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Covariation in Life-History Traits: Differential Effects of Diet on Condition, Hormones, Behavior, and Reproduction in Genetic Finch Morphs

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ABSTRACT: The relative contribution of genetic and environmental factors in determining variation in life-history traits is of central interest to evolutionary biologists, but the physiological mechanisms underlying these traits are still poorly understood. Here we experimentally demonstrate opposing effects of nutritional stress on immune function, endocrine physiology, parental care, and reproduction between red and black head-color morphs of the Gouldian finch (*Erythrura gouldiae*). Although the body condition of black morphs was largely unaffected by diet manipulation, red birds were highly sensitive to dietary changes, exhibiting considerable within-individual changes in condition and immune function. Consequently, nutritionally stressed red birds delayed breeding, produced smaller broods, and reared fewer and lower-quality foster offspring than black morphs. Differences in offspring quality were largely due to morph-specific differences in parental effort: red morphs reduced parental provisioning, whereas black morphs adaptively elevated their provisioning effort to meet the increased nutritional demands of their foster brood. Nutritionally stressed genetic morphs also exhibited divergent glucocorticoid responses. Black morphs showed reduced corticosterone-binding globulin (CBG) concentrations and increased levels of free corticosterone, whereas red morphs exhibited reduced free corticosterone levels and elevated CBG concentrations. These opposing glucocorticoid responses highlight intrinsic differences in endocrine sensitivities and plasticity between genetic morphs, which may underlie the morph-specific differences in condition, behavior, and reproduction and thus ultimately contribute to the evolution and maintenance of color polymorphism.

Keywords: life-history traits, hormones, nutrition, parental care, polymorphism, immune function.

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Introduction

A major challenge to evolutionary theory is understanding the selective factors and proximate mechanisms that generate and maintain genetic and phenotypic variation. Although evolution tends to promote an optimal phenotype for each species, in a few species evolution has instead resulted in extreme phenotypic diversity, where individuals of a given sex display a number of discrete and heritable phenotypes. The persistence of such genetically determined polymorphisms presents a difficult evolutionary question because unless alternative genetic forms (morphs) use different strategies to achieve equal fitness (Schuster and Wade 2003) or have an advantage when rare (Gross 1996; Sinervo and Lively 1996), directional selection on a strongly heritable trait should eliminate conspicuous variation and instead lead to monomorphism. Consequently, species that exhibit discrete phenotypes provide ideal systems to explore the interaction between genotypes and the environment as well as the behavioral and physiological basis of individual life-history variation (Ketterson and Nolan 1992; Sinervo et al. 2000a; Svensson et al. 2002; Galeotti et al. 2010). However, despite increasing research effort to understand the mechanisms that generate and maintain color variation, we have only a basic understanding of the physiological mechanisms that underlie morph differentiation and how genotypes interact with the environment to affect morph fitness (reviewed in Galeotti et al. 2003; Roulin 2004; Gray and McKinnon 2007). Because discrete color morphs may show genetic covariation between coloration and a range of other morphological, behavioral, and physiological traits (e.g., Sinervo et al. 2000b; Knapp 2004; Spinney et al. 2006), it is likely that alternative genetic morphs will also differ in their endocrine and physiological sensitivities to environ-

mental challenges. Such differences are likely to underlie the variable life-history solutions adopted by competing morphs attempting to maximize their fitness.

Hormones often provide a mechanistic link between the environment, behavior, and reproductive success (McGlothlin and Ketterson 2008; Ketterson et al. 2009) and thus are a key proximate factor involved in regulating covariation in life-history traits and the performance of color morphs (e.g., Sinervo et al. 2000a; Roulin 2004). In particular, glucocorticoids, including corticosterone (the primary glucocorticoid in reptiles, birds, and many mammals), are activated by stressful events and have a variety of physiological and behavioral effects, all of which strongly affect life-history allocations. In response to environmental challenges, corticosterone enhances energy acquisition and utilization (Porterfield 2001; Ramage-Healey and Romero 2001; Moore and Jessop 2003), foraging activity (Wingfield et al. 1990), locomotory activity (Breuner et al. 1998; Breuner and Wingfield 2000), and feeding activity (Wingfield et al. 1998) and thus an individual's overall energetic state (Kitaysky et al. 2003; Love et al. 2004; Blas et al. 2007; Love and Williams 2008). This redistribution of resources (away from physiological processes unnecessary for immediate survival) minimizes the effects of stressors (Wingfield et al. 1998) and allows individuals to adaptively respond to short-term food shortages and environmental perturbation (Wingfield et al. 1997). However, when corticosterone remains elevated at high levels for long periods of time (weeks to months), a wider range of negative consequences can occur, including reduced immunocompetence (Munck et al. 1994) and reproductive suppression (Sapolsky 1992). Thus, although stress responses may vary considerably between individuals (Romero and Luke 2007), corticosterone secretion is often considered to mediate a trade-off between self-maintenance and reproduction (reviewed in Wingfield and Sapolsky 2003), with increasing corticosterone levels favoring the reallocation of resources to self-maintenance at the expense of reproduction.

Investigations into the endocrine basis of life-history variation and the plasticity of stress responses have largely focused on the regulation of plasma corticosterone levels. Indeed, it is common to regard an individual's corticosterone level as representative of their main response to stressors. However, multiple factors downstream of glucocorticoid secretion, such as receptor number or the abundance of specific plasma-binding proteins, may be regulated to alter behavioral and physiological outcomes to stress (Breuner and Wingfield 2000; Breuner and Orchinik 2002). Corticosterone-binding globulin (CBG) binds corticosterone with high affinity in the plasma of birds and mammals (Westphal 1971; Breuner and Orchinik 2002; Breuner et al. 2003; Lynn et al. 2003) and may dy-

namically regulate the availability of unbound (free) steroids (Breuner and Orchinik 2002) in response to changing physiological and environmental conditions. This binding of corticosterone to CBG buffers tissues against potentially deleterious effects of elevated corticosterone, regulates the availability of free hormone to target tissues, and alters corticosterone clearance rates (Breuner and Orchinik 2002). Historically, CBG concentrations were assumed to be relatively constant; however, recent studies have shown that CBG concentrations change dynamically and that free and bound corticosterone may vary independently (Breuner and Orchinik 2002; Lynn et al. 2003; Love et al. 2004). The factors that affect CBG levels are largely unknown, but emerging studies suggest that CBG levels may decrease following food deprivation (Lynn et al. 2003) and vary during different reproductive stages (Romero et al. 1998; Love et al. 2004). Therefore, quantification of CBG (bound) and free corticosterone (unbound), rather than total corticosterone alone, may provide a better understanding of the actions of glucocorticoids during reproduction (Love et al. 2004).

To assess the relative interaction of genetic and environmental effects on hormonal regulation, physiological plasticity, and life-history behavior during reproduction, we experimentally altered nutritional quality in the Gouldian finch (*Erythrura gouldiae*), a bird characterized by a heritable and conspicuous head-color polymorphism. Head color in this species is genetically determined (Southern 1945), with both sexes expressing red and black phenotypes. The Gouldian finch is unusual in that both red and black morphs appear to coexist at relatively stable frequencies, both temporally and spatially, throughout their entire geographic range (Gilby et al. 2009): black birds (~70% males, ~80% females) outnumber red birds (~30% males, ~20% females). In both captive and wild populations, females and males show genetically determined assortative mate preferences for partners of the same head color (Pryke and Griffith 2009a; Pryke 2010), presumably because postzygotic genetic incompatibilities between the morphs lead to high offspring mortality in mixed-morph pairs (Pryke and Griffith 2009b).

Head color is also genetically correlated with several disparate behavioral, physiological, and reproductive traits (Pryke and Griffith 2006, 2009c; Pryke et al. 2007). Both red males and red females are dominant to black birds (Pryke and Griffith 2006; Pryke 2007, 2009), and red males will aggressively outcompete black males for access to limited food and nest sites (Pryke and Griffith 2006, 2009c). These differences in aggressive behavior between the male morphs also correspond to differences in hormonal regulation and immune performance. Although red males defend higher-quality resources, in highly competitive environments they show highly elevated hormone levels as-

sociated with aggression (testosterone) and stress (corticosterone; Pryke et al. 2007) and they reduce or abandon parental investment, which results in the production of lower-quality and fewer surviving offspring (Pryke and Griffith 2009c). In contrast, although the nonaggressive black males are excluded from limited or high-quality resources, they follow a more passive behavioral strategy that buffers them against social stresses (Pryke et al. 2007; Pryke and Griffith 2009c).

By experimentally manipulating the quality of the diet available to birds before and during breeding, we explored the optimal physiological and behavioral strategies adopted by both the red and the black morphs. In particular, we investigated within-individual changes in body condition (body mass, hematological measures), immune function (cutaneous immune response to phytohemagglutinin [PHA]), and endocrine characteristics (testosterone, corticosterone, free corticosterone, CBG) in response to low- and high-quality diets before and during breeding. We also determined the relative genetic and environmental effects of diet treatments on parental effort and aspects of reproduction, including brood production, timing of reproduction, and clutch size. Furthermore, we assessed how the level of parental effort adopted by individuals receiving the different diet treatments affected offspring growth, development, immune function, and survival.

Methods

Experiments were run from January to September 2006, using wild-type Gouldian finches from a large, captive-bred colony (Martinsville, New South Wales, Australia). All birds were naive (virgin) birds of the same age (all in their first year of adult plumage). All birds were banded with a unique combination of color bands and housed in small groups (8–12 birds) in single sex and morph cages (i.e., males and females and red and black morphs visually isolated from one another). From hatching, all experimental birds were reared and maintained on the same diet, which consisted of mixed finch seed (red millet, white millet, Japanese millet, Siberian rye, and canary seed), mineralized grit, and a daily supplement of freshly sprouted seeds (red millet, white millet, and Japanese millet).

Breeding and Diet Treatments

Interbreeding between red and black head-color morphs results in high offspring mortality (Pryke and Griffith 2009b). Therefore, to eliminate interacting genetic effects, birds were paired to genetically compatible partners of the same head-color morph (i.e., same genotype). Sex-linked genotypes of head-color morphs can be directly inferred

from the phenotype of females (i.e., red females are Z^R and black females are Z^r) and black males (i.e., homozygous recessive: Z^rZ^r). Because heterozygous red males (Z^RZ^r) are genetically incompatible with red females (Pryke and Griffith 2009b), only homozygous-dominant males (Z^RZ^R) were used in these experiments (as determined from established pedigrees).

In total, 60 black and 60 red birds were assortatively paired together (i.e., 30 red pairs, 30 black pairs) in single, visually isolated breeding cages (1.2 m³) and randomly assigned to either a low-quality (15 black, 15 red pairs) or a high-quality diet (15 black, 15 red pairs) containing approximately 8% or 20% protein, respectively. The low-quality diet consisted of a dry seed mix containing three different seeds in equal proportions (red millet, Siberian rye, and Japanese millet) and mineralized grit. In contrast, the high-quality diet included a dry seed mix of nine different seeds (red millet, white millet, Japanese millet, Siberian rye, canary seed, silk sorghum, niger, and black lettuce), mineralized grit, and a daily supplement (10 g) containing freshly sprouted seeds (red millet, white millet, and Japanese millet) and a specially designed supplement (egg, wheat germ extract, and multimineral powder). All birds were allowed unlimited access to the dry seed mix. For both males and females assigned to the breeding experiments, initial body weight did not differ between the morphs (males: $F_{1,59} = 2.23$, $P = .14$; females: $F_{1,59} = 0.93$, $P = .34$) or the diet treatments (males: $F_{1,59} = 0.16$, $P = .69$; females: $F_{1,59} = 0.08$, $P = .78$).

All birds were fed the experimental diets for 3 weeks before being given nest boxes and thus allowed to breed; Gouldian finches are obligate cavity nesters and are unable to build their own freestanding nests. Custom-built nest boxes were used that mimic the small, deep tree hollows used by birds in the wild (see Pryke and Griffith 2009c for details). Nest boxes were checked each morning, and progress was recorded. One to 2 days after the chicks hatched (mean \pm SD = 1.1 ± 0.6 days), all broods were cross-fostered to isolate the effects of genetic morph and maternal condition (i.e., prebreeding diet quality) at the time of laying from the posthatching rearing environment. We did not perform partial cross-fostering (i.e., one-half of the offspring swapped between nests), to avoid the possibility that parents preferentially feed larger or smaller chicks of different origins. The swapped broods were, as much as possible, of the same age (1.1 ± 0.8 days) and size (0.91 ± 0.53 g). Broods were swapped either between the two diet treatments ($n = 28$ broods) or within the same diet treatment ($n = 26$; 10 broods receiving the low-quality diet and 16 broods receiving the high-quality diet). Offspring were cross-fostered between the color morphs receiving each diet treatment (i.e., 28 of the 54 broods were swapped between morphs); there are no visual dif-

ferences (e.g., coloration, size) between chicks produced from the two different genetic morphs. Of the 28 broods that were cross-fostered between diet treatments, 16 broods were also swapped between red and black morphs (eight from low to high quality and eight from high to low quality). Of the 10 and 16 broods that were swapped within the low- and high-quality diet treatments, respectively, four (low quality) and eight (high quality) of the broods were also swapped between head-color morphs. No broods were abandoned during this experiment. All adults and offspring were fed their experimental diets (low or high quality) until offspring reached independence (60 days old) and were separated from their foster parents.

Immunological and Hematological Measures

To evaluate physiological and morphological changes, birds were weighed, measured, and blood sampled before breeding (i.e., after 3 weeks of receiving different diets) and during chick rearing (offspring age, 8 days old; receiving the diet for $\sim 9.6 \pm 1.3$ weeks). To standardize the procedures and minimize potential time effects, all physiological measurements were taken at the same time of day (0600–0900 hours). Blood samples were taken within 2 min of capture (42.5 ± 4.9 s) from the jugular vein (using 29-gauge needles), and ~ 150 μ L of blood was transferred to heparinized microhematocrit tubes. Blood samples were immediately centrifuged, and the plasma was stored at -20°C .

Hematocrit, or packed cell volume in relation to the whole volume of blood, reflects the erythrocyte content of blood and is an increasingly used index of avian condition and physiological performance (Saino et al. 1997; Potti et al. 1999; Fair et al. 2007). Hematocrit was calculated as the ratio of the packed cell volumes of whole blood (%) from the two centrifuged capillaries; within-individual measurements were highly repeatable ($r = 0.99$, $F_{119,240} = 168.42$, $P < .001$). As an index of nutritional condition (Bańbura et al. 2007; Pryke and Griffith 2009c), whole-blood hemoglobin concentration (g/dL) was also measured from a 5- μ L blood sample, using a HemoCue photometer (HemoCue, Ängelholm, Sweden).

Cutaneous immune responsiveness was evaluated by measuring the extent of cutaneous swelling following injection of birds with a nonpathogenic antigen, phytohemagglutinin (PHA-P; Sigma). PHA is a lectin that stimulates innate and adaptive immune responses, including those that give rise to a measurable inflammatory response (Martin et al. 2006). Each bird's right and left wing web were injected with either PHA or sterile phosphate-buffered saline (PBS), respectively (Pryke et al. 2007; Pryke and Griffith 2009c), and the consequent swellings were measured with pressure-sensitive digital calipers (0.01

mm) 24 h later. The PHA response was calculated as the difference in swellings between the left wing web (control for the effect of injection on web wing thickness: PBS only) and the right wing web (0.2 mg PHA-P dissolved in 0.04 mL PBS).

Hormone Assays

To determine within-male variation in testosterone concentrations, testosterone samples were analyzed in duplicate, using a Cayman Enzyme Immunoassay kit (582701; Ann Arbor, MI; details in Pryke 2009). Each sample was spiked with <1 pg of tritiated testosterone (Amersham [1,2,6,7-3H]) to allow the calculation of recoveries following extraction in diethyl ether, and samples were redissolved in buffer dilutions optimized for the standard curve (1 : 20). Two samples (from black males) were excluded from analyses because lower buffer dilutions were required to measure testosterone levels. Percentage testosterone recovery was $97.8\% \pm 2.4\%$, and intra- and interassay variation were 6.3% and 10.5%, respectively.

Total corticosterone concentration was measured in duplicate from plasma samples, using a Cayman Enzyme Immunoassay kit (500651). Kit instructions were followed, but each sample was also initially spiked with <1 pg of tritiated corticosterone (Amersham [1,2,6,7-3H]) to determine percentage recovery. After steroid extraction in dichloromethane, samples were reconstituted in buffer dilutions optimized for the standard curve (1 : 40). Final hormone values were adjusted for individual sample recovery (mean recoveries were $91.3\% \pm 1.2\%$). Intra-assay variation was 6.4% and interassay variation was 11.2%; these were assessed using a chicken plasma pool. The time from capture to bleeding (within a 0–2-min interval) had no effect on corticosterone levels ($F_{1,107} = 0.02$, $P = .89$), and there were no differences in capture times between birds receiving the low- and high-quality diets ($F_{1,107} = 0.19$, $P = .66$).

Plasma CBG capacity was measured using a radioligand-binding assay with tritiated corticosterone (details provided in Lynn et al. 2003). Plasma was stripped of steroids in dextran-coated charcoal buffer for 30 min and then diluted in buffer (1 : 200) for the CBG binding assessment. Samples (in triplicate) were incubated in solution for 2 h at 4°C to allow competitive binding between sample CBG and either standardized aliquots of tritiated corticosterone or unlabeled corticosterone to determine total or non-specific binding, respectively. Solutions were rinsed and CBG was harvested on 0.3% polyethyleneimine-soaked glass filters, using vacuum filtration through a Brandel cell harvester. The amount of tritiated corticosterone bound to filters was measured as DPM, using a liquid scintillation beta counter (Ultima Gold Scintillant, Tricarb LS Analyzer,

Perkin Elmer). CBG concentrations (nM) were calculated as mean DPM per sample \times plasma dilution (200) \times K , where K is a correction factor determined by label-specific activity (SA) and assay volume:

$$K = \frac{\text{DPM}}{0.00015 \text{ L}} \times \frac{\text{uCi}}{2.22 \times 10^6 \text{ DPM}} \\ \times \frac{1 \times 10^{-3}}{\text{SA as } 10^6 \text{ uCi/mMol}} \times \frac{1 \times 10^9 \text{ nMol}}{\text{M}}.$$

Pooled plasma from captive Japanese quail served as a reference standard for all assays of CBG capacity and was tested along with individual finch plasma samples on all filters. Mean intra- and interassay variability were <5% and <10%, respectively. Equilibrium saturation binding analysis was conducted using samples from nonexperimental finches to determine the affinity (K_d) and capacity (B_{max}) of Gouldian finch CBG. Pooled stripped plasma samples (in triplicate) were incubated in nine concentrations of 0.23–12 nM of tritiated corticosterone, and CBG-bound labeled hormone was harvested and counted as above. The affinity (K_d) was 2.12 ± 0.63 nM, and the capacity (B_{max}) was 0.09 ± 0.009 nM (goodness of fit: $df = 24$, $R^2 = 0.94$). Free corticosterone concentrations were estimated using the equation of Barsano and Bauermann (1989).

Parental-Provisioning Rates

Parental-provisioning rates were quantified using a remote-monitoring system that recorded all visits to the nest (Magrath and Elgar 1997; Pryke and Griffith 2009c). Individual birds were identified by uniquely coded passive integrated transponder (PIT) tags (Trovan, The Netherlands) that were glued to the leg band. These transponders are small (11×2 mm), passive devices that emit a unique identification code when in close proximity to powered antennae. Antennae (i.e., powered wire coils) with a 4-cm detection range were placed centrally (i.e., 7 cm from the nest entrance) around the outside of the entrance tunnel to the nest. Two pairs of infrared sensors, positioned 4 cm apart (less than a bird length) on either side of the antennae, were used to detect the direction of the bird's movement (i.e., into or out of the nest). The antennae were activated (at 1-s intervals) when either of the two infrared beams was interrupted. For analyses, parental visits were quantified only when both beams were interrupted, to exclude any incomplete movements or visits by birds (e.g., bird moves from inside the nest toward the entrance and then back to the nest). In addition, only nest visits that resulted in feeding (>10 s; 97.3% of all recordings) were included in analyses. For each nest, visitation rate was averaged across a 72-h period starting at 0600

hours on day 8 (chick age, 8–12 days), which coincides with the period in which nestling food demand is the highest (Pryke and Griffith 2009c).

Offspring Growth Rate, Condition, and Survival

Chicks were weighed (to the nearest 0.1 g; using an electronic balance) at hatching and then every second day until they were 18 days old, and then on the day they fledged (left) the nest (range, 18–33 days old). To estimate growth rates, we used a logistic equation developed by Royle et al. (2006) to measure offspring growth in another estrilid finch: $M = A/[1 + \exp(-KT + B)]$, where M is chick mass (in grams), A is the asymptotic mass, K is the growth-rate constant, T is chick age (in days), and B is a constant determining the initial mass (at $T = 0$). Values for the parameters A , K , and B were derived for each chick, using a nonlinear regression (Royle et al. 2006; details in Pryke and Griffith 2009c). Only chicks that reached asymptotic mass (fledged the nest) were included in analyses of growth.

Every second day after hatching, the lengths of fully developed tail feathers (R2) and the part that was still covered with a waxy sheath (0.1 mm) were measured. Chicks that develop faster have a relatively smaller waxy sheath. Therefore, we used $1 - (\text{length of waxy sheath})/(\text{total tail feather length})$ as an estimate of development.

As an index of nutritional condition, whole-blood hemoglobin concentration (g/dL) was measured on 5- μ L blood samples from 12-day-old nestlings, using a HemoCue photometer. To assess nestling immune function, PHA responses were measured in 12-day-old nestlings (as described for adults above). Overall offspring survival was calculated as the relative proportion of hatched nestlings that successfully fledged from the nest.

Statistical Analyses

Data were analyzed in Genstat 9 (Rothamsted Experimental Station, Harpendon, United Kingdom). Changes in male and female condition, cutaneous immune function, and hormonal responses (prebreeding and breeding) to the dietary treatments were analyzed using repeated-measures ANOVA with a structured blocking function (i.e., repeated measures for within-individual comparisons).

Clutch size and latency to breed were analyzed using generalized linear models (GLMs) with Poisson distributions and logarithmic link functions. Similarly, parental-provisioning rates of both males and females were analyzed using a GLM. The significance of the predictor variables in these models was tested by the change in deviance of the different models, using a χ^2 approximation. Chick

growth, development, and immune measures were analyzed using generalized linear mixed models (GLMMs). Mixed models allow both fixed and random components to be fitted to a model; random components in this case take into consideration the repeated measures of offspring (siblings) from the same nest. Offspring survival (number of nestlings fledging/total number fostered after hatching) was analyzed at the brood level by fitting a GLMM. The error distribution was binomial, with a logit link weighted by brood size. The significance of the explanatory terms in mixed models were assessed by the Wald statistics, which are distributed as χ^2 . In all models of offspring condition and survival, nest identity was included and retained as a random repeated subject but did not constitute a significant random component ($P > .19$).

For all models, all possible effects, combinations, and interactions (two- and three-way interactions) were initially modeled. Second-order Akaike Information Criterion (AIC_C) weights were calculated for each model. The AIC_C (used for smaller sample sizes) balances the fit of the model against the number of parameters and was used to objectively compare different models (i.e., it indicates the probability that a model is the best among the tested models). Since all models tested had an AIC_C weight of at least 79.2% compared with other potential models, only final models are reported. Significant probability values were derived from having all relevant terms fitted in the final model together, whereas those of nonsignificant terms were obtained from having all significant terms in the final model and each nonsignificant term fitted individually. Means \pm SD are presented throughout.

Results

Condition and Immune Function

Red and black birds differed significantly in several condition-related indices. First, although there were no overall differences in body weight among red and black males ($F_{1,59} = 2.23$, $P = .14$) receiving different diets before breeding ($F_{1,59} = 0.16$, $P = .69$), diet treatment differentially affected within-male body weight in the two color morphs during breeding (morph \times diet: $F_{3,50} = 10.35$, $P < .001$; morph: $F_{1,53} = 12.05$, $P < .001$; diet: $F_{1,53} = 15.11$, $P < .001$). In particular, whereas both red and black males receiving the high-quality diet maintained comparable body weights, red males receiving the lower-quality diet lost more weight than black males (fig. 1A). Similarly, although preexperimental red and black female body weights did not differ ($F_{1,59} = 0.93$, $P = .34$), red females receiving the low-quality diet lost significantly more weight than black females receiving the low-quality diet

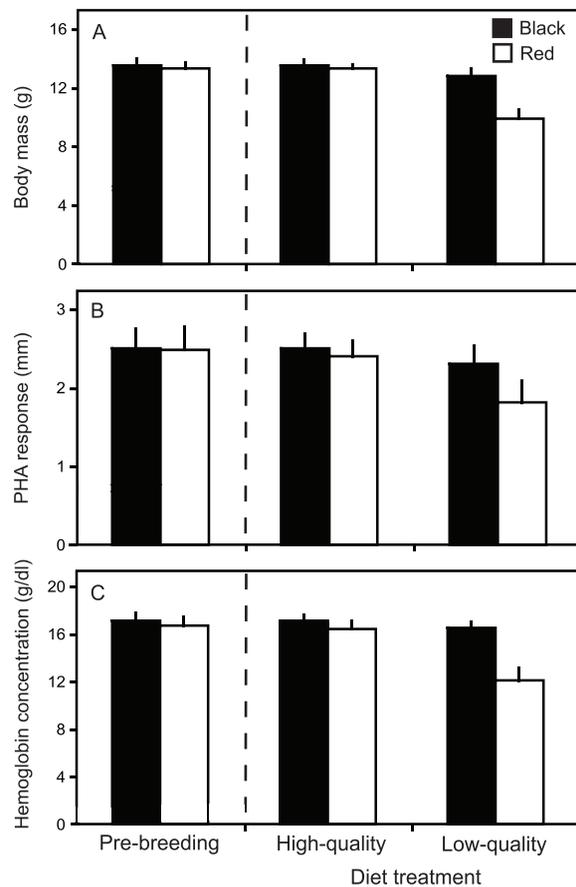


Figure 1: In response to nutritional stress, red and black males expressed significantly different within-individual changes in body mass (A), cell-mediated immune function (PHA response; B), and hemoglobin concentrations (C) before (prebreeding) and during breeding. All values are presented as predicted means (\pm SE) from the best-fitting generalized linear model.

(morph \times diet: $F_{3,50} = 68.29$, $P < .001$; morph: $F_{1,53} = 48.50$, $P < .001$; diet: $F_{1,53} = 74.83$, $P < .001$; fig. 2A).

Second, diet treatment differentially affected immune function between the two male morphs (fig. 1B). Immune responsiveness did not differ between red and black males before breeding ($F_{1,59} = 0.79$, $P = .38$), but breeding red males receiving the low-quality diet experienced reduced PHA responses (morph \times diet: $F_{3,50} = 5.29$, $P = .008$; morph: $F_{1,53} = 10.47$, $P < .01$; diet: $F_{1,53} = 20.01$, $P < .001$), whereas there was no such effect for black males (fig. 1B). Similarly, breeding red males receiving the low-quality diet also exhibited lower whole-blood hemoglobin concentrations (morph \times diet: $F_{3,50} = 7.56$, $P = .001$; morph: $F_{1,53} = 19.01$, $P < .001$; diet: $F_{1,53} = 43.11$, $P < .001$; fig. 1C) and reduced hematocrit (morph \times diet:

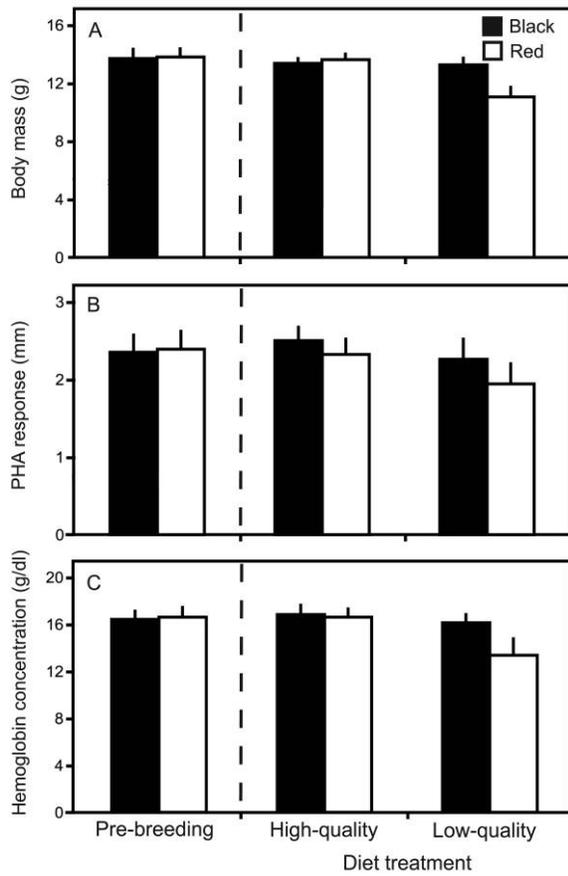


Figure 2: Red and black females showed different within-individual changes to diet treatment in body mass (A), cell-mediated immune function (PHA response; B), and hemoglobin concentrations (C) before (prebreeding) and during breeding. All values are presented as predicted means (+SE) from the best-fitting generalized linear model.

$F_{3,50} = 9.13, P < .001$; morph: $F_{1,53} = 16.20, P < .001$; diet: $F_{1,53} = 53.05, P < .001$) compared with breeding birds receiving the high-quality diet.

Similar effects were also found for females (fig. 2). Although red and black females showed similar PHA responses before breeding ($F_{1,59} = 0.33, P = .57$), breeding red females receiving the low-quality diet tended to have lower responses (morph \times diet: $F_{3,50} = 2.73, P = .08$; morph: $F_{1,53} = 12.02, P < .001$; diet: $F_{1,53} = 14.53, P < .001$) than black females (fig. 2B). Red females also exhibited lower whole-blood hemoglobin concentrations when receiving the low-quality diet (morph \times diet: $F_{3,50} = 23.62, P < .001$; morph: $F_{1,50} = 31.02, P < .001$; diet: $F_{1,53} = 52.34, P < .001$; fig. 2C). However, hematocrit measures did not differ between red and black females receiving either the low- or the high-quality diet (morph \times diet: $F_{3,50} =$

0.66, $P = .52$; morph: $F_{1,53} = 1.93, P = .16$; diet: $F_{1,53} = 1.94, P = .15$).

Endocrine Responses

Before breeding, red males had higher levels of circulating testosterone than black males ($F_{1,59} = 36.68, P < .001$; fig.

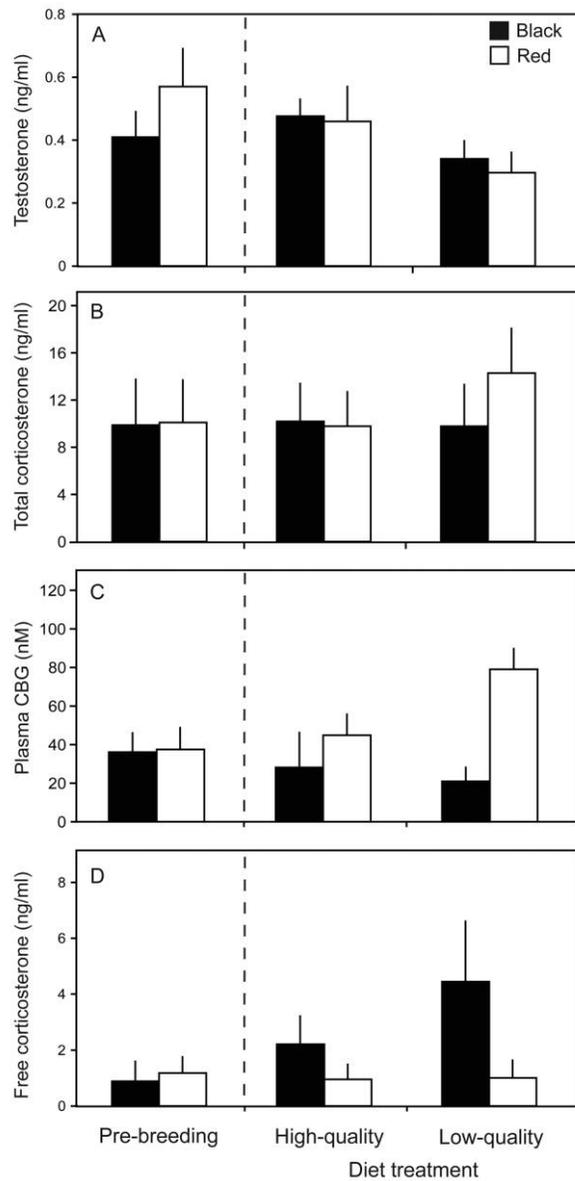


Figure 3: Between the two different diet treatments, red and black males had similar plasma testosterone levels (A) but different total corticosterone (B), corticosterone-binding globin (CBG) concentrations (C), and free corticosterone levels (D). All values are presented as predicted means (+SE) from the best-fitting generalized linear model.

3A). When breeding, however, testosterone levels of individual males declined with the low-quality but not the high-quality diet ($F_{1,51} = 11.13$, $P = .002$). There were no morph-specific testosterone responses to either the high-quality or the low-quality diet (morph: $F_{1,51} = 2.49$, $P = .12$; morph \times diet: $F_{3,48} = 0.52$, $P = .47$), and thus both color morphs showed a similar decrease in testosterone in response to the diet treatments (fig. 3A).

Overall, circulating plasma corticosterone (i.e., total corticosterone) did not differ between the red and the black morphs before ($F_{1,59} = 0.06$, $P = .80$) or during breeding ($F_{1,53} = 2.78$, $P = .10$). However, diet treatments had different effects in breeding red and black males receiving the low-quality diet (morph \times diet: $F_{3,50} = 5.65$, $P = .006$; morph: $F_{1,53} = 4.41$, $P = .02$), with red but not black males showing a significant increase in circulating plasma levels of total corticosterone (fig. 3B). Similarly, although female morphs had comparable corticosterone levels before breeding ($F_{1,59} = 0.33$, $P = .57$), red females receiving the low-quality diet had higher total corticosterone levels than black females (morph \times diet: $F_{3,50} = 6.11$, $P = .004$; morph: $F_{1,53} = 2.56$, $P = .08$; diet: $F_{1,53} = 2.72$, $P = .07$; fig. 4A).

Diet also had significant but opposite effects on the CBG levels of the color morphs (figs. 3C, 4B). Although the CBG concentrations of red and black males were similar before breeding ($F_{1,59} = 0.24$, $P = .62$), the CBG levels diverged between the morphs during breeding ($F_{1,53} = 53.92$, $P < .001$). In particular, the CBG levels of red males increased during breeding, especially in those receiving the low-quality diet, whereas the CBG levels of black males decreased, especially in those receiving the low-quality diet (morph \times diet: $F_{3,50} = 16.46$, $P < .001$; diet: $F_{1,53} = 8.76$, $P < .01$; fig. 3C). Similarly, the CBG levels of red females significantly increased when they received the low-quality diet, while those of black females decreased (morph \times diet: $F_{3,50} = 17.78$, $P < .001$; morph: $F_{1,53} = 162.18$, $P < .001$; diet: $F_{1,53} = 0.54$, $P = .59$; fig. 4B).

Diet affected circulating levels of free corticosterone in black but not red morphs (figs. 3D, 4C). During breeding, free corticosterone levels of black males increased from prebreeding levels ($F_{1,53} = 5.51$, $P = .007$), especially in those receiving the low-quality diet (morph \times diet: $F_{3,50} = 4.88$, $P = .01$; morph: $F_{1,53} = 22.99$, $P < .01$), whereas free corticosterone levels of red males were unaffected by breeding or diet quality (fig. 3D). In females, free corticosterone levels decreased for breeding red females but increased for breeding black females ($F_{1,53} = 29.82$, $P < .001$; fig. 4C). Breeding black females receiving the low-quality diet had particularly high free corticosterone levels, whereas free corticosterone levels of red females were unaffected by diet quality (morph \times diet: $F_{3,50} = 12.38$, $P < .001$; diet: $F_{1,53} = 8.18$, $P < .01$).

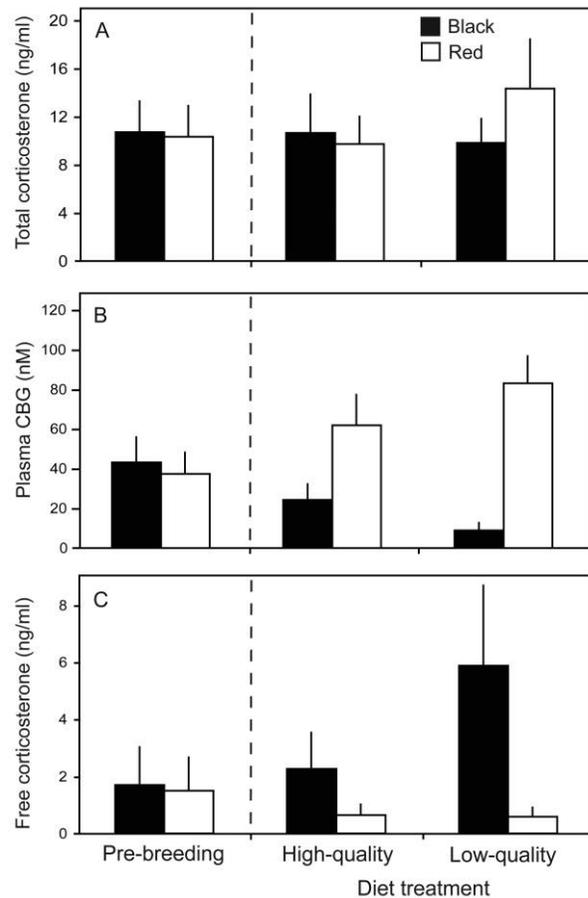


Figure 4: Red and black females differed between the two diet treatments in total corticosterone (A), corticosterone-binding globin (CBG) concentrations (B), and free corticosterone levels (C). All values are presented as predicted means (\pm SE) from the best-fitting generalized linear model.

Clutch Initiation and Size

Of the 30 red and 30 black breeding pairs, one black pair and five red pairs did not breed (all were receiving the low-quality diet). Clutch initiation time was faster for breeding birds receiving the high-quality diet (7.57 ± 2.58 days) than for those receiving the low-quality diet (19.45 ± 8.59 days; $F_{1,53} = 51.89$, $P < .001$). Black birds receiving the low-quality diet initiated egg-laying earlier (14.21 ± 5.07 days) than red pairs (26.81 ± 6.97 days; morph \times diet: $F_{3,50} = 26.08$, $P < .001$; morph: $F_{1,53} = 27.79$, $P < .01$). Furthermore, red pairs receiving the low-quality diet produced fewer eggs (3.20 ± 0.79) than black pairs (5.14 ± 1.17 ; morph \times diet: $F_{3,50} = 9.03$, $P = .004$; morph: $F_{1,53} = 13.65$, $P = .005$; diet: $F_{1,53} = 11.78$, $P = .01$), but there were no clutch-size differences for red

Parental Effort and Hormone Responses

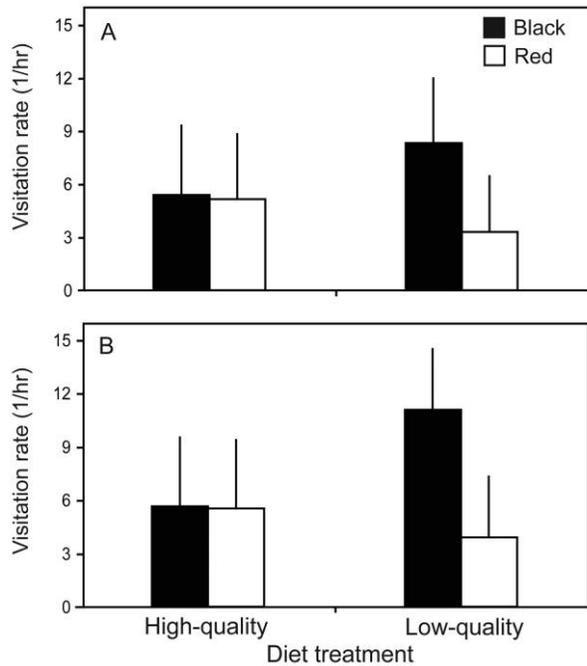


Figure 5: Proportion of time spent provisioning offspring by red and black males (A) and females (B) on the low- and high-quality diet treatments. All values are presented as predicted means (+SE) from the best-fitting generalized linear model.

(5.07 ± 1.09) and black birds (5.27 ± 1.03) breeding while receiving the high-quality diet.

Parental Effort

Red and black parents provisioned offspring differently between the two diet treatments (fig. 5). While receiving the low-quality diet, red females fed their fostered offspring significantly less frequently, while black females significantly increased their provisioning rates (morph \times diet: $F_{3,50} = 13.95$, $P < .001$; morph: $F_{1,53} = 14.95$, $P < .001$; diet: $F_{1,53} = 4.01$, $P = .05$). Provisioning rate was not influenced by the original clutch size ($F_{1,53} = 0.13$, $P = .72$) or partner visitation rate ($F_{1,53} = 0.72$, $P = .39$).

Similarly, red and black males differed in their provisioning rates (fig. 5). While red and black males provided comparable parental care while receiving the high-quality diet, red males receiving the low-quality diet significantly reduced their visitation rates, whereas black males significantly increased their provisioning rates (morph \times diet: $F_{3,50} = 6.17$, $P = .01$; morph: $F_{1,53} = 6.49$, $P = .01$; diet: $F_{1,53} = 0.23$, $P = .64$). These effects were independent of original clutch size ($F_{1,53} = 0.01$, $P = .94$) and partner visitation rate ($F_{1,53} = 0.36$, $P = .55$).

Although overall female visitation rates were not predicted by variation in total baseline corticosterone ($F_{1,53} = 1.94$, $P = .16$), female effort was related to both free baseline corticosterone ($F_{1,53} = 78.95$, $P < .001$) and CBG levels ($F_{1,53} = 18.21$, $P < .001$). In particular, there were morph-specific effects (fig. 6); visitation rates of black (but not red) females were predicted by increased free baseline corticosterone (morph \times free corticosterone: $F_{1,53} = 6.94$, $P = .01$; morph: $F_{1,53} = 4.01$, $P = .05$; free corticosterone: $F_{1,53} = 0.46$, $P = .49$) and decreased CBG levels (morph \times CBG: $F_{1,53} = 9.26$, $P = .003$; morph: $F_{1,53} = 4.17$, $P = .05$; CBG: $F_{1,53} = 16.72$, $P < .01$). Similarly, black males with relatively higher levels of free corticosterone (morph \times free corticosterone: $F_{1,53} = 8.67$, $P = .005$; morph: $F_{1,53} = 8.16$, $P = .006$; free corticosterone: $F_{1,53} = 2.88$, $P = .09$) provisioned their offspring more than red males did (fig. 6). CBG levels were also related to overall male provisioning rate ($F_{1,53} = 8.25$, $P = .006$), but there was no effect of total corticosterone ($F_{1,53} = 2.00$, $P = .16$) or testosterone levels ($F_{1,51} = 0.09$, $P = .77$) on visitation rates.

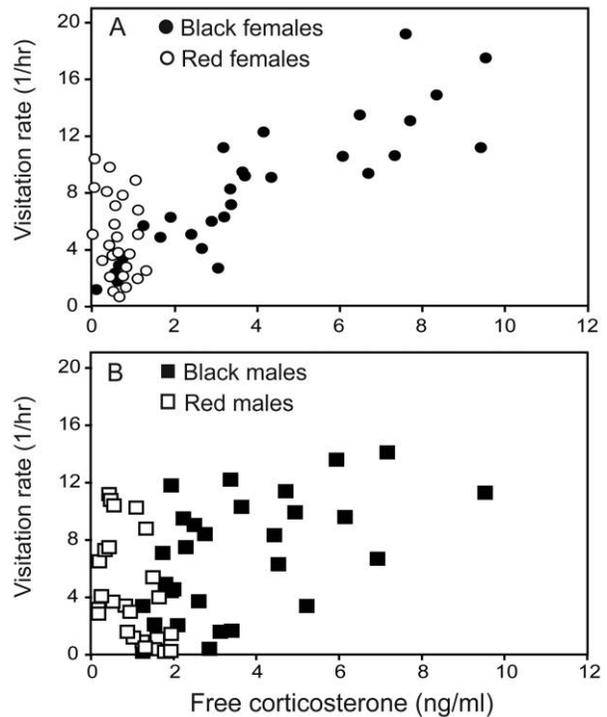


Figure 6: Relationship between parental-provisioning rate and circulating levels of free baseline corticosterone for black and red females (A) and black and red males (B).

Offspring Growth Rates, Condition, and Survival

At hatching, offspring produced by birds receiving the high-quality diet were heavier (0.91 ± 0.21 g; $n = 155$) than offspring produced by birds fed the low-quality diet (0.71 ± 0.17 g; $n = 104$; $F_{1,53} = 66.88$, $P < .001$), independent of head-color morph ($F_{1,53} = 0.09$, $P = .76$). However, after cross-fostering offspring within and between nests of high-quality and low-quality parents, there were no significant differences observed in the masses of offspring experimentally placed into the nests of foster parents receiving the high-quality (0.83 ± 0.20 g; $n = 147$) and the low-quality diets (0.84 ± 0.23 g; $n = 112$; $F_{1,53} = 0.14$, $P = .71$).

Foster offspring receiving the high-quality diet grew significantly faster than offspring receiving the low-quality diet ($F_{1,51} = 88.11$, $P < .001$; fig. 7A). Offspring growth rate was also affected by the foster parents' color morph ($F_{1,51} = 13.92$, $P < .001$). In particular, offspring in red foster nests reared on the low-quality diet grew significantly more slowly than offspring reared in black nests (morph \times diet: $F_{3,48} = 7.26$, $P = .008$; fig. 7A). The difference in body mass remained significant at fledging (morph \times diet: $F_{3,48} = 33.94$, $P < .001$; morph: $F_{1,51} = 46.37$, $P < .001$; diet: $F_{1,51} = 108.23$, $P < .001$), despite offspring from low-quality nests fledging later (27.73 ± 3.08 days) than offspring from high-quality nests (21.61 ± 1.72 days; $F_{1,51} = 125.99$, $P < .001$). In particular, offspring reared on the low-quality diet by red parents fledged later (30.06 ± 3.72 days) than offspring reared by black parents (26.79 ± 2.22 days; morph \times diet: $F_{3,48} = 20.01$, $P < .001$; morph: $F_{1,51} = 21.75$, $P < .001$). However, offspring growth was unaffected by genetic origin (i.e., red or black: $F_{1,51} = 0.36$, $P = .55$) or the diet of the genetic parents ($F_{1,51} = 0.13$, $P = .71$).

Using tail feather development as an index, we found that foster offspring reared on the high-quality diet also developed faster (0.36 ± 0.08 mm) than foster offspring reared on the low-quality diet (0.20 ± 0.06 mm; $F_{1,51} = 63.14$, $P < .001$). There was also a tendency for offspring reared by black parents to develop faster than those reared by red parents ($F_{1,53} = 4.23$, $P = .04$), but there was no significant difference in the developmental rates of offspring originating from genetic red or black parents ($F_{1,51} = 0.59$, $P = .44$) or an effect of prehatching diet ($F_{1,51} = 0.73$, $P = .39$).

Similarly, foster offspring reared on the low-quality diet had lower immune function than those reared on the high-quality diet (fig. 7). In particular, offspring reared by red parents receiving the low-quality diet had reduced PHA responses compared with offspring reared by black parents (morph \times diet: $F_{3,50} = 40.61$, $P < .001$; morph: $F_{1,53} = 48.06$, $P < .001$; diet: $F_{1,53} = 78.27$, $P < .001$), and lower

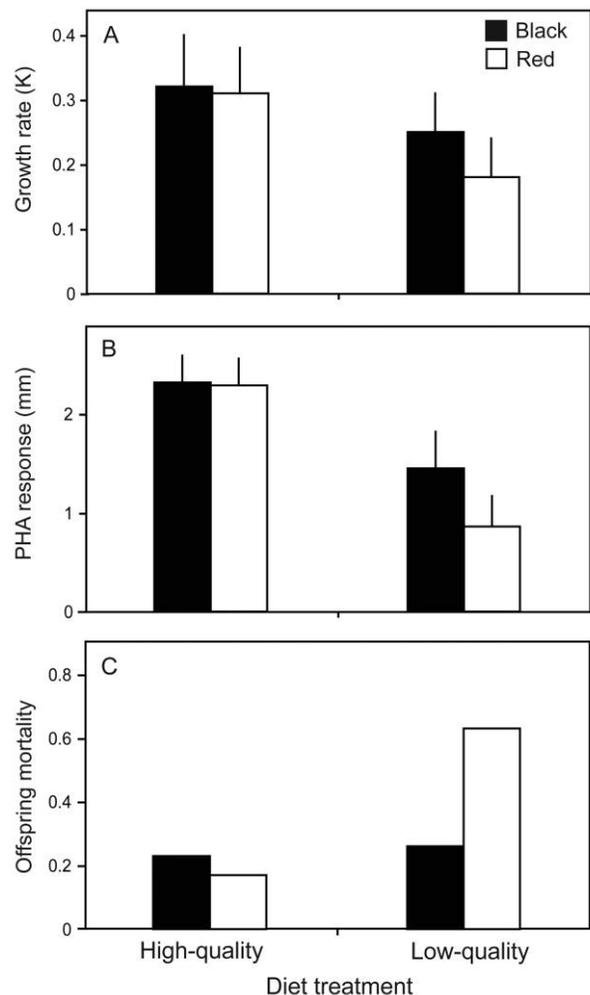


Figure 7: Between the two different diet treatments, foster offspring reared by red and black birds had significantly different growth rates (A), cell-mediated immune function (PHA responses; B), and survival rates (proportion of offspring dying from hatching to fledging; C). All values are presented as predicted means (\pm SE) from the best-fitting generalized linear mixed model.

whole-blood hemoglobin concentrations (morph \times diet: $F_{3,50} = 18.88$, $P < .001$; morph: $F_{1,53} = 20.03$, $P < .001$; diet: $F_{1,53} = 27.05$, $P < .001$). There were no effects of genetic parents ($F_{1,53} < 2.31$, $P > .13$) or prehatching diet ($F_{1,53} < 2.27$, $P > .13$) on measures of immune responsiveness.

In total, 77 of 259 nestlings died between hatching and fledging. Proportionately more offspring reared on the low-quality diet (42.9%) died than those reared on the high-quality diet (19.7%; $\chi^2 = 15.42$, $P < .001$). This effect was particularly severe for foster offspring reared by red parents receiving the low-quality diet (morph \times diet:

$\chi^2 = 10.92$, $P < .001$; morph: $\chi^2 = 4.05$, $P = .04$; fig. 7C). However, an offspring's mortality was independent of its genetic origin ($\chi^2 = 0.09$, $P = .92$) and the diet of its genetic parents ($\chi^2 = 1.20$, $P = .27$).

Discussion

The two genetic color morphs differ in the degree of plasticity in physiological traits, behavior, and reproduction. While there were no differences in individual condition, immune function, parental effort, or reproductive output between the morphs receiving the high-quality diet, red birds were highly sensitive to nutritional stress, with compromised condition, immune responsiveness, and decreased parental effort when receiving the low-quality diet. In contrast, black birds increased parental effort to meet the demands of a lower nutritional diet and were largely unaffected by the diet treatments. This divergent response to nutritional stress reinforces the contrasting physiological and behavioral strategies employed by red and black birds (Pryke et al. 2007; Pryke and Griffith 2009c), and it suggests that there may also be morph-specific differences in nutritional requirements. Although maintenance energy requirements do not differ between the red and the black morphs (W. A. Buttemer, E. Chappell, and S. Pryke, unpublished data), there may be morph-specific differences in digestive assimilation efficiencies and daily nutrient requirements that underlie their differential response to dietary quality.

Although red males have a dominance advantage and gain access to higher-quality food and nest sites (Pryke and Griffith 2006; Pryke and Griffith 2009c), their sensitivity to changes in the social environment (Pryke and Griffith 2009c) and resource quality during breeding results in large fitness costs. When provided nutritionally deficient diets, red males and females lost condition (body mass, hematocrit, and hemoglobin content) and had reduced immune function (PHA cutaneous immune response). Presumably as a result of the low-nutrition diet, red females also had severely delayed clutch initiation, produced fewer eggs, or failed to breed altogether (16.7%). When receiving the low-quality diet, red birds also provided relatively little parental care to their broods. Although one parent is expected to fully or at least partially compensate for decreased provisioning by the other parent (e.g., Hegner and Wingfield 1987; Ketterson and Nolan 1992), red males and females did not adjust their provisioning relative to their partner's effort. Consequently, foster offspring reared by red parents receiving the low-quality diet had reduced condition (lower body mass, reduced hemoglobin levels) and cutaneous immune function (PHA immune response) compared with red broods reared on the high-quality diet. PHA responses in nestlings have been

found to predict survival probability in a number of free-living bird species (e.g., Saino et al. 1997; Tella et al. 2000; Christe et al. 2001), presumably because they are reflective of increased disease susceptibility of poorer-quality offspring. Consistent with this, we found that broods reared by red parents receiving the low-quality diet had severely delayed fledging dates and successfully fledged only 37.2% of their offspring.

This contrasts with black morphs, which appeared to be largely unaffected by diet treatment, maintaining comparable body condition and immune function while receiving both the high- and the low-quality diets. Furthermore, despite the nutritional differences, black parents successfully bred while receiving both diet treatments, although foster offspring reared by black parents receiving the low-quality diet had reduced growth rates and immune function compared with those reared by black parents receiving the high-quality diet. Nevertheless, black parents produced more surviving offspring (74.4%) than red parents receiving the low-quality diet, and this was relatively comparable to the number of successfully fledged offspring reared by black (77.8%) and red parents (83.6%) receiving the high-quality diet. This discrepancy in offspring survival between the morphs is likely related to the higher investment in parental effort by black but not red parents. In response to receiving the low-quality diet, black birds substantially increased their provisioning rates, presumably to meet the higher nutritional demands of the offspring being reared on the low-quality diet. Therefore, black birds appear to be less sensitive to dietary changes in the environment, increasing their parental effort when receiving nutritionally deficient diets by heavily investing in parental provisioning to increase the quality and survival of their offspring. In contrast, the red morphs are less able to maintain their own condition or successfully rear broods while receiving a low-quality diet; this suggests that there may be fundamental differences in nutritional needs or nutritional uptake between the morphs.

Morph-Specific Regulation of Glucocorticoids

The endocrine system provides a proximate link between physiology, behavior, and fitness (e.g., Marler and Moore 1988; Sinervo and DeNardo 1996; Sinervo and Svensson 1998; McGlothlin and Ketterson 2008; Ketterson et al. 2009). In response to environmental stressors, including nutritional stress, the hypothalamic-pituitary-adrenal axis is activated, and this typically increases circulating glucocorticoid levels (Woodward et al. 1991; Heath and Dufty 1998; Kitaysky et al. 2003). In the short term (hours to days), elevation of corticosterone increases blood glucose levels and mobilizes energy stores to the muscles (Sapolsky 1992; Wingfield et al. 1998), adaptively serving energeti-

cally demanding behaviors such as foraging activities (Sapolsky 1992; Wedekind and Folstad 1994; Wingfield and Ramenofsky 1997; Wingfield et al. 1997). In contrast, in the longer term, sustained or chronic levels of elevated corticosterone can lead to severe immunosuppressive effects (Wingfield et al. 1997; Råberg et al. 1998; Buchanan 2000; but see Rodríguez et al. 2001) and reproductive abandonment (Sapolsky 1992). We found that after considerable nutritional stress (~9.2 weeks), breeding red males and females had higher total corticosterone levels than black birds (and red or black birds receiving the high-quality diet), which is consistent with their reduced immune function (Pryke et al. 2007). Although testosterone is often associated with immunosuppressive effects (Saino et al. 1995; Peters 2000; Buchanan et al. 2003; but see Owen-Ashley et al. 2004), testosterone is unlikely to be responsible for the compromised immune function of nutritionally stressed red males, because testosterone levels of both red and black males decreased to comparable levels with the low-quality diet.

However, nutritional stress not only affects total corticosterone levels, it may also independently influence CBG capacity (Woodward et al. 1991; Tinnikov et al. 1996), thus altering the amount of unbound or free corticosterone available to enter tissues. Recent studies have shown that CBG concentrations are reduced in fasting birds (Lynn et al. 2003) and in birds with poor reproductive performance (Williams et al. 2008), suggesting that variation in CBG content may be directly affected by nutritional stress. Our results, therefore, are paradoxical. Male and female black morphs showed significant reductions in CBG content while breeding and receiving both the low- and the high-quality diets, but their body mass and hemoglobin content remained invariant between treatments (i.e., suggesting nutritional insensitivity to dietary quality). In contrast, red males and females had significant reductions in body mass and hemoglobin content while receiving the poorer diet, responses that are typical of nutritional stress. Yet despite this, red morphs consistently mounted significant increases (not decreases) in CBG levels, implying that CBG elevation was a priority and, therefore, may have functional benefits. Unfortunately, the experimental design used here (which provides only a snapshot within progressive effects of the dietary treatment) does not allow us to evaluate whether black and red morphs are in different positions along a similar temporal trajectory or if these effects are both more pronounced and occur earlier in red than black birds. Nevertheless, the consistent but opposing CBG responses of breeding red and black morphs to nutritional stress invites speculation about its functional significance.

To date, there has been some debate about the functional distinctions between free and CBG-bound corticosterone.

Traditionally, it was thought that only free corticosterone was biologically active (Breuner and Orchinik 2002; Malisch and Breuner 2010), but there is increasing evidence that CBG-bound corticosterone may target and selectively release corticosterone at sites undergoing inflammatory reactions (Hammond 1995; Petersen et al. 2006; Lin et al. 2009). This may explain the more suppressed PHA response of breeding red morphs receiving the low-quality diet (despite having lower free corticosterone levels than black morphs). Following PHA injection, immune cells such as heterophils infiltrate the injection site and greatly increase in abundance (Martin et al. 2006). Heterophils rapidly change the affinity of CBG for corticosterone, subsequently releasing free corticosterone at the site of inflammation (Lin et al. 2009). During inflammation, the endothelial surfaces of vessels become more permeable, allowing CBG-bound corticosterone in the plasma to reach inflamed tissues (Raza et al. 2010), and thus all bound and unbound corticosterone is functionally available. Because inflammatory cytokines will promote reallocation of proteins toward immune functions (Lochmiller and Deerenberg 2000), enhanced corticosterone availability should protect against further muscle wasting during periods of nutritional insufficiency. In this context, the response of red morphs to elevated total corticosterone but lower free corticosterone may be viewed as a self-protective mechanism. Potentially, in red morphs, the elevated CBG-bound fraction relative to free-corticosterone levels functions as an immunomodulatory mechanism that allows the energetically more affordable noninflammatory immune responses to function fully while suppressing the more nutrient-demanding inflammatory immune functions. Furthermore, such selective immune inhibition would also reduce inflammation-related increases in susceptibility to oxidative damage (Bertrand et al. 2006).

In black morphs, increased levels of free corticosterone were associated with increased parental provisioning. In contrast, the feeding rates of nutritionally stressed red birds were unaffected by variation in free corticosterone levels, perhaps because of differences in corticosterone receptor densities or variations in other interacting hormones associated with reduced body condition (e.g., prolactin secretion; O'Dwyer et al. 2006). The decreased CBG content in breeding black morphs has also been noted in other species. For example, female European starlings (*Sturnus vulgaris*) show progressively increasing baseline levels of free corticosterone between egg laying and chick rearing and higher levels with increased brood sizes (Love et al. 2004). Such elevations in combination with coincident increases in prolactin have been suggested to stimulate parental feeding rates to chicks (Angelier and Chastel 2009; Miller et al. 2009). Indeed, there is increasing empirical support showing a link between corticosterone elevation

and successful brood provisioning (i.e., corticosterone-adaptation hypothesis; Bonier et al. 2009, 2011). Overall, it appears that the responses of individuals to changes in circulating corticosterone are context dependent and will vary in relation to the fitness cost of balancing resources between self and offspring (Bonier et al. 2009). Nevertheless, because the interrelations between corticosterone and other important reproductive hormones (e.g., prolactin) are unknown in the Gouldian finch, we do not know whether the correspondence between plasma free corticosterone and chick provisioning rates in the black morphs are causative or simply correlative.

Nutritional Stress and the Persistence of the Color Polymorphism

Genetic differences in endocrine and physiological sensitivities have been documented in a variety of polymorphic taxa, including fish, reptiles, and birds (e.g., Sinervo et al. 2000b; Knapp 2004; Spinney et al. 2006; Sacchi et al. 2007; Huyghe et al. 2009). For such alternative strategies to be evolutionarily stable, a mechanism must exist for the alternative forms to gain equal fitness benefits over evolutionary time (Maynard Smith 1982). Previous work on this system has shown that the alternative reproductive strategies adopted by red and black birds are maintained by negative frequency-dependent selection in the local social environment (Pryke et al. 2007; Pryke and Griffith 2009c). Aggressive red males have a competitive advantage over black males (Pryke and Griffith 2006), but in highly competitive environments they compromise their reproductive fitness by heavily investing in aggression, to the detriment of parental provisioning and the quality and survival of their offspring (Pryke and Griffith 2009c). In contrast, although nonaggressive black birds are excluded from limited or high-quality food and nest sites (Pryke and Griffith 2006), they gain larger fitness benefits in socially competitive environments than red birds by investing heavily in parental care (Pryke and Griffith 2009c). Opposing physiological responses to the social environment (Pryke et al. 2007; Pryke and Griffith 2009c) and nutritional stress (this study) are likely to underlie the divergent behaviors adopted by red and black birds and to help explain their continued persistence. For example, over the last 50 years, both the range and the size of wild populations of these birds have dramatically declined, and the Gouldian finch is now classified as endangered (<2,500 individuals; O'Malley 2006). Large-scale changes in habitat quality, and particularly the availability and quality of seedling grasses, are thought to be a primary cause (O'Malley 2006). Potentially, if nutritional quality continues to decline, the nutritionally less sensitive black birds are likely to gain the largest fitness benefits, which may further in-

crease their relative frequencies in natural populations (they currently comprise 70%–90% of populations) and potentially reduce the relative frequencies of the red morph (or outcompete it altogether). On the other hand, however, the elevated corticosterone levels and increased mobilization of CBG capability in red birds may provide alternative or other short-term survival benefits (e.g., increased foraging, dispersal), which may provide additional advantages to aggressive red males and allow them to continue to persist in wild populations. Furthermore, it is likely that morph-specific susceptibility to other environmental stressors, such as predators, diseases, or parasite infections, will also interact and affect the long-term persistence and fitness of these genetic morphs.

Together, these results suggest that different life-history strategies adopted by red and black birds are maintained by genetic differences in physiological sensitivities (plasticity) to nutritional stress. Nutritionally stressed red birds experienced high stress levels and translated glucocorticoid elevation toward self-maintenance (i.e., elevated CBG to potentially help protect themselves from detrimental corticosterone effects) but still experienced high health and reproduction costs. In contrast, nutritionally stressed black birds appear to be able to tolerate higher free corticosterone levels, and they heavily invested in parental provisioning to minimize reproductive costs. Morph differences in hormone regulation—and specifically, plasma glucocorticoid levels—may also translate to a range of other behavioral alterations (e.g., changes in locomotory activity, dispersal, and foraging) and fitness effects (e.g., survival). Although more work is needed to gain a full understanding of the relative fitness effects of morph-specific variation in physiological and behavioral plasticity (and the interaction with other life-history traits), these findings have important implications for the maintenance of color polymorphism in this species, since indirect selection is likely to act differentially on physiological attributes (e.g., immune response, resistance to stressors) that vary among color morphs and affect their long-term persistence.

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Redheaded male Gouldian finch (*Erythrura gouldiae*) feeding his chicks. Photograph by Sarah Pryke.