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Original Article

Melanin-based coloration reflects alternative strategies to cope with food limitation in pigeons

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Variation in genetic-based coloration is widespread among vertebrates, yet the underlying mechanisms explaining this polymorphism maintenance remain poorly known. Gene-by-environment interactions on fitness traits in a fluctuating environment are often invoked to explain the maintenance of such color diversity. According to this hypothesis, variation in coloration could signal alternative life-history strategies to cope with variable environmental conditions. However, empirical studies testing this hypothesis are still rare. Here, we aimed at comparing the variation of body mass maintenance behavior and reproductive traits between differently colored individuals in alternative environments. We exposed differently colored captive feral pigeons to different food conditions and immune challenges and measured their investment in body mass maintenance, egg laying, and offspring quality. Under food restriction, darker eumelanic females had a higher egg production, but darker adults tended to lose more body mass than paler conspecifics. Moreover, offspring reared in food-limited conditions had a higher body mass at fledging when sired by darker biological fathers, suggesting a positive genetic effect of a darker eumelanic coloration in harsh food conditions. In contrast, when food was abundant, pale- and dark-colored females had a similar egg production, but darker adults lost significantly less body mass than paler conspecifics. The immune challenge had no effect on adult body mass maintenance and reproduction. Differently colored individuals may thus display alternative reaction norms to different food conditions, suggesting that eumelanin-based coloration reflects how animals cope with variations in food availability. Spatiotemporal heterogeneity of food availability in natural systems may thus play a central role in the evolution of melanin-based coloration in wild animal populations. *Key words:* coloration, color polymorphism, feral pigeon, food restriction, life-history strategies, melanin. *[Behav Ecol]*

INTRODUCTION

Variation in genetic-based coloration is widespread among vertebrates, yet the underlying mechanisms explaining this polymorphism maintenance in the wild remain poorly known. Theoretical studies have demonstrated that fluctuations of selective pressures over time or space could promote the maintenance of genetic diversity in natural populations (e.g., Gillespie and Turelli 1989). Differently colored individuals may have different performances in alternative environments, and the spatiotemporal heterogeneity of the environment could participate in the maintenance of color diversity through genotype-by-environment interactions (Hedrick 2006). When gene flow is limited, such gene-by-environment interactions can promote local adaptation (Kawecki and Ebert 2004). However, empirical demonstrations in natural populations remain limited to a few cases (for instance in the peppered moth *Biston betularia*, Majerus 1998; reviewed in Hedrick 2006). A key step to investigate the role of environmental heterogeneity on polymorphism maintenance is to investigate the adaptive value of alternative genotypes and their reaction norms in different conditions (genotype-by-environment interactions) (Gillespie and Turelli 1989).

Melanin-based coloration is a good candidate to investigate such question. First, this type of coloration is mainly genetically determined (reviewed in Roulin 2004). Moreover, numerous studies support the existence of covariations between such coloration and fitness-related traits, such as parasite resistance (Roulin et al. 2001; Jacquin et al. 2011), reproduction (Krüger and Lindström 2001), or energy homeostatis (Dreiss et al. 2010) (reviewed in Ducrest et al. 2008). However, the sign and magnitude of these covariations vary according to environmental conditions (e.g., Piault et al. 2009; Sirkiä et al. 2010). This suggests that melanin-based coloration could reflect alternative genetic life-history strategies to cope with different social or environmental conditions (Roulin 2004; Roulin et al. 2008). Until now, most studies focused on the evolution of coloration and strategies in the context of alternative social conditions (e.g., Sinervo et al. 2000; Pryke et al. 2007; Vercken et al. 2007), but still little is known on the evolution of genetic-based coloration and strategies in the context of variable environmental conditions (Brockmann 2001; Roulin 2004; but see Piault et al. 2009; Sirkiä et al. 2010).

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In this study, we aimed at investigating the role of alternative food and parasite conditions on coloration polymorphism maintenance, by testing whether eumelanin-based coloration in feral pigeons was associated with differential body mass maintenance, reproductive effort, and offspring quality in different environmental conditions. Feral pigeons provide an excellent system to investigate such questions, as they display a continuous variation in melanin-based coloration, which is genetically determined (Johnston and Janiga 1995; Jacquin L, Haussy C, Bertin C, Karimi B, Laroucau K, Gasparini J, unpublished data) and which covaries with parasite resistance (Jacquin et al. 2011) and sexual activity (Murton et al. 1973). Moreover, differently colored feral pigeons have been shown to exploit alternative habitats differing in urbanization rate (Obukhova 2007). As urbanization is known to induce heterogeneous conditions regarding resource availability and parasite pressures (Shochat 2004; Bradley and Altizer 2007), it is possible that differently colored individuals have different performances in alternative food and parasite conditions.

To investigate this question, we experimentally manipulated food availability and injected antigens in captive feral pigeons displaying different melanin-based colorations. We then compared their performance with respect to body mass loss, egg production, and offspring quality (growth and survival until fledging). To disentangle adult performance with respect to egg production and parental care, we cross-fostered eggs between nests. If different-colored genotypes are differently adapted to alternative environment, we expect to observe genotype-by-environment interactions, that is, we expect differently colored adults to have different reproductive performances and/or body mass loss under alternative food or immune conditions.

MATERIALS AND METHODS

Coloration measurement

Color variation (black, red, or brown) in feral pigeons is due to the deposition of 2 different types of melanin pigments: yellow to red phaeomelanins and black eumelanins (Haase et al. 1992). In this study, we focused on eumelanic black coloration because it is the most widespread cause of variation in coloration in feral pigeon populations (Johnston and Janiga 1995). Feral pigeons display continuous variation in this eumelanin-based coloration from white to black (Johnston and Janiga 1995), and its expression is under strong genetic control (Johnston and Janiga 1995), with estimated heritability from parent–offspring regressions 0.82 ± 0.12 (mean ± standard error; Jacquin L, Haussy C, Bertin C, Karimi B, Laroucau K, Gasparini J, unpublished data). Patterns of eumelanin-based coloration differ with respect to the surface area of dark pigmentation on the wings, corresponding to different levels of melanin deposition in feathers (Haase et al. 1992). In this study, we calculated a continuous coloration score as the percentage of dark surface on the wing compared with gray surface (see Materials and Methods in Jacquin et al. 2011). All birds were photographed with a digital camera (Sony Cyber-shot DSC-HX1, Weybridge, United Kingdom) under a standardized white light in a photo light tent. The coloration score (percentage of dark surface) was calculated as the number of black/white pixels × 100 after binary transformation of the picture on the wings of birds using ImageJ software (Rasband 2011). The percentage of dark surface was highly repeatable within individuals ($N = 30$, 4 photographs per individual, $F_{29,90} = 40.28$, $P < 0.001$, $r_I = 0.91$) and was correlated to a global visual scoring of coloration (Spearman correlation test: $N = 111$, $\rho = 0.94$, $P < 0.001$; for method validation, see Jacquin et al. 2011).

Experimental design

One hundred and twenty adult feral pigeons showing variation in melanin-based coloration (60 females and 60 males) were captured at 3 locations of the Parisian suburbs and kept in 10 outdoor aviaries (2.20 m × 2.20 m) in 2010 at the CEREEP field station (Centre d'Ecologie Expérimentale et Prédictive [CEREEP]-Ecotron Ile-de-France, UMS 3194, Ecole Normale Supérieure, Saint-Pierre-lès-Nemours, France). Each aviary contained 6 males and 6 females. The day before the beginning of the experiment, birds were weighed to the nearest grams and their tarsus was measured to the nearest millimeters. Body condition was calculated as the residual of the body mass on tarsus length regression (both log transformed) (Jakob et al. 1996). Differently colored adults did not differ according to site of capture (linear model: $t_{117} = 0.15$, $P = 0.87$), initial body mass ($t_{117} = -0.55$, $P = 0.57$), or initial body condition ($t_{117} = -1.56$, $P = 0.12$). Birds housed in different aviaries did not differ significantly according to coloration (linear model: $t_{116} = -0.31$, $P = 0.75$), site of capture ($t_{116} = 1.29$, $P = 0.19$), initial body mass ($t_{116} = -1.31$, $P = 0.19$), or initial body condition ($t_{116} = -0.13$, $P = 0.89$).

Birds were submitted to 2 different treatments: a "food" treatment and an "immune" treatment in a cross design. The food treatment was initiated 2 weeks before the immune treatment and lasted 6 months. In the "food-limited" treatment, 60 birds from 5 aviaries were provided with 30 g of wheat per day per animal, corresponding to a basal diet necessary to maintain nonbreeding captive pigeons (Hawkins 2001). During reproduction, 15 g of wheat per day were added per chick older than 1 week and 30 g per chick older than 2 weeks. In the "ad libitum" treatment, 60 other pigeons (5 aviaries) were fed ad libitum with a mix of corn, wheat, and peas. Pigeons from both groups were supplemented with the same minerals and vitamins.

For the immune treatment, we first identified breeding pairs by behavioral observations (repeated observations of courtship feeding and mating). Two weeks after the beginning of the food treatment, 3 pairs were randomly chosen in each aviary to receive an antigen injection ("antigen-injected" treatment), whereas the 3 remaining pairs received an injection of saline ("sham-injected" treatment). In the antigen-injected treatment, 60 birds were injected subcutaneously with 50 µg of Keyhole Limpet Hemocyanin (KLH) and 100 µl of tetanus antigens (tetanus vaccine, Sanofi Pasteur). KLH is an artificial protein used to stimulate immune responses to a novel antigen in birds (Hasselquist et al. 1999). Tetanus vaccine contains tetanus toxoids and additives causing a production of specific antibodies (Gasparini et al. 2009). In the sham-injected treatment, the 60 remaining birds were injected with saline (phosphate buffered saline). A second injection was performed 2 weeks later to ensure that antigen- and sham-injected treatment groups differed in term of immune solicitation. Nests were then opened and reproduction started. All protocols were approved by the French Veterinary Department of Seine-et-Marne (authorization no. 77-05).

Food and immune experiments were crossed: 31 adults were food limited and antigen injected, 29 adults were food limited and sham injected, 30 were fed ad libitum and antigen injected, and 30 were fed ad libitum and sham injected. Experimental groups did not differ according to coloration score (linear model: $F_{3,116} = 0.59$, $P = 0.21$), initial body mass ($F_{3,116} = 0.46$, $P = 0.38$), or initial body condition ($F_{3,116} = 0.23$, $P = 0.87$). Five adults died during the experiment, causing variation in the degrees of freedom. Experimental groups did not differ according to adult survival (effect of the immune treatment: $t_{118} = 0.99$, $P = 0.32$, effect of the food treatment: $t_{118} = -0.32$, $P = 0.75$). One month after the second injection, reproduction started.

Body mass maintenance and egg laying measurements

To compare the body mass maintenance between differently colored individuals, we recorded body mass to the nearest 5 g with a spring balance (Medio-Line 40600; Pesola, Baar, Switzerland) every 2 weeks and calculated the total body mass change over the experiment as body mass at the end of the experiment minus body mass at the beginning of the experiment. To compare their egg production, we monitored the number of eggs laid every day and weighed each egg to the nearest milligrams on the day of egg laying. Because eggs are very costly for females to produce, the total number of eggs produced and the mass of eggs laid are a good proxy for the energy invested by females in egg laying (Nager et al. 2001). Hatching success of eggs was also recorded and chick growth was recorded.

Chick growth and survival measurements

To disentangle the effects of the biological parents' coloration and foster parent's coloration and treatments on chick growth and survival, we swapped eggs between nests. On the day of a clutch completion, the 2 eggs of each clutch were randomly assigned to another nest with a similar laying date. Body mass of chicks was monitored each day from the age of 1 day to the age of 21 days, then each week to the age of 56 days, allowing us to construct accurate growth curves for each individual. Young feral pigeons can fledge between 30 and 40 days of age (Johnston and Janiga 1995), but they can stay around the nest after fledging and are still fed by parents until the age of 50–60 days, making the determination of fledging and independence date difficult (Johnston and Janiga 1995). Body mass growth of nestlings was described using the logistic growth curves in the form: $W = \frac{A}{1+\exp(-K(t-t_i))}$, where W = body mass, t = age, A = asymptote (final body mass at the end of growth), K = growth rate constant (slope of the linear regression between mass and time in the beginning of the growth), and t_i = the inflexion point of the curve (Newbrey and Reed 2009). As t_i and K are usually highly correlated (Newbrey and Reed 2009), we only studied K (body mass growth rate) and A (final body mass) parameters. Growth rate K often reflects the conditions in which the chick has grown (Starck and Ricklefs 1998), whereas final body mass A at the end of growth is often linked to survival prospect after fledging and to fitness (e.g., Krementz et al. 1989; Monros et al. 2002). Estimation of the final body mass from a growth curve is more accurate than a direct estimation of body mass at the end of growth because body mass of pigeons is likely to fluctuate throughout time when fledglings reach independence (Johnston and Janiga 1995). Chick survival until the end of growth (56 days) was also recorded. It was not possible to obtain the sex, coloration, or growth parameters of dead chicks due to early death (12 ± 2 days of age on average, $N = 25$ dead chicks of 142 hatched chicks).

Behavioral observations

Behavioral observations started 12 weeks after the beginning of the food treatment and 8 weeks after the second antigen injection. Groups of 4 individuals were randomly chosen in each aviary and their behavior was recorded during 20 min every week during 6 weeks by a single observer (P.B.) by scanning observations (Altmann 1974; Vézina et al. 2010). Groups were observed in a random order to avoid bias due to circadian or meteorological factors. We recorded the total number of chick-feeding behaviors displayed by adults during all the sessions. We also recorded the total number of male aggressive behaviors (territorial behaviors such as intimidation or pursuit as described by Johnston and Janiga 1995) and courtship behaviors (ritualized bowing and vocalization, wing quivering, courtship feeding, heteropreening, nestbuilding, and copulation as described by Johnston and Janiga 1995) displayed by males during the sessions.

Statistical analyses

To compare the investment of differently colored females in egg production, we used linear models with female coloration, food treatment, immune treatment, male coloration, and all second-order interactions as explanatory variables. For body mass changes in adults, we also included the number of chicks reared, the number of eggs laid as response variables in the linear model. Sex was included as a covariate. To compare the number of chick-feeding behaviors (Poisson distribution) displayed by differently colored individuals under different conditions, we used generalized linear models (GLMs) with sex, coloration, treatments, and second-order interactions as response variables, with the number of chicks reared as a covariate. To compare the total number of male aggressive and courtship behaviors (log transformed), we used linear models with coloration, treatments, and second-order interactions as response variables. Before each model selection, we compared a full linear mixed model with a random effect of aviary and a full model without this random effect. In all models, including aviary as random effect in models resulted in a higher AICc. Models without aviary random effect were therefore preferred (Zuur et al. 2009).

To analyze the effects of parent coloration on chick growth (growth rate and final body mass extracted from growth curves) and survival (binomial distribution), we used linear models with the coloration of the biological father and mother, the coloration of the foster father and mother, the treatments of foster parents (which determine the environment of growing chicks), and the second-order interactions as explanatory variables. Chick sex and chick coloration were included in growth analyses but not in survival analyses because chicks died before coloration and sex measurements. In all models, including the identity of biological or foster parents or nest identity, as random effects resulted in models with higher AICc than linear models without random effects. Models without random effects were therefore preferred (Zuur et al. 2009).

All best models were selected following the AICc criterion (MuMIn package of the R software). When several models had similar AIC (ΔAICc < 2), the model containing the lower number of explanatory variables was chosen (Zuur et al. 2009). When an interaction between coloration and treatment had an effect on a response variable, we performed post hoc analyses testing the effect of coloration on the response variable in each treatment separately. Homoscedasticity and normal distribution of residuals were checked for each linear model. All tests were conducted using the R software (R Development Core Team 2010; version 2.13). Significance levels were set to 0.05, and tests were two-tailed.

RESULTS

Adult body mass changes

Female and male coloration scores of each breeding pair were not significantly correlated (Spearman correlation test: $N = 48$ pairs, $\rho = -0.011$, $P = 0.93$). Egg laying started 1 month after the second injection of antigens. Immune challenge did not affect body mass change before egg laying ($t_{117} = 0.44$, $P = 0.66$) or at the end of the experiment ($t_{108} = -0.59$,

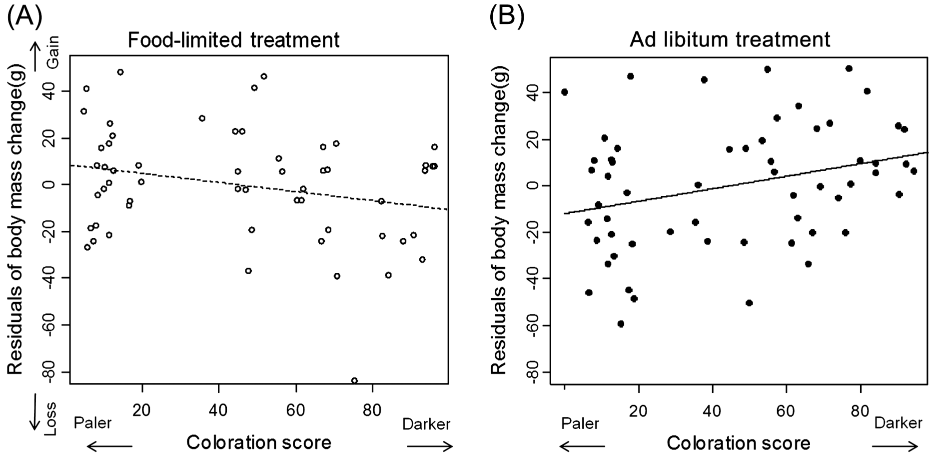

Figure 1
Residuals of total body mass change over the experiment as a function of coloration score after correction for the number of chicks reared and sex. Lines represent the predicted values of the linear models. (A) In the limited food treatment, darker eumelanic adults tended to lose more body mass than paler ones when food restricted (linear model: estimate = −0.23 ± 0.12, t_{53} = 1.95, P = 0.055). (B) In the ad libitum treatment, darker eumelanic adults lost significantly less body mass than paler ones (linear model: estimate = 0.26 ± 0.12, t_{53} = 2.18, P = 0.033).

P = 0.55). Food limitation decreased body mass at the beginning of egg laying (estimate = −8.7 ± 3 g, t_{117} = 2.83, P = 0.005). At the end of the experiment, males had lost significantly less body mass than females (Table 1). The number of chicks reared differentially affected body mass change depending on the food treatment, as shown by the significant interaction between the number of chicks reared and food treatment (Table 1). Chick rearing decreased parent body mass when they were food limited (effect of the number of chicks reared: estimate = −0.29 ± 0.12, t_{53} = 2.44, P = 0.02) but not when they were fed ad libitum (estimate = 0.039 ± 0.12, t_{54} = 0.32, P = 0.75). However, coloration did not affect this relationship (linear model: effect of coloration × number of chicks on body mass changes: t_{108} = −1.42, P = 0.16). Independent of this effect of reproduction on body mass, food availability differentially affected body mass change in dark and pale adults as shown by the significant interaction between food treatment and coloration on total body mass change (Table 1). The interaction remained significant after removing the outlier (Figure 1A) (estimate = 0.51 ± 0.18, t_{107} = 2.78, P = 0.006). Indeed, darker adults tended to lose more body mass than paler adults when food limited (post hoc test after correction for reproduction and sex: effect of coloration: estimate = −0.23 ± 0.12, t_{53} = 1.95, P = 0.055; without the outlier: t_{52} = 1.95, P = 0.13) (Figure 1A). Darker adults lost significantly less body mass than paler ones when food was provided ad libitum (post hoc test after correction for reproduction and sex: effect of coloration: estimate = 0.26 ± 0.12, t_{53} = 2.18, P = 0.033) (Figure 1B). Mixed models with repeated body mass measures as response variables and individual identity as a random effect gave similar results. There was no significant effect of the mass of eggs laid (t_{108} = −0.065, P = 0.95), the mean number of days between successive clutches (t_{77} = −1.01, P = 0.31), or other interactions ($P > 0.20$) on body mass changes.

Number of eggs laid

Food availability differentially affected the total number of eggs laid by dark and pale females as shown by the significant interaction between female coloration score and food treatment (Table 2). Darker females had a higher egg production than paler ones when food was limited (post hoc test, GLM with a Poisson distribution: estimate = 0.034 ± 0.01, t_{28} = 2.51, P = 0.018) (Figure 2A). This relationship was marginally significant when removing females having laid no egg (N = 6 of

Table 1

Best linear model explaining the total body mass change over the experiment (body mass at the end of the experiment minus body mass at the beginning of the experiment)

	Total body mass change			
	Estimate ± SE	df	t	P
Sex	0.68 ± 0.17	1,108	4.08	<0.001
Food treatment	−15.1 ± 8.3	1,108	1.83	0.07
Number of chicks reared	−0.31 ± 0.13	1,108	2.31	0.024
Coloration	−0.21 ± 0.12	1,108	1.85	0.077
Food treatment × chicks reared	0.34 ± 0.17	1,108	2.00	0.05
Coloration × food treatment	0.52 ± 0.17	1,108	3.06	0.004

Food availability differentially affected body mass changes in dark and pale adults as shown by the significant interaction between food treatment and coloration. SE, standard error; df, degrees of freedom.

Table 2

Best GLM explaining the total number of eggs laid by females over the experiment (Poisson distribution)

	Egg production			
	Estimate ± SE	df	t	P
Food treatment	0.22 ± 0.13	1,42	1.65	0.098
Female coloration	0.14 ± 0.092	1,42	1.57	0.12
Male mate coloration	0.09 ± 0.072	1,42	1.25	0.21
Female coloration × male coloration	−0.15 ± 0.06	1,42	−2.34	0.019
Female coloration × food treatment	−0.36 ± 0.13	1,42	−2.75	0.006

Food availability differentially affected the reproduction of pale and dark females as shown by the significant interaction between food treatment and female coloration. Immune treatment, female body mass or other interactions were not kept in the final model. SE, standard error; df, degrees of freedom.

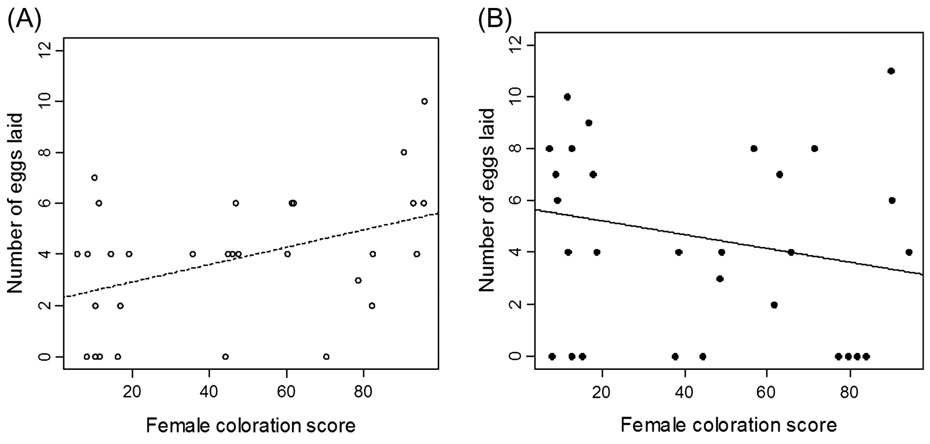

Figure 2
Egg production of females (number of eggs laid over the experiment) as a function of female coloration score. Lines represent the predicted values of the GLMs. (A) In the limited food treatment, darker eumelanic females produced more eggs than paler eumelanic females under food restriction (GLM: 0.034 ± 0.01, $t_{28} = 2.51$, $P = 0.018$). (B) Egg production of females at the end of the experiment as a function of female coloration score in the ad libitum food treatment, dark and pale females produced a similar number of eggs when fed ad libitum (GLM: estimate = −0.026 ± 0.023, $t_{28} = 1.12$, $P = 0.27$).

30 females, food-limited females did not lay eggs) (estimate = 0.020 ± 0.011, $t_{22} = 1.73$, $P = 0.09$). There was no such relationship in the ad libitum treatment (estimate = −0.026 ± 0.023, $t_{28} = 1.12$, $P = 0.27$) (Figure 2B). Moreover, the coloration of the male mate differentially affected dark and pale females as shown by the significant interaction between female coloration and male coloration (Table 2). Indeed, when paired with a dark male (male coloration score > median [50.8%]), darker females had a lower egg production than paler females (estimate = −0.006 ± 0.003, $t_{22} = -2.14$, $P = 0.032$). When paired with a pale male (coloration score < 50.8%), all females had a similar egg production ($t_{22} = 1.25$, $P = 0.21$). There was no significant effect of the immune treatment ($t_{41} = -0.79$, $P = 0.43$), female body mass ($t_{41} = -0.25$, $P = 0.79$), or other interactions (all P values >0.40). Similar results were obtained when analyzing the number of eggs laid (linear model, interaction between female coloration and food treatment: $t_{42} = -2.75$, $P = 0.006$; interaction between female and male coloration: $t_{42} = -2.34$, $P = 0.019$). The higher number of clutches laid by darker females under food restriction was likely due to a shorter delay between successive clutches (mixed model with a random effect of female identity, effect of female coloration score when food limited on the delay between clutches: estimate = −0.192 ± 0.092, $t_{12} = 2.09$, $P = 0.06$; when fed ad libitum: $t_{29} = 0.07$, $P = 0.93$).

Egg mass and hatchability

The food treatment differently affected the mass of each egg laid by dark and pale females as shown by the significant interaction between female coloration and food treatment (mixed model on the mass of each egg with a random effect of female identity; interaction between female coloration and food treatment: estimate = −0.025 ± 0.012, $t_{211} = 2.07$, $P = 0.039$). Similar results were obtained with the average mass of each clutch (mixed model: effect of female coloration × food treatment: estimate = −0.024 ± 0.012, $t_{85} = -2.01$, $P = 0.047$). However, post hoc tests were not significant (effect of female coloration on egg mass with limited food: $t_{91} = 1.23$, $P = 0.22$; with ad libitum food: $t_{120} = -1.45$, $P = 0.15$). The interaction between female and male coloration had no significant effect on egg mass ($t_{210} = -1.50$, $P = 0.13$). There was no effect of the immune treatment ($t_{43} = 0.16$, $P = 0.86$), male coloration ($t_{43} = -0.66$, $P = 0.51$), or other interactions (all $P > 0.40$) on egg mass. 14% of the eggs failed to hatch (23 eggs of 163). The hatching probability of eggs was not significantly related to female coloration (generalized linear mixed model: $z_{116} = -1.28$, $P = 0.20$), food treatment ($z_{41} = 0.99$, $P = 0.32$), the interaction between female coloration and food treatment ($z_{115} = 0.34$, $P = 0.73$), immune treatment ($z_{41} = -0.54$, $P = 0.58$), or male coloration ($z_{41} = 0.53$, $P = 0.59$).

Chick growth rate

The body mass growth rate of chicks (linear growth rate extracted from growth curves) was significantly affected by the interaction between the food and the immune treatments of the foster parents (linear model: effect of food × immune treatment of foster parents on growth rate: estimate = 0.055 ± 0.019, $t_{110} = 2.81$, $P = 0.0059$). Food restriction of foster parents decreased chick growth rate but only for sham-injected

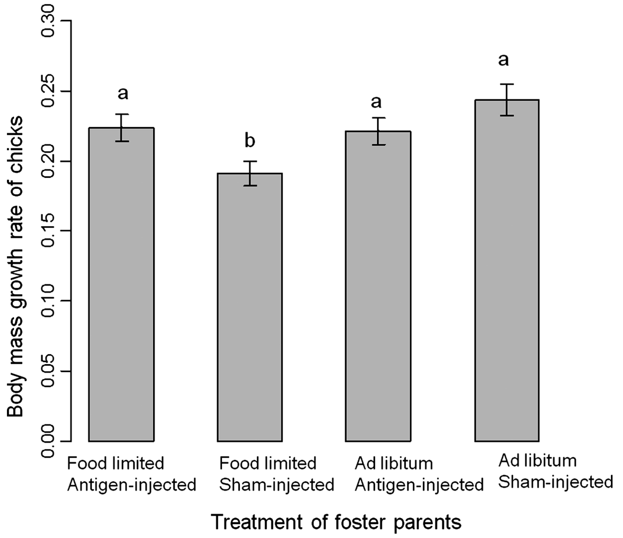

Figure 3
Mean ± standard error body mass growth rate of chicks as a function of foster parent treatments. Letters above bars represent significant differences following post hoc tests.

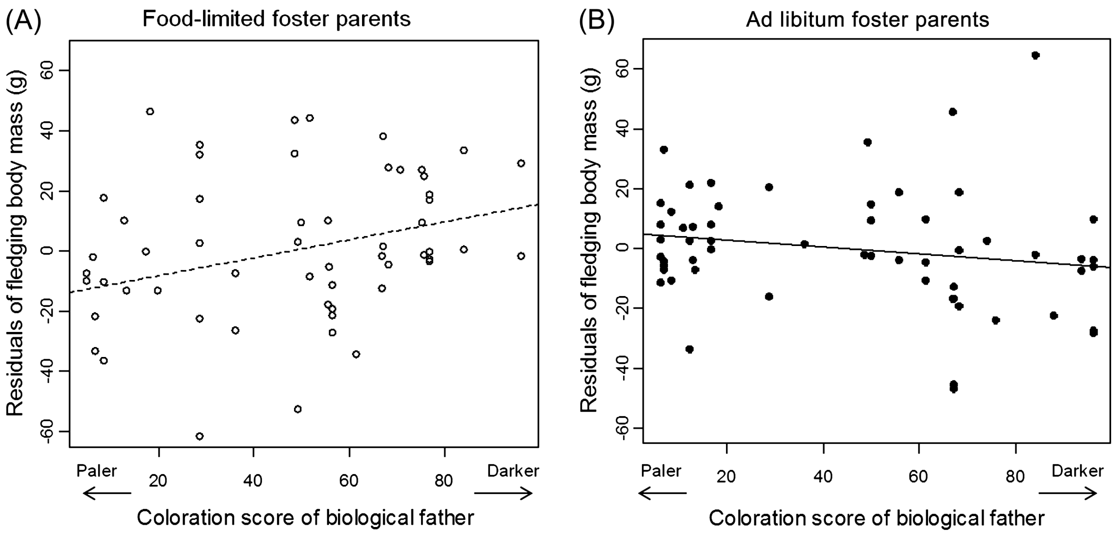

Figure 4
Residuals of final body mass of chicks (asymptotic body mass at the end of growth) as a function of the coloration score of the biological father. Residuals were obtained after correction for sex and coloration of foster parents. Lines represent the predicted values of the linear models. (A) Chicks reared by food-limited foster parents had a higher final body mass when sired by a darker biological fathers compared with a paler biological father (linear model: estimate = 0.29 ± 0.12, t_{55} = 2.41, P = 0.019). (B) The final body mass of chicks reared by ad libitum foster parents was not influenced by the coloration of their biological father (linear model: estimate = −0.11 ± 0.08, t_{55} = −1.40, P = 0.17).

foster parents (estimate = −0.053 ± 0.013, t_{56} = 3.81, $P <$ 0.001) and not for antigen-injected foster parents (t_{54} = −0.15, P = 0.88). The immune challenge of foster parents significantly increased chick growth rate but only for food-limited foster parents (estimate = 0.033 ± 0.013, t_{55} = −2.49, P = 0.016) and not for ad libitum foster parents (t_{55} = 1.52, P = 0.13) (Figure 3). There was no effect of chick sex (t_{109} = −1.08, P = 0.28), chick coloration (t_{109} = 0.29, P = 0.77), or parent coloration (coloration of the biological father: t_{109} = −0.23, P = 0.81; biological mother: t_{109} = 0.65, P = 0.52; foster father: t_{109} = 1.27, P = 0.20; foster mother: t_{109} = −0.73, P = 0.47) on chick growth rate.

Chick final body mass

The final body mass of chicks (asymptotic body mass at the end of growth extracted from growth curves) and chick growth rate were not significantly related (Pearson's correlation test: t_{112} = 1.14, P = 0.25). Male chicks had a higher final body mass than female chicks (estimate = 15.8 ± 4.1, t_{106} = 3.8, $P <$ 0.001) (Table 3). Food restriction differently affected chicks sired by dark and pale biological fathers as shown by the significant interaction between the coloration of the biological father and the food treatment of the foster parents on chick final body mass (Table 3). Chicks reared by food-limited foster parents had a higher final body mass when sired by a darker biological father than when sired by a paler biological father (post hoc linear model after correction for sex and coloration of foster parents: effect of biological father coloration under food limitation: estimate = 0.31 ± 0.12, t_{55} = 2.65, P = 0.010) (Figure 4A). The coloration of the biological father had no effect on the mass of chicks reared by parents fed ad libitum (t_{55} = −1.09, P = 0.27) (Figure 4B). Moreover, the coloration of both the foster mother and the foster father affected the final body mass of chicks (Table 3). Post hoc tests reveal that chicks reared by a pale mother (coloration score < 50) had a higher final body mass when reared by a darker father than when reared by a paler father (effect of foster father coloration after correction for other effects: estimate = 0.34 ± 0.11, t_{60} = 3.21, P = 0.0023). The final body mass of chicks reared by a dark mother (coloration score > 50) was not influenced by the coloration of the foster father (t_{60} = −1.26, P = 0.21). There were no effect of the immune treatment (t_{105} = −1.22, P = 0.22), biological mother coloration (t_{105} = 0.012, P = 0.99), or chick coloration (t_{105} = −0.03, P = 0.97) on chick final body mass.

Table 3
Best linear model explaining the final body mass of chicks (asymptotic body mass at the end of growth)

	Final body mass of chicks			
	Estimate ± SE	df	*t*	*P*
Sex	15.8 ± 4.1	1,106	3.84	<0.001
Food treatment of foster parents	42.4 ± 7.8	1,106	5.51	<0.001
Coloration of foster father	0.56 ± 0.13	1,106	4.14	<0.001
Coloration of foster mother	0.43 ± 0.10	1,106	4.00	<0.001
Coloration of biological father	0.37 ± 0.11	1,106	3.41	<0.001
Coloration of biological father × food treatment of foster parents	−0.46 ± 0.14	1,106	3.18	0.0012
Coloration of foster father × coloration of foster mother	−0.01 ± 0.002	1,106	−4.41	<0.001

Food availability differentially affected the final mass of chicks sired by dark and pale fathers as shown by the significant interaction between food treatment and biological father coloration. SE, standard error; df, degrees of freedom.

Chick survival

82% of the chicks survived until the age of 56 days. The best model explaining chick survival included only the coloration score of the biological father. Chicks sired by a darker biological father had a lower survival than chicks sired by a paler biological father (effect of biological father coloration: estimate = -0.021 ± 0.008, $z_{139} = -2.73$, $P = 0.006$). There was no significant effect of the coloration score of the foster father ($z_{139} = -1.05$, $P = 0.29$), foster mother ($z_{139} = -0.96$, $P = 0.34$), biological mother ($z_{139} = -0.53$, $P = 0.59$), of the food treatment of foster parents ($z_{139} = 0.015$, $P = 0.98$), or their immune treatment ($z_{139} = 1.36$, $P = 0.18$) on chick survival.

Adult behavior

Food-limited parents displayed a lower number of chick-feeding behaviors than parents fed ad libitum (GLM: effect of food treatment after correction for the number of chicks reared: estimate = -0.73 ± 0.33, $t_{116} = 2.19$, $P = 0.028$), but differently colored adults displayed a similar number of feeding behaviors during the observations (GLM: effect of coloration: $t_{115} = -0.94$, $P = 0.35$). There was no effect of sex ($t_{115} = 1.11$, $P = 0.27$) or immune treatment ($t_{115} = -1.17$, $P = 0.24$) on the total number of feeding behaviors observed.

The total number of male courtship behaviors was not significantly linked to male coloration (linear model: $t_{58} = -1.49$, $P = 0.14$), food treatment ($t_{58} = 0.37$, $P = 0.71$), or immune treatment ($t_{58} = -0.68$, $P = 0.5$). Antigen-injected males displayed a higher number of aggressive behaviors than sham-injected males (linear model: effect of antigen injection: estimate = 0.40 ± 0.19, $t_{58} = -2.09$, $P = 0.040$), but there was no significant effect of coloration ($t_{58} = -0.83$, $P = 0.41$) or food treatment ($t_{58} =-0.28$, $P = 0.78$) on the total number of male aggressive behaviors.

DISCUSSION

Melanin-based coloration and body mass maintenance

The aim of this study was to compare the variation in reproduction and body mass maintenance between differently colored adults in alternative environments. First, our results show that chick rearing had a cost in terms of body mass loss in our experiment, but only when food was limited. In addition to this, food conditions differently affected body mass changes in different-colored phenotypes, after correction for the effect of reproduction on body mass. Darker eumelanic adults tended to lose more body mass than paler conspecifics in food-limited conditions, whereas they lost significantly less body mass than paler conspecifics in ad libitum conditions. This result shows that differently colored genotypes display alternative body mass change reactions norms depending on food conditions, suggesting a "genotype-by-environment" interaction on body mass maintenance.

Melanin-based coloration and egg laying

In addition to this, food conditions differentially affected the reproduction of pale and dark females. Darker eumelenic females had a higher egg production than paler ones when food was limited, showing that eumelanin-based coloration is positively associated with prenatal reproductive effort of females in feral pigeon under harsh environmental conditions. Although this result might be confounded by different age or experience of differently colored females captured in natural populations, it is consistent with previous studies showing that darker melanin-based coloration is associated with higher levels of sexual activity in females (Roulin 2009; Vergara et al. 2009). As hatching success did not vary between differently colored females, difference in egg production between pale and dark females in the food-limited treatment was not due to a higher clutch failure rate but rather to a shortening of reproductive cycles, which is very plastic in this species (Jacquin et al. 2010). In contrast, under conditions of abundant food, all females laid a similar number of eggs, maybe because they have sufficient energy to perform equally well.

Melanin-based coloration and offspring quality

Chick growth rate was only affected by parental treatments and not by the coloration of biological or foster parents. This suggests that experimental treatments affected the ability of foster parents to provide food to their offspring, whatever their coloration. Indeed, food limitation of sham-injected foster parents decreased chick growth rate, potentially due to a decrease in energy available for foster parents and a decrease in parental investment (Martin 1987). According to this, food-limited parents displayed a lower number of feeding behaviors than adults fed ad libitum. In contrast, antigen injection had a positive effect on chick growth for food-limited foster parents. This could be due to a terminal investment of parents facing both food shortage and immune solicitation. Immune activation may indeed be perceived as a cue of diminishing residual reproductive value, leading to an enhanced investment toward current reproduction (e.g., Bonneaud et al. 2004). According to this, antigen-injected males displayed more courtship behaviors compared with sham-injected males in this study. However, courtship behavior, feeding behaviors, and chick growth rate were not linked to parent coloration, suggesting that differently colored parents had the same behavioral response to food limitation and immune activation regarding parental investment.

In contrast, the coloration of biological fathers affected the final body mass of chicks at the end of their growth. Indeed, chicks sired by a darker biological father had a higher body mass at fledging under food restriction than chicks sired by a paler father. As fledging body mass is often linked to survival prospect in wild birds (Krementz et al. 1989; Monros et al. 2002) and to reproductive success in feral pigeons (Johnston and Janiga 1995), this suggests that being a darker male entails genetic advantages in terms of offspring quality when food is scarce. Interestingly, this result is consistent with a previous study in the alpine swift *Apus melba* showing that nestling swifts sired by blacker fathers grew longer wings than nestlings sired by paler fathers but significantly so only when reared under stressful conditions (enlarged brood) (Bize et al. 2006). As proposed by Roulin et al. (2008), a darker eumelanic coloration could thus reflect a better ability of chicks to cope with food limitation compared with a paler coloration, which could be due to genetic effects. However, this result should be taken with caution. Indeed, chicks coming from darker biological fathers had also a lower chance to survive until the end of growth, which could confound the results on body mass (if chicks with a lower body mass had a lower chance to survive). Further studies are thus necessary to test the effects of coloration on offspring quality. Long-term data will be useful to assess the effect of parent coloration on offspring fitness.

Proximate mechanisms of the covariation between melanin-based coloration and traits

Taken together, these results show that differently colored feral pigeons display different responses regarding body mass maintenance, egg laying, and offspring quality when facing alternative food conditions. The links between melanin-based coloration and these traits could be mediated by several different mechanisms, like differential aggressive behavior and competitive abilities to access food (Mennill et al. 2003) or differential sexual activity of differently colored adults (reviewed in Ducrest et al. 2008). However, we found no evidence of behavioral differences between differently colored adults in this study. Differences between colorations could also be explained by differences in age, experience, or previous habitat before capture or pleiotropic effects of hormones (Ricklefs and Wikelski 2002) or melanocortin genes (Ducrest et al. 2008). As the melanocortin system has been shown to affect energy homeostasis (Boswell and Takeuchi 2005) and reproduction (Ducrest et al. 2008), differently colored individuals could display different abilities to reproduce and to manage energy intake and expenditure due to pleiotropic effects of genes coding for melanocortin ligands or receptors (e.g., Dreiss et al. 2010; reviewed in Ducrest et al. 2008). The fact that the coloration of the biological father affects chick traits supports this explanation, but further studies are now necessary to test this hypothesis.

Captivity is also a stressful environment, which entails specific conditions. It is thus possible that differences in traits between differently colored individuals stem from different abilities to cope with the stress of captivity under different food conditions. Indeed, there is some evidence that darker colored birds are more resistant to stressful events than paler ones, which could be due to pleiotropic effects of the melanocortin system on both eumelanogenesis and regulation of the physiological stress response (for instance, release of steroid hormones) (e.g., Almasi et al. 2010). Such a mechanism could account for the lower body mass loss of darker adult pigeons under ad libitum food conditions and their better reproduction under food-limited conditions compared with paler conspecifics in this study. Further mechanistic studies investigating the genetics of melanin-based coloration and the link between coloration and neuroendocrine pathways are now needed to disentangle these explanations and will help understanding the underlying mechanisms linking coloration and life-history traits.

Evolution of melanin-based coloration diversity in natural systems

Whatever their precise underpinnings, this study shows that different-colored phenotypes display different reaction norms with respect to body mass maintenance, egg production, and offspring phenotype in different food conditions, suggesting a role of resource heterogeneity on the evolution of genetic-based coloration in natural systems (e.g., Roulin et al. 2008). In contrast, the immune treatment had no effects on egg laying and body mass loss, maybe because the immune challenge occurred long before reproduction or because the cost of mounting an immune response was not high enough to influence egg laying and body maintenance of adults in this study. Consequences of heterogeneous parasite conditions on fitness and the evolution of coloration remain thus to be determined.

Under harsh food conditions, darker females had a higher egg production and darker males produced chicks of higher quality, but darker melanic adults tended to have a higher body mass loss compared with paler conspecifics. This suggests that costs and benefits of differently colored individuals are equally balanced under harsh conditions (e.g., Calsbeek et al. 2010). In contrast, under relaxed food conditions, all morphs laid a similar number of eggs, but darker adults had an advantage with respect to body mass change compared with paler ones, which could provide them with an advantage compared with paler conspecifics. This suggests that both differential performance under different circumstances and balanced costs and benefits on different life-history traits may play a role in the evolution of genetic-based coloration in natural populations (Roulin 2004).

Moreover, the fact that the coloration of both members of the breeding pair had an influence on reproduction also suggests that frequency-dependent selection could play a role in the maintenance of coloration diversity within populations. In this study, dark female pigeons mated with dark males had a lower egg production than other females and pale females mated with pale fathers reared chicks with a lower final body mass than other females. Mating with a dissimilarly colored mate would then bring reproductive advantages in this species and disassortative mating should occur (e.g., Tuttle 2002). Rare morphs should be preferentially chosen to achieve disassortative mating and enjoy reproductive advantages compared with common morphs. In that case, negative frequency-dependent selection should occur and could participate in the maintenance of color polymorphism within populations (e.g., Sinervo and Lively 1996). However, it is still unclear whether melanin-based coloration is used as a signal for sexual selection in feral pigeons (Johnston and Janiga 1995), and coloration of male and female of breeding pairs was not significantly correlated in this study. Mate choice experiments are now necessary to understand how coloration is used as a cue of individual quality in this species.

Further studies should now evaluate how the variations of reproductive traits, body mass maintenance, and mate choice impact the fitness of differently colored individuals in the wild to determine whether environment heterogeneity could result in balanced selection on genetic-based coloration and participate in the maintenance of coloration polymorphism in natural populations.

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