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The relative role of male vs. female mate choice in maintaining assortative pairing among discrete colour morphs

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Abstract

Mate choice has important evolutionary consequences because it influences assortative mating and the level of genetic variation maintained within populations. In species with genetically determined polymorphisms, nonrandom mate choice may affect the evolutionary stability and maintenance (or loss) of alternative phenotypes. We examined the mating pattern in the colour polymorphic Gouldian finch (*Erythrura gouldiae*), and the role of mate choice, both female and male, in maintaining the three discrete head colours (black, red and yellow). In both large captive and wild populations, Gouldian finches paired assortatively with respect to head colour. In mate choice trials, females showed a strong preference for mates with the most elaborate sexually dimorphic traits (i.e. more chromatic UV/blue plumage and longer pin-tail feathers), but did not discriminate assortatively. Unexpectedly, however, males were particularly choosy, associating and pairing only with females of their own morph-type. Although female mate choice is generally invoked as the major selective force maintaining conspicuous male colouration in sexually dichromatic species, and is typically thought to drive nonrandom mating, these findings suggest that mutual mate choice and male mate choice in particular, are an important yet neglected component of selection.

Introduction

Understanding genetic colour polymorphism has proved a major challenge, both in terms of the underlying genetic mechanisms (e.g. Nachman *et al.*, 2003; Mundy, 2005) and the evolutionarily forces maintaining such genetic variation (Krüger *et al.*, 2001; Lank, 2002; Fowlie & Krüger, 2003; Galeotti *et al.*, 2003; Roulin, 2004). In this context, an important evolutionarily force, which can substantially affect the magnitude and stability of genotypes under selection, is nonrandom mating (Allard *et al.*, 1968), where certain combinations of genotypes or phenotypes will occur more often than expected by chance. For example, disassortative mating (i.e. pairing of different morphs) may prevent the loss of rare phenotypes (e.g. Thorneycroft, 1975; Knapton & Falls, 1983), whereas assortative mating (i.e. pairing of same-type morphs) may result in reduced gene flow among the morphs, leading to phenotypic divergence and potentially even reproductive isolation (Kirkpatrick, 2000; Salzburger *et al.*, 2006).

Nonrandom mating with respect to colouration is a commonly observed mating pattern among birds (Hill, 2006). In colour polymorphic systems, while mating in several species is thought to be random with respect to morph (e.g. northern fulmar *Fulmarus glacialis*: Hatch, 1991; little shag *Phalacrocorax melanoleucos brevirostris*: Dowding & Taylor, 1987; tawny owls *Strix aluco*: Brommer *et al.*, 2005), the majority of studied species exhibit assortative (e.g. brant *Branta bernicula*: Abraham *et al.*, 1983; lesser snow geese *Anser caerulescens caerulescens*: Cooke *et al.*, 1976; barn owl *Tyto alba*: Roulin, 1999; buzzard *Buteo buteo*: Krüger *et al.*, 2001) or disassortative pair formations (variable oystercatcher *Haematopus unicolor*: Baker, 1973; white-throated sparrows *Zonotrichia albicollis*: Knapton & Falls, 1983), although conclusions for particular mating patterns within species are often unclear (O'Donald, 1983; Phillips & Furness, 1998; Janssen *et al.*, 2005). The frequent occurrence of assortative or disassortative mating among polymorphic species suggests that phenotypic traits are important in mate

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selection. Yet, in contrast to condition-dependent traits, where individuals are predicted to exert mainly directional sexual selection by preferring the most attractive mate (e.g. most colourful; Hill, 2006), in polymorphic species mate choice decisions may differ between individuals of different morph-types (e.g. if the preferred mate for one individual is a different colour morph than the preferred mate for another individual). Identifying between-morph variation in mate choice decisions is important for understanding selection pressures on alternate colour morphs and the mechanisms maintaining non-random mating patterns.

To date, evidence for a role of mate choice in determining assortative or disassortative mating among colour morphs is scarce (reviewed in Roulin, 2004) and often unclear (e.g. Phillips & Furness, 1998; Fox *et al.*, 2002). One of the most-studied polymorphic avian species is the snow goose, in which female imprinting on the parental colour type (Cooke *et al.*, 1972) determines their future mate preference (Cooke & McNally, 1975; Cooke *et al.*, 1976), and explains the strong assortative mating on plumage colouration (Cooch & Beardmore, 1959). As colour polymorphisms are commonly present in both sexes, theoretically, mutual mate choice should be adaptive and prevalent. However, while few studies have experimentally investigated the role of female mate choice (e.g. Knapton & Falls, 1983; Johnson & Janiga, 1995; Fox *et al.*, 2002), even fewer have examined the relative importance of male mate choice (Knapton & Falls, 1983; Johnson & Janiga, 1995; Roulin, 1999). Although females are typically considered to be the choosier sex (Andersson, 1994), it has recently been demonstrated that males may also benefit from mate choice discrimination (Kokko & Johnson, 2002), especially in systems where males contribute to offspring care (Amundsen & Pärn, 2006).

The present study experimentally investigates the relative role of both female and male mate choice in determining breeding pair formation in the socially monogamous Gouldian finch (*Erythrura gouldiae*). Gouldian finches possess three genetically determined and naturally co-occurring coloured head morphs (yellow, red and black) in both sexes. The genetics of this head colour polymorphism is probably one of the best resolved among birds (Cooke & Buckley, 1987) and is determined by the interaction of two genes (one sex-linked and one autosomal), which together stimulate or suppress the production of carotenoid and melanin plumage pigments, resulting in the three completely discrete head morphs (Southern, 1945; Murray, 1963). A truly discrete polymorphism such as this is extremely rare in birds – most colour polymorphic species studied to date, such as artic skuas (*Stercorarius parasiticus*: O'Donald, 1983), snow geese (*Chen caerulescens*: Cooch & Beardmore, 1959), red-billed queleas (*Quelea quelea*: Dale, 2000), ruffs (*Philomachus pugnas*: Lank, 2002), tawny (*Strix aluco*: Roulin *et al.*, 2003) and barn owls (*Strix alba*: Roulin, 1999) and common buzzards (*Buteo buteo*: Krüger *et al.*, 2001) display continuous, or at least overlapping, variation in genetically determined phenotypic colour/pattern expression, where intermediates are often unable to be reliably assigned to a specific phenotypic colour/pattern morph. The discrete colour polymorphism is further distinctive in that all three colour morphs coexist together; estimates from the wild indicate that the black-headed morphs are the most common (*c.* 70%), red-headed moderately common (*c.* 30%) and yellow-headed extremely rare (< 0.1%; Brush & Seifried, 1968; Franklin & Dostine, 2000). Here we investigate the mating pattern of Gouldian finches with respect to colouration in both large captive and wild populations, and further experimentally test the relative role of male and female mate choice in maintaining nonrandom mating.

Methods

Study species

The Gouldian finch (family Estrilidae) is an endemic Australian finch of savannah woodland in tropical northern Australia. In addition to the three discrete head morphs (black, red and yellow), Gouldian finches are also one of the most brightly coloured passerines, with a bright yellow belly, an ultraviolet/violet breast, green back and wings, and an ultraviolet/blue head collar (see Pryke & Griffith, 2006). Sexual dimorphism is pronounced, with males displaying brighter body plumage and longer pin-tail feathers. Gouldian finches are socially monogamous, and breed in nest hollows of *Eucalyptus* trees during the dry season (Tidemann *et al.*, 1999). Both sexes participate in nest building, incubation, brooding and feeding the nestlings (S.R. Pryke, personal observation). Over the past 30 years, Gouldian finch populations have suffered major declines and the species is now listed as endangered (Garnett, 1992). To investigate the mating pattern among colour morphs of Gouldian finches in the wild, a breeding population was studied at Mornington Sanctuary in the central Kimberley of Western Australia, between March and June 2005.

For the captive experiments, the wild-type birds (n = 278) were obtained from a large number of aviculturists ($n = 23$) distributed throughout Australia. None of these birds displayed any of the plumage mutations or anomalies that have arisen since the domestication of the species, with the coloured patches of these birds all indistinguishable (using reflectance spectrometer) from those of wild birds (S.R. Pryke, unpublished data). Prior to the experiments, birds were housed in single sex and separate morph aviaries (2.1 m^3), each holding eight to 10 birds and providing visual isolation from birds from different localities. Although mate choice trials (see below) of all individuals were tested prior to their introduction into the free-flying aviary experiment, each experimental aviary housed unfamiliar birds with no prior experience or exposure to each other.

Pair formation by head colour

To assess assortative mating by head colour, we analysed pair formation in both wild and captive populations. In the wild, active nests (eggs or nestlings) were located and the parents of 12 nests were banded and measured. Because of the low sample size of actively breeding pairs (and the highly endangered status of the finch in the wild), birds were also monitored at watering holes. During the first 3 h of sunrise, pairs were surveyed at 13 frequently visited water holes. Only visiting pairs with dependent fledglings were considered to be breeding pairs. To further minimize repeatedly sampling the same pairs visiting the same water holes daily, only a single temporal survey from each water hole was used (total = 47 pairs).

In captive populations, 40 (in 2005) and 20 (in 2006) unfamiliar birds were introduced into a number of large aviaries (12 × 5.5 × 3.5 m) and allowed to freely interact with each other, including courting, mating and breeding (total 200 birds). Although an equal number of head colours from each sex were used, each aviary (population) consisted of different proportions of head colour morphs. In 2005 populations comprised of: four yellow-head, 12 red-head and 24 black-head; four yellow-head, 24 red-head and 12 black-head; and 12 yellow-head, 14 red-head and 14 black-head. In 2006, two populations contained 14 black-head and six red-head pairs, whereas another two aviaries consisted of six black-head and 14 red-head. In each aviary, birds were given a unique combination of coloured bands for individual identification, and only birds which bred together were considered to have paired.

To test for assortative pairing by head colour, we first categorized each male–female pair as red–red, red–black, red–yellow, black–red, black–black, black–yellow, yellow–red, yellow–black or yellow–yellow, and tested whether the observed proportions differed from random by a contingency table χ^2 test.

Mate choice experiments

To experimentally assess mate choice, we used a mate choice design in which birds were encouraged to select short-term mates in a standardized mate choice apparatus (e.g. Hunt *et al.*, 1997). The cross-shaped wooden apparatus comprised a square main test chamber (0.6 m^3) with four stimulus arms (1.2 × 0.4 × 0.6 m). Each arm was separated from the main chamber by a removable partition of transparent Plexiglas (Perspex; Plexiglas®, Röhm, Germany), which transmits light in the full avian visual range (i.e. 300–700 nm) (Arnold *et al.*, 2002). The central part of the chamber contained food and water, and from which the test birds had an unobstructed view of all of the stimulus chambers. For the stimulus bird, each arm contained a single perch, positioned in front of the Plexiglas partition (12 cm). The time spent by test birds in front of each stimulus bird was recorded by the test bird's movement through an infrared light beam (activated by an infrared LED modulator), which was set up across each of the perches in front of the stimulus birds, and all linked to a computer (recording the time, in milliseconds, a test bird spent on perches in each stimulus compartment). The apparatus was placed in a dark room, visually and acoustically isolated from other birds. Illumination was provided from daylight-balanced fluorescent ballasts (with greater emission of ultraviolet wavelengths than standard artificial lighting and a spectral emission similar to natural daylight), placed behind the stimulus chamber (and separated from the stimulus bird by a Plexiglass partition) in each of the four arms.

Prior to the experiments, a number of body size measurements were taken. Tarsus, culmen and tail length (excluding pin-tail feathers) were measured to the nearest 0.1 mm and wing length to the nearest 0.5 mm. Body mass was measured to the nearest 0.1 g. Because the strong sexual dimorphism in tail length and plumage colouration (i.e. males have significantly more intense colouration, larger collar and chest patches and longer pin-tail feathers) suggests that they may be targets for female mate choice, these traits were also quantified. Total tail length was estimated from the longest of the two tail pin-feathers. Fluctuating asymmetry (FA) of the pin-tail feathers was calculated as the absolute left–right difference divided by their average length (Møller & Höglund, 1991). Birds with broken, missing, or growing feathers were excluded from the FA analyses. The size of the violet chest patch was quantified (to the nearest 0.1 mm) by the maximum width (across the chest) and vertical height of the patch while holding the head in a standard position. Similarly, the size of the blue head collar was quantified from measurements of the width of the collar on the head (head blue) and throat (throat blue). Repeatabilities of the patch measurements were highly significant ($r = 0.88–0.93$, $P < 0.001$ for all; see Pryke & Griffith, 2006).

Spectral reflectance from the blue collar, violet chest, green rump, yellow breast, and yellow, red or black head were measured using a USB2000 spectrometer (Ocean Optics, Dunedin, FL, USA) and fibre-optic reflectance probe in relation to a xenon light source (Ocean Optics PX-2; Dunedin, FL, USA). Preceded by a reference scan (WS-2 white standard), three consecutive scans were taken from the centre of each patch. Objective indices of the three main dimensions of colour signals (spectral intensity, location and purity), were computed from the three scans and averaged for each individual. Brightness (spectral intensity) was estimated as the sum of reflectance from 320 to 700 nm. Hue (spectral location) was estimated as $\lambda(R_{50})$, the wavelength at which reflectance is halfway between its minimum ($R_{\min}$) and its maximum ($R_{\max}$). Using $\lambda(R_{50})$ as the individual segment divider, we calculated overall chroma (spectral purity: C_{R50}) as $(R_{320-\lambda(R50)} - R_{\lambda(R50)-700})/R_{320-700}$. To address the strong contribution of ultraviolet (UV) to the blue and violet-coloured patches, we also included a measure of UV chroma, which is the relative reflectance ratio of UV to total reflectance ($R_{320-400}/R_{320-700}$). As cone absorbances from a number of birds suggest that the UV/Violet (320–420 nm) channel may be more appropriate (Bowmaker *et al.*, 1997), we also calculated a UV/V chroma measure based on this segment ($R_{320-420}/R_{320-700}$) (full details on colorimetric measurements and analyses are described in Pryke *et al.*, 2001; Pryke & Griffith, 2006).

Mate choice trials were conducted between January and March 2005, coinciding with the start of the birds' natural breeding season (Tidemann *et al.*, 1999) and all birds used in the trials were in breeding condition (signified by a blackening of the beak). In total we conducted 435 mate choice trials, 255 of which used females as the choosy/test bird, and 180 using males as the choosy sex. For female mate choice experiments, three males were randomly drawn from different housing locations and sequentially assigned to the stimulus chambers. In the first experiment, to test female preferences among the three morphs ($n = 85$ females), one male from each of the three morphs was used as a stimulus bird (i.e. each female had a choice of a red-, yellow- and black-headed male). In the second experiment, females ($n = 85$) were presented with three males of the same morph-type as the female. In both experiments, approximately equal numbers of females expressing each head-colour ($n = 25$ yellow; $n = 30$ red; $n = 30$ black) were used as the test birds. Similarly, in the male mate choice experiments, male preferences among ($n = 90$ trials; $n = 30$ of each morph) and within ($n = 90$) different female head-colour morphs were tested. To determine whether test birds were making a sexual (rather than social) choice, in each trial a stimulus bird of the same sex and morph, but from a different housing cage (i.e. location), was included in the trial. Furthermore, to identify whether females consistently target the same male traits or morphs, the repeatability of mate choice decisions was retested (as in experiment one) by providing each female with a different set of stimulus birds displaying all three head-colour morphs. Therefore, each test bird was used three times: in one experiment the test bird chose among birds of the same head morph (and a same sex and morph conspecific), and in the other two experiments (the second repeated with a different set of birds), the test birds chose among the three different head morphs. Both the sequence of the experiments (i.e. within or between morphs) and the trials (i.e. order in which a test bird took part in the experiments) were randomized. In addition, the presentation order and placement of stimuli birds in the chambers were sequentially altered among trials.

At the beginning of each trial, test birds were placed into the main chamber of the apparatus and allowed 15 min to acclimatize. Following this acclimatization period, the four stimuli birds were introduced into the four stimulus chambers. Trials began only when the test bird was in the central (neutral) part of the main chamber and mate choice was determined by the total amount of time spent with each of the four stimuli birds. Each trial lasted 45 min to determine whether birds were able to rapidly assess one another primarily on their plumage signals. Trials were considered successful only if the test bird visited all stimuli birds during the first 15 min of the trial. Birds that remained in the central part of the main chamber ($n = 13$) or with only a single bird ($n = 7$) for the entire trial were excluded from subsequent analyses. Following a trial, all birds were returned to their housing aviaries.

Mate choice analyses

Analyses were conducted in GENSTAT 7.1.0 (VSN, Hemel Hempstead, UK). Outcomes from all the experiments were initially analysed using residual maximum likelihood models, which are algorithms of generalized linear models (GLM), except that that they also allow random components to be fitted (Schall, 1991); random components in this case take into consideration the use of the same bird in more than one trial (e.g. some birds were used in both the between and within head morph experiments). A significant random term suggests that inherent properties of the term (individuals) affect the outcome of mate choice decisions. Because the random term (individuals) had a negative component of variance (indicating that it explained none of the variance in the model), we used GLM to analyse the outcomes of the mate choice experiments. Using the most parsimonious model (i.e. GLM) is appropriate when random terms have nonsignificant effects.

For all analyses, GLM were modelled with a Poisson distribution and a logarithmic link function, and all possible effects, combinations and interactions of the measured variables: body size variables (culmen, tarsus, wing and tail length), body mass, colourimetrics of all patches (brightness, hue, chroma, and/or UV chroma, UV/V chroma depending on the patch), pin-tail length, pin-tail FA, test bird head morph, stimulus bird head morph, test bird identity, stimulus bird identity, housing locality, trial number, time and date, and position (chamber) in experimental apparatus. The significance of these predictor variables was tested by the change in deviance of the different models using a chi-squared approximation. Akaike's Information Criterion (AIC) was used to objectively select the best-fitting and most parsimonious model (i.e. the model only included terms those terms for which elimination would have significantly reduced the explanatory power of the model). AIC balances the fit of the model against the number of parameters used in the model, and the model with the lowest AIC value (and a difference of at least two AIC units from the other models) is accepted as the final model (Anderson & Burham, 2001). As all models tested had an AIC weight of at least 92% compared with other potential models, for simplicity, only the final models are presented.

To estimate the repeatability of an individual female's preference (between the first and repeated among-morph experiments) we calculated the variance components (among-individual variation vs. within-individual variation) using the mean squares from a one-way ANOVA (Lessells & Boag, 1987) for each of the significant traits in the GLM. In order to generate a univariate female preference measure, we ranked both the female's preference in each trial (i.e. time spent with male, in descending order) and the target trait among the three stimulus males (in descending order), before calculating the sum of the differences between these ranks (i.e. rank correlation or differential). As females typically associated with a single preferred male (see *Results*), female preference ranks were weighted towards their most preferred choice [i.e. differential = preference 1 + (preference 2 + preference 3)/2]. Because male morph could not be ranked hierarchically and is an absolute and unchanging factor in these experiments (i.e. unlike colour which depends on the relative differences among the stimulus birds), the repeatability of female preferences for morph-type in each trial were simply estimated from the male morph the female preferentially associated with.

To investigate the repeatability of an individual's mate preference between the initial mate choice trials and their subsequent pair formation in free-flying populations, we calculated repeatabilities (as above) for each individual (both females and males) using colour morph preference (choice trials) and colour morph of breeding partner (free-flying populations).

Results

Assortative pairing by head colour

Overall, 137 breeding pairs (59 wild, 78 captive) were assessed. Most pairs consisted of black- and red-headed birds; none of the rare yellow-headed birds were recorded breeding in the wild and only two bred in captivity (both assortatively). With respect to head colour, Gouldian finches pair positive assortatively; more pairs had birds with the same head colour than would be expected if pairing was random (108/137; $\chi^2_8 = 63.69$, $P < 0.001$). Assortative pairing was observed both within wild (47/59; $\chi^2_4 = 17.49$, $P < 0.001$) and captive populations (61/78; $\chi^2_8 = 46.03$, $P < 0.001$). When disassortative pairing occurred (21.1%), it was most common among black-headed females and red-headed males (23/29 disassortative pairs), and much less common between red-headed females and black-headed males (6/29 disassortative pairs).

Female mate choice

Females actively visited all of the stimulus males within the first 15 min of a trial, except for eight females (9.4%; three red-, three black- and two yellow-headed birds) which were removed from subsequent analyses. Overall, females spent an average of 23.7 ± 8.4 min of the trials in front of males and only three females (1.2%) spent more time with the stimulus female than with the average stimulus male. Furthermore, females appeared to preferentially associate (> 90% of time) with a particular male: during the 255 trials, females associated with a single male in 67.4% of the trials, two different males in 22.7% and three males in 4.3%.

Female mate choice within morphs

Using a GLM to test for female mate preferences among birds of the same morph (as the test female), the best-fitting model (AIC = 2628, $\chi^2_{312} = 13.37$, $P > 0.001$) identified a preference for males (i.e. sex of stimulus bird: $F = 28.75$, $P < 0.001$), and an effect of head collar UV/blue chroma ($F_{1,322} = 186.32$, $P < 0.001$; Fig. 1) and tail length ($F_{1,322} = 6.98$, $P = 0.007$). None of the other potential variables (see *Methods* for list) had any effect on mate preferences. In particular, there were no significant differences among females of the different morphs in the time they spent with males ($F_{1,322} = 0.11$, $P = 0.89$), indicating that there were no morph-related differences in responsiveness. Furthermore, there were no interacting effects of female colour morph (red, yellow and black) on the preferred male traits (collar UV/blue chroma: $F_{1,322} = 0.60$, $P = 0.55$; tail length: $F_{1,322} =$

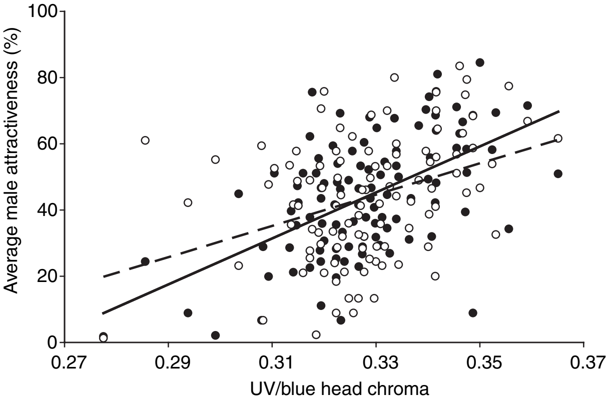

Fig. 1 The relationship between head collar UV/blue chroma and male attractiveness ($n = 104$ for each experiment), calculated as the average proportion of time females spent with the male (each male was used in three different trials), for both within-morph (closed circles, solid line: $y = 694.1x - 183.7$; $F_{1,102} = 43.81$, $R^2 = 30.1\%$, $P < 0.001$), and among-morph experiments (open circles, dashed line: $= 472.x - 111.4x - 111.4$; $F_{1,102} = 15.67$, $R^2 = 13.3\%$, $P < 0.001$).

1.51, $P = 0.19$), and thus, irrespective of female morph, males displaying more intense UV chromatic blue collars and longer tails were the most attractive.

Female mate choice among morphs

Similar to the outcome of female choice within morphs, females given a choice of three different morphs (and a female of the same morph) spent significantly longer with males ($F_{1,322} = 44.97$, $P < 0.001$), especially those displaying more chromatic UV/blue plumage ($F_{1,322} = 24.46$, $P < 0.001$; Fig. 1) and longer tails ($F_{1,322} = 7.06$, $P = 0.007$). This best-fitting model (AIC = 2093, $\chi^2_{309} = 160.34$, $P < 0.001$) also identified an effect of colour morph ($F_{2,321} = 58.38$, $P < 0.001$; Fig. 2): overall, females demonstrated a slight preference for red- over black-headed males ($t = 1.53$, $P = 0.07$), but discriminated against yellow-headed males ($t = -6.64$, $P < 0.001$). In particular, both red- ($t = 7.04$, $P < 0.001$) and black-headed females ($t = 1.96$, $P = 0.04$) preferentially associated with red-headed males (Fig. 2), whereas yellow-headed females demonstrated no detectable preferences among the three male morphs ($t = 0.73$, $P = 0.47$).

Furthermore, the observed female preference was consistent. When the same test females ($n = 85$) were presented with a similar choice of four (but new and unfamiliar) stimulus birds (three males of different morphs and a female of the same morph), females once again (AIC = 1685, $\chi^2_{309} = 136.11$, $P < 0.001$) targeted males ($F_{1,322} = 23.91$, $P < 0.001$) with more intense UV/blue chromatic plumage ($F = 35.51$, $P < 0.001$) and longer tails ($F_{1,322} = 3.91$, $P = 0.04$), and discriminated against yellow-headed males ($F_{2,321} = 27.33$, $P < 0.001$). In addition, the preferences of individual females between the two experiments (with a different set of males) were repeatable. Although female preferences for tail length showed significant but low repeatability ($r = 0.17 \pm 0.106$, $F_{1,84} = 1.41$, $P = 0.05$), female preferences for both UV/blue chroma ($r = 0.48 \pm 0.083$, $F_{1,84} = 2.89$, $P < 0.001$) and in particular, morph-type were more repeatable ($r = 0.56 \pm 0.067$, $F_{1,84} = 3.41$, $P < 0.001$). This repeated female preference for particular male colour morphs was observed among each of the three female morphs: red-headed ($r = 0.53 \pm 0.079$, $F_{1,29} = 3.21$, $P = 0.001$), black-headed ($r = 0.61 \pm 0.055$, $F_{1,29} = 4.77$, $P < 0.001$) and yellow-headed females ($r = 0.54 \pm 0.077$, $F_{1,24} = 3.35$, $P = 0.002$).

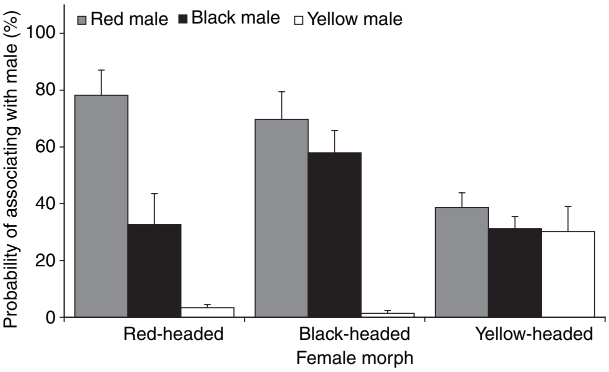

Fig. 2 The probability (%) of females (of each morph) associating with red-, black- and yellow-headed males. Probabilities are calculated from the coefficients of the best-fit generalized linear model [probability = $e^{(\text{coeff})}/1 + (e^{(\text{coeff})})$] and error bars represent the 95% confidence levels of the coefficients.

Male mate choice

During the first 15 min of the mate choice trials, most males (86.7%) actively visited all three females. Overall, males spent an average of 21.8 ± 5.6 min with the stimulus females, but they rarely visited ($n = 7$; 7.8%) or associated (0.8 ± 0.3 min) with the stimulus male of the same morph. Males typically spent the majority of the trial (> 90%) with a single female (69.6%), less often with two (18.7%) and rarely with all three females (9.4%).

Male mate choice within morphs

The best-fitting GLM explaining male preferences among females of the same morph-type (AIC = 3216, $\chi^2_{359} = 22.60$, $P < 0.001$) included discriminating against conspecific males (sex of stimulus bird: $F_{1,359} = 17.64$, $P < 0.001$) and a morph-related difference in male responsiveness ($F_{2,358} = 7.23$, $P = 0.008$); black-headed males spent significantly more time with the stimulus females compared to the yellow- ($t = 2.81$, $P = 0.005$) and red-headed males ($t = 2.09$, $P = 0.03$), although red- and yellow-headed males did not differ ($t = 1.42$, $P = 0.15$).

Male mate choice among morphs

The GLM best explaining male mate preferences among females of the three different morphs (AIC = 3016, $\chi^2_{359} = 118.02$, $P < 0.001$) identified a highly significant preference for conspecific females ($F_{1,359} = 77.4$, $P < 0.001$) with males preferentially associating with females of their own morph-type (female morph × male morph interaction: $F_{2,358} = 15.19$, $P < 0.001$; female morph: $F_{2,358} = 1.45$, $P = 0.24$; male morph: $F_{2,358} = 1.15$, $P = 0.32$). This assortative mate preference was evident among all three of the male morphs, but weaker in red-headed males ($t = 3.11$, $P < 0.001$; see Fig. 3) compared with both yellow- ($t = 17.84$, $P < 0.001$) and black-headed males ($t = 11.51$, $P < 0.001$). Thus, overall, males prefer to associate with females of their own colour morph (Fig. 3).

Mate choice and assortative mating by head colour

Between the two experiments (mate choice trials and free-flying populations), individuals demonstrated

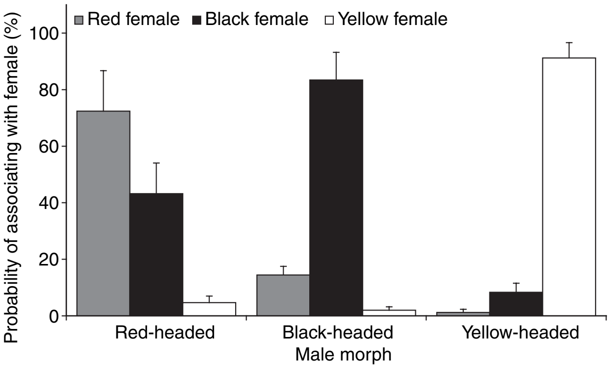

Fig. 3 The probability (%) of males (of each morph) associating with red-, black- and yellow-headed females. Probabilities are calculated from the coefficients of the best-fit generalized linear model [probability = $e^{(\mathrm{coeff})}/1 + (e^{(\mathrm{coeff})})$] and error bars represent the 95% confidence levels of the coefficients.

repeatable preferences for partners of the same head colour morph (i.e. individuals initially associating with a particular colour morph in mate choice trials generally paired with that same colour morph in the large aviary populations). In particular, compared with female mate preferences ($r = 0.85 \pm 0.029$, $F_{1,78} = 12.74$, $P < 0.001$), male mate preferences were highly repeatable ($r = 0.99 \pm 0.002$, $F_{1,78} = 672.33$, $P < 0.001$) with most males (75/78) pairing with their preferred female colour morph.

Discussion

In both wild and captive populations, Gouldian finches paired assortatively with respect to head colour. This is in line with other studies demonstrating nonrandom mating with respect to colour morph (Baker, 1973; Cooke *et al.*, 1976; Abraham *et al.*, 1983; Knapton & Falls, 1983; Krüger *et al.*, 2001; Roulin, 2004). Mate choice is a powerful evolutionary mechanism, often proposed to drive assortative or disassortative mating (Knapton & Falls, 1983; Johnson & Janiga, 1995), as well as influence the stability and maintenance of colour polymorphisms within a species (Cooke & McNally, 1975; Kingston *et al.*, 2003). In particular, female mate choice is a particularly strong selective force in the evolution and maintenance of sexually dimorphic colouration in birds (reviewed in Griffith & Pryke, 2006; Hill, 2006) and is also often thought to be the driving force maintaining assortative mating among polymorphic species (Cooke *et al.*, 1976; Phillips & Furness, 1998; Fox *et al.*, 2002). Yet, our results suggest that not all females choose to associate with males of the same colour morph as themselves. Although red-headed females preferred red-headed males, black-headed females associated with both red- and black-headed males, and yellow-headed females exhibited no strong preference among the male morphs. Despite this variability in female preferences, both within and between the female morphs, individual females consistently chose different males of the same head morph. Furthermore, irrespective of both the choosing female's colour morph and the stimulus male morph (i.e. experiments with the same and different colour morphs), females preferred males with more intense UV/blue head collars and longer pin-tail feathers. Thus, these results suggest that females target sexually dimorphic traits (head collars and tail length), and that female mate choice is not the main selective mechanism promoting assortative mating in the Gouldian finch (Fox *et al.*, 2002).

In contrast to the variable mate preferences demonstrated by females, males of all three colour morphs preferred females of the same morph colour and strongly discriminated against females of different morph-types. However, red-headed males did associate (although to a lesser degree) with black-headed females, which together with female mate preferences for red-headed males may explain why when disassortative pairing does occur, it is often biased towards black-headed females and red-headed males (see Figs 2 and 3). Overall, male, not female, mate choice may be particularly important in maintaining the observed patterns of assortative mating in this species. Indeed, although 78% of breeding birds paired assortatively, males ultimately paired with a female of their preferred female morph-type in 75 of 78 (96.2%) of these breeding pairs (only 70.5–73.4% females paired with their preferred male morph colour). This appears surprising, given that female, rather than male, mate preferences are typically considered to be the major driving force in the evolution of extravagant colouration (Hill, 2006). However, males investing heavily into parental care should be particularly choosy about their potential mates (Amundsen & Pärn, 2006). Studies of male mate choice have been relatively rare in birds, with some studies demonstrating male preferences for particular female traits (Hill, 1993; Amundsen *et al.*, 1997; Jones & Hunter, 1999; Griggio *et al.*, 2005), but a number of others finding no evidence (e.g. Muma & Weatherhead, 1989; Dale & Slagsvold, 1994; Cuervo *et al.*, 1996). In polymorphic systems, males have also been shown to exhibit preferences for particular female morphs, such as male white-throated sparrows *Zonotrichia albicollis* (Houtman & Falls, 1994) and feral pigeons *Columba livia* (Johnson & Janiga, 1995), but in both these species females were choosier about morph type than males. In barn owls, *Tyto alba*, males tend to be more selective than females (Roulin, 1999), however, sexual dimorphism (i.e. spottiness) in these owls is more pronounced in females than conspecific males (Roulin, 1999). Although the relative role of mate choice in polymorphic populations remains unclear (Roulin, 2004), theoretically, male discrimination is adaptive in species where offspring fitness (and hence colour morph) is largely dependent on the parental phenotypes (genotypes).

At present, the mechanisms underlying the observed variability in mate preferences, both between and within the sexes, are unknown, but there are a few possibilities. First, because two different loci control the expression of head colour (Murray, 1963; Cooke & Buckley, 1987), mate preferences between yellow-headed (autosomal linked), and red- and black-headed (sex-linked) females may be genetically linked to the expression of the morph. However, this pattern does not fully explain why, for example, female yellow-headed morphs show no mate preferences for any particular morph males (irrespective of morph), or males strongly target like-coloured female morphs. Another possibility is that individuals acquire their mating preferences through sexual imprinting on the parental phenotypes (e.g. Immelmann, 1972; ten Cate & Vos, 1999). The functional value of imprinting lies in choosing a mate of the same species (i.e. hybridization avoidance), and as a result, may strongly affect assortative mating in polymorphic species (Cooke *et al.*, 1972; Cooke & McNally, 1975). In Gouldian finches, sexual imprinting may explain mate preference as some assortative head colour pairs (e.g. red-headed parents) can produce offspring of all three head morphs.

In addition to the mechanisms outlined above, the observed mate preferences may also reflect alternative behavioural strategies adopted by the different colour morphs. Mate choice preferences are often highly adaptive, with mate choice decisions' varying within individuals, especially if the expected benefit derived from mating with a differently coloured mate varies in space and/or time (e.g. Griffith *et al.*, 1999; Qvarnström *et al.*, 2000). In polymorphic species, between-individual variation in mate choice may arise when individuals employ different tactics in an attempt to maximize their fitness (e.g. Lank *et al.*, 1995; Gross, 1996). The dominance-related behavioural differences among Gouldian finch colour morphs (Pryke & Griffith, 2006; Pryke 2007) suggests that alternative behavioural strategies are employed by the different colour morphs, but further studies are needed to investigate how such strategic variation exists in maintaining the colour polymorphism.

Given the observed assortative mating between head morphs of Gouldian finches, what are the fitness consequences of this mating pattern? Although disassortative mating may function as a mechanism maintaining multiple morphs within a species (Thorneycroft, 1975; Knapton & Falls, 1983), assortative mating typically depletes gene flow between individuals in a population, and is commonly invoked as a mechanism leading to the evolution of reproductive isolation and speciation (van Oppen *et al.*, 1998; Kirkpatrick, 2000; Salzburger *et al.*, 2006). Therefore, in order for genetically determined alternatives to coexist, additional selective mechanisms must be present to maintain genetic variation between the morphs. The three colour morphs of the Gouldian finch coexist together at different frequencies where black-headed birds are the most common, yellow-headed birds extremely rare and red-headed of intermediate status (Brush & Seifried, 1968; Franklin & Dostine, 2000). The low frequency of yellow-headed birds may partially be explained by the assortative mating pattern (and the chance of finding a like-coloured mate), together with the discrimination by red- and black-headed females against yellow-headed males. Yellow-headed birds are also strongly dominated by the other two colour morphs in competition for food and nest sites (Pryke & Griffith, 2006). However, the observed mating patterns do not completely explain the frequency of red- and black-headed birds; the highly dominant red-headed birds (Pryke & Griffith, 2006) are generally more attractive to both red- and black-headed females, yet black-headed birds are the most common morph in wild populations.

How the pattern of mate choice and assortative mating contributes to the maintenance of this polymorphism is unclear at present. Although mating preferences and the evolutionarily forces influencing these preferences may be partially responsible for maintaining the colour polymorphism, it seems probable that additional selective pressures (e.g. genetic pleiotrophy, sexual imprinting, extra-pair paternity, frequency-dependent selection) may also vary across the colour morphs, balancing out net morph fitness, and contributing to the maintenance of the three colour morphs at their different frequencies. Although most studies have focused exclusively on the role of female mate preferences in maintaining or promoting colour variation, this study highlights the importance of accounting for both female and male components of mate choice.

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