SEXUAL SELECTION

Female preference for rare males is maintained by indirect selection in Trinidadian guppies

Tomos Potter[1]*†, Jeff Arendt[2]†, Ronald D. Bassar[3], Beth Watson[4], Paul Bentzen[4], Joseph Travis[1], David N. Reznick[2]

When females prefer mates with rare phenotypes, sexual selection can maintain rather than deplete genetic variation. However, there is no consensus on why this widespread and frequently observed preference might evolve and persist. We examine the fitness consequences of female preference for rare male color patterns in a natural population of Trinidadian guppies, using a pedigree that spans 10 generations. We demonstrate (i) a rare male reproductive advantage, (ii) that females that mate with rare males gain an indirect fitness advantage through the mating success of their sons, and (iii) the fitness benefit that females accrue through their "sexy sons" evaporates for their grandsons as their phenotype becomes common. Counter to prevailing theory, we show that female preference can be maintained through indirect selection.

Whether female preference for rare males can sustain genetic polymorphisms in nature has long been controversial (*1*). Rarity, as an attractive trait, complicates sexual selection theory for the evolution of female preference because it introduces negative frequency-dependence. Negative frequency-dependent selection occurs when the fitness of a trait decreases as it becomes more common and increases as it becomes rarer. In the absence of negative frequency-dependence, female preference for certain male traits can be explained if the sons of attractive males are also attractive (*2*, *3*) or if paternal attractiveness correlates with enhanced viability in offspring (*4*, *5*). However, when attractiveness is frequency-dependent, as is the case when rare male phenotypes have an advantage, sons can become victims of their father's success. Specifically, the progeny of successful rare males are doomed to become common and thus unattractive. Although there is robust theory describing conditions under which female preference for rare males may evolve (*6*), it is unclear whether the costs of such a preference will outweigh its benefits (*7*).

Numerous laboratory studies have demonstrated a rare-male mating advantage in several taxa [reviewed in (*8*, *9*)]. However, we know of only five such studies in nature (*10*–*14*). As is common in laboratory studies, all studies in nature except one (*12*) reduced the options for female choice to just two male morphs (albeit in some cases noting variation within morphs) (*11*, *14*). These studies were further limited to a single mating season, precluding the detection of long-term fitness consequences for females that mate with rare males. As a result, although these studies have documented rare-male advantage and demonstrated its proximate mechanism through female preference behaviors, the ultimate, evolutionary explanation of why females prefer to mate with rare males remains unknown.

We show an advantage for rare color patterns in males under natural conditions in the highly polymorphic Trinidadian guppy (*Poecilia reticulata*) and quantify the fitness consequences of this advantage over multiple generations. Male color patterns in guppies are reliably transmitted from father to son (*15*–*18*). We identified 27 distinct color patterns in our study population and have confirmed that all males within a patriline share the same pattern (Fig. 1) (*19*). During courtship, males display their pattern to potential mates (*20*). Numerous laboratory studies (*15*, *20*–*30*) and one field manipulation (*31*) have demonstrated female preference for rare or unfamiliar male patterns. Whether those results apply to the much wider level of unmanipulated variation in wild male color patterns is unknown.

We tested these ideas as part of an experimental study of evolution in a natural stream in Trinidad (*19*, *32*, *33*). We used monthly mark-recapture data to determine the presence and movement patterns of individuals within the population over this period. We used a microsatellite-based pedigree to determine the relatedness of individuals and the reproductive success for each individual every month over this period (*19*). Our dataset includes monthly observations of 7173 individuals spanning 10 generations (*34*). We used generalized linear mixed effects models (GLMMs) to test the effects of male pattern rarity and novelty (defined below) on components of fitness in guppies.

Measuring rarity and novelty

We assigned a "rarity" score monthly to each male. We calculated the rarity of a focal pattern (r_i) as a function of the total numbe individuals with that pattern (n_i), the t number of individuals of all patterns (N_p), and the degree of polymorphism, i.e., the number of patterns (P):

$$r_i = ln\left(\frac{n_i}{N_p}P\right)$$

Weighting the relative frequency of patterns ($^{n_i}/_{N_p}$) by P allows meaningful comparison across localities with different degrees of polymorphism. Another useful aspect of this approach is that log-transformation results in rare patterns having negative values, common patterns having positive values, and patterns that are neither rare nor common ($^{n_i}/_{N_p} = {}^1/_P$) having a value of zero. This makes linearized model coefficients directly interpretable. To illustrate our results, we define a "rare" male as one with a pattern half as frequent as expected given the total degree of polymorphism [i.e., $r_i = \ln(0.5)$], and a "common" male as one with a pattern twice as frequent as expected [i.e., $r_i = \ln(2)$]. These illustrative values fall well within the observed distribution of male pattern rarity (fig. S1).

A female's assessment of male rarity will depend upon the males that she regularly encounters, which may be a spatial subset of the total population. The stream habitat is subdivided into discrete pools connected by riffles. Our spatially explicit mark-recapture censuses allowed us to reconstruct patterns of movement and make inferences about population structure. To assess the possibility that female mate preference is shaped by that structure, we calculated rarity at three spatial scales: the local level (e.g., the pool or riffle where the fish was caught that month), the neighborhood, and the whole population. Neighborhoods were defined using network analysis of movement of male guppies between pools (*19*). We identified four distinct multipool neighborhoods characterized by high movement within but low movement between. Males moved around considerably (62% were new arrivals to pools each month, 17% were new arrivals to neighborhoods, fig. S2), whereas females moved around much less (28% in pools, 4% in neighborhoods, fig. S2). When a female assesses how rare a male is, she is likely to see all those males that we collected in the pool with her that month. Although we did not observe this directly, our neighborhood-level analyses include males likely to have passed through the pool in the previous month.

In addition to an advantage to rarity, several studies have demonstrated the advantage of novelty in the form of female preference for unfamiliar males (*15*, *22*, *23*, *26*, *35*), regardless of their color pattern. Female guppies may identify novel males through olfactory cues (*36*). As such, males that are new arrivals in a

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[1]Department of Biological Science, Florida State University, Tallahassee, Florida, USA. [2]Department of Evolution, Ecology and Organismal Biology, University of California, Riverside, California, USA. [3]Department of Biological Sciences, Auburn University, Auburn, Alabama, USA. [4]Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada.
*Corresponding author. Email: tomos.potter@protonmail.com
†These authors contributed equally to this work.

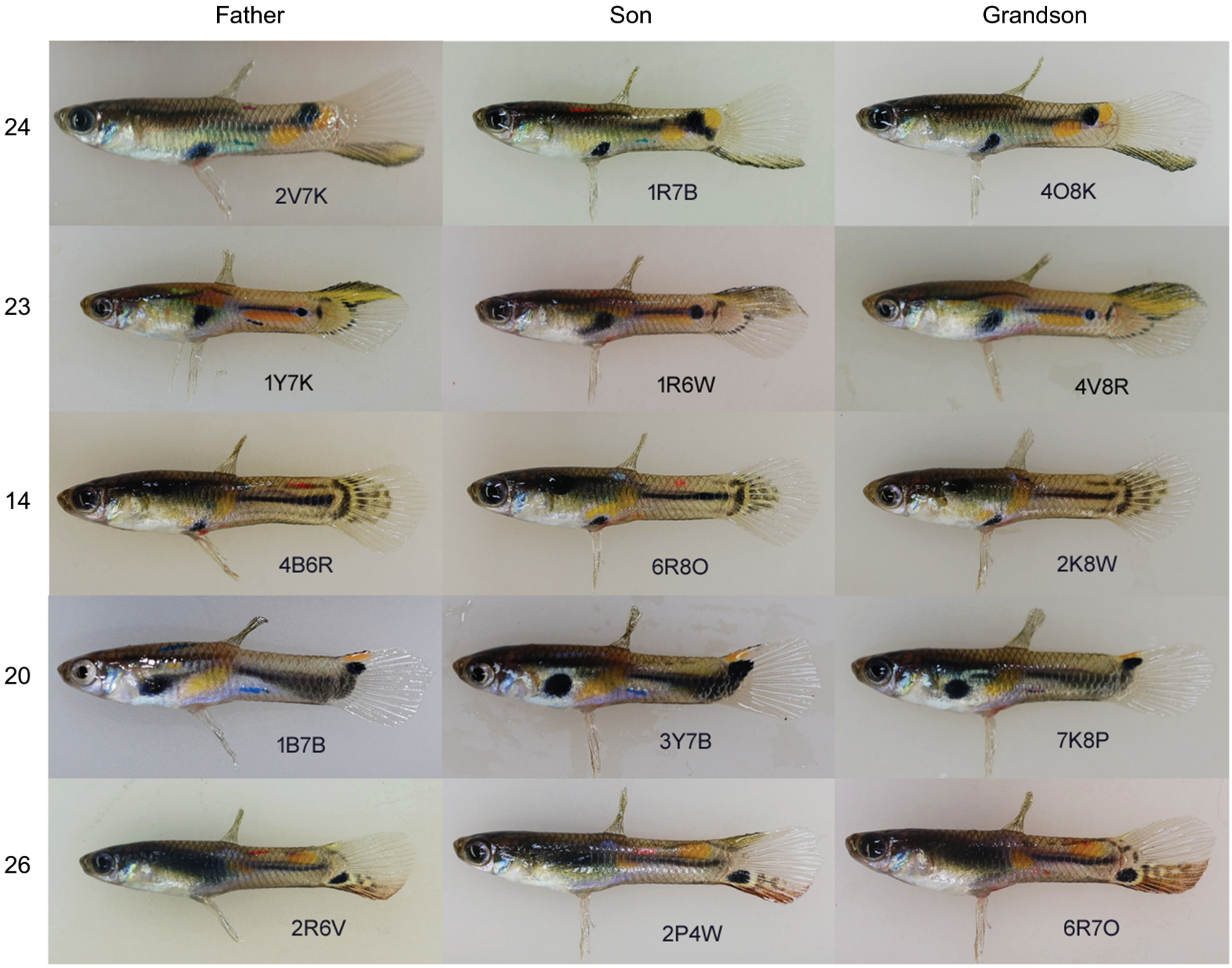

Fig. 1. Male color patterns in guppies are reliably transmitted from father to son. Here, we show three generations (father, son, and grandson) for five example patrilines showing consistency in color pattern within a Y-lineage. Numbers to the left indicate which lineage of 27 are being shown. Some elastomer marks, used to identify individuals, are clearly visible. For example, the son of lineage 14 has a red mark on the dorsal side of the caudal peduncle.

pool or neighborhood may experience a reproductive advantage. To test this, we defined males as "novel" if they were new arrivals to the pool or neighborhood in which they were caught that month. By this definition, males cease to be novel one month after arriving in a locality.

Results

We found evidence for negative frequency-dependent selection operating on male color patterns, resulting in a rare-male advantage (Fig. 2 and table S1). These effects were significant over all three spatial scales over which male rarity was calculated but were strongest and weakest at the neighborhood and local levels, respectively, as determined by Akaike information criterion (AIC) scores (table S1). Each month, males with rarer patterns at the neighborhood level had 36% more mating partners (GLMM, $n = 6248$, P-value = 2.43×10^{-6}) and ultimately sired 38% more offspring that recruited into the population (GLMM, $n = 6248$, P-value = 4.75×10^{-6}) (Fig. 2). These results, observed over multiple generations, provide strong evidence that negative frequency-dependent sexual selection is occurring through rare-male advantage.

Novel males (new arrivals to a pool or neighborhood, regardless of their color pattern) also had a large reproductive advantage over residents (Fig. 2 and table S1), with the effect strongest at the local level. Compared with residents of equivalent rarity, new arrivals to pools had 45% more mating partners (GLMM, $n = 6248$, P-value = 2.77×10^{-4}) and sired 50% more offspring each month (GLMM, $n = 6248$, P-value = 3.30×10^{-4}).

In line with earlier studies (*15*, *20*–*30*), our results indicate that female guppies prefer to mate with rare color-patterned and/or unfamiliar males. One potential explanation for this preference is inbreeding avoidance: rare or novel males may be less likely to be kin (*37*). However, we found no evidence that male rarity was associated with the relatedness of mating partners (linear model, $n = 1259$, P-value = 0.273) (*19*). Surprisingly, resident males were more likely to be unrelated to their partners than novel males who were new arrivals to pools [$P(\text{unrelated}|\text{resident} = 0.28)$, $P(\text{unrelated}|\text{novel} = 0.15)$, logistic regression, $n = 1580$, P-value = 5.74×10^{-8})]. This could occur if males are more likely to remain in pools with unrelated females. Nevertheless, the higher relatedness of novel (and thus attractive) males and the absence of any association of relatedness with rarity contradict the inbreeding-avoidance hypothesis (table S2).

We found no direct benefit for females' preference for rarity or novelty. Mating with rare males did not result in more recruited offspring (table S3 and Fig. 2C, GLMM, $n = 2290$, P-value = 0.448), nor did mating with new arrivals to neighborhoods (P-value = 0.969) or pools (P-value = 0.397). Our measure of recruited offspring refers to those that survived to be large enough to be individually marked (~2 months old) (*19*), meaning that

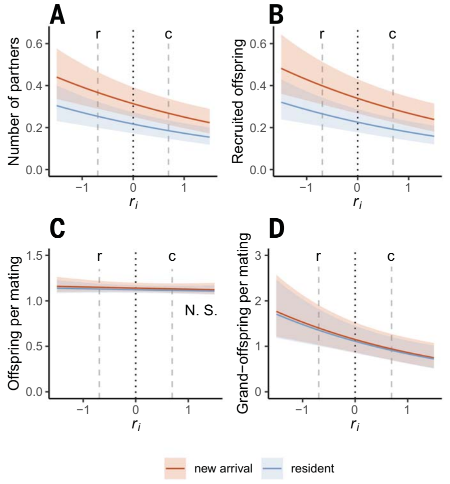

Fig. 2. Rare and novel males have higher reproductive fitness, and females that mate with rare males have more grand-offspring. Effects of male pattern rarity (r_i) and novelty (new arrival or resident) on components of fitness. (**A**) Number of mating partners per month (for males); (**B**) monthly number of offspring recruited into the population (for males); (**C**) number of recruited offspring per mating (for males and females); (**D**) number of grand-offspring that ultimately recruit into the population from a single mating (for males and females). Values for "rare" [$r_i = \ln(0.5)$] and "common" [$r_i = \ln(2)$] males are indicated with dotted lines annotated *r* and *c*, respectively; the dashed line indicates $r_i = 0$. Shaded areas are 95% confidence intervals, N.S. indicates that the slope is not significant ($P > 0.05$). Predictions are based on models where rarity was calculated at the neighborhood level (A) and (B) or the population level (C) and (D).

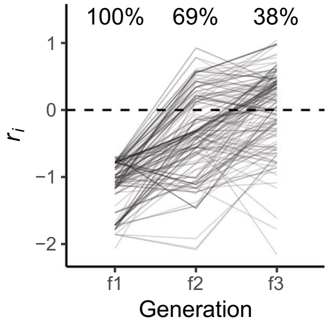

Fig. 3. Rare males have rare sons but common grandsons. In this figure, we track the trajectory of male pattern rarity (r_i, at the population level, *x* axis) over three generations (*y* axis), focusing on rare males [f1, $r_i < \ln(0.5)$], their sons (f2), and grandsons (f3). Lines connect male to sons to grandsons. Percentages describe individuals in each generation where $r_i < 0$, i.e., that are rarer than expected.

variation in offspring viability is captured in this metric. This indicates that preference for rare or novel males is not under direct selection through mechanisms that enhance offspring viability, such as inbreeding avoidance or so-called "good genes" (*4*, *5*).

What then is the ultimate benefit of mating with rare or novel males? Although we detected no indirect fitness benefits for females that mated with novel males [i.e., that were new arrivals to neighborhoods (GLMM, n = 1951, *P*-value = 0.439) or pools (*P*-value = 0.573)], matings with rare males (at the population level) ultimately resulted in 48% more grand-offspring recruited into the population than matings with common males (*P*-value = 3.42×10^{-4}). This is a substantial indirect fitness benefit for those females (table S3 and Fig. 2D).

Females that mated with rare males gained this indirect fitness advantage through the enhanced reproductive success of their sons: a so-called "sexy sons" effect [in the sense of Kokko (*6*)]. The sons of rare males (at the population level) sired more offspring per month (table S4, GLMM, n = 9807, *P*-value = 0.0039), but there was no such effect in daughters (*P*-value = 0.575). This occurred because the sons of rare males were also rare (albeit less so than their fathers) and thus still attractive (Fig. 3). This advantage was short-lived, however; after two generations of rare-male advantage the grandsons of rare males became victims of their forefathers' success and were more likely to be common (Fig. 3). Consequently, for males with equivalently rare fathers, having a rare grandfather reduced reproductive success (*P* = 0.0082).

Discussion

Female guppies that mated with rare males gained no direct fitness advantage in doing so. Their offspring did not have increased viability due to any genetic advantage of their attractive fathers, nor were they less inbred. Instead, females that mated with rare males derived substantial indirect fitness through the attractiveness of their sons. This is at odds with the long-held prediction that such indirect selection cannot maintain female preference (*7*, *38*–*40*). This prediction is not an ineluctable consequence of theory. It is based on assumptions about the genetic variances and covariances of female preference and male traits, which imply that indirect selection must always be overwhelmed by the direct costs of female choice (*7*). We suggest that this may not hold true when the desirable trait is rarity and physical traits are arbitrary. Our study shows that female preference can be maintained by indirect selection when negative frequency-dependence is operating.

Our findings offer a resolution to the "lek paradox" (*39*). To maintain female preferences through indirect selection, there must be a sustained supply of genetic variation in male traits (*39*, *41*). The crux of the lek paradox is that selection on male traits will erode that genetic variation, ultimately resulting in the loss of female preference (*39*). However, when females prefer rare males—regardless of male genotype—negative frequency-dependent selection occurs, ensuring the necessary maintenance of genetic variation.

A notable result is the absence of any detectable fitness benefit, direct or indirect, for females that mated with novel males. Novel males (new arrivals to pools or neighborhoods) had substantially higher reproductive success than residents, regardless of the rarity of their color pattern. Our results illustrate that mating with rare and novel males has distinct fitness consequences for females: Rare males conferred a single-generation reproductive advantage to their sons, driving indirect selection for female preference, whereas novel males conferred no fitness advantages to their partners, either directly or through their offspring. Why then do females prefer novel males?

One possibility is that female preferences for rare and novel males stem from a single, simple mechanism: habituation to familiar males, i.e., females preferring males that are unlike those they have recently encountered (*21*, *22*, *27*). Males with rare color patterns or that are new arrivals to pools (i.e., novel) are both likely to fit this criterion. In this scenario, selection for choosy females is driven by the indirect fitness advantage gained when they mate with rare males. By contrast, preference for novel males emerges as a nonadaptive byproduct of the simple behavioral mechanism under selection.

Female choosiness is likely also under frequency-dependent selection (*6*, *42*). Consider what would happen if all females mated with a single male bearing the rarest color pattern: the sexy son benefit would be lost because all male offspring would have the same pattern, making it common and thus unattractive. As a result, selection for choosy females would evaporate. Although we do not know the mean frequency of choosy females in our population, theory suggests that it is likely to be high. Female preference alleles evolve to higher frequencies when the ability to express choice is hindered (*6*). Here, female choice was hindered by the different movement patterns of males and females. The optimum scale on which females should choose rare males is at the level of the population: Males frequently change location so the rarity of sons, upon which the indirect benefits to females depend, is best predicted by the rarity of fathers at the population level (tables S3 and S4). However, females can only assess the rarity of males they encounter. The more limited movement of females meant that they chose males that were rare at the level of the neighborhood (table S1). This mismatch between the optimum and realized exercises of choice creates the hindrance that could sustain a high frequency of choosy females.

In conclusion, our results challenge the theoretical arguments against the role of sexy sons in sexual selection (*7*, *38*–*40*) by showing that this indirect form of selection can sustain female preference. At the same time, we show that female preference for rare male phenotypes resolves the lek paradox. Both results are a consequence of negative frequency-dependent selection operating on sexual signals and preferences in guppies. Female preference for rare males is well documented in a diversity of organisms (*8*–*14*), but detecting indirect selection in the wild is uncommon because it requires multigenerational studies. The replication of such studies in other organisms will test the generality of our results and determine the broader importance of sexual selection in maintaining, rather than depleting, genetic variation in the wild.

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SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.ade5671
Materials and Methods
Figs. S1 to S4
Tables S1 to S5
References (*43*–*55*)
MDAR Reproducibility Checklist

View/request a protocol for this paper from *Bio-protocol*.

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A passing advantage
Female choice plays a large role in shaping populations. Across many species, females have been shown to prefer males with traits that are rare or uncommon. How this preference is maintained over time has remained an open question. Potter *et al*. looked across generations in Trinidadian guppies and found that females do have a clear preference for rare males, and that they acquire a further fitness benefit through sons that also have these rare traits. Once rare traits become more common, however, this fitness benefit dissipates such that rare traits in the father eventually become common, leaving the grandsons to be less preferred. —SNV

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