Genetic and environmental effects on the covariation between colour polymorphism and a life-history trait

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ABSTRACT

Variation in coloration with a strong underlying genetic basis is frequently found within animal populations but little is known about its function. Covariation between colour polymorphism and life-history traits can arise because morphs perform differently among environments or because they possess alternative alleles coding for key life-history traits. To test these two hypotheses, we studied a population of tawny owls *Strix aluco*, a bird displaying red, brown and grey morphs. We assessed the colour morph of breeding females, swapped eggs or hatchlings between pairs of nests, and examined how body condition in 3-week-old nestlings covaries with coloration of foster and genetic mothers. Redder foster and genetic mothers produced young in better condition. Because in two other years we observed that greyish females produced offspring in better condition than those of red females, the present study suggests that colour polymorphism signals genetic and phenotypic adaptations to cope with a fluctuating environment.

Keywords: colour polymorphism, disruptive selection, ecological niche, frequency-dependent selection, genetic–environment interaction, *Strix aluco*.

INTRODUCTION

The display of genetically inherited colour morphs within a population is frequently found in animals and plants (Kay, 1978; Ferguson-Lees and Christie, 2001). Evolutionary biologists have long been interested in understanding colour polymorphism. Such insights shed light on maintenance of genetic variation in populations and on the possible adaptive value of alternative phenotypes (e.g. Fisher, 1930; Ford, 1945; Huxley, 1955; Mather, 1955;

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O'Donald, 1983). Some notable failures to find adaptive benefits of colour polymorphism in the past (e.g. Cooke *et al.*, 1995) have led to scepticism that most polymorphisms are adaptive. However, several key studies have shown that colour polymorphism is not neutral with respect to life-history components (for example, see reviews in Jones *et al.*, 1977; Hoffman and Blouin, 2000; Roulin, 2004). For example, in birds, reproductive success, survival prospects, physiology and behaviour are morph-dependent. Furthermore, the sign and magnitude of these relationships can vary both spatially and temporally (Roulin, 2004), suggesting that colour polymorphism is under disruptive selection with each morph being adapted to specific environmental factors that can fluctuate over time and space (Losey *et al.*, 1997; Skúlason and Smith, 1995). In many species, colour morphs can therefore be viewed as adaptations to cope with specific ecological factors.

   Two mutually non-exclusive mechanisms can explain how covariation between colour polymorphism and life-history traits can arise. First, coloration may play a direct role in the display of a particular adaptation, and hence be naturally selected. For example, pale and melanistic morphs may absorb varying amounts of solar radiation (Berry and Willmer, 1986) or be cryptic in different habitats (Greco and Kevan, 1999). Therefore, whatever the offspring coloration, young raised by parents of the selectively favoured morph should receive more food, and thus be in better condition than young raised by parents of the morph that is selected against (phenotypic effect). In this first scenario, such covariation is therefore environmentally mediated. Secondly, coloration may play an indirect role in the expression of a given adaptation if differently coloured individuals vary at key genes that determine performance under specific environmental conditions (genotypic effect). Such a situation may arise when genes responsible for variation in coloration and important life-history traits are located close on the same chromosome, when they are located on different chromosomes that are inherited not independently from each other (i.e. non-random pairing with respect to these two traits), or when color-ation is coded by genes having pleiotropic effects on life-history traits. As a consequence, differently coloured individuals may be adapted to alternative environmental conditions as demonstrated in several organisms (Brodie, 1989; Armbruster, 2002; Forsman *et al.*, 2002; Ahnesjö and Forsman, 2003). In this second scenario, therefore, covariation between colour polymorphism and life-history traits has a genetic basis, and colour polymorphism may evolve or be maintained as a correlated response to selection exerted on the genetically correlated life-history traits. The existence of such a genetic correlation ensures that colour polymorphism signals individual quality, and may explain why conspecifics assess each others' colour morph in social and sexual interactions (Roulin, 2004).

   Using the tawny owl (*Strix aluco*) as a model organism, our aim was to determine whether covariation between colour polymorphism and life-history traits has a phenotypic and/or a genotypic basis. This nocturnal bird of prey is ideal because individuals belonging to the same population display one of several morphs, and because female morphs were observed to produce offspring in different condition in different years (Roulin *et al.*, 2003a). To test experimentally whether the covariation between mother colour polymorphism and offspring body condition is environmentally mediated or genetically determined, we performed a cross-fostering experiment. For this purpose, we assessed mother colour morph, swapped eggs or hatchlings between pairs of nests, and then weighed cross-fostered offspring at fledging. The hypothesis of an environmental determination of the covariation between mother colour polymorphism and offspring condition predicts a correlation

between body condition of cross-fostered offspring and colour morph of the foster mother. The hypothesis of a genetic basis predicts a similar correlation but with colour morph of the genetic mother.

MATERIALS AND METHODS

The study organism

The tawny owl is monogamous, highly philopatric and can live for more than 20 years. It is relatively large, with individuals weighing between 335 and 780 g, females being heavier than males (Baudvin and Dessolin, 1992). This bird preys upon a large number of animals, including mammals, birds, frogs and reptiles. Although it can breed in a variety of sites, it is mainly found in forests where it breeds in tree holes and sometimes in the abandoned nests of raptors and crows. Reproduction takes place between January and May, with brood size ranging between one and seven nestlings. Only the female incubates the clutch, and half way through the nestling period she starts to help her partner collect food for the offspring. Nestlings abandon their nest before being able to fly, at an age of 25–30 days. The back and ventral body sides of owls vary in coloration from red to grey, a variation that can be easily classified into colour morphs. This trait is not sexually dimorphic (Baudvin and Dessolin, 1992; Galeotti and Cesaris, 1996) and pairing with respect to colour morph is not assortative (Roulin *et al.*, 2003a). Observations made in Switzerland showed that colour polymorphism is not neutral with respect to life-history components. Although greyer females had shorter tarsi, they produced heavier offspring in two out of three years, and over 14 years the proportion of all breeding females that were reddish-brown was greater in years when the breeding density was lower. Capture–recapture analyses showed that the latter result is explained by the fact that greyish females bred less often than red ones, although their survival probability was similar (Roulin *et al.*, 2003a). The finding that in Italy grey individuals suffered a higher mortality in warm/dry years, whereas red owls were found dead more often in cold/wet years (Galeotti and Cesaris, 1996), confirms that colour polymorphism is associated with life-history strategies. Furthermore, in the same Italian population red owls hosted more endoparasites than grey owls, and with respect to coloration birds were not distributed among forests at random (Galeotti and Sacchi, 2003).

Experimental procedure

The study was carried out in western Switzerland using 18 nests. We controlled nest-boxes to record clutch size (mean = 3.4; range = 2–5), egg volume using the formula length × breath^2 $\Pi/6$ (mean = 39.25 cm^3; range = 29.21–39.25 cm^3), hatching date (mean = 6 April; range = 21 March to 25 April) and to capture breeding females. One of us classified them in one of five colour morphs (1 = red, 2 = red-brown, 3 = brown, 4 = brown-grey, 5 = grey). In total, we had a sample of two red, six red-brown, four brown, four brown-grey and two grey females. To assess the reliability of our scoring system, we recaptured 16 females one week later. A repeatability analysis (one-way ANOVA, repeatability = 0.87, $F_{17,16}$ = 14.98, $P < 0.0001$) shows that the assessment of colour morph is reliable, as already shown in an earlier study (Roulin *et al.*, 2003a). We weighed each female to the nearest gram, and measured the length of one wing, one tarsus and central tail feather to the nearest millimetre. In this sample, none of these traits were correlated with female colour

morph (Pearson correlations, P-values > 0.33). Female colour morph was also not significantly associated with hatching date ($r = -0.26$, $n = 18$, $P = 0.30$), clutch size ($r = 0.20$, $n = 18$, $P = 0.44$) and mean egg volume ($r = 0.03$, $n = 18$, $P = 0.91$). Males were not in the nest when we captured females, and hence we could not investigate the relationship between nestling body condition and colour morph of genetic and foster fathers. Even though among owls males provide most prey items to their brood, we had already showed that female colour morph is related to major life-history traits (Roulin *et al.*, 2003a), justifying the present study on female colour polymorphism.

We matched the 18 nests in nine pairs with the criteria that nests of a pair had a similar hatching date ($r = 0.95$, $n = 9$, $P = 0.0001$) and clutch size ($r = 0.71$, $n = 9$, $P = 0.033$). We cross-fostered all eggs ($n = 6$ nests) or newly hatched chicks ($n = 12$ nests) between pairs of nests. A blood sample was taken from each nestling to identify gender using blood cell DNA. Foster and genetic mothers did not resemble each other with respect to colour morph ($r = -0.13$, $n = 9$, $P = 0.75$; Fig. 1). Proportion of male offspring (i.e. sex ratio) was not significantly associated with colour morph of foster ($r = -0.04$, $n = 17$, $P = 0.87$) or genetic mothers ($r = 0.05$, $n = 17$, $P = 0.84$). Brood size at hatching was also not significantly associated with colour morph of foster mother ($r = 0.40$, $n = 18$, $P = 0.10$). When nestlings were on average 22 days of age (range = 17–30), one of us weighed them to the nearest gram, and measured wing length to the nearest millimetre and tarsus length to the nearest 0.1 mm. We calculated an index of nestling body condition as the residuals extracted from a four-way ANOVA with nestling body mass as the dependent variable, and with nestling wing length ($F_{1,41} = 0.75$, $P = 0.39$, $\beta = 0.16$), tarsus length ($F_{1,41} = 12.88$, $P < 0.0001$, $\beta = 0.64$), hour of the day when nestlings were weighed ($F_{1,41} = 8.65$, $P = 0.005$, $\beta = -0.19$) and sex ($F_{1,41} = 26.20$, $P < 0.0001$, $\beta = -0.29$) as four independent variables. We introduced wing length into the model because this trait is closely associated with nestling age. Relationships between nestling body mass and both wing and tarsus length were linear (data not shown). To avoid pseudo-replication, for each nest we computed a mean residual value, denoted 'nestling body condition', that we used in the statistical analyses.
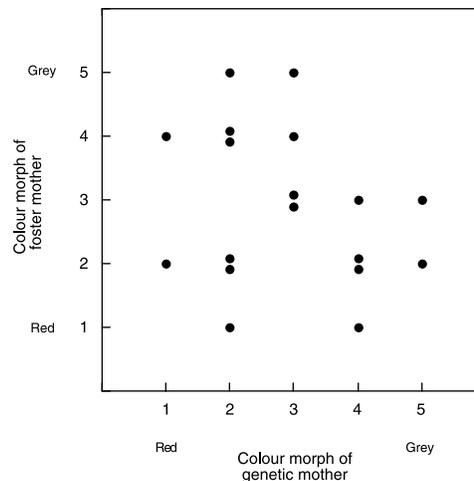

Fig. 1. Relationship between colour morph of genetic and foster mothers. For example, eggs of a grey female (colour score 5) were exchanged with those of a brown female (colour score 3).

Statistical analysis

For unknown reasons, one nest was abandoned a couple of days after eggs were cross-fostered. The sample size is therefore 17 experimental nests. Statistical tests are two-tailed and P-values less than 0.05 are considered significant.

RESULTS

In a multiple linear regression analysis, nestling body condition was negatively associated with colour morph of both foster ($F_{1,14} = 7.69$, $P = 0.015$, $\beta = -0.53$) and genetic mothers ($F_{1,14} = 9.64$, $P = 0.008$, $\beta = -0.60$). Redder foster and genetic mothers produced heavier offspring than greyish mothers (Fig. 2). Inclusion of number of hatchlings in the foster nest ($F_{1,12} = 1.63$, $P = 0.23$) and mean volume of eggs out of which nestlings hatched ($F_{1,12} = 1.81$, $P = 0.20$) did not modify the results, with the effect of colour morph of foster ($F_{1,12} = 4.91$, $P = 0.047$, $\beta = -0.54$) and genetic mothers ($F_{1,12} = 10.71$, $P = 0.007$, $\beta = -0.78$) on nestling body condition remaining significant.

DISCUSSION

In a previous study, greyish mothers produced heavier offspring in two out of three years but skipped reproduction more often than red individuals (Roulin *et al.*, 2003a). One interpretation of this result is that grey individuals breed less often but equalize fitness with red conspecifics by investing more effort in reproduction. The results of the present study
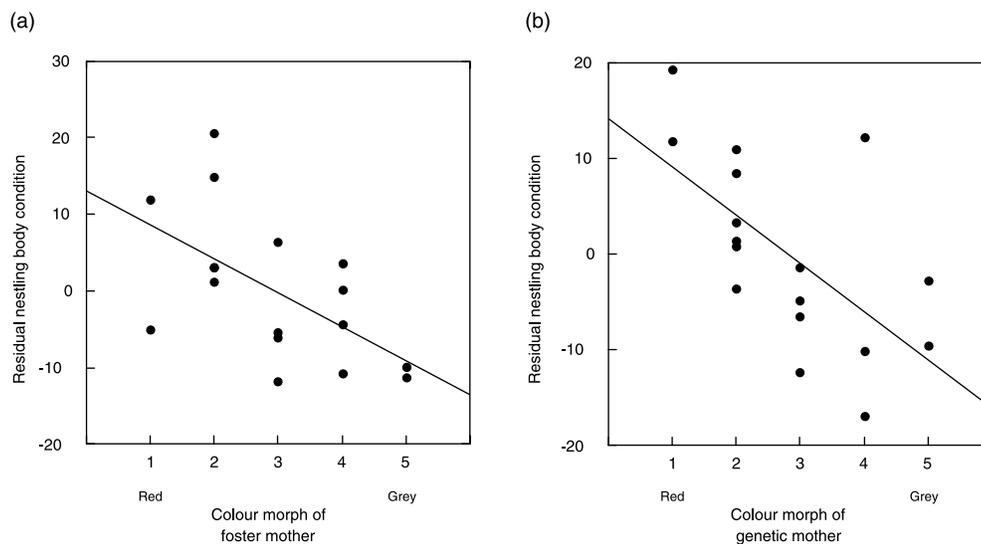

Fig. 2. Relationship between residual nestling body condition and colour morph of foster mother (a) and genetic mother (b). For (a), residuals were extracted from an ANOVA with nestling body condition as the dependent variable and colour morph of genetic mother as the independent variable. For (b), the independent variable was the colour morph of foster mother. Regression lines are shown.

are also consistent with the alternative hypothesis that the magnitude and sign of the covariation between offspring body condition and female colour morph can vary temporally and spatially depending on prevailing environmental conditions. Apparently, conditions met in 2003 were particularly favourable to red individuals, whereas grey birds appeared to be penalized. A similar situation was also observed in the barn owl *Tyto alba*, in which the covariation between nestling body condition and a genetically inherited plumage trait displayed by their mother (i.e. the proportion of the plumage surface covered by black spots, so-called plumage spottiness) varied from one year to the next. In years when broods were larger, lightly spotted mothers produced offspring that were in better condition than the ones of heavily spotted mothers (Roulin *et al.*, 2003b). Therefore, annual variation in the relationship between offspring body condition and colour polymorphism may be a general phenomenon as suggested by a recent review (Roulin, 2004). A major issue is now to determine the key environmental factors that determine sign and magnitude of such covariation. This will shed light on the mechanism that maintains genetic variation in colour polymorphism.

The cross-fostering experiment showed that nestling body condition was associated with coloration of their genetic mother. This suggests that differently coloured females pass on to their offspring different genes that influence major life-history components such as body condition. Alternatively, red and grey females may devote to their eggs different amounts of nutrients or hormones that influence the development of young (maternal effect). However, the absence of a significant effect of egg volume on nestling body condition is not consistent with the hypothesis of a role of maternal effect. The cross-fostering experiment also demonstrated that nestling body condition is sensitive to the colour morph of their foster mother. Two hypotheses can account for this relationship. First, in 2003 a red coloration may have facilitated foraging if, for instance, this coloration was particularly cryptic to prey species that were abundant that year. Alternatively, differently coloured females may occupy different habitats, and in 2003 redder individuals exploited habitats of higher quality. This is a plausible hypothesis because tawny owls are not randomly distributed among habitats with respect to colour morph (Galeotti and Sacchi, 2003). Secondly, red females may possess genes that were beneficial to them in 2003, allowing them to produce high-quality offspring.

Two general conclusions drawn from the present study are particularly likely to interest evolutionary ecologists. First, to our knowledge this is the first study to show that coloration of both foster and genetic mothers is associated to a similar extent with an important life-history component, namely nestling body condition. Therefore, the covariation between colour polymorphism and life-history traits can have both a phenotypic and genotypic basis. Secondly, in the tawny owl, the sign and magnitude of the covariation between nestling body condition and female colour morph varies between years (present study versus Roulin *et al.*, 2003a). This is an important finding because it contradicts theoretical models suggesting only a minor role of temporal fluctuation in selection regimes in the maintenance of genetic polymorphism (Hedrick, 1986; Barton and Turelli, 1989; but see Ellner and Hairston, 1994). Hence, our empirical study (see also Roulin *et al.*, 2003a) indicates that more emphasis should be placed on the importance of temporal variation in ecological factors in the maintenance of colour polymorphism and, more generally, in the maintenance of genetic variation (Suiter *et al.*, 2003).

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