J.A. & Zeh, D.W. 1996. Evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proc. R. Soc. B* **263**: 1711–1717.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Ordination plots showing the multivariate dispersion in the phenotypes at population level for (a) sons produced in the monandrous (red) vs. polyandrous mating treatment (green), (b) fathers allocated to the monandrous (red) vs. allocated to the polyandrous mating treatment (green), (c) fathers (red) vs. sons in the polyandrous mating treatment (green) and d) fathers (red) vs. sons in the monandrous mating treatment (green).

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

Received 18 January 2010; revised 22 July 2010; accepted 3 August 2010