



Influence of mating preferences on yolk testosterone in the grey partridge

V. Garcia-Fernandez^{a,*}, B. Guasco^{b,1}, A. Tanvez^{b,1}, A. Lacroix^{c,2}, M. Cucco^{b,1}, G. Leboucher^a, G. Malacarne^{b,1}

^a Laboratoire d'Ethologie et Cognition Comparées, Université Paris Ouest Nanterre La Défense

^b Dipartimento di Scienze dell'Ambiente e della Vita, Università degli Studi del Piemonte Orientale Amedeo Avogadro

^c Laboratoire d'Analyses Biologiques, CNRS-UPR1934, Centre d'Etude Biologiques de Chizé

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In precocial bird species, the eggs constitute most of the maternal investment because parents do not feed the young after they hatch. Maternal testosterone in egg yolk influences the embryo's and chick's development. Females deposit testosterone in the eggs as a response to the environment experienced during the laying period, including the quality of their mate. To assess the relevance of the female's mate selection on egg characteristics in the grey partridge, *Perdix perdix*, we tested breeding females in a choice trial where they were allowed to choose between two males. After the choice trials, females were mated either with their preferred male (P group) or with the nonpreferred one (NP group). Although eggs laid by females of the two groups did not differ significantly in mass, females of the P group laid eggs with a higher yolk testosterone concentration than females of the NP group. This study agrees with previous work pointing out that partner attractiveness may play an important role in the transfer of maternally derived egg components.

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Maternal effects imply that the environment and the phenotype of the mother affect the progeny's phenotype (Mousseau & Fox 1998). In oviparous vertebrates, all the resources needed by the embryo to develop must be present in the egg laid by the female. After the eggs are laid, no further adjustments to their components are possible so maternal resource allocation is limited, therefore, to a narrow window of time, in contrast to what occurs in viviparous species. Hence, in oviparous species, the prelaying parental effort plays a determinant role in the development of the embryo. Moreover, the allocation of resources to eggs could potentially influence the expression of genes and the fitness of offspring (Bolton 1991; Clutton-Brock 1991; Williams 1994; Lovern & Wade 2003; Pilz et al. 2004; Mansour et al. 2007). Resource allocation should be costly for the female (Gil et al. 1999; Pilz et al. 2003); hence a differential allocation can be related to the expected fitness of the young (Stearns 1992).

The quality of eggs is influenced by environmental conditions experienced by the mother before and during egg formation

(Schwabl 1996), and also by the characteristics of the partner (Burley 1988; Sheldon 2000). Features of the mother's environment may be translated into the differential deposition of several substances in eggs, such as corticosterone (Pike & Petrie 2005; Rubolini et al. 2005), androgens (Schwabl 1993; Gil et al. 1999), carotenoids (Blount et al. 2002; Williamson et al. 2006) and lysozymes (important albumen antimicrobial components; Saino et al. 2002; Cucco et al. 2007).

There is growing evidence that female investment is influenced by male quality reflected by physical and behavioural characteristics. For instance, female canaries, *Serinus canaria*, laid more eggs after listening to males with a large repertoire of songs than after listening to monotonic and repetitive songs (Kroodsma 1976) and female zebra finches, *Taeniopygia guttata*, mated to males whose quality was artificially increased by red leg rings deposited more carotenoids and vitamin E in their eggs than females mated with males whose quality was artificially decreased by green leg rings (Williamson et al. 2006). In the opposite way, female barn swallows, *Hirundo rustica*, deposited a lower lutein concentration in their eggs when mated with artificially tail-elongated males than with control and tail-shortened males (Saino et al. 2002). Some studies have found that manipulation of male attractiveness induces changes in maternal yolk hormone deposition in female birds (canary: Gil et al. 2004; reviewed in Kingma et al. 2009). In contrast, studies on the consequences of female choice between potential mates in more naturalistic conditions, that is, without

* Correspondence: V. Garcia-Fernandez, Université Paris Ouest Nanterre La Défense, LECC, 200, avenue de la République, 92000 Nanterre, France.

E-mail address: violaine.garcia_fernandez@u-paris10.fr (V. Garcia-Fernandez).

¹ B. Guasco, A. Tanvez, M. Cucco and G. Malacarne are at the Università del Piemonte Orientale, DiSAV, via Bellini 25, I-15100 Alessandria, Italy.

² A. Lacroix is at the Laboratoire d'Analyses Biologiques, CNRS-UPR1934, CEBC, Villiers-en-Bois, 79360 Beauvoir-sur-Niort, France.

modification of mate attractiveness, are rather scarce (Rintamaki et al. 1998; Cunningham & Russell 2000; Loyau et al. 2007).

Clearly, females might adjust their investment as a function of the perceived quality of their mates. The differential allocation hypothesis predicts an increased investment in offspring in females paired with high-quality males. In contrast, the reproductive compensation hypothesis predicts an increased investment with low-quality males (reviewed in Gowaty 2008; Harris & Uller 2009; Ratikainen & Kokko 2010).

The aim of this study was to determine the extent to which allocation of maternal resources is affected by active mate choice in the grey partridge, *Perdix perdix*, which lays numerous and relatively large eggs. The chicks are precocial and hatch well developed, so posthatching investment is weak and the main investment occurs during egg formation (Potts 1986). Consequently, the grey partridge is a good model for the study of differential allocation of prenatal maternal resources.

After a choice trial, each individual of one group of females (P group) was paired with the male she preferred, while the other group of females (NP group) was paired with the male she did not prefer. We measured males' body characteristics and an index of health (haematocrit and cellular immunity) to determine to what extent these characteristics could influence the female's choice.

METHODS

We studied 18 males and 18 females, randomly selected from a breeding farm stock in Alessandria, northwest Italy (Cucco et al. 2006a, 2007). The animals were provided for a period of 5 months by the breeding farm owned by Dr G. De Vito. At the end of the experiment, the animals were given back to the breeder for eventual release into the wild. The study was conducted at the breeding farm under licence from the Provincia di Alessandria administration and ASL 20 veterinary agency.

All birds were 1 year old and in their first reproductive season. Throughout the year, the birds were maintained in natural light and temperature conditions. Partridges were housed in small groups of four individuals, in outdoor aviaries (6 × 3 m and 2 m high). Groups were exclusively composed of individuals of the same sex; females and males had only visual or acoustic contact during the pre-experimental period. The floor of the aviaries was the natural ground with grass clumps. Each aviary was provided with food and water containers and two wooden shelters (0.5 × 0.5 m and 0.5 m high) and was covered with a roof of synthetic material. Ash was provided in a hollow placed in a corner of the aviary for dustbathing. The rearing food was a powdered mixture, commonly used by aviculturists to provide proper nutrition during egg laying. Food and water were provided ad libitum.

Choice Tests

At the beginning of the breeding season (from 14 February to 22 March 2006), we conducted trials on female mate choice. Following Leonard & Zanette (1998), each female was allowed to choose between two males in a test cage. The experimental test aviary was the same size as the breeding aviaries. Wire-mesh partitions divided the aviary into a front compartment, enclosing the test females, and two back compartments, housing the males. The back part was divided into two sectors by an opaque partition that did not allow the males to see each other. During each trial, two observers sat 1 m outside the aviary, the first near the aviary's back and the other in front. The two males were placed in the compartments in the back of the aviary 5 min before the trial to allow familiarization with the apparatus. Males quickly became accustomed to their surroundings and consistently displayed normal behaviour. The females were

placed, one at a time, in the front part of the aviary. Before the trial, the female was kept waiting 5 min in a small enclosure positioned in the middle of the front of the aviary. During this period, she could freely see and hear the two males. The choice trial began with the removal of the enclosure. For 20 min, the female could freely move in the whole front part of the aviary. The two observers recorded the amount of time she spent in front of each male. Each female was tested three times, on different days, with the same pair of males and we switched the males' positions at each trial. We did not find any significant effect resulting from the position of the males (the difference in time spent by the female in each side during the three choice trials; repeated measures two-way ANOVA: effect of side: $F_{1,16} = 0.94$, $P = 0.35$; effect of test number: $F_{2,16} = 0.64$, $P = 0.53$; interaction: $F_{2,16} = 0.58$, $P = 0.56$). We considered the male near which the female spent most time to be the preferred one. Female preference did not change significantly from one trial to another (the difference in time spent by the female in front of the preferred male during the three choice trials; repeated measures ANOVA, with the test number as repeated factor: $F_{1,7} = 1.02$, $P = 0.46$; repeatability according to Lessells & Boag 1987: $r = 0.893$, $P < 0.001$).

For trials, the birds were grouped into nine sets of two males and two females. In seven sets of birds, females consistently chose the same male. In each of these sets, one female was paired with her preferred male and the other female with the nonpreferred male. In the other two sets, the females chose different males. In one of these sets, the two females were given their preferred male whereas in the second set, the two females were given their non-preferred male. Finally, we obtained two breeding groups: nine females paired with their preferred males (P group: females of this group spent on average \pm SE $72.6 \pm 5.2\%$ of their trial time in front of the preferred male) and nine females paired with the non-preferred males (NP group: females of this group spent less trial time in front of their mate, on average $34.5 \pm 4.7\%$ of time).

We placed each pair in a breeding aviary (6 × 6 m and 2 m high) on 29 March 2006. In May, the hens started to lay their eggs.

Male Characteristics

We measured some male characteristics to check whether these were related to the female's preference. All measures were made 2 weeks before choice trials: (1) tarsus length (with a calliper, ± 0.1 mm accuracy); (2) body mass (with an electronic balance, ± 1 g accuracy); (3) haematocrit, according to Béguin et al. (1998), 100 μ l of blood were collected in heparinized capillary tubes, from the wing vein, then capillaries were centrifuged for 5 min at 10 000 rpm and afterwards the length of the tube's layer of packed red blood cells, ± 1 mm accuracy, was divided by the total length of the blood sample to obtain the haematocrit value; (4) cellular immunity was evaluated by the cutaneous response to an injection of phytohaemagglutinin (PHA), a foreign antigen that causes T lymphocyte proliferation and local swelling (Lochmiller et al. 1993). A small area on the right wing web was marked with nontoxic ink. The thickness of the wing web was measured with a gauge (Alpa S.p.A., Milan, Italy, accuracy of 0.01 mm), then the web area was injected intradermally with 0.25 mg of PHA (Sigma L 8754, St Louis, MO, U.S.A.) dissolved in 0.05 ml of phosphate-buffered saline (PBS). After 24 h, the wing web thickness in the marked area was remeasured. The subcutaneous injection with PHA produces only a local inflammation without any other adverse effect, and the increased wing web thickness is directly related to the condition of the immune system (Merino et al. 1999; Smits et al. 1999).

Egg Collection and Analyses

We inspected the aviary and collected eggs ($N = 287$) daily during the laying period. To simulate natural conditions, eggs

removed from the nest were replaced by others obtained from commercial breeders. Females laid a mean of 15.9 eggs. All eggs were weighed with an electronic balance (± 0.1 g accuracy).

A number of these eggs were taken for biochemical analyses (laboratory eggs, $N = 119$). We took the first, second, fifth, eighth, 11th, 14th, 17th and 20th eggs of all females, with the exception of two females that stopped laying before the eighth egg, three other females before the 11th and another one before the 14th. After having separated the yolk from the albumen, we froze eggs at -20°C . Sampled eggs were attributed a random number to avoid any effect of an indication of their group during the analyses.

Yolk testosterone concentration was determined by radioimmunoassay at the CEBC laboratories (CNRS Chizé, France) according to Tanvez et al. (2004). Each yolk was homogenized before analysis (Lipar et al. 1999). Testosterone was extracted from the yolk with diethyl ether and then assayed in the same assay using $[1,2,6,7,^3\text{H}]$ -Testosterone (Amersham, Les Ulis, France) and a rabbit-specific testosterone antibody (sensitivity: 5.4 pg/mg of yolk, intra-assay coefficient of variation: 8.0%). Bound and free fractions were separated by adsorption using the dextran-coated charcoal method and the bound fractions were counted with a Packard 1600 liquid scintillator counter.

Statistical Analysis

We used t tests to analyse the difference in numbers of eggs laid by females of the P or NP groups, latencies between the pair formation and the first egg laying, and the total duration of the laying period.

The effects of mate preference on egg mass and testosterone concentration in the yolk were analysed using random slopes linear mixed models (LMM procedure in Systat version 12, Systat Software Inc., San Jose, CA, U.S.A.; Wilkinson 2007; Schielzeth & Forstmeier 2009), with the preference group as a fixed effect. Because each female laid several eggs, characteristics of both eggs and hatched siblings may not be independent. In addition, the number of possible male–female combinations decreased because in the choice tests the adults were grouped into nine sets of two males and two females. Hence, to control for the potential effects of parents and sets in this experiment, we inserted the parent identity and the choice set as random effects in all the analyses. To test for possible effects of egg position along the laying sequence (Soma et al. 2009), laying order was inserted as a covariate in all mixed models. Values are reported as mean \pm SE.

RESULTS

Male Characteristics and Female Preference

Physiological and anatomical characteristics of males did not differ between preferred and nonpreferred males (tarsus length: P males: 46.22 ± 0.72 mm; NP males: 46.00 ± 0.83 mm; t test: $t_{16} = 0.20$, $P = 0.84$; body mass: P males: 354.78 ± 15.43 g; NP males: 352.33 ± 10.07 g; t test: $t_{16} = 0.13$, $P = 0.90$; haematocrit: P males: 45.75 ± 1.23 ; NP males: 43.63 ± 0.86 ; t test: $t_{16} = 1.41$, $P = 0.18$; cellular immunity: P males: 0.89 ± 0.16 mm; NP males: 0.91 ± 0.14 mm; t test: $t_{16} = 0.11$, $P = 0.92$).

Laying and Egg Characteristic

Females paired with preferred males (P group females) or nonpreferred males (NP group females) did not differ significantly in the number of eggs laid (P group: 17.33 ± 2.20 eggs; NP group: 15.11 ± 2.20 ; t test: $t_{16} = 0.77$, $P = 0.45$), the latency between pair formation and the laying of the first egg (P group: 30.44 ± 3.63

days; NP group: 29.89 ± 2.78 days; t test: $t_{16} = 0.12$, $P = 0.90$) or the total duration of the laying period (P group: 26.56 ± 3.35 days; NP group: 25.89 ± 3.37 days; t test: $t_{16} = 0.14$, $P = 0.89$).

Eggs laid by P or NP group females did not differ significantly in total mass (LMM: treatment: $F_{1,264} = 0.08$, $P = 0.78$; laying order: $F_{1,264} = 2.20$, $P = 0.16$), but were significantly different in yolk testosterone concentration (LMM: treatment: $F_{1,97} = 4.34$, $P = 0.040$; laying order: $F_{1,97} = 1.59$, $P = 0.21$). Specifically, the P group females laid eggs with a greater concentration of testosterone than NP group females (Fig. 1). No correlation was found between testosterone and mass values ($r_{119} = 0.07$, $P = 0.473$).

DISCUSSION

Our study was designed to examine whether a female's active choice between two males whose characteristics were not artificially modified affected the allocation of maternal resources into the eggs (Burley 1988; Sheldon 2000). Each female was allowed to choose between two males. Chosen males were not the most preferred males in the population, just the best of a random pair. In natural conditions females must deal with the few available males (unmated neighbours) and do not have the opportunity to check an infinite number of potential mates; in this respect our experiments are close to naturalistic conditions.

In this study, male characteristics (body mass, tarsus length) and male health measures (haematocrit, immune response) were not related to female choice. It is likely that other traits, not investigated in this experiment, could explain female preferences. For instance, vocal performance and vigilance behaviour have been shown to affect female preferences (Dahlgren 1990; Beani & Dessi-Fulgheri 1995).

Despite their clear-cut and repeated preferences for a male, the active choice of females did not affect subsequent maternal investment in terms of egg mass. The lack of significant differences in this species could be caused by an absence of any female preference effect on mass, but also by the high repeatability of egg mass as a reproductive trait (Christians 2002; Cucco et al. 2006b). Our

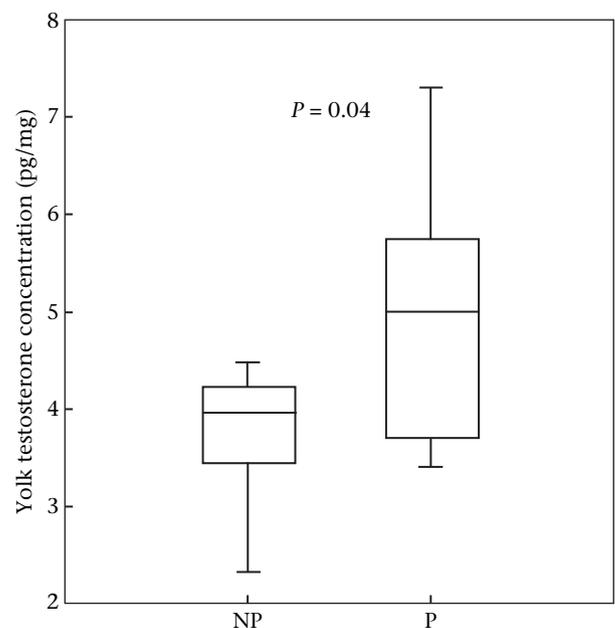


Figure 1. Yolk testosterone concentration of eggs laid by females mating with their nonpreferred mate (NP) or their preferred mate (P). Box plots show the median and upper and lower quartiles; whiskers show the minimum and maximum values.

result is congruent with studies on the collared flycatcher, *Ficedula hypoleuca*, the domesticated canary, and the house sparrow, *Passer domesticus* (Mazuc et al. 2003; Tanvez et al. 2004; Michl et al. 2005), but contrasts with data obtained on the mallard, *Anas platyrhynchos*, the Chinese quail, *Coturnix chinensis*, the peafowl, *Pavo cristatus*, and the zebra finch, which laid larger eggs when mated with more attractive males (Cunningham & Russell 2000; Uller et al. 2005; Gilbert et al. 2006; Loyau et al. 2007).

In contrast, our results indicate that testosterone concentration in grey partridge egg yolk was influenced by female mate preference. Eggs laid by females of the preferred P group contained significantly more testosterone in their yolk than those laid by females of the nonpreferred NP group. This result matches those obtained in other species showing that females paired with attractive males deposit more testosterone in their eggs (reviewed in Kingma et al. 2009). Female barn swallows increased yolk androgen concentration when mated to males with experimentally elongated tails (Gil et al. 2006) or to more colourful males (Safran et al. 2008); female canaries deposited significantly more testosterone when exposed to song with attractive repertoires than when exposed to unattractive ones or maintained in acoustic isolation (Gil et al. 2004; Tanvez et al. 2004); blue tit, *Cyanistes caeruleus*, females laid eggs with higher testosterone concentration when mated with control males than with males with an artificial reduction of the crown UV coloration (Kingma et al. 2006); zebra finches and peafowl increased yolk testosterone when paired with attractive males (Gil et al. 1999; Loyau et al. 2007).

In conclusion, there are some studies showing that mothers invest more in their offspring when paired to an attractive male, but also a few examples of the opposite, higher investment in response to an unattractive mate. There are reasons to believe that both strategies can be beneficial for the individuals, depending on the circumstances (Ratikainen & Kokko 2010). In our study, we found that testosterone concentration in egg yolk is positively influenced by maternal preference in the grey partridge. This result, combined with the previous observation that egg testosterone is beneficial for chicks in the early period of life (Cucco et al. 2008), lends support to the hypothesis of an increased investment in offspring by females paired with preferred mates, which are thought to be attractive (differential allocation hypothesis, reviewed in Gowaty 2008). In contrast, this result is not in line with the opposite prediction of a higher investment by females paired with unattractive males (reproductive compensation hypothesis, reviewed in Harris & Uller 2009). Future studies could clarify whether the allocation of other yolk or albumen substances can be influenced by female choice as well.

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