LETTER

doi:10.1038/nature12717

Mating advantage for rare males in wild guppy populations

Kimberly A. Hughes[1], Anne E. Houde[2], Anna C. Price[3] & F. Helen Rodd[3]

To understand the processes that maintain genetic diversity is a long-standing challenge in evolutionary biology, with implications for predicting disease resistance, response to environmental change, and population persistence[1–3]. Simple population genetic models are not sufficient to explain the high levels of genetic diversity sometimes observed in ecologically important traits[2]. In guppies (*Poecilia reticulata*), male colour pattern is both diverse and heritable, and is arguably one of the most extreme examples of morphological polymorphism known[4,5]. Negative frequency-dependent selection (NFDS), a form of selection in which genotypes are favoured when they are rare[6], can potentially maintain such extensive polymorphism, but few experimental studies have confirmed its operation in nature[7,8]. Here we use highly replicated experimental manipulations of natural populations to show that males with rare colour patterns have higher reproductive fitness, demonstrating NFDS mediated by sexual selection. Rare males acquired more mates and sired more offspring compared to common males and, as previously reported, had higher rates of survival[8]. Orange colour, implicated in other studies of sexual selection in guppies, did predict male reproductive success, but only in one of three populations. These data support the hypothesis that NFDS maintains diversity in the colour patterns of male guppies through two selective agents, mates and predators. Similar field-based manipulations of genotype frequencies could provide a powerful approach to reveal the underlying ecological and behavioural mechanisms that maintain genetic and phenotypic diversity.

Populations of organisms exhibit enormous genetic diversity. Explaining this diversity has been challenging, particularly when variation occurs in traits under strong natural selection[1,3]. High genetic variation in ecologically important traits cannot be explained by standard population genetic models that incorporate directional natural selection, genetic drift and mutation[2]. Instead, various kinds of balancing selection, in which genotype fitness varies temporally, spatially, or as a function of genotype frequency, have been proposed to explain this diversity[2,3]. However, the ecological mechanisms that generate balancing selection are generally unknown[3].

The colour patterns displayed by adult male guppies (Fig. 1) are highly heritable, but also highly variable within populations[5,9]. Female mate choice has been proposed to account for this diversity because laboratory studies indicate a strong preference for rare or novel colour patterns[10–12]. If this preference occurs in nature, then it would result in NFDS on male colouration and promote variation. However, it can be difficult to detect NFDS in nature because the equilibrium frequencies of different phenotypes under this form of selection are those at which fitnesses are equal[13]. Consequently, a rare-male advantage will be detectable only when phenotype frequencies are perturbed from their equilibrium values.

To determine whether male guppies with rare colour patterns have a reproductive advantage in nature, we conducted 17 separate manipulations in 3 different populations in Trinidad[8]. Over two field seasons, we manipulated frequencies of naturally occurring colour patterns within replicate pools in the Mausica River (5 replicates) and in two separate tributaries of the Quare River (Quare River 1 (6 replicates); and Quare River 7 (6 replicates)). At each site, we sorted males into groups that were nearly equal in abundance, based on caudal fin colour, as 'uncoloured' (>75% of the caudal fin transparent) or 'coloured' (>50% of fin coloured); males with intermediate colouration were excluded from the experiment. Males were then re-introduced into pools in a ratio of 3:1 (in which 1 is 'rare'), with each morph being rare in half of the pools. After 16 to 17 days, depending on the site, all adults were collected, separated by sex, and identified by pool-specific tattoos and pre- and post-release photographs. Adult females ($n = 193$) were taken to the laboratory, where we collected their first two broods (2 to 24 offspring per female, mean = 7.6). We genotyped these females, 166 experimental males, 693 first-brood and 777 second-brood offspring at 9 to 14 variable microsatellite loci[14,15]. To avoid confounding reproductive success with differential survival, only experimental males that survived to the end of the experiment, and that did not move between pools during the experiment were considered as candidate fathers. Using a conservative 95% confidence level for paternity assignment[16], the number of offspring assigned to candidate fathers ranged from 0 to 12, and mating success (the number of females with which a male produced at least one offspring) ranged from 0 to 8 females (Supplementary Table 1).

Among first-brood offspring, males bearing rare colour patterns had more than twice as many mates and offspring as males bearing common patterns, based on the paternity assignment (Fig. 2a). Other experimental factors such as population, morph (the particular colour pattern chosen to be rare or common within a replicate), and population-by-morph interaction did not significantly affect the number of mates or offspring (Extended Data Table 1). Males with rare colour patterns thus had a reproductive advantage over those with common patterns, and this advantage did not depend on population or on the specific colour pattern that was rare or common in a given replicate (Extended Data Fig. 1). When we relaxed the criterion for paternity

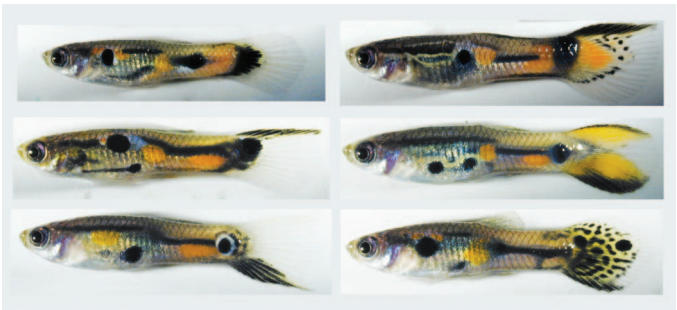


Figure 1 | Colour pattern variation among males from a single population. Male offspring of Quare River 7 tributary fish, reared in a common environment and showing heritable colour-pattern variation. Males on the left have a caudal fin that is representative of the 'uncoloured' group, those on right are have a caudal fin that is representative of the 'coloured' group. Criteria for classification are described in ref. 9.

[1]Department of Biological Science, 319 Stadium Drive, Florida State University, Tallahassee, Florida 32306, USA. [2]Department of Biology, Lake Forest College, 555 North Sheridan Road, Lake Forest, Illinois 60045, USA. [3]Department of Ecology and Evolutionary Biology, University of Toronto, 25 Harbord Street, Toronto, Ontario M5S 3G5, Canada.

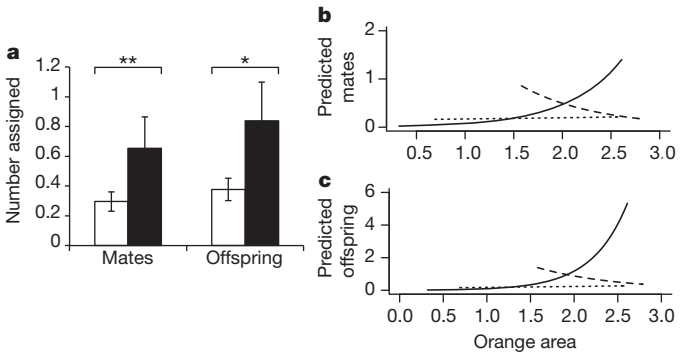


Figure 2 | Rare colour patterns have higher mating and reproductive success. a, Number of mates and offspring assigned to common (white, $n = 124$) or rare (dark grey, $n = 42$) males. Centre values are marginal means from the full generalized linear mixed model; bars indicate s.e.m. adjusted for model covariance parameters. $^{**}P < 0.005$ and $^{*}P = 0.01$, respectively; see Extended Data Table 1. **b**, **c**, Association between square-root orange area and reproductive success in Quare River 7 (solid line, $n = 34$), Quare River 1 (dashed line, $n = 72$) and Mausica River (dotted line, $n = 60$), predicted from the full model. **b**, Predicted mates. **c**, Predicted offspring; see Extended Data Table 2. Minimum and maximum values along abcissa indicate range of values recorded.

confidence to >85%, the effects of all experimental factors were similar to the original analysis (Extended Data Table 2). The results are therefore robust to the specific criterion for paternity assignment.

We also examined effects of variation in male size (total body area), and colour (area of orange and black body colouration, Supplementary Table 1) because these traits have been implicated in previous studies of sexual selection in guppies[4,17]. In generalized linear mixed models (GLMMs, see Methods), main effects of variation in these traits were not significant, but the population-by-orange area interaction was associated with both mate and offspring number (Extended Data Table 1). In both cases, the significant interaction was due to a positive association between reproductive success and orange area that was unique to the Quare River 7 population; the association was negative in Quare River 1, and close to zero in the Mausica River population (Fig. 2b, c and Extended Data Table 3). This population dependence is consistent with laboratory studies that document variation in the existence and strength of female preference for orange among natural guppy populations[4]. Again, these patterns were robust when we relaxed the criterion for paternity assignment (Extended Data Table 2).

Female guppies give birth to live offspring every 27 to 30 days and will fertilize eggs with stored sperm if they have not mated recently[4,17]. To distinguish the effects of recent insemination from long-term sperm storage, we separately analysed the second broods produced by females following the field experiment. These broods were produced at least 47 days after the end of the field experiment, and a full reproductive cycle after the first broods; they were therefore fertilized by sperm stored for at least 47 days because guppies do not carry embryos at multiple stages of development[18]. For these second broods, several aspects of male colouration, but not rarity, significantly influenced male reproductive success (Extended Data Table 4). Males with more highly coloured caudal fins had more assigned mates and offspring than those with little colour, while the number of orange spots on the body was negatively related to mate number (GLMM; $\beta = -1.0 \pm 0.5$, $\chi^2 = 4.83$, d.f. = 1, $P = 0.03$), and non-significantly related to offspring number (GLMM; $\beta = -1.3. \pm 0.6$, $\chi^2 = 3.53$, d.f. = 1, $P = 0.06$). As in first broods, the area of orange body colour interacted significantly with population to predict male reproductive success. Again, the interaction occurred because orange was positively associated with reproductive success in Quare River 7, but not in the other two populations (Extended Data Table 5).

These results for second broods are consistent with laboratory data indicating that male colour can predict sperm competitive ability and/or cryptic female choice[19]. Nevertheless, if females mate at least once per reproductive cycle in nature, the first-brood data should better reflect male success because these data capture the effects of recent matings and short-term sperm storage. Two lines of evidence indicate that females do mate at least once per cycle: first, a high proportion of wild females have recently-deposited sperm in their gonoducts[20,21]; and second, all post-partum females re-mate within 48 h in laboratory studies[10,22]. These data suggest that nearly all females mate at least once (and probably more than once) per cycle.

Our results support the prediction of the 'rare-male effect' hypothesis that males with phenotypes that are rare in the local environment have a reproductive advantage over males with common phenotypes[23]. It is possible that we observed unusually strong NFDS because we perturbed morph frequencies in our experiment, and thereby made it more difficult to detect selection on other aspects of male phenotypes. However, it is precisely this dependence of selection on morph frequency that maintains variation. Our results indicate that if colour pattern frequencies deviate sufficiently from their equilibrium values, NFDS will dominate the evolutionary dynamics.

Although we cannot be certain that the rare-male advantage we observed was caused by female mate preference, abundant evidence from laboratory studies indicates that females prefer males with rare phenotypes[11,12,24]. Moreover, female preference is more important than male–male competition in determining mating outcomes in this species[4,17]. These data indicate that rare-male advantage in the field is likely to be mediated by female mate preference. In contrast, male–male competition[7,25,26] and sexual conflict[27] have been implicated in the maintenance of discrete sex-limited polymorphism in other species. In each of those cases, the polymorphism involves two or three discrete morphs, compared to the much larger numbers of morphs found within guppy populations. The relative importance of female preference and other kinds of interactions in driving NFDS and in maintaining polymorphism is an intriguing direction for future research.

Despite strong evidence for the rare-male effect in guppies, the evolutionary processes that account for its prevalence are not known. Mate preference for males with unusual colouration might have evolved as a mechanism for inbreeding avoidance[15], as a consequence of generalized neophilia[10], or because females avoid remating with previous mates and also reject males with colouration similar to that of previous mates[12]. It has been previously proposed[28] that a survival advantage to rare morphs, as demonstrated in ref. 8, could also drive the evolution of mate preference for rare phenotypes, even though rarity itself is not heritable.

Understanding whether, and when, balancing selection maintains genetic variation in ecologically important traits is a central challenge for modern evolutionary biology, with profound implications for medical, agricultural and ecological genetics[3]. Manipulative experiments in nature have demonstrated balancing selection in guppies[8] and other species[7,29]. In guppies, these experiments have been powerful enough to reveal multiple ecological mechanisms that contribute to balancing selection and to reveal directional and balancing selection that act on the same phenotypes. We suggest that manipulations in natural populations will be useful for discovering the ecological processes that maintain other polymorphisms including those, like the vertebrate major histocompatibility complex, that have been refractory to other approaches[30].

METHODS SUMMARY

At each site, we used pools of similar size, structure, substrate and water clarity to form replicate experimental pools[8]. After collecting all adult guppies from each experimental pool, all fish were photographed and given pool-specific elastomer tattoos before being used in the experiment. We also kept density and sex ratio close to natural levels. All procedures complied with animal care standards of the Canadian Council on Animal Care and were approved by University of Toronto's Animal Care Committee.

The candidate fathers were the 166 marked experimental males that survived to the end of the experiment (60 from Mausica River, 72 from Quare River 1, and 34 from Quare River 7). We used CERVUS 3.03 (ref. 16) for paternity assignment. For 135 first-brood offspring, paternity was assigned to an experimental male with >85% confidence; 93 were assigned with >95% confidence (Supplementary Tables 1 and 2). For the rest, the difference in log-likelihoods between the first and second most likely fathers indicated <85% probability that the most likely father was the true father, and these paternity assignments were not used in our analysis. For second broods, 158 offspring were assigned with >85% confidence and 102 with >95% confidence. We fit generalized linear mixed models to counts of assigned mates and offspring, using a negative binomial distribution and Laplace approximation for estimating the marginal likelihood. Pools nested within populations were modelled as random effects. Fixed effects included population, treatment (rare versus common), morph (coloured versus uncoloured caudal fin), body size (area of a two-dimensional image, excluding all fins), body area covered by orange, number of discrete orange and black spots, and all two-way interactions. Area covered by black and three-way interactions were also tested, but never approached significance (all $P > 0.1$) and were not included in the final models.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 10 June; accepted 26 September 2013.
Published online 30 October 2013.

1. Lewontin, R. C. *The Genetic Basis of Evolutionary Change* (Columbia Univ. Press, 1974).
2. Turelli, M. & Barton, N. H. Polygenic variation maintained by balancing selection: pleiotropy, sex-dependent allelic effects and $G \times E$ interactions. *Genetics* **166,** 1053–1079 (2004).
3. Mitchell-Olds, T. Willis, J. H. & Goldstein, D. B. Which evolutionary processes influence natural genetic variation for phenotypic traits? *Nature Rev. Genet.* **8,** 845–856 (2007).
4. Houde, A. E. *Sex, Color and Mate Choice in Guppies* (Princeton Univ. Press, 1997).
5. Brooks, R. & Endler, J. A. Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution* **55,** 1002–1015 (2001).
6. Ayala, F. J. & Campbell, C. A. Frequency-dependent selection. *Annu. Rev. Ecol. Syst.* **5,** 115–138 (1974).
7. Bleay, C., Comendant, T. & Sinervo, B. An experimental test of frequency-dependent selection on male mating strategy in the field. *Proc. R. Soc. B* **274,** 2019–2025 (2007).
8. Olendorf, R. *et al.* Frequency-dependent survival in natural guppy populations. *Nature* **441,** 633–636 (2006).
9. Hughes, K. A., Rodd, F. H. & Reznick, D. N. Genetic and environmental effects on secondary sex traits in guppies (*Poecilia reticulata*). *J. Evol. Biol.* **18,** 35–45 (2005).
10. Hughes, K. A., Du, L., Rodd, F. H. & Reznick, D. N. Familiarity leads to female mate preference for novel males in the guppy, *Poecilia reticulata*. *Anim. Behav.* **58,** 907–916 (1999).
11. Zajitschek, S. R. K. & Brooks, R. C. Distinguishing the effects of familiarity, relatedness, and color pattern rarity on attractiveness and measuring their effects on sexual selection in guppies (*Poecilia reticulata*). *Am. Nat.* **172,** 843–854 (2008).
12. Hampton, K. J., Hughes, K. A. & Houde, A. E. The allure of the distinctive: reduced sexual responsiveness of female guppies to 'redundant' male colour patterns. *Ethology* **115,** 475–481 (2009).
13. Maynard Smith, J. *Evolution and the Theory of Games* (Cambridge Univ. Press, 1982).
14. Olendorf, R., Reudi, B. & Hughes, K. A. Primers for 12 polymorphic microsatellite DNA loci from the guppy *(Poecilia reticulata*). *Mol. Ecol. Notes* **4,** 668–671 (2004).
15. Johnson, A. M. *et al.* Inbreeding depression and inbreeding avoidance in a natural population of guppies (*Poecilia reticulata*). *Ethology* **116,** 448–457 (2010).
16. Kalinowski, S. T., Taper, M. L. & Marshall, T. C. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* **16,** 1099–1106 (2007).
17. Magurran, A. E. *Evolutionary Ecology: The Trinidadian Guppy* (Oxford Univ. Press, 2005).
18. Pires, M. N., Arendt, J. & Reznick, D. N. The evolution of placentas and superfetation in the fish genus *Poecilia* (Cyprinodontiformes: Poeciliidae: subgenera *Micropoecilia* and *Acanthophacelus*). *Biol. J. Linn. Soc.* **99,** 784–796 (2010).
19. Evans, J. P., Zane, L., Francescato, S. & Pilastro, A. Directional postcopulatory sexual selection revealed by artificial insemination. *Nature* **421,** 360–363 (2003).
20. Matthews, I. M. & Magurran, A. E. Evidence for sperm transfer during sneaky mating in wild Trinidadian guppies. *J. Fish Biol.* **56,** 1381–1386 (2000).
21. Evans, J. P., Pilastro, A. & Ramnarine, I. W. Sperm transfer through forced matings and its evolutionary implications in natural guppy (*Poecilia reticulata*) populations. *Biol. J. Linn. Soc.* **78,** 605–612 (2003).
22. Rosenthal, H. L. Observations on reproduction of the poeciliid *Lebistes reticulatus* (Peters). *Biol. Bull.* **102,** 30–38 (1952).
23. Petit, C. & Ehrman, L. in *Evolutionary Biology* Vol. 3 (eds Dobzansky T., Hecht M. K. & Steere W. C.) Sexual Selection in *Drosophila.* (North-Holland, 1969).
24. Farr, J. A. Male rarity or novelty, female choice behavior and sexual selection in the guppy *Poecilia reticulata* Peters (Pices: Poeciliidae). *Evolution* **31,** 162–168 (1977).
25. Shuster, S. M. & Wade, M. J. Equal mating success among male reproductive strategies in a marine isopod. *Nature* **350,** 608–610 (1991).
26. Dijkstra, P. D., Seehausen, O. & Groothuis, T. G. G. Direct male–male competition can facilitate invasion of new colour types in Lake Victoria cichlids. *Behav. Ecol. Sociobiol.* **58,** 136–143 (2005).
27. Takahashi, Y., Yoshimura, J., Morita, S. & Watanabe, M. Negative frequency-dependent selection in female color polymorphism of a damselfly. *Evolution* **64,** 3620–3628 (2010).
28. Kokko, H., Jennions, M. D. & Houde, A. Evolution of frequency-dependent mate choice: keeping up with fashion trends. *Proc. R. Soc. B* **274,** 1317–1324 (2007).
29. Gigord, L. D. B., Macnair, M. R. & Smithson, A. Negative frequency-dependent selection maintains a dramatic flower color polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soó. *Proc. Natl Acad. Sci. USA* **98,** 6253–6255 (2001).
30. Spurgin, L. G. & Richardson, D. S. How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. *Proc. R. Soc. B* **277,** 979–988 (2010).

Supplementary Information is available in the online version of the paper.

Acknowledgements We thank R. Olendorf for help with field work and genotyping; D. Punzalan, K. Dixon, M. Bryant, T. Pitcher, C. Baril, A. Inman and C. Hurt for field work; C. Gibson, K. McGhee for photographs in Fig. 1 and for animal rearing; J. Koprivnikar for animal rearing; and A. Vanesyan for collecting morphology data. D. Reznick provided advice on field experiments, and D. Punzalan, K. Dixon and M. Fitzpatrick commented on manuscript drafts. In Trinidad, we thank the Sinanan family for accommodation, and I. Ramnarine and the government of Trinidad for permission to collect guppies and to work in the Quare watershed. This work was supported by grants from the National Science Foundation (to K.A.H. and A.E.H.) and the Natural Sciences and Engineering Research Council (NSERC) of Canada (to F.H.R.).

Author Contributions K.A.H. and F.H.R conceived and designed the experiment and conducted all data analyses. A.E.H. consulted on experimental design and A.C.P reared live animals. All authors participated in field experiments and in writing the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to K.A.H. (kahughes@bio.fsu.edu).

METHODS

Field experiment. The sections of the Mausica River, Quare River 1 and Quare River 7 streams that we used had a pool-and-riffle structure that naturally limits migration between pools, particularly during the dry season, which is the time of year when our experiments were conducted[31]. We also built temporary barriers between pools to reduce migration further. At each site, we used five to six pools of similar size, structure, substrate and water clarity to form replicate experimental pools[8]. We collected all adult guppies from each pool and sorted males into two groups that were nearly equal in abundance within sites, based on classification of caudal fin colour morphs as 'uncoloured' (>75% of the caudal fin transparent) and 'coloured' (>50% of fin coloured); males with intermediate amounts of colour were excluded from the experiment. Fish were photographed and given pool-specific marks using a small elastomer injection. Males were reintroduced into pools in a ratio of 3:1 with each morph as the rare type in half the pools. Females that were used in the experiment were always reintroduced into the same pool from which they had been collected. We controlled for the density and size of the pools by maintaining the original, or slightly reducing, the numbers of adults that had been in the pools; we also maintained the natural sex ratio. Detailed methods and the number of males of each type and the number of females reintroduced into each pool are described in ref. 8. All procedures complied with animal care standards of the Canadian Council on Animal Care and were approved by University of Toronto's Animal Care Committee.

Experimental males. We genotyped 166 marked experimental males that survived to the end of the experiment (60 from Mausica River, 72 from Quare River 1, and 34 from Quare River 7); these were the recaptured 'rare' and 'common' males described in ref. 9. Of these, 124 were classified as common (43 in Mausica River, 53 in Quare River 1, and 28 in Quare River 7) and 42 were classified as rare (17 in Mausica River, 19 in Quare River 1, and 6 in Quare River 7). We also genotyped 193 adult females that survived the field experiment (55 from Mausica River, 83 from Quare River 1, and 55 from Quare River 7), 693 first-brood offspring of these females, and 777 second-brood offspring. Animals from Quare River 1 and Mausica River were from the field experiment that was conducted in 2003, and animals from Quare River 7 were from the field experiment that was conducted in 1999. Sixty-one other males caught at the end of the experiment were unmarked and presumed to have matured during the experiment or to have migrated into the study area. Eight marked males migrated between pools during the experiment. We genotyped all mature males that were collected at the end of the experiment so that offspring could be assigned to them, but we did not include the unmarked males or the eight migrant males in subsequent analyses because they were present in the experimental pools or were sexually mature for an unknown period of time. In addition, 58 marked males did not survive to the end of the experiment[8]. These males were not genotyped and therefore not included in the paternity analysis.

Paternity analysis. We genotyped 14, 11, and 9 loci, respectively, from fish of the Quare River 1, Quare River 7 and Mausica River populations, which had a mean number of 11, 16.2 and 11.6 alleles per locus. The individual who scored genotypes was blind to the experimental treatment group of the individuals. For paternity analysis, we used CERVUS 3.0.3 to simulate 100,000 offspring, assuming a mistyping rate of 0.02, that 80% of candidate fathers were genotyped, a minimum of 5 loci typed, and a strict confidence level of 95%. The combined exclusion probabilities were 3.8×10^{-9} in Quare River 7, 3.9×10^{-5} in Quare River 1, and 4.7×10^{-4} in Mausica River. To assign paternity to a male, the difference in LOD (logarithm of odds) scores between the most likely candidate father and the second most likely candidate father (delta) had to exceed the 95th percentile of values produced from 100,000 simulations.

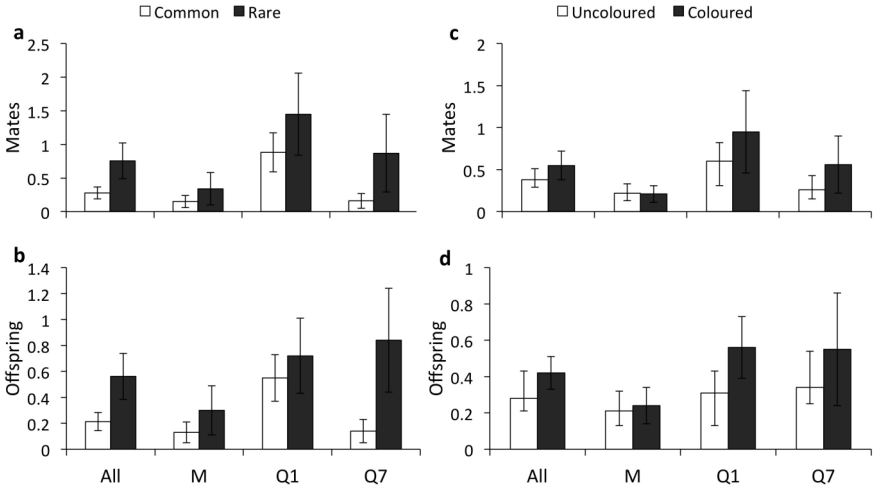
For 424 first-brood offspring, the most likely father was an experimental male; for the remainder the most probable father was a migrant or was unassigned. For 135 offspring, paternity was assigned to an experimental male with >85% confidence; 93 were assigned with >95% confidence. For the rest, the difference in log-likelihoods between the first and second most likely fathers corresponded to <85% probability that the most likely father was the true father, and these paternity assignments were not used in subsequent analyses. For second broods, 435 offspring were assigned to experimental males, 158 of these were assigned with >85% confidence and 102 with >95% confidence (Supplementary Table 2).

Morphological measurements. Images were measured using ImageJ[32]. Areas were measured by outlining the region with the freehand tool. The area of the entire body, excluding the fins and gonopodium, was measured. Individual coloured spots were each outlined and measured separately. These measures of area were square root transformed before analysis to conform to model assumptions. For three males (two in Quare River 7 and one in Mausica River), pictures taken in the field were of such low quality that reliable morphological measures could not be obtained. These males were excluded from the analyses of quantitative traits, but were included in the analyses that only considered experimental treatment factors.

Statistical analysis. We fit generalized linear mixed models to counts of assigned mates and offspring, using a negative binomial distribution, and Laplace approximation for estimating the marginal likelihood. Replicate experimental pools nested within populations were modelled as random effects. Fixed effects included population, treatment (rare versus common), morph (coloured versus uncoloured caudal fin), body size (area of a two-dimensional image, excluding all fins), body area covered by orange, numbers of discrete orange and black spots, and all two-way interactions. Body area covered by black, and three-way interactions were also tested, but never approached significance (all $P > 0.1$) and were not included in the final models. All analyses were conducted in SAS 9.3 (ref. 33); generalized linear mixed models were implemented in Proc Glimmix.

GPS coordinates of sites. The natural populations used in these experiments were located at: Mausica, PS 685749 1176906; Quare 1, PS 696972 1180687 (called Quare 2 in ref. 34); Quare 7, PS 697407 1179935.

31. Reznick, D. N., Butler, M. J. IV., Rodd, F. H. & Ross, P. Life-history evolution in guppies (*Poecilia reticulata*): 6. Differential mortality as a mechanism for natural selection. *Evolution* **50,** 1651–1660 (1996).
32. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9,** 671–675 (2012).
33. SAS Institute. *SAS/STAT 9.3 User's Guide*. (SAS Institute, 2011).
34. Endler, J. A. A predator's view of animal color patterns. *Evol. Biol.* **11,** 319–364 (1978).



Extended Data Figure 1 | Population means for effects of rarity and morph on reproductive success. Centre values are marginal means from generalized linear mixed models; bars indicate s.e.m. adjusted for model parameters. *$P < 0.01$ and **$P < 0.05$, respectively. **a**, Mates assigned to common (white bars) and rare (dark grey bars) males in all populations (All, $n = 124$ common, 42 rare) and by population (M, Mausica, $n = 43$ common, 17 rare; Q1, Quare 1, $n = 53$ common, 19 rare; Q7, Quare 7, $n = 28$ common, 6 rare). **b**, Offspring for common and rare males. **c**, Mates assigned to males with uncoloured (white bars) or coloured (dark grey bars) tails for all populations (All, $n = 79$ uncoloured, 87 coloured) and by population (M, Mausica, $n = 26$ uncoloured, 34 coloured; Q1, Quare 1, $n = 35$ uncoloured, 37 coloured; Q7, Quare 7, $n = 18$ uncoloured, 16 coloured). **d**, Offspring for males with uncoloured and coloured tails.

Extended Data Table 1 | Effect of experimental factors and quantitative traits on reproductive success in first-brood offspring

Mates	DF	χ^2	P
Population	2	3.82	0.148
Rarity	**1**	**9.09**	**0.003**
Population*rarity	2	4.25	0.120
Morph	1	1.75	0.185
Population*morph	2	0.43	0.805
Body size	1	0.01	0.919
Population*size	2	3.58	0.167
Orange area	1	0.16	0.689
Population*orange area	**2**	**6.15**	**0.046**
Number orange spots	1	1.24	0.266
Population*orange spots	2	0.91	0.634
Number black spots	1	1.31	0.251
Population*black spots	2	5.31	0.070
Offspring			
Population	2	3.10	0.213
Rarity	**1**	**6.69**	**0.010**
Population*rarity	2	1.57	0.457
Morph	1	1.21	0.271
Population*morph	2	0.78	0.677
Body size	1	0.01	0.908
Population*size	2	2.54	0.281
Orange area	1	0.87	0.352
Population*orange area	**2**	**6.52**	**0.035**
Number orange spots	1	2.75	0.098
Population*orange spots	2	1.36	0.506
Number black spots	1	1.18	0.278
Population*black spots	2	3.18	0.204

Significant effects are shown in bold.

Extended Data Table 2 | Effect of experimental factors and quantitative traits on reproductive success

Mates	DF	χ^2	P
Population	2	2.89	0.269
Rarity	**1**	**4.50**	**0.036**
Population*rarity	2	4.68	0.101
Morph	1	0.60	0.440
Population*morph	2	2.83	0.247
Body size	1	0.13	0.721
Population*size	2	2.86	0.244
Orange area	1	0.00	0.986
Population*orange area	**2**	**6.92**	**0.034**
Number orange spots	1	0.62	0.434
Population*orange spots	2	0.65	0.722
Number black spots	1	2.58	0.111
Population*black spots	2	3.38	0.189
Offspring			
Population	2	2.39	0.332
Rarity	1	2.94	0.089
Population*rarity	2	3.03	0.223
Morph	1	0.55	0.462
Population*morph	2	3.26	0.200
Body size	1	0.16	0.689
Population*size	2	2.00	0.370
Orange area	1	0.27	0.602
Population*orange area	**2**	**6.37**	**0.045**
Number orange spots	1	1.43	0.235
Population*orange spots	2	1.23	0.542
Number black spots	1	2.31	0.131
Population*black spots	2	2.37	0.309

First-brood offspring, paternity confidence >85%. Significant effects are shown in bold.

Extended Data Table 3 | Population-specific estimates of the association between area of orange body colour and reproductive success

Mates	Estimate	SE	DF	t	P
Q7	1.8	1.0	128	1.86	0.066
Q1	-1.3	0.8	128	-1.70	0.095
M	0.1	1.0	128	0.14	0.893
Offspring					
Q7	2.5	1.2	128	2.13	0.035
Q1	-1.1	0.8	128	-1.40	0.164
M	0.2	1.1	128	0.24	0.813

First-brood offspring and paternity confidence >95%. Estimates derived from the generalized linear mixed model.

Extended Data Table 4 | Effect of experimental factors and quantitative traits on reproductive success

Mates	DF	χ^2	P
Population	2	1.24	0.539
Rarity	1	0.90	0.344
Population*rarity	2	1.38	0.503
Morph	**1**	**7.20**	**0.007**
Population*morph	2	1.34	0.511
Body size	1	1.04	0.308
Population*size	2	1.74	0.418
Orange area	1	2.97	0.085
Population*orange area	**2**	**7.63**	**0.022**
Number orange spots	**1**	**4.83**	**0.028**
Population*orange spots	2	1.44	0.488
Number black spots	1	0.00	0.974
Population*black spots	2	3.68	0.159
Offspring			
Population	2	0.00	0.999
Rarity	1	0.11	0.743
Population*rarity	2	1.00	0.606
Morph	**1**	**7.37**	**0.007**
Population*morph	2	2.12	0.347
Body size	1	1.38	0.241
Population*size	2	0.08	0.960
Orange area	1	2.06	0.152
Population*orange area	**2**	**9.76**	**0.008**
Number orange spots	1	3.53	0.063
Population*orange spots	2	3.96	0.142
Number black spots	1	0.22	0.643
Population*black spots	2	5.10	0.082

Second-brood offspring, paternity confidence 95%. Significant effects are shown in bold.

Extended Data Table 5 | Parameter estimates for effects of morph (coloured – uncoloured) and the area of orange body colour, on number of assigned mates and assigned offspring

Mates	Estimate	SE	DF	t	P
Morph	1.5	0.8	128	-2.05	0.043
Q7	3.4	1.3	128	2.60	0.010
Q1	-0.7	0.7	128	-1.01	0.310
M	0.5	1.2	128	0.41	0.682
Offspring					
Morph	2.0	0.9	128	-2.17	0.030
Q7	4.0	1.4	128	2.81	0.006
Q1	-1.2	0.9	128	-1.40	0.165
M	0.3	1.3	128	0.22	0.826

Second-brood offspring, paternity confidence >95%.