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Frequency-dependent physiological trade-offs between competing colour morphs

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Evolutionary theory suggests that alternative colour morphs (i.e. genetically controlled phenotypes) may derive similar fitness under frequency-dependent selection. Here we experimentally demonstrate opposing effects of frequency-dependent social environments on plasma hormone levels (testosterone and corticosterone) and immune function between red- and black-headed male morphs of the Gouldian finch (*Erythrura gouldiae*). Red-headed males are highly sensitive to changes in the social environment, especially towards the relative density of their own aggressive morph, exhibiting high stress responses and immunosuppression in socially competitive environments. In contrast, the non-aggressive black-headed males follow a more passive strategy that appears to buffer them against social stresses. The differential effect of hormones on aggressive behaviour and immune performance reinforces the contrasting behavioural strategies employed by these colour morphs, and highlights the importance of the social environment in determining the individual basis of behavioural and physiological responses.

Keywords: colour polymorphism; steroid hormones; immunocompetence; alternative strategies; *Erythrura gouldiae*

1. INTRODUCTION

A central challenge for evolutionary theory is to understand the underlying mechanisms that generate and maintain individual variation. Species that exhibit extreme phenotypic diversity, where individuals within a sex display a number of discrete and heritable phenotypes, provide ideal systems to explore the interaction between genotypes and the environment, as well as the physiological and developmental mechanisms underlying individual variation. Because alternative morphs may also vary in behaviour and physiology (in addition to appearance), such genetically determined phenotypes are often reinforced by alternative behavioural strategies where competing morphs use alternative tactics to maximize their fitness (Gross 1996; Sinervo & Lively 1996). Most studies on the coexistence of multiple morphs typically focus on negative frequency-dependent selection resulting from processes such as predation (Olendorf *et al.* 2006) and sexual selection (Sinervo & Lively 1996), where rare morphs have a fitness advantage. However, social interactions among different morphs can also generate frequency-dependent effects in the local environment, which may directly affect individual behaviour, physiology and fitness (Svensson *et al.* 2001; Comendant *et al.* 2003).

Hormones provide a physiological link between the social environment, behaviour and health. Testosterone, for example, is a well-known modulator of aggressive behaviour (Wingfield *et al.* 1987), whereas corticosterone is a stress hormone that responds to a variety of environmental and social stressors (Creel 2001). There is considerable variation in how individuals and species respond to environmental stressors and how these adjustments lead to changes in behaviour and physiology (Hillgarth *et al.* 1997; Sapolsky *et al.* 2000; Creel 2001). In colour polymorphic species, social interactions between individuals of different morphs can directly affect physiology and fitness (Svensson *et al.* 2001; Comendant *et al.* 2003; Spinney *et al.* 2006).

Here, we evaluate the physiological basis of individual variation in response to different competitive contexts between red- and black-headed male morphs (figure 1*a*) of the Gouldian finch (*Erythrura gouldiae*). Head colour in this species is genetically determined (from alleles segregating at a single autosomal and sex-linked locus) and previous studies show that head colour is correlated with distinctive dominance behaviours: red-headed males are dominant and overtly aggressive, while black-headed birds are submissive (Pryke & Griffith 2006). To experimentally examine the proximate basis for this difference, we placed birds in a range of social environments, differing only in the relative frequency of non-aggressive black-headed and aggressive red-headed morphs, and assessed the interactions between social competition, hormonal status and immune function.

2. MATERIAL AND METHODS

(a) *Experimental design*

Experiments were run between December 2005 and March 2006, using 120 males of the same age (all in their first year of adult plumage). All birds were socially naive, in that they had never encountered other coloured birds (apart from their parents), because after fledging and through their moult into coloured plumage, individuals were housed alone in complete social isolation. Body size was estimated from the first principal component (PC1) of a PCA, explaining 75.4% of the variance in wing, tail, tarsus and bill lengths.

For these experiments, six males were placed in an unfamiliar cage (2.1 m^3) and allowed to interact for 6 days. Five replicates of four different social environments were created by altering the frequency of red- to black-headed birds (R/B: 0/6; 2/4; 4/2; 6/0). Male dominance rank was inferred daily from two well-established competitive contests for this species: standardized contests for access to limited food and relative position in a linear dominance hierarchy (details in Pryke & Griffith 2006). These two measures were highly correlated (91%; $r=0.97$, $n=112$, $p<0.001$), and dominance rank remained consistent for the duration of the experiment (repeatability=0.83, $F_{111,672}=23.41$, $p<0.001$). In line with previous results (Pryke & Griffith 2006), red-headed

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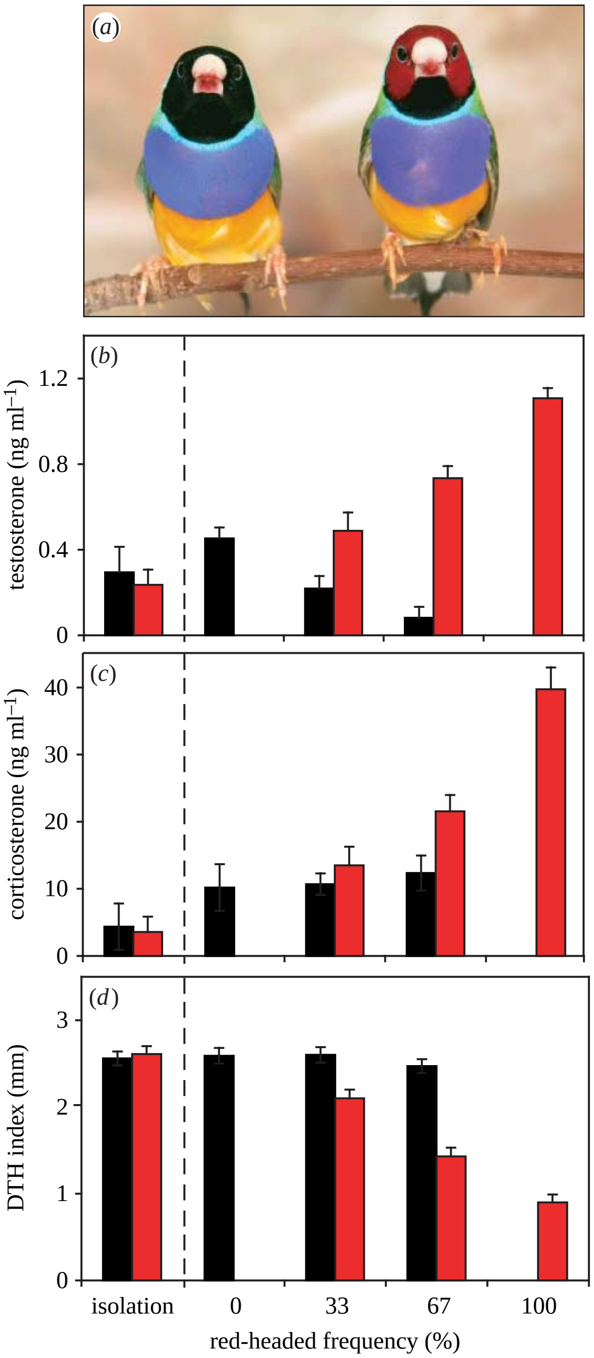

Figure 1. (*a*) Black-headed (left) and red-headed (right) male morphs of the Gouldian finch. Under varying social environments, red-headed (red bars) and black-headed (black bars) morphs expressed significantly different (*b*) plasma testosterone and (*c*) corticosterone levels, and (*d*) cell-mediated immune responses (DTH index). Prior to the experiments, birds were housed alone in complete visual isolation (isolation). All values are presented as least-square means (+s.d.) from the best-fitting GLM (see §3).

males aggressively dominated black-headed males in mixed groups ($F_{5,52}=12.16$, $p<0.001$).

To evaluate physiological changes, birds were weighed and blood samples (*ca* 150 μl; two capillaries) taken on the day prior to the start of the social tests (day 0; in isolation) and 7 days later (day 6). Blood samples were collected between 09.00 and 09.30, and within 2 min of capture (mean±s.d.=46.2±3.2 s). Samples were centrifuged and plasma stored at −20°C.

(b) *Hormone assays*

Testosterone and corticosterone levels were measured in duplicate from plasma samples using Cayman Enzyme Immunoassay kits (nos. 582701 and 500651, respectively; Ann Arbor, MI, USA). Kit instructions were followed, but each sample was also initially spiked with tritiated testosterone (Amersham [1,2,6,7-^{3}H]) or corticosterone (Amersham [1,2,6,7-^{3}H]) to determine percentage recovery (following Clotfelter *et al.* 2004; preliminary tests determined appropriate plasma dilutions). After steroid extraction in diethyl ether (testosterone) or dichloromethane (corticosterone), samples were reconstituted in buffer dilutions optimized for the standard curve (typically 1 : 10 or 1 : 20 for testosterone and 1 : 40 for corticosterone, but lower dilutions were sometimes used for very small sample volumes). Final hormone values were adjusted for individual sample recovery; mean recoveries were 90.29±0.85% for testosterone and 82.1±1.19% for corticosterone. Interplate variation for six plates was 14.9% for testosterone and 13.9% for corticosterone, assessed using a chicken plasma pool.

(c) *Immune measures*

Haematocrit, an index of physiological condition, was calculated as the ratio of packed cell volume to whole blood (%) from the two centrifuged capillaries; within-individual measurements were highly repeatable ($r=0.99$, $F_{119,480}=169.87$, $p<0.001$). Whole-blood haemoglobin concentration (g dl^{-1}) was measured on a 20 μl blood sample using a HemoCue photometer (HemoCue AB, Ängeholm, Sweden). T-cell-mediated immune responsiveness was evaluated using delayed-type hypersensitivity (DTH) tests. On test day 5, each bird's right and left wing web was injected with either phytohaemagglutinin solution (PHA-P, Sigma) or sterile phosphate buffered saline (PBS), respectively, and the consequent swellings measured with pressure-sensitive digital callipers (0.01 mm) 24 h later. A group of red-headed ($n=15$) and black-headed birds ($n=15$) were also tested during the isolation period. The DTH index was calculated as the difference in swellings between the left wing web (control: PBS only) and the right wing web (0.2 mg PHA-P dissolved in 0.04 ml PBS); within-individual measures were highly repeatable ($r=0.99$, $F_{119,360}=183.85$, $p<0.001$).

(d) *Statistical analyses*

Generalized linear models (GLMs) with a logarithmic link function and the Poisson distribution were constructed (GenStat v. 7.1.0), incorporating all potential combinations of the effects and their interactions (e.g. experiment, group, individual, morph, dominance status, body size (PC1), mass, testosterone, corticosterone, haematocrit, haemoglobin concentration, DTH index). Akaike's information criterion (AIC$_{C}$) was used to objectively compare different models; since the best-fitting models had an AIC$_{C}$ weight of at least 92% compared with other potential models, only final models are reported (see the electronic supplementary material, table S1).

3. RESULTS

This experiment revealed striking physiological differences between black- and red-headed morphs. During social isolation, there were no differences in the plasma levels of circulating testosterone between black- and red-headed males ($t_{83}=1.49$, $p=0.14$; figure 1*b*). When placed in socially competitive environments; however, testosterone levels of the two morphs changed dramatically (figure 1*b*). The relative density of red-headed males had significant, but opposite, effects on the testosterone levels of the colour morphs (morph×red-head frequency: $F_{3,101}=15.06$, $p<0.001$). That is, as the relative density of red-headed males increased, testosterone levels of black-headed males decreased significantly ($t=7.93$, $p<0.001$), whereas testosterone levels of red-headed males increased significantly ($t=9.29$, $p<0.001$).

Red-headed, but not black-headed, birds also showed a progressive increase in corticosterone levels with increasingly competitive environments (morph× red-head frequency: $F_{3,101}=6.46$, $p=0.01$; figure 1*c*). Although corticosterone levels did not differ between the morphs during isolation ($t_{83}=0.89$, $p=0.38$), corticosterone levels of red-headed males increased dramatically (over 11 times the isolation level) with increased densities of aggressive red-headed males ($t=7.90$, $p<0.001$), whereas the corticosterone levels of black-headed males were unaffected by the social environment ($t=1.23$, $p=0.22$). Additionally, the morphs also differed in the degree to which social competition affected immune function and metabolic competence. While immune responsiveness did not differ between red- and black-headed males in isolation ($t_{15}=1.96$, $p=0.23$; figure 1*d*), with increasing competition, red-headed males experienced reduced cell-mediated immune responses (morph×red-head frequency: $F_{3,101}=13.88$, $p<0.001$; red: $t=7.93$, $p<0.001$), whereas there was no such effect for black-headed males ($t=1.20$, $p=0.23$; figure 1*d*). Similarly, red-headed males also exhibited lower whole-blood haemoglobin concentrations (morph×red-head frequency: $F_{3,101}=7.71$, $p=0.006$; red: $t=3.91$, $p<0.001$; black: $t=1.25$, $p=0.21$) and reduced haematocrit (morph×red-head frequency: $F_{3,101}=13.41$, $p<0.001$; red: $t=4.22$, $p<0.001$; black: $t=1.33$, $p=0.19$), indicating that higher densities of red-headed morphs lead to greater immunosuppression and reduced physiological capacity among red-headed males.

4. DISCUSSION

Red- and black-headed males differed dramatically in hormonal and immune responsiveness to the social environment. While there were no differences in the levels of hormones (testosterone and corticosterone) and immune performance between the morphs when socially isolated, in socially competitive environments, red-headed males responded aggressively, elevating their testosterone levels to over five times their isolation level, whereas black-headed males exhibited reduced testosterone levels in response to higher frequencies of aggressive red-headed males. This divergent testosterone response not only reinforces but also provides a physiological basis for the contrasting behavioural dominance strategies employed by the highly aggressive red-headed males and the submissive black-headed morphs (Pryke & Griffith 2006). The elevated testosterone levels of red-headed males are presumably advantageous during dominance interactions, where red-headed males aggressively dominate black-headed males in competition for essential resources, such as food and nest sites (Pryke & Griffith 2006).

However, the increased aggression of red-headed birds is not without costs. In socially competitive contests, red-headed males have a highly elevated stress response (corticosterone secretion) and cannot maintain normal levels of immunocompetence. Corticosterone has well-known immunosuppressive effects when chronically elevated (Buchanan 2000; Sapolsky *et al.* 2000), and together with the elevated levels of plasma testosterone, it is likely to negatively affect immunocompetence (Hillgarth *et al.* 1997; Braude *et al.* 1999). This contrasts with the submissive black-headed males that show little or no stress response or immunosuppression to socially competitive environments.

Therefore, although red-headed males have the dominance advantage over black-headed males, they face steep physiological trade-offs and potential health risks at high frequencies. This could limit the relative abundance of aggressive red-headed males, and together with the observed patterns of assortative mating by head colour (Pryke & Griffith 2007), may explain the higher densities of black-headed birds (70%) compared with red-headed birds (30%) in wild populations.

In summary, head colour plays an important role in the maintenance of alternative dominance strategies in male Gouldian finches (Pryke & Griffith 2006), and our results suggest that these contrasting strategies could be maintained by genetic differences in endocrine responsiveness and sensitivity to socially competitive environments. Together with furthering our knowledge of the poorly understood physiological mechanisms that generate and maintain phenotypic variation, this study also highlights the importance of the social environment in determining the individual basis of behavioural and physiological responses.

All protocols were approved by the UNSW Animal Care and Ethics Committee (04/108A).

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