Interaction between female mating preferences and predation may explain the maintenance of rare males in the pentamorphic fish *Poecilia parae*

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Abstract

Variation in mating preferences coupled with selective predation may allow for the maintenance of alternative mating strategies. Males of the South American live-bearing fish *Poecilia parae* fall in one of five discrete morphs: red, yellow, blue, stripe-coloured tail (parae) and female mimic (immaculata). Field surveys indicate that the red and yellow morphs are the rarest and that their rarity is consistent across years. We explored the role of variable female mating preference and selective predation by visual predators in explaining the rarity of red and yellow males, and more generally, the maintenance of this extreme colour polymorphism. We presented wild-caught *P. parae* females and *Aequidens tetramerus,* the most common cichlid predator, with the five male colour morphs in separate trials to determine mating and prey preferences, respectively. We found that a large proportion of females shared a strong preference for the rare carotenoid-based red and yellow males, but a distinct group also preferred the blue and parae morphs. The cichlid predator strongly preferred red and yellow males as prey. Together, these results suggest that the interaction between premating sexual selection favouring and predation acting against the red and yellow morphs may explain their rarity in the wild. The trade-off between sexual and natural selection, accompanied by variation in female mating preferences, may therefore facilitate the maintenance of the striking colour polymorphism in *P. parae*.

Introduction

Colour polymorphism, genetically based discrete colour patterns expressed by interbreeding individuals within a population, is widespread in nature (Ford, 1965; Oxford & Gillespie, 1996; Roulin, 2004). In theory, colour polymorphism can be maintained by different mechanisms such as disruptive selection, mutation-selection balance, heterosis and balancing selection (Ford, 1965; Levins, 1968; Endler, 1986; Roulin, 2004; Gray & McKinnon, 2007). However, when colour polymorphism is sex limited and linked to sexual function, the evolution and maintenance of such variation may be explained by

the interaction between sexual and natural selection (Houde & Endler, 1990; Endler, 1992; Sinervo *et al.*, 2001; Kingston *et al.*, 2003; Seehausen & Schluter, 2004; Pierotti *et al.*, 2009).

Whenever competition for mates is intense, males could evolve variation in behavioural, morphological, physiological and life history strategies to maximize reproductive success (Gross, 1996; Shuster & Wade, 2003; Oliveira *et al.*, 2008). If this variation is genetically based, they are referred to as alternative mating strategies (Gross, 1996), and their occurrence has been documented in several species (Zimmerer & Kallman, 1989; Shuster & Wade, 1991; Thompson *et al.*, 1993; Lank *et al.*, 1995; Sinervo & Lively, 1996; Hurtado-Gonzales & Uy, 2009). Frequency-dependent selection is often invoked to explain the stable coexistence of alternative strategies (Gross, 1996), but far less attention has been paid to the interaction between female mating preferences (but see

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Brooks & Endler, 2001a; Morris et al., 2003; Rios-Cardenas et al., 2007) and risk of predation (Endler, 1986; Merilaita & Lind, 2005; Bond & Kamil, 2006).

Females often prefer males with bright and colourful signals because these signals are honest indicators of male quality (Andersson, 1994; Stein & Uy, 2006). However, female mating decisions may favour alternative strategies depending on a number of morph-related or environmental factors (Barlow, 1973; Alonzo & Sinervo, 2001; Brooks & Endler, 2001a; Kingston et al., 2003; Rios-Cardenas et al., 2007). For instance, under high risks of predation, bright and colourful male guppies (*Poecilia reticulata*) are less preferred by females than drab males (Gong & Gibson, 1996; Houde, 1997), suggesting that susceptibility to visual predators offset the advantages of mating with conspicuous males. Such interaction between female mating preferences and susceptibility to predation may in turn allow alternative mating strategies to invade and persist within populations (Bond & Kamil, 2006; Gamble et al., 2003).

Males of the live-bearing fish *Poecilia parae* display one of the most prodigious examples of intraspecific colour polymorphisms (Liley, 1966; Bourne et al., 2003; Lindholm et al., 2004; Hurtado-Gonzales & Uy, 2009). Males in this species occur in five discrete, Y-linked colour morphs (Fig. 1 in Hurtado-Gonzales & Uy, 2009): the drab-coloured immaculata (i.e. female mimic), the stripe-coloured tail parae, the structural-coloured blue and the carotenoid-based red and yellow morphs. Each of the five colour morphs employs alternative mating strategies that ranges from males exploiting female mate choice to males winning access to females via male–male competition (i.e. red, blue, yellow and parae) to males mimicking juvenile females and using a sneaker tactic to gain forced copulations (i.e. immaculata) (Bourne et al., 2003; Hurtado-Gonzales & Uy, 2009). Previous studies have shown that females of *P. parae* strongly prefer the carotenoid-based red and yellow morphs (Bourne et al., 2003; Lindholm et al., 2004), leading to the expectation that red and yellow males should be the most abundant males in the population. However, our field surveys from 2006 through 2009 (this study) along with published data from 2002 to 2005 (Lindholm et al., 2004; Watson-Rodney, 2005) indicate that the red and yellow morphs are consistently the least abundant males. With such a precopulatory mating advantage, why are red and yellow males consistently rare?

In this study, we conducted a series of controlled experiments and extensive field surveys to test whether individual variation in female mating preferences and trade-offs between female mating preference and selective predation can help explain the maintenance of the colour polymorphism, as well as the persistence of red and yellow males at low frequencies. First, we re-examined female mate choice because previous studies did not control for intrasexual interactions (Bourne et al., 2003; Lindholm et al., 2004), which can influence the outcome of female choice (Radesater & Halldorsdottir, 1993; Nilsson & Nilsson, 2000; Shackleton et al., 2005). We then explored the role of visual predators in keeping attractive males rare by (i) comparing the frequency of the most common predator (*Aequidens tetramerus*: Cichlidae) with the frequency of each colour morph from our field surveys, and (ii) conducting laboratory experiments to determine whether predators preferentially approach and attack certain colour morphs.

Methods

Collection and husbandry

Our collection sites are located on the east (6° 47.2′ N, 58° 09′ W) and west (6° 41′ N, 58° 12′ W) sides of the Demerara River, Guyana. Individuals of *P. parae, P. retic-*
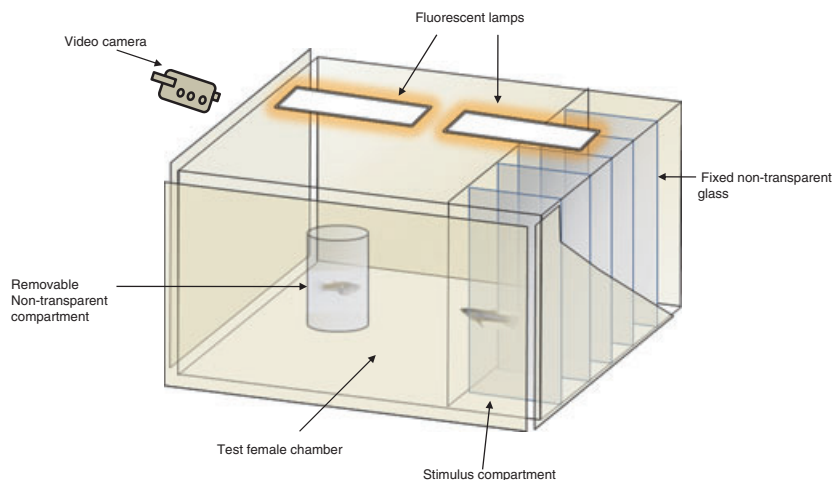

Fig. 1 Experimental tank design.

ulata and the cichlid predator *A. tetramerus* were collected in 2007 and 2008. Pregnant females of *P. parae* were individually housed to monitor their breeding status and used for our mate choice experiments within 4 days after parturition. Cichlid predators were individually housed to reduce stress and aggression among them, whereas morphs of *P. parae* (immaculata, parae, blue, red and yellow) and males of *P. reticulata* were housed in communal tanks. All fish were maintained at 12L: 12D light cycle, at 28 ± 1 °C, and fed with commercial flake food and live brine shrimp (for poeciliids) or commercial pellets (for cichlids).

Morph frequencies and predator relative abundance

To estimate morph frequencies in the wild, we combined published and unpublished surveys of morph abundances from the east and west populations as follows: data from counts collected by Lindholm *et al.* (2004) for east and west populations (January–February 2002), Watson-Rodney (2005) for west population (May–August 2003–2004) and data collected by J.L.H.G. (this study) for east and west populations (January–March, May–August, December 2006–2009). We sampled transects in the east ($n = 15$ sites, mean ± SD: 25.8 ± 2.18 m length, 0.76 ± 0.07 m width, 0.93 ± 0.04 m depth) and west ($n = 15$ sites, mean ± SD: 29.97 ± 2.97 m length, 0.96 ± 0.06 m width, 0.88 ± 0.06 m depth) populations from 2006 to 2009. These transects were used only for survey purposes. To capture individuals, we blocked segments in each of our sampling transects with fish nets, resampling each transect for three consecutive times or until no additional *P. parae* were caught. All captured individuals were placed in containers with treated water and constant aeration and immediately sorted and counted. Because male morphs of *P. parae* resemble each other during their juvenile stage, we only used mature males that displayed fully developed colouration (i.e. for parae, blue, red, and yellow morphs) and/or with total body length of ≥ 18 mm (for immaculata). At this body length, all coloured morphs express adult characteristics (Watson-Rodney, 2005; Hurtado-Gonzales & Uy, 2009).

The relative abundances of aquatic predators were quantified using transects conducted during the summer of 2007 in the east and west populations. We only recorded the presence of *Rivulus stagnalis* (Rivulidae), *Apistogramma steindachneri, A. tetramerus* (Cichlidae) and *Hoplias malabaricus* (Erythrinidae). With the exception of *R. stagnalis* and *A. tetramerus*, the two other predators were only caught occasionally. *R. stagnalis* feed mostly on fry, whereas *A. tetramerus* feed on adult poeciliids (Watson-Rodney, 2005). We, therefore, only tested for an association between the relative abundances of *A. tetramerus* and the five colour morphs.

General experimental protocol

Experimental tank (Fig. 1) dimensions were 50 cm × 45 cm × 28 cm (L × W × H) filled to a depth of 14 cm with water for female choice experiments (but up to 20 cm for the predator experiments) and divided across its width by a glass partition that split the aquarium into two sections. The shorter section contained six compartments of approximately 8 cm × 7 cm × 20 cm each. The sides of these compartments were blocked with an opaque noncoloured thin mesh, to prevent adjacent fish from establishing visual contact. Tanks were supplied with a thin layer of gravel, illuminated with full spectrum light (70 cm above the tank) and were covered on four sides with brown, kraft paper. Before trials, stimuli individuals selected from the stock populations were anaesthetized with MS-222 (3-aminobenzoic acid ethyl ester) and photographed using a digital camera. As a metric reference, we include a 100-mm ruler in each photograph. The total length (tip of the upper jaw to tip of caudal fin) for each individual was obtained from the digital images using Sigmascan Pro®v5.0.0 (San Jose, CA, USA). With the exception of test predators that were deprived of food for 24 h prior to testing, test females and stimuli in both the female choice and predator–prey preference experiments were fed to satiation before experiments started. Immediately after, stimuli individuals were randomly assigned to one of the six compartments by a third party who did not participate in scoring the trials. To prevent visual contact between the test and stimuli individuals during acclimation time (i.e. 20 min period), a removable barrier was fitted, which was then lifted at the start of each trial. Trials were run for 20 min and were continuously recorded with a Samsung camcorder (SCL-810 Hi8) on a tripod positioned about 50 cm above the tank.

From the video tapes, we scored active association time in seconds (females facing morphs, and moving left and right smoothly and repeatedly) as an index of female mating preferences. We excluded periods of time in which females remained stationary facing a male compartment. Laboratory experiments in other fish species have confirmed that this index is an effective assay of female mating preferences (Godin & Dugatkin, 1996; Kodric-Brown & Nicoletto, 1996; Houde, 1997; Kingston *et al.*, 2003; Morris *et al.*, 2003; Cummings & Mollaghan, 2006). For predators, time spent near the stimulus (sec) was used as a measure of predator discrimination or preference for a particular prey type. We recorded the time in which *A. tetramerus*, once habituated to the artificial conditions of the tank, moved slowly to approach and inspect a male and then bump softly into the glass to attack. Several studies in poeciliids and other fish taxa confirm that this type of experimental approach effectively measures prey preferences by visual predators (Rosenthal *et al.*, 2001; Godin & McDonough, 2003).

Experiment 1: female mate choice

Female mate choice trials were run in 2007 (east: $n = 32$ females; mean ± SE: 29.11 ± 0.7mm total length, west: $n = 18$; 28.83 ± 0.65 mm) and 2008 (east: $n = 34$; 29.91 ± 0.21 mm, west: $n = 32$; 29.13 ± 0.11 mm). Because females may preferentially associate with other fish for schooling (Liley, 1966; Houde, 1997), we included a female as a part of the experimental stimuli to control for schooling behaviour. Thus, the stimuli consisted of the five male morphs (immaculata, parae, blue, red and yellow) and a female. With the exception of immaculata males, which are usually 15% smaller than the other morphs (Hurtado-Gonzales & Uy, 2009), males used in female preference trials were similar in total body length (i.e. within 7%). To estimate repeatability of female preferences, trials were repeated after 24 h using the same set of males and females (see statistical analyses for further details). Repeatability (Lessells & Boag, 1987; Boake, 1989) quantifies the proportion of phenotypic variation among individuals, which can also be an indication of the upper limit of heritable variation for female preference (Bakker & Pomiankowski, 1995; Brooks & Endler, 2001b).

Experiment 2: predator prey preference

Experiments to assay predator preference for *P. parae* morphs were run in 2007 (east: $n = 16$ cichlids; mean ± SE: 164.1 ± 3.38 mm total length, west: $n = 16$; 138.3 ± 2.86 mm) and 2008 (east: $n = 32$; 164.7 ± 2.08 mm, west: $n = 32$; 150.7 ± 2.66 mm). Each predator test included the five male morphs (immaculata, parae, blue, red and yellow) and a male of a sympatric prey species, the guppy *P. reticulata*. Guppies are the most common prey in the wild, and so predators may have formed a search image for this prey. The inclusion of guppies therefore represents a control for the most common prey model for the cichlid predator. We did not estimate repeatability for predator preferences. Thus, each predator was tested only once.

Statistical analyses

Total association time (sec) that each female or predator spent during each trial with a potential mate or prey was converted to proportions (Figs S1 and S2). Because the analysis of proportions is subjected to a constant sum constraint (i.e. equal to 1 or 100%), the nature of the data is nonindependent (Aitchison, 1986). Thus, any attempt in analysing proportions using standard parametric or nonparametric multivariate statistical approaches can lead to erroneous conclusions (Jackson, 1997). Hence, we analysed our data using compositional log-ratio techniques, following the methods of compositional data analysis (Aitchison, 1986; Buccianti *et al.*, 2006; Pierotti *et al.*, 2009).

We used the package CoDaPack (http://ima.udg.edu/Recerca/EIO/inici_cat.html), which runs as an Excel® add-Ins. CoDaPack implements the basic methods of compositional data based on log-ratios (Thió-Henestrosa & Martín-Fernández, 2006). In brief, zero values in our data (i.e. no interactions between test subject and stimuli) were replaced by using the 'random zero replacement' command in CoDaPack. This is an important step because zero values would preclude the log-ratio transformations. Random zero replacement value can be assigned by default or manually. We set the value as 0.003, which did not affect the total compositional values expressed by either females or predators. Second, we transformed our data to the isometric-log-ratios (ilr) (Egozcue *et al.*, 2003) to represent a compositional value as a real vector. As a result, the transformed data are said to fluctuate (i.e. the closure of the data is opened) (Buccianti *et al.*, 2006). We determined whether there was any statistical difference for individual female preferences for potential mates depending on population (east and west) and year (2007 and 2008) by running a multivariate analysis of variance (MANOVA). Population and year did not factor in the model (both main effect and interaction; all $P > 0.191$), and so we pooled all female preference data ($n = 116$ females). We estimated repeatabilities of female preference (Lessells & Boag, 1987; Boake, 1989) for all the females tested and considering the groups defined by the cluster analysis. For this purpose, we used the equation developed by Pierotti *et al.* (2009) to calculate repeatability index in multivariate data [detail explanation for the applicability of modified estimator of repeatability index see pg. 2387–2388, appendix B, Pierotti *et al.* (2009)].

To determine whether predators differ in their preference for a potential prey, we run an analysis of variance (ANOVA). We used population (east and west) and years (2007–2008) as factors, and the ilr-transformed predator preference values as the dependent variable. Again, population and year did not factor in the model (all $P > 0.182$), and so we pooled all the predator preference data ($n = 96$). Our aim was to test for differences in the geometric means of preferences expressed by females and predators for potential mates and prey, respectively. Because of the nature of the ilr transformations, it is difficult to infer the direction of the preference (i.e. which males are the most preferred). However, if the tests are nonsignificant, it may represent a general consensus for predators to display the same preferences as females do as mating preferences. Therefore, a cluster analysis is a robust way to explore the possibility that females and predators share similar preferences for particular morphs. The ilr-transformed data of individual females and predators were clustered using mahalanobis distance (Aitchison, 1986; Pierotti *et al.*, 2009) following the Ward clustering algorithm (Ward, 1963). We obtained two clusters representing female mating preferences and predator prey preferences. Cluster validation

indices are presented in Table S1 (supporting electronic materials).

Tables of multiple ternary diagrams, which included the compositional geometric mean and confidence regions for each of the resulting clustering groups, were graphed and analysed with the package 'compositions' (http://www.stat.boogaart.de/compositions) (van den Boogaart & Tolosana-Delgado, 2008) within the statistical package 'R' (http://www.cran.R-project.org, R development Core Team Ver. 2.92 as in 2010). The ternary diagrams help in identifying the trends and relationships between the levels of preferences by either females or predators for particular morph(s). In our case, the tables of multiple ternary diagrams allowed the identification of subgroups of preferences supporting the cluster analysis (see Figs S3 and S4 and for a basic explanation of how to interpret the resulting ternary diagrams see Appendix S1). For all other statistical analyses, unless indicated, we used the software SYSTAT® 12 Software, Inc. (San Jose, CA, USA).

Results

Colour morph and predator relative abundances

The sex ratio (female: male) of *P. parae* was nearly 1:1 for the east (1.07: 0.93) and west (1.03: 0.97) populations. Surveys across years (Fig. 2) indicate that the immaculata and parae morphs are consistently the most abundant colour morphs, with the blue males typically being the third most abundant morph. The red and yellow are the least abundant morphs within and between populations, with the red morph being the rarest in the west population and the yellow in the east population (Fig. 2).

The relative abundance of the most common cichlid predator *A. tetramerus* was 2.74 ± 0.73 individuals per sampling transect. The relative abundance of *A. tetramerus* negatively correlated with the relative abundance of the red morph ($r = -0.55$, $P = 0.03$). A similar negative trend was seen between *A. tetramerus* and yellow morph abundances ($r = -0.48$, $P = 0.06$). No significant correlations were found between the abundances of *A. tetramerus* with that of females ($r = -0.15$, $P = 0.58$), immaculata ($r = -0.27$, $P = 0.33$), parae ($r = -0.13$, $P = 0.63$) and blue ($r = -0.24$, $P = 0.37$) morphs.

Female mate choice

Female association time with each morph was highly variable, ranging from 4 to 506 s. In all the behavioural trials, females spent more time with each of the five males than with control females (geometric mean = 6.9% of total time), indicating that females associated with males for reasons other than schooling. The MANOVA indicated that females vary in their preferences for potential mates (Wilks $\lambda = 0.76$, $F_{8,}$
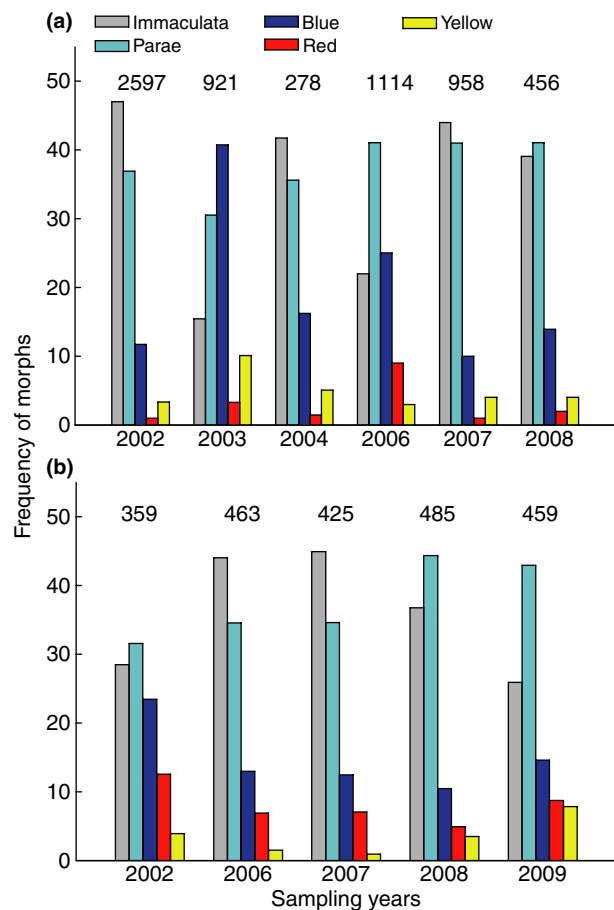

Fig. 2 Frequencies of *P. parae* colour morphs for the (a) west and (b) east populations across years. Total number of individuals sampled for each year is given above the bars [2002, Lindholm *et al.* (2004); 2004, Watson-Rodney (2005); and this study (2006–2009)].

$_{1148} = 21.46$, $P < 0.001$). The homogeneity of the log-ratio variances and covariances was also statistically significant, indicating differences among groups in female mating preferences (Box's M test = 234.74, $F_{12,}$ $_{2450515} = 19.38$, $P < 0.001$). The resulting cluster of individual female mating preferences identified three major groups (Fig. 3a): females with a preference for (i) both carotenoid red and yellow males, (ii) red males only, and (iii) parae, blue, red and yellow males (Figs 3a and S3). Our analysis detected a weak preference for females to associate with immaculata males and control females. Repeatability of female mating preferences was moderately high for all the clustering groups (group 1 = 0.36, group 2 = 0.29 and group 3 = 0.21).

Predator prey preference

The time that the cichlid predator *A. tetramerus* spent with specific *P. parae* morphs ranged from 2 to 336 sec.
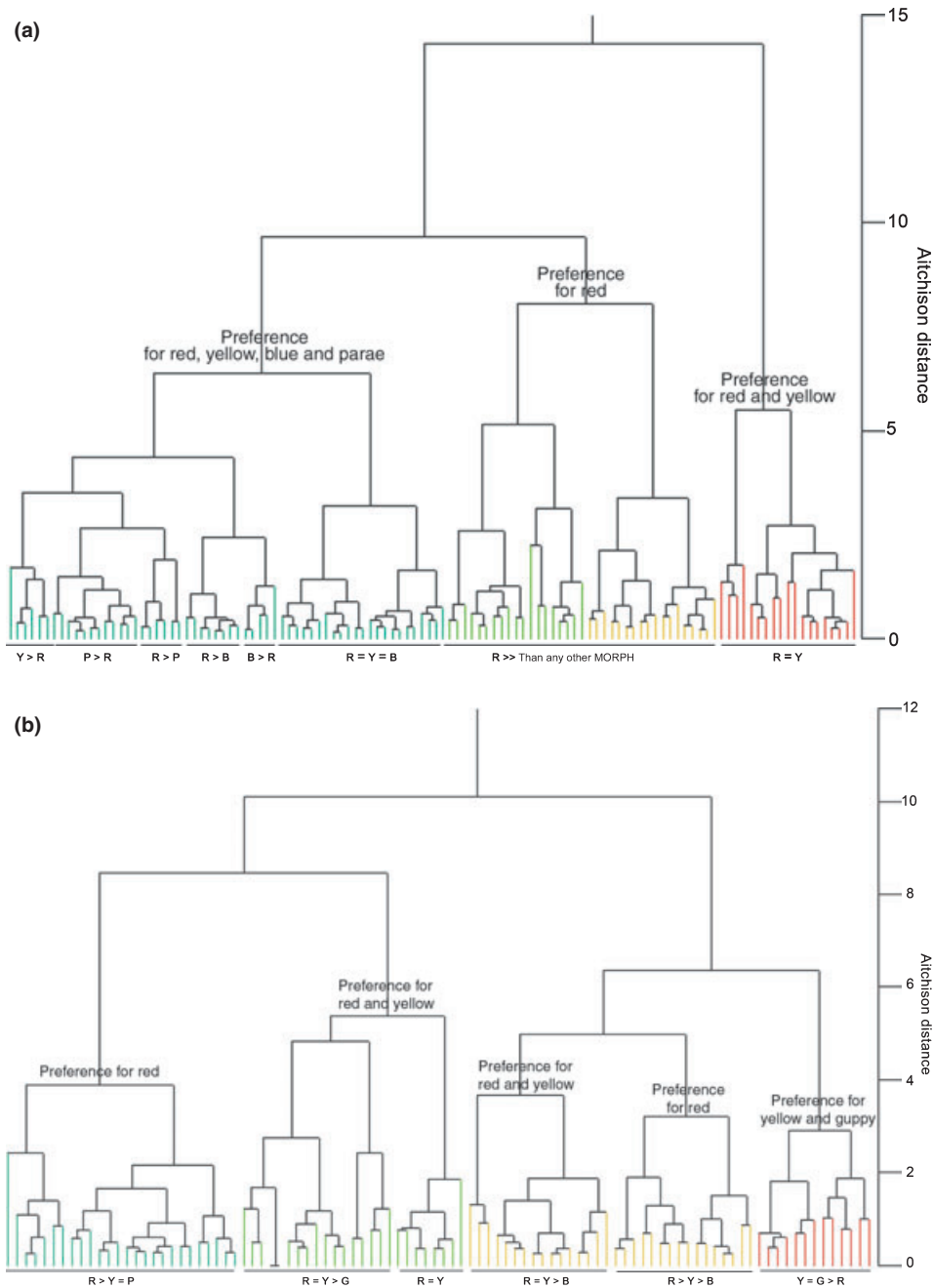
Fig. 3 Dendograms of geometric means of individual (a) female mating preferences and (b) predator prey preferences using the Ward hierarchical clustering algorithm based on Aitchison distance (log-mahalanobis distance) between the variables. Underlined cluster groups of preferences for morphs (P = parae, B = Blue, R = red, Y = yellow, G = guppy) are as defined from the resulting matrices of ternary diagrams (Figs S3 and S4).

We found that the amount of time *A. tetramerus* spent associated with the different *P. parae* morphs and a congener, the guppy (*P. reticulata*), differed significantly (ANOVA: $F_{5, 475} = 27.70$, $P < 0.001$). The resulting cluster of predator prey preferences identified three major groups (Fig. 3b): predator preference for (i) the yellow and guppy males, (ii) red males only and (iii) red and yellow males. In general, the cichlid predators preferred fish with carotenoid-based colouration (Fig. S4).

Discussion

Our experiments demonstrate variation among *P. parae* females in their mating preferences for each colour morph. The compositional clustering analysis indicated that a large group of females preferred the rare carotenoid-based red and yellow males, but another group shared preferences for blue and parae males. These distinct female mating preferences were also highly repeatable, indicating consistency in premating behaviour of individual females. These results indicate that variable female mating preferences and the consistent variation among females in the strength of their preferences may partially explain the persistence of the four colourful male morphs (red, yellow, blue, and parae). However, the compositional analysis detected only a weak female mating preference for the drab immaculata males (Supporting Information Material, Fig. S3), suggesting that mating preference does not keep this morph type in high frequency. Alternatively, our previous work indicates that immaculata males have larger relative testes and primarily use a sneaker mating strategy (Hurtado-Gonzales & Uy, 2009). That is, the immaculata morph foregoes courtship and attempts force copulations with females, suggesting that their high frequency in the population may be a result of their effectiveness in post-copulatory sexual selection. Together, these results suggest that variable female mating preferences and alternative mating strategies by males may allow for the persistence of the five *P. parae* colour morphs.

Although females varied in their mating preferences, the majority preferred red and yellow males, and this is consistent with previous studies (Bourne *et al.*, 2003; Lindholm *et al.*, 2004). If increased mating success leads to increased reproductive success, then red and yellow males should be consistently the most abundant morphs in the wild. However, based on our and previously published surveys, rarity of red and yellow males has been consistent and stable since the 1960s (Liley, 1966; Lindholm *et al.*, 2004; Hurtado-Gonzales & Uy, 2009). One possibility is that red and yellow males persist in low frequencies because the strong female mating preference for these males is offset by elevated risks to visual predators (e.g. guppies; Endler, 1980). Indeed, our field surveys indicate a negative association between the abundances of the most common cichlid predator *A. tetramerus* and the abundances of the red and yellow colour morphs. Predator abundance, however, does not predict the frequency of immaculata, parae and blue morphs. More directly, our predator experiments showed that *A. tetramerus* preferentially approached red and yellow *P. parae* males. The compositional cluster analysis further identified a group with a preference for the common co-occuring prey, the guppy *P. reticulata* (Fig. S4). In our study site, *P. parae* is sympatric with three other poeciliids (*P. reticulata*, *P. picta* and *P. vivipara*). Males of *P. reticulata* and *P. picta* also display carotenoid-based colour patches,

and *P. reticulata* is the most abundant poeciliid. Therefore, cichlid predators may prefer red and yellow males because predators have developed a general search image for carotenoid colour patches or share similar sensory biases as the majority of female *P. parae* (Reznick & Endler, 1982; Rosenthal *et al.*, 2001; Godin & McDonough, 2003).

Frequency-dependent selection tends to be the most common mechanism for the maintenance of colour polymorphisms (Gray & McKinnon, 2007); nevertheless, *P. parae* seems to follow different evolutionary dynamics. While a major visual predator may be affecting the relative abundances of red and yellow males, there is a strong female preference for these rare morphs. The positive and negative selective pressures on these males may therefore create a balance favouring the maintenance of red and yellow morphs in natural conditions. In addition, rarity of the most preferred morphs via predation may allow for the invasion and maintenance of alternative mating strategies (e.g. Bond & Kamil, 2006). This trade-off between natural and sexual selection can then be accompanied by variation in female mating preference to further facilitate the persistence of alternative strategies (see also Rios-Cardenas *et al.*, 2007).

The main result for frequency-dependent selection is that the relative abundances of morphs should fluctuate overtime (Gray & Mckinnon, 2007). In the case of side-blotched lizard, for instance, there is evidence for the fluctuation of the frequency of morphs across years, with a clear advantage for rare phenotypes (Sinervo & Lively, 1996; Alonzo & Sinervo, 2001). In contrast, the abundances of the five morphs of *P. parae* are relatively stable, with no evidence of cycling and with a clear trend for the red and yellow males to remain rare (Liley, 1966; Lindholm *et al.*, 2004; this study). The same frequency patterns are also found across the geographical distribution of this species (Lindholm *et al.*, 2004; Hurtado-Gonzales, unpublished data). A further exploration of female mating preferences under experimentally manipulated frequencies of red and yellow males will clearly contribute to clarify the relative importance of frequency-dependent selection in the maintenance of the five colour morphs in the wild.

Although our study provides evidence that the interaction between variable female mating preferences and selective predation can explain the frequencies of both carotenoid-based morphs (red and yellow) and that of the immaculata males, the persistence of the blue and parae morphs needs further explanation. Our results suggest that some females indeed prefer parae and blue males, and this may partially explain their maintenance. The parae morph is typically larger than other males and often more aggressive (Hurtado-Gonzales & Uy, 2009; Hurtado-Gonzales & Uy, unpublished data). It is, therefore, possible that parae males primarily rely on overt male-male aggression in monopolizing females and securing matings, resulting in high abundances in the

wild. Blue males have unique colour patches that reflect in the ultraviolet and short wavelengths. As the ditches, where *P. parae* are found show variation in ambient light (Hurtado-Gonzales & Uy, unpublished data), these males may persist in the wild by exploiting microhabitats rich in short wavelength light, thereby increasing their conspicuousness and attractiveness to females. Our ongoing field observations and experiments are exploring these possibilities and should provide a more complete explanation for the maintenance of the striking colour polymorphism in *P. parae*.

Acknowledgments

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Supporting information

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

Appendix S1 Interpretation of matrices of ternary diagrams.

Table S1 Cluster validity algorithms for female mating and predator preferences.

Figure S1 Box plots showing distribution of raw data for female preferences (proportion of association time) by population and year.

Figure S2 Box plots showing distribution of raw data for predator preferences (proportion of association time) by population and year.

Figure S3 Matrix of ternary diagrams of the geometric mean and confidence regions of a five sub compositions of individual female mating preferences for color males.

Figure S4 Matrix of ternary diagrams of the geometric mean and confidence regions of a six sub compositions of individual predator prey preferences for color morphs and male guppy.

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