



Evening up the score: sexual selection favours both alternatives in the colour-polymorphic ornate rainbowfish

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Sympatry of multiple, interbreeding colour morphs within a single population requires equilibrium between the various agents of selection acting on alternative colour morphs. This colour polymorphism is an area of growing interest in evolutionary biology as it contradicts many assumptions of natural and sexual selection. Owing to the strong role of colour in intraspecific communication, sexual selection is a primary candidate for balancing selection and its potential is supported by a growing body of both empirical and theoretical evidence. We examined patterns of mate choice in the ornate rainbowfish, *Rhadinocentrus ornatus*, a small freshwater fish species from the wallum habitat of east coast Australia, to see whether nonrandom mating contributes to colour polymorphism maintenance in this system. Populations from Fraser Island and the adjacent mainland comprise a common blue morph and a rare red morph. These colour morphs are present in both males and females, with red individuals accounting for approximately 18% of the individuals in our focal population from Fraser Island. In dichotomous laboratory mate choice experiments, we found that female *R. ornatus* preferred males of the opposite colour morph to themselves, a mating pattern known as negative assortative mating, which may provide indirect benefits to females through the generation of outbred offspring. In contrast, males preferred red females irrespective of their own colour morph, suggesting females may possess red morph-specific traits that are the target of sexual selection by males. Our findings support an emerging pattern that suggests that multiple selection pressures may interact to maintain alternative colour morphs.

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Colour ornamentation is often a key element in intraspecific communication (Brooks & Endler 2001; Maan et al. 2006; Kokko et al. 2007), with behavioural processes such as mate recognition and mate choice frequently relying upon the information provided by colour signals (Alexander & Breden 2004; Dijkstra et al. 2007). In particular, sexual selection based on colour ornamentation can result in the fixation of a single phenotype, or colour morph, leading to monomorphic populations comprised entirely of an optimal phenotype. Colour polymorphism, where more than one colour morph persists within a population, is not predicted under traditional selection theory and is a rare occurrence in natural populations (Gray & McKinnon 2007; Roulin & Bize 2007). However, sexual selection has the potential to maintain within-population colour polymorphism by equalizing the fitness of different colour morphs (Galeotti et al. 2003; Gray & McKinnon 2007; Roulin & Bize

2007). Nonrandom mating through linear sexual selection, positive assortative mating and negative assortative mating have strong potential to maintain colour polymorphism (Gray & McKinnon 2007; Roulin & Bize 2007).

Traditional sexual selection theory has focused on linear selection, where all individuals prefer the same phenotype (Roulin 2004; Gray & McKinnon 2007; Roulin & Bize 2007). In this instance, we use linear selection to refer to a pattern of nonrandom mating where selection favours one colour morph where alternative morphs are present. This is intended to distance this idea conceptually from that of directional selection, which typically refers to selection for exaggeration of individual traits in a specific direction. In colour-polymorphic species, linear selection is most likely to act as a compensatory mechanism for the relative infrequency of that phenotype in the population (Gray & McKinnon 2007). Known as negative frequency-dependent selection or the 'rare male effect', this type of linear selection is yet to receive empirical support (Gray & McKinnon 2007; Roulin & Bize 2007), with the closest known process being a preference for novel males in the guppy, *Poecilia reticulata* (Brooks & Endler 2001; Eakley & Houde 2004; Kokko et al. 2007). The assumed benefit of mating with rare partners is the production of outbred or heterozygous offspring, which may outweigh the cost of finding the rare morph and securing

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mating opportunities (Roulin & Bize 2007). Alternatively, individuals may select rare partners not for complementary genes but for other beneficial traits associated with the morph itself (Roulin & Bize 2007), in which case its rarity does not itself predict mate choice. Preference for common morphs, however, is not undocumented. For example, males of several species within the dragonfly order Odonata adjust their mating preference to male-like or female-specific colour morphs, whichever is more common (Van Gossum et al. 1999, 2007).

Circumstances in which the colour-polymorphic trait is exhibited by both sexes may foster more complex patterns of sexual selection. The role of colour in mate recognition has led to strong support for positive assortative mating as a mechanism for the maintenance of within-population colour polymorphism. Assortative mating occurs through pairing of similarly coloured individuals and may occur where there is selection against intermorph hybrid inferiority (if colour morphs are adapted to different conditions e.g. visual habitats) or where genetic compatibility between morphs is reduced (Gray & McKinnon 2007). Positive assortative mating is well documented in avian species, with strong evidence in the case of the Gouldian finch, *Erythrura gouldiae*. Pryke & Griffith (2007) demonstrated strong assortative pairing in ca. 80% of breeding pairs in field and aviary observations, reinforced by their subsequent finding that partial reproductive isolation reduces hybrid viability, particularly in females (Pryke & Griffith 2008).

Far less common is the process of negative assortative mating, where individuals preferentially mate with colour morphs that are different from their own (Gray & McKinnon 2007; Roulin & Bize 2007). Still poorly understood, negative assortative mating has strong potential to maintain within-population colour polymorphism. Heterozygote advantage has long been touted as a mechanism for the maintenance of within-population genetic diversity. If hybrid offspring have increased fitness because of heterozygosity, the most beneficial strategy of mate choice would be to select partners based on genetic complementarity. If colour is a reliable signal of underlying genotype, this may provide strong selection for negative assortative mating. However, the preference of one morph for partners of the opposite morph is still yet to be empirically demonstrated, and this may be because of the focus of previous studies on systems that have the potential for gene flow or introgression between populations (see Gray & McKinnon 2007; Roulin & Bize 2007 for examples), or where spatial segregation is facilitated. Conversely, individuals from isolated populations may be more likely to encounter close relatives when searching for potential mating partners, whereby selection of partners based on phenotypic similarity would further increase this risk.

Freshwater fish species are ideal for examining sexual selection (Hughes et al. 1999; Brooks & Endler 2001; Alexander & Breden 2004; Eakley & Houde 2004; Wong et al. 2004; Kokko et al. 2007), often displaying strong preference for secondary sexual characters, including colour ornamentation. Furthermore, obligate freshwater species often represent discrete populations, being restricted to drainages or even to specific sites with little chance for migration and introduction of new alleles (McGlashan & Hughes 2002). This places great importance on within-population processes. To date, studies of colour-polymorphic freshwater fish species have been largely restricted to examining the maintenance of male polymorphism, owing to sexual dimorphism in the trait among the majority of these species (Fuller 2002; Eakley & Houde 2004). This restricts the role that assortative mating, either positive or negative, can play in these systems. Where colour polymorphism is expressed in both sexes, study systems represent sympatric sister or incipient species where polymorphism is partitioned between subspecies. As a result, empirical support is overwhelmingly in favour of positive assortative mating in freshwater fish.

The ornate rainbowfish, *Rhadinocentrus ornatus*, is a unique freshwater fish species in that it displays a within-population colour polymorphism that is expressed in males and females. Populations of *R. ornatus* from Fraser Island, Queensland possess a known colour polymorphism comprising a common blue morph and a rare red morph (Fig. 1; Allen & Cross 1982; Allen et al. 2002; Pusey et al. 2004). This colour polymorphism provides an ideal system for examining the pattern of sexual selection in both sexes, and how this may differ between colour morphs. Using a focal population, we assessed the relative frequency of red and blue morphs in each sex and the proportion of red coloration expressed within the red morph, which is known to vary among individuals. We then conducted dichotomous mate choice experiments to test competing hypotheses of nonrandom mating, and examine mating preferences between male and female ornate rainbowfish.

METHODS

Study System

Habitat

The ornate rainbowfish is a small, obligate freshwater fish species restricted to the coastal wallum habitat of eastern Australia (Page et al. 2004). The streams and lakes inhabited by *R. ornatus* are situated in coastal scrub and heath and are generally fed by natural springs, making their water soft and acidic (pH 3.5–6; D. Hancox, C. J. Hoskin & R. S. Wilson, unpublished data). Individual sites also vary in the level of tannin stain that remains after filtration by aluminium compounds as the water is drawn to the surface. This results in



Figure 1. (a) Blue and (b) red male ornate rainbowfish from Gerowweea Creek, Fraser Island.

between-population differences in the visual habitat, from far 'red-shifted' habitats created by highly tannin-stained water to 'white' habitats where tannin compounds are absent. Populations of *R. ornatus* vary in the ratio of red and blue individuals, from all-red to all-blue and the range between (D. Hancox, C. J. Hoskin & R. S. Wilson, unpublished data). Our focal population from Gerowweea Creek on the eastern coast of Fraser Island, Queensland, represents the 'white' visual habitat and comprises both colour morphs but with a bias towards blue. The relationship between habitat variation and between-population divergence in phenotype and colour polymorphism forms part of our ongoing examination of this system.

General and reproductive behaviour

Based on direct field observations of *R. ornatus* from our focal population and various other locations, this species forms small shoals of approximately 6–20 individuals that comprise adult males and females of a range of body sizes. These aggregations are found in the top to middle range of the water column, from approximately 10 to 150 cm depth. Large males (to ca. 8 cm) can be found foraging further down in the water column. Juveniles (individuals less than ca. 2 cm in total length) form loose aggregations close to the water's surface and generally remain separate from adults. Red and blue individuals display no spatial segregation that is discernable through observation or catching success. Reproductive behaviour is initiated by male *R. ornatus* which identify and defend suitable spawning sites among aquatic vegetation (hairgrass, *Eleocharis* spp. being the most common substrate at Gerowweea Creek) within the top 30 cm of the water column. Males attempt to solicit visits by females by displaying their fins and a red nuptial blaze (common to all Melanotaeniid rainbowfish; Allen & Cross 1982) while in active pursuit, and lead females back to their chosen spawning site. This temporary territory is defended against rival males, which attempt to intercept receptive females, by vertical fin extension and increased melanin expression that often escalates to biting and chasing. Successful spawning is achieved through external fertilization and no parental care is given to offspring, meaning that the reproductive investment of both males and females is wholly represented by premating investment (either physiological, such as in gamete production, or through energy and time costs, such as behaviour).

Collection and Husbandry

Adult *R. ornatus* were collected from Gerowweea Creek from 15 to 17 May 2007 by R.W. and D.H. Fish were collected using long-handled dip nets and bait traps, and all activities were conducted in compliance with permits from the Queensland Department of Primary Industry and Fisheries and Queensland Environmental Protection Agency/Parks and Wildlife Service. Fish were transported in 60-litre bins with constant aeration via air-conditioned 4WD to the School of Biological Sciences, University of Queensland. Housing consisted of 75-litre closed system group aquaria containing an air-powered filter, a 2.5 cm gravel bed and woollen spawning mops for structural complexity. Water changes of approximately 25% of the aquarium volume were conducted twice a week using reverse osmosis-purified water with 10 g of added synthetic aquarium salt. Water quality was tested for ammonia, nitrites, nitrates and pH fluctuations at each water change. The aquatic temperature at Gerowweea Creek fluctuates seasonally between approximately 15 and 25 °C (unpublished data), so our study aquaria were maintained at 25 ± 1 °C in a constant temperature room with a 14:10 h light:dark regime to approximate a summer (primary spawning season) temperature regime and photoperiod. Fish were fed daily on newly hatched brine shrimp (*Artemia*) until foraging behaviour ceased. The behaviour and body condition of all individuals were monitored

daily, and all individuals were allowed 2 weeks to become established before the experiments started. Males and females were housed separately for the duration of the study, with each tank containing approximately 15 individuals. At the completion of our study, all fish continued to be housed at the School of Biological Sciences under the conditions described above for subsequent use in additional experiments. All housing conditions and experimental activities were approved by the University of Queensland Animal Ethics and Welfare Committee.

Quantifying Variation in Phenotypes

We estimated the frequency of the red morph as a proportion of the Gerowweea Creek population in a sample of 152 adult fish. We found variation in the red morph for the extent of expression of red coloration, which we calculated as the proportion of the total lateral area of the fish including the body and head, as well as the caudal, dorsal and anal fins. Each red individual was sedated by lowering its body temperature to 6 °C by immersion in chilled aquarium water (Endler 1991; Alexander & Breden 2004; Aparico et al. 2005; Hankison et al. 2006). We are aware that concern has been raised about the use of this technique as a method of immobilization, largely because of the possibility of ice crystal formation and the discomfort this would cause during rewarming. However, our 6 °C treatment would not result in ice formation and all fish involved in this study were closely monitored for signs of distress throughout the procedure. A recent study by Ashley et al. (2007) examining nociception in the rainbow trout, *Oncorhynchus mykiss*, found no cold nociceptors (those that exhibit a 'pain' response when exposed to -7 to +4 °C temperatures) in the temperate species, meaning that exposure to 6 °C conditions is unlikely to be painful in our subtropical study species. We used a two-step method where fish were first placed in 15 °C aquarium water until their mobility slowed, which generally occurred within 1 min, before being transferred to 6 °C. This resulted in faster recovery of individuals during preliminary trials of this technique than straight transference from 25 °C to 6 °C. When loss of equilibrium occurred, generally within 10 s of being placed in aquarium water, each individual was transferred to a 7.5 cm petri dish containing approximately 15 ml of 6 °C water. Placing water in the petri dish helped keep fish sedated during photography, and active gill ventilation was observed in most individuals. We believe this is also beneficial to fish as they were not mechanically dried or placed in contact with a dry surface, and it facilitated estimation of the proportion of red on individuals as the fins were in a natural, relaxed posture. Photographs were taken using a Canon PowerShot A540 digital still camera mounted on a small tripod with a reference grid included in each image. Photography was achieved within 15 s for each individual, after which point it was transferred back to 15 °C until equilibrium was regained before being returned to the 25 °C group aquarium. Normal behaviour (shoaling, foraging) resumed within 15 min for most individuals, and no short- or long-term effects were observed during the study. Photographs were analysed using the image analysis program SigmaScan Pro 5 (Systat Software Inc., San Jose, CA, U.S.A.). The iridescent nature of blue coloration in *R. ornatus* requires that a receiver must be at a specific angle of reflectance, relative to the ambient light source, to view this signal. Further investigation is being made into the geometric component of this signal; however, it is apparent that no observable reflectance of ambient light occurs at 0° (when the light source and viewer are in the same position), as when photographing a horizontal fish from above (RGB values for blue caudal, dorsal and anal fins calculated in Adobe Photoshop are not significantly different from the background against which fish are photographed; unpublished data). Therefore, blue coloration is not visible in our photographs and analysis of these images was

based on the area of red visible on the total lateral area of individuals (as defined above).

Sexual Selection for Colour Morph

Female mate choice was assessed through traditional dichotomous choice tests (Seehausen & van Alphen 1998; Maan et al. 2006; Pierotti & Seehausen 2006; Wong & Rosenthal 2006). Our design replicated that used by Wong (2004) and Wong et al. (2004) in studies of dichotomous mate choice in the Pacific blue-eye, *Pseudomugil signifer*; mate choice under these conditions was a strong predictor of mating success. In the present study a red or blue focal female was able to interact visually, through a clear Perspex divider, with two males (one red, one blue) separated from each other by an opaque divider. The behavioural response of focal females to stimulus males was recorded over a 20 min observation period, commencing when the observer was confident that visual contact had been made between the female and both males. During this period the female was classified as associating with a male when she was within 5 cm of the clear divider with her body oriented towards the male at an angle of $<45^\circ$. We measured the time spent associating with each male by entering the start and end times of each association event in the behavioural software program ETHOM (Shih & Mok 2000). Observation aquaria were illuminated by commercially available Sylvania Gro-lux fluorescent tubes, with males viewed against a neutral (dull white) background.

Our focal individuals consisted of eight red females (those that displayed red pigmentation) and eight blue females (those that displayed no red pigmentation), where the red phenotype was treated as a categorical variable. However, by measuring the extent of expression in each red individual (see above) we were able to analyse the red morph as both a categorical and a continuous variable. We tracked individual identity by housing each female and red male in a fry-rearing enclosure (16 × 12 cm and 10 cm high) within the group aquaria to account for the repeated use of the focal females and red males. This allowed female and red male identity to be incorporated into our statistical analyses. The majority of females were observed over three trials; however, one red female was in two trials and one blue female in only one, resulting in a total of 22 mate choice trials for red females and 21 for blue females. For each trial, a blue male of equal size to each red male ($<5\%$ difference in total length) was chosen from the group aquaria containing approximately 70 blue males. No female was ever retested with the same red male, and blue males were treated as random. Male mate choice for seven red and nine blue individuals was assessed using the same design, and all focal fish were observed over three trials, resulting in a total of 21 trials for red males and 27 for blue.

Statistical Analyses

We conducted a beta regression (Ferrari & Cribari-Neto 2004) in the statistical program R to model the proportion of the observation period focal individuals spent associating with stimulus fish. This is a useful regression model for analysing data that are continuously distributed and bound between the values of 0 and 1 (Ferrari & Cribari-Neto 2004), such as the response data in our study. We used the pooled response of each focal fish across trials to remove the effect of replication in our data caused by the repeated use of focal individuals. As a result, each individual, rather than each trial, is used as our unit of replication. We also performed a beta regression to test for differences in the proportion of total association time each morph received from males and females, again by taking the mean proportion for the focal individual. All results are presented as means and 95% confidence intervals calculated from beta regressions unless otherwise stated.

RESULTS

Quantifying Variation in Phenotypes

We quantified the amount of red coloration for 71 males and 81 females from Gerowweea Creek. Within this sample, 28 (18.5%) expressed some level of red coloration and were assigned to the red morph, with each sex containing the same number of red individuals (14 each). As a proportion of the total number of individuals, 19.7% of males were red and 17.3% of females were red. Thus, there was no difference between the sexes in the proportion of red individuals in our sample (chi-square test: $\chi^2_1 = 0.149$, $P = 0.669$). We found that the red coloration represented a mean \pm SE of $24.8 \pm 5.5\%$ and $20.7 \pm 3.0\%$ of the lateral area of red males and red females, respectively, which was not significantly different between the sexes (t test: $t_{20} = -0.065$, $P = 0.522$). The phenotypic distribution of this red coloration varied from 0.5 to 83% in males and 7 to 48% in females, with a mode at approximately 16% for both.

Female Selection for Male Colour Morph

Females exhibited a strong response in dichotomous mate choice trials, spending an average of 554.6 s (416.1, 698.3) or 46.2% (34.7, 58.2) of the 20 min observation period associating with either male. We found no difference in response to stimulus males between red (583.3 s (455.6, 793.9)) and blue (526.0 s (338.8, 729.1)) female morphs ($Z = 0.427$, $P = 0.669$). Females displayed a distinct preference for one of the stimulus males during behavioural observations, spending 82.47% (71.3, 89.92) of their total association time with the preferred male ($Z = 5.19$, $P = 0.001$).

We found no overall preference for one male colour morph, with red males receiving 47.8% (34.2, 61.8) of female association time, which was not significantly different from 50% ($Z = -0.325$, $P = 0.745$). However, females displayed a strong preference for males of the opposite morph to their own. Blue females spent 65.9% (48.8, 82.7) of their association time with the red male ($Z = 2.54$, $P = 0.011$) while red females spent only 30.0% (11.4, 58.0; $Z = -2.04$, $P = 0.041$). Thus, red and blue females differed greatly in their choice of preferred male colour morph ($Z = -3.13$, $P = 0.002$).

Female preference for red or blue males was not influenced by the proportion of red displayed by each individual female (within the red morph; $Z = -1.093$, $P = 0.274$), nor was the proportion displayed by red stimulus males ($Z = 1.22$, $P = 0.222$). This was the case for both blue ($Z = 1.502$, $P = 0.133$) and red ($Z = -0.233$, $P = 0.82$) females.

Male Selection for Female Colour Morph

As with females, males exhibited a strong response in dichotomous mate choice trials, spending 698 s (567.0, 820.1) or 58.2% (47.2, 68.3) of the 20 min observation period associating with stimulus females. Unlike females, however, there was a near significant difference in the responsiveness of males ($Z = 1.95$, $P = 0.051$), where red males spent 77.9% (57.35, 90.2) of the observation period associating with stimulus females and blue males spent only 50.2% (37.19, 63.19). Males displayed a strong and significant preference for one of the stimulus females, spending 84.5% (71.56, 89.4) of their total association time with their preferred female ($Z = 8.3$, $P < 0.0001$) with no significant difference between male morphs ($Z = 1.53$, $P = 0.126$).

Overall, we found a preference for the red female morph, which received 70.5% (59.3, 79.6) of male association time ($Z = 3.76$, $P < 0.001$). There was also a significant difference in the strength of preference between male morphs, where red males spent 81.4% (60.5, 92.4) with the red female, and blue males spent 63.0% (49.1,

75.0; $Z = 1.99$, $P = 0.046$). Both these results were significantly different from 50%, indicating a departure from random mate choice.

We found no effect of the proportion of red displayed by the chooser whether within the red morph ($Z = -0.183$, $P = 0.855$) or within all males ($Z = 1.40$, $P = 0.162$). The proportion of red displayed by stimulus females also had no effect on the strength of male preference ($Z = -1.70$, $P = 0.089$).

DISCUSSION

Our assessment of the frequency of the red morph within the Gerowweea Creek population is consistent with the observed colour polymorphism in this Fraser Island population of *R. ornatus*. In accordance with our own observations, the red morph occurs at a frequency much lower than the blue morph, contributing to approximately 1/5 to 1/6 of the total population. In behavioural experiments, we found male and female ornate rainbowfish displayed strong yet contrasting patterns of nonrandom mating (Fig. 2). Females significantly preferred males of the opposite morph to their own, while both male morphs preferred red females. The outcomes of our mate choice experiments are consistent with our treatment of red and blue *R. ornatus* as belonging to discrete colour morphs, as opposed to representing continuous variation between entirely blue and entirely red individuals. Given the variation in the amount of red displayed by individuals from our focal populations, it is perhaps surprising that mate choice was predicted by the presence or absence of red, rather than the amount of red expressed by the focal individual. It is possible that the proportion of red could be under natural selection or correlated with other traits that are not under direct sexual selection. Alternatively, the amount of red may be an important trait in identifying preferred mates in the complex visual habitat of Gerowweea Creek, even though we found no effect under controlled aquarium conditions. The use of a larger number of individuals may allow the relationship between an individual's proportion of red and the direction and strength of its mating preference to be more comprehensively tested by presenting focal fish with a greater range of phenotypes from which to choose, and

also allowing further examination of the chooser's phenotype and possible correlated traits (such as mate choice).

It is possible that the variation in phenotypes in this population (between blue and red morphs, as well as within the red morph) may be a signature of phenotypic plasticity and associated with a shift in reproductive behaviour. However, we believe the scope for plasticity is limited in this system. Wild *R. ornatus* maintain their adult phenotype under aquarium conditions with no observable alteration to the quality or extent of their coloration (discounting reversible plasticity), and while we have made no concerted effort to breed this species in captivity, spawnings outside our experiments have resulted in large variation in adult phenotype despite all individuals being raised under identical conditions. Furthermore, we have observed no temporal variation in the visual habitat of Gerowweea Creek (also subject to ongoing investigation) that would provide selection for developmental plasticity, and if this were the case environmental cues would need to be reliable predictors of the environment an individual will inhabit as an adult up to 6 months in advance. We therefore concentrate on possible genetic determinants of phenotypic variation and their interaction with processes of sexual and natural selection.

Within the red morph, variation in the expression of red coloration could be the result of functional trade-offs. For example, an increase in red pigmentation may affect risk of predation or reduce the area available for expression of other phenotypic traits involved in intraspecific communication. The lakes and streams of Fraser Island are home to few fish species capable of exerting selection pressure through selective predation (we have observed no piscivorous species larger than *R. ornatus*); however, large numbers of long-arm prawns (*Macrobrachium* spp.) are present at Gerowweea Creek (D. Hancox, C. J. Hoskin & R. S. Wilson, personal observation). The presence of the predatory *Macrobrachium crenulatum* has been shown to increase orange coloration in populations of the Trinidadian guppy, owing to the poor perception of red by *M. crenulatum* (Kemp et al. 2008). However, we would expect to see less variation in the proportion of red displayed by individuals if predation were generating directional selection for greater red coloration in the Gerowweea Creek population of *R. ornatus*. Physiology and resource allocation could also be involved. Many red pigments are carotenoid based and can require considerable resources to maintain, as demonstrated in the Gouldian finch (Pryke et al. 2007; see also Reimchen 1989; Albert et al. 2007; Kemp et al. 2008; Lewandowski & Boughman 2008). A direct relationship between the amount of red displayed by an individual and either its mate choice or success would increase the likelihood of carotenoid-linked correlated traits. However, because colour appears to be a strong predictor of mate choice in *R. ornatus*, the pattern of inheritance of colour variation within the red morph would also be worthy of examination.

In discussing possible genetic determinants of colour polymorphism in *R. ornatus*, the observed frequency of the rare morph in our focal population is not dissimilar to expected ratios under Mendelian inheritance. A recessive allele for red coloration could account for the low frequency of red individuals relative to blue, especially if there were costs associated with homozygosity as suggested by negative assortative mate choice in females. Fuller & Travis (2004) successfully demonstrated Mendelian inheritance for polymorphic traits in the bluefin killifish, *Lucania goodei*, through half-sib breeding experiments, and a similar approach could be implemented for *R. ornatus*.

Female choice experiments revealed a strong pattern of negative assortative mating in both morphs. This mating strategy has long been touted as a potential mechanism for maintaining population-level genetic diversity and, as in this case, phenotypic variation. At the level of the individual, females may increase their

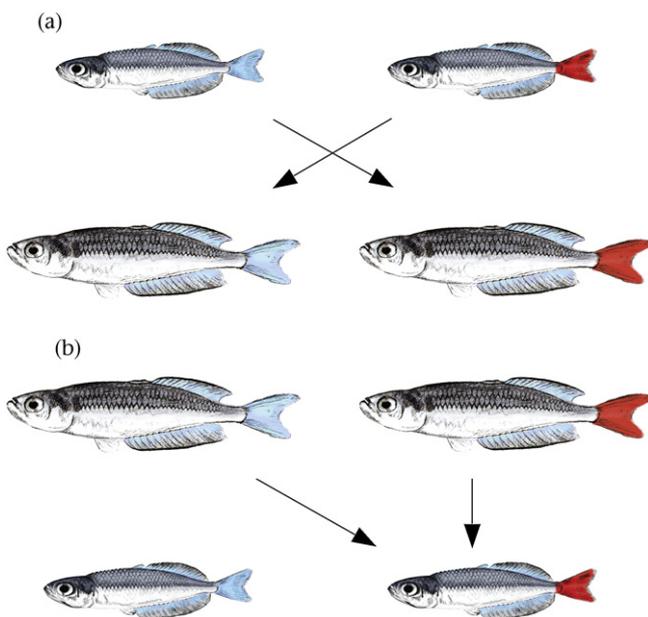


Figure 2. Summary of the mating preference of red and blue (a) female and (b) male ornate rainbowfish from Gerowweea Creek, Fraser Island. Arrows show direction of preference.

reproductive fitness by mating with males of the opposite colour morph. Females of a range of taxa are known to seek unrelated males as sexual partners (Hoffman et al. 2007; Mays et al. 2008), which is probably a mechanism for inbreeding avoidance and increased production of heterozygous offspring. Even if the red morph of *R. ornatus* were determined by a recessive allele for colour, in which case a large proportion of blue individuals could be heterozygous for colour, these blue individuals would still benefit by preferentially mating with red partners. Likewise, the average benefit for red individuals of mating with blue partners (whose underlying genotype is unknown) would still be greater than under a random mating strategy. The likelihood of a given individual encountering a reproductively mature close relative in Gerowweea Creek is unknown, and as such this interpretation is based solely on relevant evolutionary theory (see Roulin 2004; Gray & McKinnon 2007; Roulin & Bize 2007). None the less, the outcome of negative assortative mating in female mate choice trials was equal to the net selection for both male colour morphs, as the level of preference in red and blue females for their respective preferred males was almost identical. However, our focal group consisted of an even number of red and blue females, which is not representative of the frequency of each in the natural population. In reality, the selection pressure exerted on each male morph by female mate choice may be considerably skewed towards red males because of the abundance of blue females.

Unlike female *R. ornatus*, red and blue males both displayed strong preference for the red morph in mate choice trials. This result is consistent with the theory of negative frequency-dependent selection, where the cost of searching out rare partners should be offset by the benefit of avoiding inbred offspring. However, we have observed the frequency of the red morph in Gerowweea Creek to be stable over a number of years (D. Hancox & R. Wilson, personal observation). Preference for whichever morph is rare at a given point in time is therefore unlikely to account for the observed pattern of mate choice. Furthermore, blue males may gain the benefit of producing outbred offspring by mating with rare red females, but red males would not. Alternatively, red females may possess morph-specific traits that are of benefit to males as a whole, irrespective of their own phenotype. Differences in female fecundity between alternative colour morphs have been reported in a number of colour-polymorphic species. In the side-blotched lizard, *Uta stansburiana*, orange-throated females lay large clutches of small offspring, while yellow-throated female lay small clutches of large offspring (Sinervo et al. 2001). These offspring have high or low survivorship depending on overall offspring density, thus resulting in alternative reproductive strategies between competing female morphs (Sinervo et al. 2001). In the common lizard, *Lacerta vivipara*, orange and yellow females differ in their clutch to egg size ratio (as in *U. stansburiana*) and in the survivorship of their offspring in conjunction with numerous other biotic and abiotic variables (Vercken et al. 2007). Owing to their effects on offspring survival, these traits could be sexually selected by males and provide correlational selection for female colour along with higher (or more successful) reproductive output.

In addition to nonrandom mating, alternative male reproductive strategies may play a role in the maintenance of colour polymorphisms in *R. ornatus*. Red males displayed a greater response to stimulus females than blue males, which may indicate any number of behavioural differences. The three male colour morphs of the side-blotched lizard are associated with alternative strategies; Bleay et al. (2007) defined the orange-throated males as 'territorial usurpers', blue-throated males as 'monogamous mate guards' and yellow-throated males as adopting a 'sneaker strategy', all of which have differential success based on the social environment. Based on the increased courting response of red males in our study, this morph

may invest more effort than blue males in courting females and thereby solicit more successful matings, perhaps at the cost of other activities such as foraging. Alternatively, red males could be displaying greater overall activity with increased courting behaviour representing just one measure. The carotenoid basis of the red coloration of many species can be linked to androgen up-regulation, resulting in higher activity levels (especially aggression e.g. *E. gouldiae*: Pryke & Griffith 2006; Pryke 2007). The competitive nature of *R. ornatus* males means that intrasexual conflict would be worth examining, especially as male dominance may influence the extent to which mating preferences are realized (Pryke & Griffith 2007).

Sexual conflict could also occur within this system; red males prefer red females, which in turn prefer blue males. Likewise, blue females prefer red males, which do not reciprocate this preference. Inbreeding avoidance (as suggested by negative assortative mating in females) combined with directional male selection for red females could instigate such conflict. Resistance of red females to red males (as an alternative mechanism for blue preference) could also provide selection for increased effort or persistence in red males. In contrast, red males should be less inclined to refuse matings from non-preferred blue females, as the relative cost of reproduction should be low compared with sacrificing potential offspring. To address these possibilities, however, the level at which individuals adhere to the above mating strategies and whether or not there is active avoidance of nonpreferred mates needs assessment.

In combination, the mating preferences of male and female *R. ornatus* have a large capacity to maintain the rare red morph. Chunco et al. (2007) demonstrated that female mate choice alone has the potential to maintain male colour polymorphism, and where both sexes are polymorphic and display nonrandom mating the potential should be greatly increased. Blue females, blue males and red males all displayed a preference for the rare morph in our study, suggesting that mate choice could make a large contribution to the fitness equilibrium between red and blue morphs.

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