

Frequency-dependent survival in natural guppy populations

Robert Olendorf¹, F. Helen Rodd³, David Punzalan³, Anne E. Houde⁴, Carla Hurt⁵, David N. Reznick⁶ & Kimberly A. Hughes^{1,2}

The maintenance of genetic variation in traits under natural selection is a long-standing paradox in evolutionary biology^{1–3}. Of the processes capable of maintaining variation, negative frequency-dependent selection (where rare types are favoured by selection) is the most powerful, at least in theory¹; however, few experimental studies have confirmed that this process operates in nature. One of the most extreme, unexplained genetic polymorphisms is seen in the colour patterns of male guppies (*Poecilia reticulata*)^{4,5}. Here we manipulated the frequencies of males with different colour patterns in three natural populations to estimate survival rates, and found that rare phenotypes had a highly significant survival advantage compared to common phenotypes. Evidence from humans^{6,7} and other species^{8,9} implicates frequency-dependent survival in the maintenance of molecular, morphological and health-related polymorphisms. As a controlled manipulation in nature, this study provides unequivocal support for frequency-dependent survival—an evolutionary process capable of maintaining extreme polymorphism.

Colour-pattern polymorphism in guppies is limited to males and consists of irregular spots of several different structural (blue, green and purple) and pigment-based (yellow, orange, red and black) colours that occur on the body, caudal fin and dorsal fin (Fig. 1). The position, number, size and hue of the spots are highly heritable^{5,10}, although the colour saturation (chroma) of orange spots can be influenced by diet^{11,12}. Male colouration is highly polymorphic despite being subject to sexual and ecological selection. Female mating preferences usually favour males with the greatest area of orange, although the strength of that preference varies among populations^{10,12,13}. Predators also exert selection on colour patterns; they preferentially prey upon males with brighter or more conspicuous colours^{14,15}. Despite the apparently strong and directional selection within populations, colour patterns are so variable that any two males are easily distinguishable based on colour pattern alone, unless they are closely related¹⁰.

Several mechanisms have been proposed to explain the maintenance of this extreme polymorphism^{10,14}. Mate-choice experiments indicate that females preferentially mate with males bearing rare or novel colour patterns^{16,17}. A trade-off (antagonistic pleiotropy) between male sexual attractiveness and offspring viability has also been reported¹⁸. Both processes could contribute to the maintenance of genetic variation in nature. However, experiments demonstrating these processes were conducted in laboratory environments, and it is not clear whether either process occurs in nature. Another process capable of maintaining polymorphism is a rare-morph survival advantage. This process has been implicated in the maintenance of colour polymorphism in some invertebrates^{19,20} and vertebrates²¹, but it has not been tested in the highly polymorphic guppy system.

We tested the hypothesis that male survival is causally related to colour-pattern rareness in three natural guppy populations in Trinidad. We used an established mark–release–recapture protocol^{22–24} to estimate the survival of wild guppies in native streams, where we manipulated the frequency of male colour patterns. Under frequency-dependent selection, genotypes have equal fitness when they are at equilibrium frequencies; therefore, we manipulated the frequencies of different morphs to move the population away from equilibrium. We conducted 34 separate manipulations across 19 replicate pools in three streams over four years. If frequency-dependent selection occurs, we expected a survival advantage for rare phenotypes relative to common phenotypes.

We conducted these experiments in two unconnected tributaries of the Quare River (Quare 1 and Quare 7), and the main branch of the Mausica River. Both Quare tributaries are small pool-and-riffle streams where the dominant predator is a killifish (*Rivulus hartii*). The Mausica River is a larger stream with pool–riffle topology, having both *R. hartii* and the pike cichlid (*Crenicichla alta*), which is thought to be the dominant predator where it occurs. *C. alta* prey predominantly on adult female and male guppies, and *R. hartii* consume juvenile and adult male guppies, but not large adult females^{23,25}. These piscivorous fish are the only major predators of guppies in the sites we used²³. Guppies show little intraspecific aggression, and intraspecific interactions are not a direct source of mortality¹⁰. Consequently, predators are the most likely sources of short-term mortality for guppies. In each stream, we used four to seven pools of similar size and structure in each of two different years. All replicate pools within a stream were similar with respect to substrate and water clarity. The presence of adult *R. hartii* and/or *C. alta* was confirmed for each pool used in the experiment.

After collecting all adult males and females from each experimental pool at a site, the males from all pools within a site were combined and then sorted based on the classification of alternate tail colour patterns, which were nearly equal in abundance within a site. We used tail colour patterns because they are distinctive, likely to be conspicuous to predators, and are assumed to have an important role in courtship. For convenience, alternate morphs were designated as ‘Coloured’ and ‘Uncoloured’, or ‘Flag’ and ‘Blob’, depending on the site and year (Fig. 1). In half of the replicates within a site, one morph was made rare and the other common, typically in a ratio of 3:1. In the remainder of the pools, rare and common morph frequencies were reversed (Supplementary Table S1). Within a morph category, males were randomly assigned to experimental pools. We released adult females into the same pool from which they had been collected, and maintained natural densities and sex ratios in each pool. All adults were given a pool-specific mark^{23,24} so that any migrants could be identified.

¹School of Integrative Biology and ²Institute for Genome Biology, University of Illinois, Urbana, Illinois 61801, USA. ³Department of Zoology, University of Toronto, Toronto, Ontario M5S 3G5, Canada. ⁴Department of Biology, Lake Forest College, Lake Forest, Illinois 60045, USA. ⁵Smithsonian Tropical Research Institute, Naos Marine Laboratory, Roosevelt Avenue (APO AA 34002), Panama City, Panama. ⁶Department of Biology, University of California, Riverside, California 92521, USA.

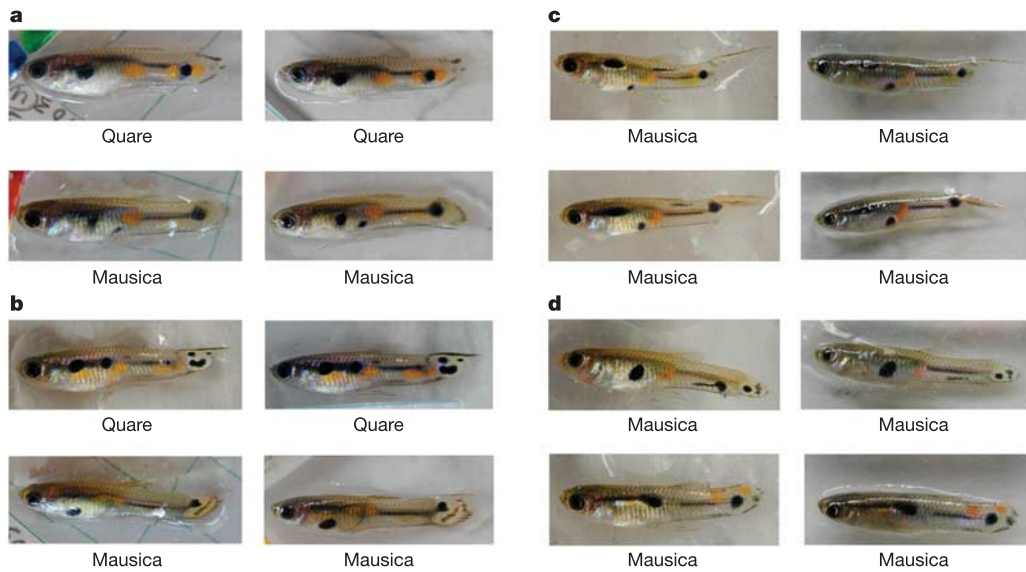


Figure 1 | Males before release and after recapture. In each panel, photographs taken before release are on the left; photographs of the same animal after recapture are on the right. Variations in hue reflect ambient light variation in the field. Tattoo marks are visible as thin horizontal lines on the caudal peduncles. **a, b**, Examples of 'Uncoloured' (**a**) and 'Coloured' (**b**) morphs; **c, d**, examples of 'Flag' (**c**) and 'Blob' (**d**) morphs (see Supplementary Methods). Panel **c** illustrates that even males with very similar colour patterns can be distinguished when matching before and after photos.

The release phase of the experiment lasted 15 (Mausica) or 17 (Quare tributaries) days. At the end of this phase, we again exhaustively searched until we had captured all adult-size guppies from each pool, and from non-experimental pools up- and downstream to identify any migrants. We classified recaptured males with respect to colour pattern and pool-specific mark, and compared photographs of released and recaptured males to determine the recapture status of individual males (Fig. 1).

Males with the rare phenotype had significantly higher recapture rates overall than did males with the common phenotype (Table 1 and Fig. 2), suggesting that rare types had a large survival advantage. Rare types also had higher recapture rates within each site; site-specific contrasts were significant in Quare 1 ($\chi^2 = 461$; degrees of freedom (d.f.) = 1; $P < 0.0001$) and Quare 7 ($\chi^2 = 347$; d.f. = 1; $P < 0.0001$), with a similar trend in Mausica ($\chi^2 = 3.30$; d.f. = 1; $P = 0.07$) (Fig. 2). Notably, we recaptured every rare male in every pool in Quare 7 (1996) and in Quare 1 (2004), whereas only 61% and 67% of common males were recaptured, respectively (Table 2). Repeated-measures analysis of variance (ANOVA) of recapture rates, treating rare and common morphs within a pool and year as repeated measures, yielded similar results (see Supplementary Notes). Rare types had higher recapture rates within years at each site, except for one site-year combination (Quare 7, 1999). Therefore, this site accounts for the significant site \times rarity \times year interaction effect in Table 1; when the data from this site are removed, the effect of rarity is still significant ($\chi^2 = 11.23$; d.f. = 1; $P = 0.0008$), but the interaction term is no longer significant ($\chi^2 = 5.8$; d.f. = 2; $P = 0.06$).

Table 1 | Model effects and likelihood-ratio tests

Effect	Degrees of freedom	χ^2	P
Site	2	3.61	0.16
Frequency	1	14.73	0.0001
Morph	1	0.70	0.40
Site \times frequency	2	2.54	0.28
Site \times year	2	10.35	0.006
Frequency \times morph	2	0.01	0.93
Site \times frequency \times year	3	17.69	0.0005
Site \times morph \times year	5	6.14	0.29

(b) morphs; **c, d**, examples of 'Flag' (**c**) and 'Blob' (**d**) morphs (see Supplementary Methods). Panel **c** illustrates that even males with very similar colour patterns can be distinguished when matching before and after photos.

Morphs did not differ in recapture rates overall or within sites (Table 1). Thus, there is no evidence of differential survival between the phenotypes we used. Total recapture rate was 73%, in accordance with estimates from other studies^{22,23}. There were no morph \times frequency interactions, indicating that the particular phenotype that was rare or common had no effect on recapture probability.

We reanalysed the data to determine whether any bias was introduced by the few males who migrated or by the unmarked males that were captured at the end of the experiment in some pools. A few marked males at Quare 7 migrated to other pools before recapture; these males were counted as survivors in the above analyses. However, if the males migrated early in the release phase, they would not have experienced the same morph-frequency environment as other males in their experimental pool. We therefore removed these males from the data set. This change had little effect on the results (Supplementary Tables S2 and S3). We also repeated the analysis after excluding four samples in which unmarked males could have altered morph frequencies substantially (Mausica 2003 pools A and D, and 2004 pool D; Quare 7 1996 pool 12). Again, results were essentially unchanged (Supplementary Table S2). We

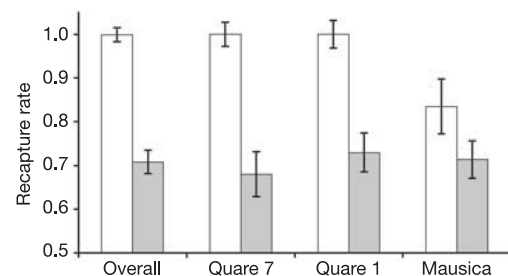


Figure 2 | Relative recapture rates for rare and common phenotypes.

Least-square mean recapture rates and standard errors for rare (open bars) and common (grey bars) phenotypes were calculated from the full model, and are therefore corrected for other terms in the model. The vertical axis should be interpreted as relative, not absolute, survival. Over all sites, the difference in recapture rates between rare and common morphs is significant. Within sites, the difference is significant for Quare 7 and Quare 1 (see the main text).

Table 2 | Recapture rates by population and year*

Site	Year	Rare	Common
Mausica	2003	0.88 (0.08)	0.72 (0.04)
Mausica	2004	0.79 (0.12)	0.66 (0.08)
Quare 1	2003	0.79 (0.07)	0.75 (0.04)
Quare 1	2004	1.00 (0.00)	0.67 (0.10)
Quare 7	1996	1.00 (0.00)	0.61 (0.10)
Quare 7	1999	0.60 (0.11)	0.74 (0.08)

*Values for rare and common morphs are means (and standard errors).

evaluated the potential for bias due to the random assignment of some males to the same pool from which they were initially captured, and found that accounting for a 'home pool' effect did not substantially change the results (Supplementary Notes).

In summary, this experiment demonstrates that frequency-dependent survival occurs in natural guppy populations, and supports the hypothesis that frequency-dependence contributes to the extreme polymorphism of male colour patterns. Although we have not measured the evolutionary response to selection in this study, the high heritability of guppy colour patterns^{4,5,10} suggests that short-term response to selection would be strong. Selection on other components of fitness could oppose survival selection on colour patterns, but the available evidence indicates that differential reproduction also favours rare colour patterns^{16,17,26} because females have a mating preference for rare or novel males¹⁷. An intriguing possibility is that frequency-dependent survival could lead to direct selection for a rare-male mating preference if the reduction in risk extends to females involved in courtship.

Given that the known guppy predators hunt visually, a possible mechanistic explanation for a survival advantage of rare morphs is that predators form a 'search image' for common morphs, resulting in an increased ability to detect familiar prey but a decreased probability of detecting alternative prey. Search image formation is thought to result from limited attention in predators; it can generate frequency-dependent predation and maintain polymorphism, as shown in experiments using artificial prey^{27,28}. If selective predation were responsible for the frequency-dependent survival we observed, our results suggest that predators were attuned to small elements of colour patterns, as we manipulated the frequency of only a small part of the overall pattern. An alternative to the search image hypothesis is that male guppies altered their behaviour in response to morph frequency, and that differential survival was related to these behavioural changes. Behavioural tests on both predators and prey will be needed to discriminate between these hypotheses, and to determine whether predators distinguish between small elements of the overall colour pattern.

METHODS

Mark–release–recapture experiments. We conducted mark–release–recapture experiments at three sites (the exact locations are given in Supplementary Notes) in different years, using a protocol that has been used successfully to estimate survival in natural guppy populations in previous investigations^{22–24}. At each site, we used adjacent or nearly adjacent pools occurring within a single section of stream (<750 m). Before each experiment, we exhaustively collected all adult and subadult guppies from each pool. Guppies were clearly visible because all pools had shallow, clear water and no aquatic vegetation. We returned to the same pools on 2–3 consecutive days until no fish were collected during 45 min of searching at each pool. Captured guppies were separated by sex and kept in aquaria for 2–8 days in our field laboratory. We marked anaesthetized fish with a small subcutaneous elastomer tattoo (Northwest Marine Technology) unique to each experimental pool. We used only white or black tattoos to avoid colours implicated in female choice¹⁴; the colour of the marks does not affect survival²³.

In a given year, males collected from different pools within a site were combined and sorted on the basis of colour pattern. In all but one replicate, males were sorted into 'Coloured' and 'Uncoloured' phenotypes (Fig. 1). In Mausica 2004, males with >50% of the caudal fin coloured were rare, so a different classification was used: 'Flag' or 'Blob' (Fig. 1; Supplementary Methods). We did not use males not clearly falling into either category, or

young males without fully developed colour patterns. Within a colour category, males were randomly assigned to release pools at the frequencies shown in Supplementary Table 1. Slight variation among pools occurred when there were insufficient numbers of one type. Each male was digitally photographed before release but after marking. We released a total of 459 marked males and recaptured 312 marked males. Fish were not mixed across sites, and were not used in multiple years within a site.

For each site in each year, equal numbers of adult females were reintroduced into all pools (8–25 females per pool), chosen to retain pre-experimental population density and sex ratio. Releases were made a minimum of 24 h (usually 2–3 days) after marking, and after removing any adult guppies that were found in experimental pools. Before release, we built nylon screen barriers at the up- and downstream ends of each pool to reduce migration of guppies into or out of the pool during the release phase of the experiment.

On recapture, we anaesthetized males and photographed them again. Three independent individuals matched 'before' and 'after' photographs (see Fig. 1). Any male that had a 'before' photo but not an 'after' photo was assumed to have died during the release phase. It is possible that a small number of the males managed to bypass our barriers and migrated beyond the pools where we checked for migrants. However, there was no significant difference between rare and common morphs in the few marked migrant males that we did capture (all in Quare 7): 3/29 rare versus 7/86 common (likelihood ratio $\chi^2 = 0.11$; $P = 0.74$). The slight tendency for higher migration rates in rare males would have led us to underestimate rare male survival. In order to affect our conclusions, there would have to be a rare-male bias in long-distance migration, but not in short-distance migration—a pattern that seems unlikely and for which there is no evidence.

Statistics. The proportions of recaptured males in each category in each pool were analysed using SAS Proc Genmod with logit link, binomial distribution function, and type 3 likelihood ratio tests²⁹. This procedure provides a generalized linear modelling framework for the analysis of categorical data. We fitted the following model of categorical effects: $\text{logit}(\pi_{ijkl}) = \mu + \alpha_i + \beta_j + \delta_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{il} + (\beta\delta)_{ik} + (\alpha\beta\gamma)_{ijl} + (\alpha\delta\gamma)_{ikl}$, where π_{ijkl} is the proportion of males recaptured at site i , in rarity category j and morph category k in year l . Parentheses denote interaction terms. This model passed goodness-of-fit tests with no evidence of overdispersion (Pearson $\chi^2 = 59.0$; d.f. = 49; $P > 0.15$), and additional terms did not improve goodness of fit. We used CONTRAST statements to test the significance of recapture rates between frequency categories within sites. Means and standard errors of recapture rates for sites and years (Table 2) were calculated by averaging over the pool-specific proportions from that site and year. Model-corrected means (Fig. 2) were calculated using inverse logit transformations, $(\bar{\pi} = e^{\bar{\eta}} / (1 + e^{\bar{\eta}}))$. Standard errors (s.e.) were determined using the delta method, $\text{s.e.}(\bar{\pi}) = \bar{\pi}(1 - \bar{\pi}) \times \text{s.e.}(\bar{\eta})$; because the delta method produces standard error estimates of zero when $\bar{\eta} = 1$, we replaced values of $\bar{\eta} > 0.95$ with $\bar{\eta} = 0.95$ to calculate approximate standard errors. Parametric linear models were fitted to arc-sine square-root-transformed proportions.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions All authors collected field data. K.A.H., F.H.R. and A.E.H. designed the experiment. K.A.H. and F.H.R. supervised the field work. D.P. conducted reliability analysis; R.O. scored survival; and R.O. and K.A.H. analysed the data and wrote the manuscript. All authors discussed and commented on the manuscript, and suggested revisions.

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