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## GOLDEN EGGS: MATERNAL MANIPULATION OF OFFSPRING PHENOTYPE BY EGG ANDROGEN IN BIRDS

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**SUMMARY.**—*Golden eggs: maternal manipulation of offspring phenotype by egg androgen in birds.* Avian eggs contain, among many components, significant concentrations of androgens and other maternal hormones. These hormones can have profound influences in the development of the embryo and the nestling, and even shape the phenotype of the adult bird. In this review I evaluate the research that has been developed in the last ten years in this field, and I suggest several lines of research that might be worth following in the future. Evidence shows that high levels of androgens in eggs speed up embryonic development and boost muscular growth and begging rates in nestlings. A study in captivity with canaries *Serinus domesticus* has identified an influence of egg androgen in the social hierarchy of the adult bird. Research is needed to ascertain long-term effects of egg androgens in the wild, but the picture is a promising one. Several studies have found a positive correlation between female hormone levels and those of the egg. However, it is still not clear whether this is the result of passive transfer or, on the contrary, there are mechanisms that control the transfer of hormones from the mother to the egg. Whatever the mechanism, several experiments show that females lay eggs with higher concentrations of androgens when paired to attractive mates. This pattern of differential allocation suggests that egg androgens are a costly resource, and that there are limiting factors for the mother or the offspring. Research has uncovered several other interesting patterns, such as differences between male and female bearing eggs in androgen levels, or a positive covariance with breeding density in different colonies of the same species. Another exciting issue concerns the uneven distribution of egg androgen within the same clutch. It has been suggested that females could use this mechanism to control aggression and competition among nestlings. To summarise, egg androgen allocation constitutes a powerful mechanism which females could use to modify offspring phenotype. Large between-species differences in egg androgen concentrations suggest that a comparative analysis may be the best tool to understand the role of the many ecological and evolutionary factors that have shaped the evolution of this character.

**Key words:** maternal effects, egg androgen, testosterone, phenotypic plasticity, avian eggs, differential allocation, sexual selection.

**RESUMEN.**—*Huevos de oro: manipulación maternal del fenotipo de la descendencia mediante andrógenos en aves.* Entre muchos otros componentes, los huevos de las aves contienen importantes concentraciones de andrógenos y de otras hormonas de origen materno. Lejos de ser inertes, estas hormonas pueden influir en el desarrollo del embrión y del pollo, e incluso moldear el fenotipo del ave adulta. En esta revisión se analizan

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los resultados de la investigación que se ha desarrollado en los últimos diez años en este campo, y se muestran varias líneas de investigación que merecerían seguirse en el futuro. Varios estudios experimentales han demostrado que altos niveles de andrógenos en los huevos aceleran el proceso embrionario, aumentan el desarrollo muscular y la frecuencia de petición de alimento del pollo. Un estudio en cautividad en canarios *Serinus domesticus* muestra incluso una influencia de los andrógenos en la jerarquía social del ave adulta. Faltan estudios que examinen efectos a largo plazo en especies en libertad, pero el panorama resulta muy prometedor. En varios trabajos se han encontrado correlaciones positivas entre los niveles de hormonas de las hembras y los del huevo que se está formando, pero faltan estudios que examinen si esta relación es pasiva, o si existen mecanismos para controlar la transferencia de hormonas al huevo. Sea como fuere, varios experimentos muestran que las hembras producen huevos con concentraciones más elevadas de andrógenos cuando se aparean con machos atractivos. Este patrón de asignación diferencial sugiere que los andrógenos en el huevo son un recurso costoso, y que existen factores limitantes, bien para los pollos o para la hembra. Se han descrito otros interesantes patrones, como por ejemplo diferencias en andrógenos entre huevos que contienen embriones macho y hembra, o diferencias entre poblaciones de la misma especie en función de la densidad reproductora. Se han encontrado asimismo diferencias en el reparto de andrógenos en el seno de una misma puesta. Este mecanismo podría ser usado por la hembra para controlar la agresión y la competencia entre los pollos. En resumen, los andrógenos en los huevos constituyen un poderoso mecanismo del que las hembras pueden servirse para modificar el fenotipo de su descendencia. La gran variación interespecífica en la concentración de andrógenos en el huevo sugiere la necesidad de estudios comparativos para entender el papel de una serie de factores ecológicos y evolutivos que podrían influir en la evolución de este carácter

*Palabras clave:* efectos maternos, andrógenos del huevo, testosterona, plasticidad fenotípica, huevos de aves, asignación diferencial, selección sexual.

## INTRODUCTION

Unlike mammals, females of oviparous organisms can do little to influence the early development of their offspring. The embryo is enclosed within the egg membranes and the shell and (except for temperature and humidity) all essential resources for development are already packed in the albumen and the yolk (Romanoff & Romanoff, 1949). These resources are costly, and strongly influence nestling fitness and survival (Williams, 1994; Nager *et al.*, 2000). Therefore, important decisions about maternal investment and trade-offs with other investments should be made before laying, at the time of egg formation. Three conflicting interests can be identified at this time: 1) egg quality; 2) egg number; and 3) female survival and reproductive prospects (Price, 1998). To these trade-offs we could add a fourth factor: sex-ratio determination, which may interact with the previous decisions, especially in sexually dimorphic species (Badyaev *et al.*, 2002).

Maternal effects are defined as the partial regression of the offspring's phenotype on the mother's phenotype, holding genetic sources of variation constant (Kirkpatrick & Lande, 1989). Recent research has identified several areas where these effects function as adaptive

mechanisms that influence offspring adaptations to changing environments (Mousseau & Fox, 1998), showing that mothers can actively generate phenotypic plasticity in an adaptive way. However, maternal effects are affected by natural selection both at the maternal and at the offspring stages, often with differences in the magnitude and the direction of selection. This two-stage selection process is a focus of evolutionary conflict between parents and their offspring, and may generate complex interactions between genotypes.

Eggs contain all the essential proteins, fat, minerals, vitamins and water that the embryo needs to develop (Romanoff & Romanoff, 1949; White III, 1991). However, only recently have researchers started to consider other components that can have more subtle action during embryo development, and which can decisively influence offspring phenotype. This relatively recent research has focused on the hormones that females deposit in their eggs and that influence embryo development (Schwabl, 1993). More interestingly, egg hormones constitute a key mechanism of developmental plasticity, by which females can adaptively affect offspring phenotype, thereby influencing the development of behavioural and life-history strategies (West-Eberhard, 2003). Hormones are one of the main interfaces between the ge-

notype and the phenotype, having a role in the regulation of gene expression. They constitute a powerful means of manipulating whole suites of traits, especially when their action takes place in early development (Ketterson & Nolan, 1999; Dufty *et al.*, 2002).

A main issue in the study of maternal effects is to determine whether or not they are adaptive (Heath & Blouw, 1998; Shine & Downes, 1999). For instance, most modifications of offspring phenotype resulting from nutritionally deprived mothers are best regarded as physiological side effects rather than adaptations (Williams, 1966). However, there is good evidence for adaptive responses in many other cases (Mousseau & Fox, 1998; Shine & Downes, 1999). In this paper, I will review the studies carried out so far on avian egg androgens and will discuss whether they provide evidence for adaptive patterns or for physiological constraints. I will also propose research topics that need to be addressed in the future.

#### HORMONES IN EGGS

In 1942, Riddle & Dunham discovered that the chicks hatching from estradiol-treated hens *Gallus domesticus* were feminised, very much in the same way as if the eggs had been directly injected (Riddle & Dunham, 1942). From these results they concluded that females could transfer hormones to their eggs and influence their development. However, this study involved very high doses of hormones, far beyond the range found in nature, and the results were not interpreted as being the result of a normal mechanism influencing development. The picture changed significantly when Schwabl (1993) discovered in the yolks of non-manipulated Canaries *Serinus canaria* significant levels of a number of different hormones and established functional consequences from variation in yolk testosterone.

The following hormones have so far been found in avian eggs: testosterone (T), 5 $\alpha$ -dihydrotestosterone (DHT), estradiol (Schwabl, 1993), androstenedione (A4) (Schwabl, 1997b), several thyroid hormones (McNabb & Wilson, 1997), progesterone (Lipar *et al.*, 1999b) and corticosterone (Downing & Bryden, 2002). Most research so far has focused on androgens, but other hormones, especially estradiol and

corticosterone, may indeed have an important role in influencing offspring phenotype, and we will undoubtedly see in the next years an impetus of research in this area (Adkins-Regan *et al.*, 1995). However, in this paper I will refer exclusively to androgens, mainly T.

How do hormones enter the egg? Until now, few studies have directly addressed this issue. We know that several types of cells in the follicle walls are specialised in the production of steroids (Huang *et al.*, 1979; Porter *et al.*, 1989), and there is a large corpus of literature that investigates the regulation and function of these cells throughout ovulation (Johnson, 1999). For instance, very precise cycles of progesterone, estradiol and androgens are produced in different stages of follicle maturation (Johnson, 1999). However, research so far has focused on the female as a target of these hormones. It is assumed that the presence of hormones in the egg is the result of passive hormone transfer from the female ovary into the yolk (Schwabl, 1996a). Follicles are highly vascularised, and the transfer would take place thanks to the lipophilic nature of steroids, and the yolk's large concentration of lipids. As most yolk is incorporated during the last 72 hours before ovulation (Romanoff & Romanoff, 1949), hormones that are produced by the large pre-ovulatory F3 follicles should be widely represented within the egg. A peak in androgen production takes place at this stage (Hernández Vértiz *et al.*, 1993), explaining why most research has concerned these particular steroids.

However, we still do not know whether this transfer is an active or a passive one. Androgens have specific functions in regulating female avian reproduction, and a certain non-adaptive passive transfer to the egg may be present. However, the fact that these steroids are not inert in the yolk and have an effect in offspring phenotype, suggests strong selection for an accurate control of this transfer (Lipar *et al.*, 1999b). As high levels of androgens may cause deleterious effects, it is also likely that females could somehow limit the transfer of circulating steroids to their eggs. There is thus ample scope for research on the physiological mechanisms regulating steroid deposition in the egg. A recent paper has found that only 0.1% of radioactively marked steroids injected in the female at the time of egg formation enter

the egg (Hackl *et al.*, 2003), suggesting that most egg steroids are formed directly by the follicular walls.

#### PLASMA LEVELS OF ANDROGEN IN THE MOTHER

A fundamental question that we need to answer is whether the levels of hormones in the egg correlate with the female's own hormonal levels. For instance, would females with high T blood levels also lay eggs with high T concentrations? This question underlines a fundamental issue in the evolution of androgen levels in female eggs, namely whether the target of selection is in the egg or in the female.

Although we know rather little about the role of androgens in female vertebrates (Staub & De Beer, 1997), female birds show higher T levels before ovulation and during episodes of frequent female-female aggression (Dufty & Wingfield, 1986; Cristol & Johnsen, 1994; Langmore *et al.*, 2002). We may ask ourselves whether selection for high T levels in females would also drag along higher levels in eggs, at both the species and individual level. More importantly, if selection for T operates in the female, to what extent would the effects on the egg be adaptive?

Data so far do not provide a straight answer. A first study by Schwabl (Schwabl, 1996a) reported a positive correlation between yolk-T concentration and mean maternal T levels during the time when most of the yolk was being incorporated to the egg. However, this study pooled several eggs from different females, and it is not clear whether the pattern observed is due to between or within clutch variation in T concentration. More convincing is the positive correlation between the number of aggressive intrusions experienced during egg laying and the mean yolk-T in Tree Swallows *Tachycineta bicolor* (Whittingham & Schwabl, 2002). However, some detailed studies in captivity have shown that there is not a one-to-one correspondence between plasma and follicle levels in the Quail *Coturnix japonica* and in the domestic hen (Doi *et al.*, 1980; Hammond *et al.*, 1980). Also, the fact that the hormonal profile of follicle maturation is closely mirrored by the hormonal variation in different layers of the yolk (Lipar *et al.*, 1999b), suggests that yolk hormonal levels are not a true reflection of the fe-

male circulating plasma hormones. Evidence showing that steroids injected in the blood stream do not reach the egg (Hackl *et al.*, 2003) does not discard the opposite pathway, which would seem rather likely. However, a recent study in the Lesser Black-backed Gull *Larus fuscus* has found that food-supplemented females had higher plasma androgen levels than controls, while the reverse was true for egg androgen (Verboven *et al.*, 2003). This is strong evidence against a simple correspondence between androgen levels in the female and the egg, and suggests that both systems can be adaptively responding to different selection pressures.

#### EFFECTS OF YOLK ANDROGEN IN THE OFFSPRING

Several studies have experimentally manipulated androgen levels in the eggs and measured the effects on nestlings at different stages. Experimentally increased concentrations of T in the eggs resulted in a higher probability of begging at hatching and nestling growth in the Canary (Schwabl, 1993), and with neck muscle size in Red-Winged Blackbirds *Agelaius phoeniceus* (Lipar & Ketterson, 2000). Studies in the Black-headed Gull *Larus ridibundus* showed that chicks hatching from these eggs had shorter incubation period (Eising & Groothuis, *in press*), grew faster and larger, and were better at competing against other nestlings (Eising *et al.*, 2001). These studies suggest that the action of T may already have occurred in the embryo, although we would need detailed studies of in ovo embryo development as a function of experimentally modified T concentration. In contrast to the previous results, although natural variation in A4 yolk concentration is positively correlated with growth rate in Barn Swallow *Hirundo rustica* nestlings (Gil *et al.*, submitted-c), there is no correlation with nestling size at hatching.

Despite this preponderance of positive effects, a study conducted on the American Kestrel *Falco sparverius*, reported that nestlings hatching from androgen injected eggs hatched later than controls and had reduced growth and survival (Sockman & Schwabl, 2000). Moreover, these nestlings tended to have higher corticosterone levels than controls (Sockman & Schwabl, 2001). As high plasma corticosterone

levels are associated with reduced growth and stressful conditions, the latter result confirms the negative effects of yolk androgen in that experiment. These are surprising results, and may indicate species-specific patterns, suggesting that there are limits above which the effects of egg androgen are no longer positive (see section on Costs of egg androgen).

Two different mechanisms may explain the effects of egg androgens in nestling growth: (1) direct physiological effect of androgen on growth rates, increased anabolism, etc.; and (2) effects of behaviour, such as begging rate or sibling aggression. So far most studies have identified direct physiological effects (e.g. Eising *et al.*, 2001). However, effects on behaviour have also been described, such as increased begging (Schwabl, 1996b). Future studies should address the importance of these two mechanisms, as well as their interaction. For instance, egg androgens affect the growth of the 'begging muscle' in ovo, which in turn may later on affect behaviour later in the nest (Lipar & Ketterson, 2000).

What are the long-term effects of variation in egg androgen levels? The only study to have addressed this issue has shown that the hierarchy ranking of juvenile canaries is positively related to T concentration in the egg (Schwabl, 1993). We do not know if these effects are permanent. Future research should also address whether variation in phenotypic and behavioural strategies, coping styles or life-history traits is related to maternal allocation of androgens. A topic of special relevance here is the development of sexual ornaments. Sexual selection theory predicts that ornaments should be costly in order to be honest indicators of male quality (Hamilton & Zuk, 1982). It has been suggested that hormonal constraints early in development may provide condition-dependent expression of some sexual traits (Nowicki *et al.*, 1998). If egg androgens were able to influence this development, they could be used by females to shape male offspring ornaments. Such a scenario would be of critical importance to our understanding of sexual selection (Kotiaho *et al.*, 2003).

There are several issues that future studies on the effect of egg androgens in nestling fitness should address. Firstly, egg androgen should be directly modified by injection techniques. Inferences about the effects of egg an-

drogen based on natural variation (Gil *et al.*, submitted-c), or on manipulation of wide-ranging factors such as maternal condition (Verboven *et al.*, 2003), are indeed interesting, but do not provide unequivocal evidence and may be confounded by variation in many other egg components.

Secondly, all studies so far have injected a single dose of androgen, usually the population maximum. Instead, it would be preferable to examine a whole range of doses. An experiment like this could reveal complex non-linear reactions (i.e. thresholds, maxima, etc.), that are indeed to be expected physiologically.

Thirdly, studies do not usually take into account the sex of the nestling. However, benefits accrued by male and female embryos may differ (Henry & Burke, 1999). As variance among females in egg androgen is much larger than within-brood variance (Reed & Vleck, 2001; Pilz *et al.*, 2003), we can predict that females are not capable of delivering to each individual egg the optimum amount of androgen required by the sex of each egg. If male and female chicks do have different optimal concentrations of androgen, then this would impose an important constraint on sex ratio determination, as mean egg androgen levels in most broods would have to involve a compromise between the two sexual optima. This constraint might favour biased sex-ratios, at either of the two extremes. Under such a scenario, we can predict that there should be a positive correlation between sex-ratio variance and mean egg androgen among-species or populations.

#### EGG HORMONES AND NESTLING PLASMA HORMONES

Nestlings of both altricial and precocial birds show physiologically relevant levels of several hormones in plasma (Schlinger & Arnold, 1992; Silverin & Sharp, 1996), but we know very little about the function of these hormones in regulating behaviour. Recent studies have identified a role of corticosterone in begging behaviour (Kitaysky *et al.*, 2001; Saino *et al.*, 2003). Androgens have also been associated to nestling competition (Sasvari *et al.*, 1999; Naguib *et al.*, submitted), but further research is needed to know the effects, benefits and costs of different steroids at the nestling stage.

One erroneous assumption that is often made is that there is a positive correlation between egg androgen concentration and nestling androgen plasma levels (e.g. Sasvari *et al.*, 1999). This is an interesting possibility, as it is likely that the perinatal hormonal milieu could have priming effects in adult endocrinology (Clark *et al.*, 1992). Such a link would help us understand the long-lasting effects of egg androgen (e.g. Schwabl, 1993), but so far the evidence is just not there. In Barn Swallows we did not find a significant positive correlation between natural variation in the concentration of yolk A4 and nestling T plasma levels in Barn Swallow nestlings (Gil *et al.*, submitted-c). Admittedly, this study was correlational and non experimental. In the American Kestrel, nestlings from androgen injected eggs had higher levels of plasma corticosterone (Sockman & Schwabl, 2001). However, the latter result may have arisen as a consequence of the reduced growth of those nestlings rather than as a direct hormonal link.

An influence of egg androgens in nestling hormones may explain some of the long-lasting effects of egg androgen.

#### WITHIN-CLUTCH PATTERNS WITH RESPECT TO LAYING ORDER

For a behavioural ecologist, one of the most intriguing aspects of egg androgen deposition is the unequal distribution of androgen within a single clutch. This provides females with a powerful mechanism to influence sibling competition. The variable that has been more commonly associated with this variation is laying order.

Laying order strongly influences androgen concentration. What is really surprising is that the pattern changes completely depending on the species. In most species, such as in the Canary (Schwabl, 1993; Gil *et al.*, *in press*), the Red-winged Blackbird *Agelaius phoeniceus* (Lipar *et al.*, 1999a) or the European Starling *Sturnus vulgaris* (Pilz *et al.*, 2003), androgen concentration increases with increasing laying order. In other species however, such as the Cattle Egret *Bubulcus ibis* (Schwabl *et al.*, 1997) or the Zebra Finch *Taeniopygia guttata* (Gil *et al.*, 1999), the pattern is reversed, and the last eggs have fewer androgen than the first ones. Still in other species (Tree Swallow

(Whittingham & Schwabl, 2002) or House Wren *Troglodytes aedon* (Ellis *et al.*, 2001) there is no apparent trend.

It has been hypothesised that within-brood variation in androgen may serve as a mechanism to modify the effects of hatching asynchrony (see Slagsvold *et al.*, 1984 for a similar argument in the case of egg size). Hatching asynchrony is common in birds, and happens when females start to incubate before laying is complete (Clark & Wilson, 1981). This results in the last eggs to be laid hatching later than early ones, and creates a size hierarchy among nestlings. If yolk androgen enhances begging and development, increasing levels of androgen with increasing laying order would favour the last eggs of a clutch, thereby counteracting the effects of hatching asynchrony (Schwabl, 1996b). On the contrary, decreasing levels of androgens with increasing laying order would reinforce the effects of hatching asynchrony; last chicks would not only suffer from hatching later than their siblings, they would also have to bear the burden of a handicap to compete with them. To the extent that androgens influence nestling aggressiveness, this difference between eggs would contribute to brood reduction in some species (Schwabl *et al.*, 1997).

Evidence for a role of androgens in regulating competitive disadvantages due to hatching asynchrony is not conclusive. One of the first studies in the Canary showed that the positive effect of androgens in late eggs was not strong enough to completely overcome the effects of hatching asynchrony (Schwabl, 1996b), although it did decrease within-brood variation in body size. Studies in the Black-headed Gull and in the Red-winged Blackbird also suggest that the increasing levels of androgen with increasing laying order buffer somehow the effects of asynchronous hatching (Lipar & Ketterson, 2000; Eising *et al.*, 2001). However, Sockman & Schwabl's experiment on the American Kestrel suggested the opposite effect (Sockman & Schwabl, 2000). Further studies are necessary to see whether the results of the latter paper are due to an experimental artefact, or to species-specific variation in the effects of yolk androgen.

Why would it pay females to counteract hatching asynchrony with a further mechanism? Would it not be simpler to avoid hatching asynchrony altogether by delaying the onset of

incubation until the clutch was complete? I would argue that this apparent paradox may help us to better understand the functional advantage of hatching asynchrony. Many hypotheses have been proposed to explain hatching asynchrony (Stoleson & Beissinger, 1995). A first group of these has focused on the effects on offspring and parents, such as the establishment of a hierarchy within the brood, optimisation of feeding effort, favouring of brood reduction, etc. (e.g. Lack, 1954). A second group of hypotheses, however, propose that hatching asynchrony is the unavoidable consequence of selection pressures to reduce the incubation period or the amount of time that eggs are exposed to adverse thermal conditions (Clark & Wilson, 1981; Arnold *et al.*, 1987). These hypotheses are not mutually exclusive and patterns in nature are likely the result of opposing selective forces (Viñuela & Carrascal, 1999; Viñuela, 2000). However, the fact that in most species yolk androgens increase with increasing laying order, suggests that females of these species are counteracting the effects of hatching asynchrony in the brood. Thus, it would seem that the selective advantage of hatching asynchrony in those species must lie in factors related to the onset of incubation, such as egg viability or predation risk and not in establishing a hierarchy within the brood. However, differences among species in the pattern of androgen allocation with respect to laying order (increasing, decreasing or even) imply the existence of different selective pressures. I would argue that a comparative study of within-brood androgen patterns could provide insight into the selective pressures of hatching asynchrony.

#### SEXUAL SELECTION AND CRYPTIC MATERNAL ALLOCATION

Good-genes models of sexual selection propose that the expression of sexual ornaments in males is an honest signal of male genetic quality, and that females acquire genetic benefits for their offspring by mating with such males (Andersson, 1994). Evidence suggests that these genetic benefits may be a consequence of a continuously evolving genotype in a never-ending co-evolution with parasites and pathogens (Møller *et al.*, 1999). Evidence for good genes usually consists in showing that the offspring of

highly ornamented males have increased viability and reproductive success (Møller, 1994; Welch *et al.*, 1998; Johnsen *et al.*, 2000). However, no study so far in birds has completely removed the effect of females in determining offspring viability. Indeed several conflicting models of sexual selection predict that females should invest more in the offspring of attractive males (Andersson, 1994; Gil & Graves, 2001), and increased allocation in the offspring of attractive males is a common finding (Burley, 1988; Sheldon, 2000).

Females can also show differential allocation before laying, by depositing differential levels of yolk androgens in their eggs as a function of male ornament size. We have found this to be the case in three different experiments done with three different bird species. We modified male attractiveness in the Zebra Finch by ringing the birds with red (attractive) and green (unattractive) colour rings (Burley, 1988; Sheldon, 2000). The concentration of androgen in the eggs laid for red-ringed males was larger than for those laid for green-ringed males (Gil *et al.*, 1999). In a second study, female Canaries were exposed to either a complex song repertoire containing 'sexy' syllables (Vallet *et al.*, 1998) or a monotonous song. Yolk-T concentration was 37% higher in the eggs of females exposed to attractive than to unattractive song (Gil *et al.*, *in press*). These two studies were done in the laboratory, and we wished to test whether this effect also existed in the wild. In the third study, we manipulated the tail length of male Barn Swallows soon after they were paired and assayed the androgen contents of the first egg of the clutch. Again the results show a positive relationship between experimentally manipulated ornament size and androgen-yolk allocation (Gil *et al.*, submitted-c).

These studies show that females invest higher androgen concentrations in the eggs laid for attractive males. As yolk androgen seems to boost nestling growth, at least in some species, this may be taken as evidence that females are increasing the fitness of the offspring fathered by attractive males. However, why don't all females invest the same amount of androgens regardless of the father? There may be several explanations for this: 1) high levels of egg androgens may be costly to the female; 2) high levels of egg androgens may be only withstood by high-quality offspring; 3) high levels of egg

androgens may provide different benefits to male and female nestlings, and this may interfere with a male-biased sex-ratio, which is expected in broods fathered by highly ornamented fathers; or 4) high levels of egg androgens may influence the development of alternative mating strategies, whose adaptiveness is a function of male quality (Andersson, 1994).

#### NESTLING SEX AND BROOD SEX-RATIO

Petrie and co-workers (2001) found that androgen levels in the yolk of half-way incubated Peacock eggs *Pavo cristatus* differed between eggs containing male and female embryos. Male-bearing eggs had higher levels of T and A4, and lower levels of estradiol and DHT than female-bearing eggs. This result was interpreted to mean that differences between clutches in mean androgen levels were the result of a physiological mechanism designed to determine sex-ratio (Petrie *et al.*, 2001). According to this hypothesis, patterns that have been described as differential allocation (see previous section) could just be the result of biased sex-ratio, because theory predicts that sex-ratios of broods fathered by attractive males should be male-biased (Burley, 1981).

However, Petrie *et al.*'s data suffer from two important methodological problems. Firstly, all eggs were pooled in the same statistical basket, without using a nested model. This means that variation between and within clutches is confounded, and we do not know whether differences are due to: (1) clutches having different androgen content depending on their sex-ratio, or (2) individual eggs having different androgen content depending on the sex of the embryo they enclose. A second problem is that yolks were assayed at day 10 of the Peacock's 28-day incubation period. By this time endogenous embryo androgen production and uptake have already taken place, and this could influence yolk concentrations (Rogers, 1995).

We have tested the relationship between sex-ratio and average clutch egg androgen levels in the Barn Swallow (Gil *et al.*, submitted-c). With a sample size of 109 nests, we found no relationship whatsoever between these two variables. Furthermore, recent studies in captivity have shown that mean egg androgen levels are not different between sexes in the domestic hen

(Müller *et al.*, 2002; Eising *et al.*, 2003a). However, certain sex differences were found when social hierarchy was considered: high ranking females deposited higher levels of T in male than in female-producing eggs, whereas the pattern for low ranking females was inverted (Müller *et al.*, 2002).

Although further evidence is still needed, it seems that egg androgen is not related to brood sex-ratio, and that Petrie *et al.*'s results could have been an experimental artefact. However, the possibility that androgens incorporated in the yolk before ovulation may affect ovum sex is still not discarded. Male and female embryos or nestlings may have different egg androgen optima, allowing selection for sex-specific egg androgen levels. Although it is difficult to envisage a mechanism by which yolk androgen could influence which sex is selected after the primary meiotic division, differences in size between eggs bearing male and female embryos suggest that it is not a matter of science fiction (Cordero *et al.*, 2000; Cordero *et al.*, 2001). Furthermore, it has been suggested that differences in the duration of embryonic period between males and females may be set off by differences in maternal hormone allocation (Blanco *et al.*, 2003). A possible mechanism relating embryo sex to yolk androgen levels could come about by an indirect relationship of both variables with laying order (K. Pilz, *pers. com.*). Indeed, population differences in the proportion of male and female nestlings in different laying positions have been found in the House Finch *Carpodacus mexicanus*, supporting a role of this trait in adaptive population divergence (Badyaev *et al.*, 2002).

#### BREEDING DENSITY

Levels of yolk-T increased with colony size in the House Sparrow *Passer domesticus* (Schwabl, 1997a). It was interpreted that this was due to the fact that females in large colonies had higher levels of T in the blood. A possible physiological link has been identified in a study on Tree Swallows, which has reported a positive correlation between female aggression and yolk-T (Whittingham & Schwabl, 2002). Schwabl proposed that egg androgen was a mechanism of adaptive transgenerational phenotypic plasticity, by which females could sha-

pe the phenotype of their offspring to adaptively match the breeding conditions that the offspring would encounter as adults. Also, post-breeding dispersal strategy decisions could adaptively be influenced by such a mechanism (Dufty *et al.*, 2002). Further evidence for a link between breeding density and egg androgen come from studies of European Starlings (Pilz & Smith, *in press*), American Coots *Fulica americana* (Reed & Vleck, 2001) and Black-headed Gulls (Groothuis & Schwabl, 2002). However, in the Barn Swallow we did not find any relationship between colony size and yolk-T concentration, despite large variation in colony size in this species (Gil *et al.*, submitted-c).

Although Schwabl (1997a) has proposed the adaptiveness of a positive relationship between breeding density and egg androgen, it is not clear that the target of selection in this case is the egg and not the female. Unless it can be shown that both female and offspring benefit independently from higher androgen levels, a positive covariance between yolk androgen and breeding density would rather suggest a secondary consequence of selection in females (see section on Plasma levels of androgen in the mother).

#### EGG ANDROGEN AND FEMALE QUALITY

Differential allocation with respect to egg androgen suggests that this is a costly investment. If this is so, we should expect a positive covariance between female quality and mean egg androgen concentration. Several lines of evidence provide support for this prediction. In the European Starling, older females lay eggs with greater androgen concentration than young females (Pilz *et al.*, 2003), and clutch size and average egg androgen are positively related. In the Barn Swallow, there is also a positive correlation between clutch size and average egg androgen (Gil *et al.*, submitted-c). Furthermore, arriving date, which is a good correlate of quality, is strongly negatively correlated with egg androgen in this species (Gil *et al.*, submitted-c), suggesting that high quality females lay eggs with larger concentrations of androgens. This evidence is further supported by a positive correlation between egg immunoglobulins and androstenedione in the Black-

legged Kittiwake *Rissa tridactyla* (Gil *et al.*, submitted-a). In contrast to this correlational evidence, a recent experimental study has found that food supplemented Lesser Black-backed Gulls laid eggs with lower concentrations of androgens than controls (Verboven *et al.*, 2003). This is contrary to expectations, and suggests that egg androgen investment may covary in complex ways with other components of maternal investment.

Conditions experienced during early development can affect reproductive performance later in life (Lindström, 1999). If yolk androgen allocation is costly, we would expect it to be positively related to developmental stress. We experimentally manipulated brood size in the Zebra Finch, thereby modifying levels of developmental stress experienced by nestlings. We then let these female nestlings attain sexual maturity and paired them randomly to other birds. We found that concentrations of yolk testosterone were negatively related to the manipulated brood size that females had been exposed to during development (Gil *et al.*, submitted-b). This result provides strong evidence for condition-dependent allocation of egg androgen, suggesting that it is indeed a costly investment.

#### BROOD PARASITISM

Females do not always lay eggs in their own nests. Obligate and facultative brood parasites lay eggs in other females' nests where their offspring compete with non-kin (Rothstein & Robinson, 1998). As egg androgen influences growth and begging intensity, we may predict that brood parasites should deposit higher levels of androgens in their eggs than non-parasites. This could explain why nestling brood parasites are often more aggressive and have the upper hand when competing with host nestlings. A recent study has compared egg androgens in the parasitic brown-headed cowbird *Molothrus ater* and in three different host species (Hauber & Pilz, 2003). However, the results show an inconsistent pattern. Cowbird yolk-T levels were higher than in Eastern Phoebe *Sayornis phoebe*, lower than in Yellow Warblers *Dendroica petechia*, and similar to Red-winged Blackbirds. Similarly, androgen concentration in the eggs of the Great Spotted Cuckoo are lower than in its main host in

Spain, the Magpie *Pica pica* (D. Gil, J. J. Soler, A. Lacroix & A. P. Møller, *unpubl. data*).

Nevertheless, I would argue that these results do not refute the hypothesis of a link between brood parasitism and egg androgens. In an evolutionarily dynamic system such as brood parasitism, strategies and counterstrategies are co-evolving continuously (Soler & Møller, 1990). If an increased egg androgen level is selected in parasites, there would be selection for hosts to evolve in the same direction. A stringent test of this hypothesis should thus take into account the evolutionary history of the parasitic relationship. Thus, we could expect a positive correlation between host egg androgen and the amount of time that host species have been exposed to parasitism. Reversing the argument, it is expected that populations with a long history of brood parasitism would have higher egg androgen levels than populations that have recently started to be parasitised.

#### COSTS OF EGG ANDROGEN

So far, we have mentioned how egg androgen can benefit nestling fitness, but the patterns of differential allocation and covariance with female quality described above suggest that it must carry some costs. Several possibilities can be considered, either in the female or the offspring:

(1) **Immune depression.** Several lines of evidence suggest that androgens and glucocorticoids (Folstad & Karter, 1992; Da Silva, 1999) have negative effects on the immune system. This may limit the amount of hormones that females may allocate or those that offspring may tolerate. It has been proposed (von Schantz *et al.*, 1999) that this immune depression may be mediated by the impairment of the enzymatic antioxidant defences or the induction of oxidative stress in various tissues by androgens and glucocorticoids (e.g. Chainy *et al.*, 1997). One experimental study has showed that higher androgen concentration in the egg induces higher levels of corticosterone in the nestling plasma (Sockman & Schwabl, 2000). As corticosterone has negative effects in immunocompetence, this may limit the level of androgen deposition. Such a pattern could explain the careful way females use this resource

with respect to male ornamentation, as offspring fathered by attractive males have a stronger immune response (Johnsen *et al.*, 2000).

(2) **Metabolic costs.** It has been proposed that elevated levels of egg androgen may increase nestling metabolic costs (Eising *et al.*, 2003b). A recent experimental test of this hypothesis in the Black-headed Gull suggests that this is unlikely to be the case (Eising *et al.*, 2003b).

(3) **Impairment of female reproduction.** Experimentally increased testosterone levels in females reduce or stop several breeding activities such as nest building, laying or incubation (Searcy, 1988; Nelson, 2000). This constraint may impose a physiological maximum to egg androgen deposition, although several mechanisms may be used to limit the effects of plasma androgens (receptor down-regulation, hormone metabolism by specific enzymes, etc.).

(4) **Predation constraints.** Nestlings that beg more often or louder are at greater risk of predation (Haskell, 1994). If egg androgen affects this component of begging, high egg androgen allocation would be selected against in populations with high predation risk.

(5) **Maladaptive sibling aggression.** Although siblicide might be adaptive in some situations, in most species siblings seem to benefit from showing moderate levels of overt aggression (Mock & Parker, 1997). High levels of egg androgens may lead to maladaptive brood reduction and this may limit benefits brought about by faster growth. Across-species, nestling growth rate and begging intensity increase with increasing levels of multiple paternity (Briskie *et al.*, 1994; Royle *et al.*, 1999). In the same line we can predict that egg androgen concentrations should increase when there is a high probability of non-related offspring sharing the same nest.

(6) **Sex optima trade-offs.** As mentioned before, the effect of egg androgen might be sex-specific. This would constrain females producing broods of mixed-sex to trade-off costs and benefits accrued by the two sexes.

#### CONCLUSION

Studies of a single species can do little to shed light on the costs and benefits that have shaped the evolution of characters that are wi-

dely shared phylogenetically (Stearns, 1992). Only comparative studies encompassing a large number of species can provide a thorough understanding of the selective forces that underlie the evolution of these traits. In the case of egg androgens, preliminary data show that differences between species are quite large, not only in concentration but also in the type of androgen. In some species most egg androgen is androstenedione (Cattle Egret: Schwabl *et al.*, 1997), whereas in others it is testosterone (Common Canary: Schwabl, 1997). Differences in concentration also make up for a large part of the variation, just compare two closely related species such as the Canary (20-80 pg/mg: Gil *et al.*, *in press*) and the Zebra Finch (2-6 pg/mg: Gil *et al.*, 1999).

To conclude, androgen deposition in eggs can be considered a flexible maternal effect that females can use to modify offspring phenotype. Recent research has suggested a number of selective forces that may have shaped this trait, as well as several ecological and physiological constraints. Although there is ample scope for adaptive patterns of this particular maternal effect, further experiments should ascertain its benefits and constraints. Particularly necessary are studies considering long-term effects, as well as comparative analyses across a wide phylogeny. It is expected that research in this area will experience a major surge in the following years.

ACKNOWLEDGEMENTS.—I am the recipient of a Ramón y Cajal fellowship from the Spanish Ministry of Science. My research is funded by the project BOS2002-00105 from the same organism. I would like to thank Elena Bulmer, Pedro Cordero, Jeff Graves, Lucy Gilbert, Isabel López, Juan Moreno, Kevin Pilz, Joanna Rutkowska and Javier Viñuela for providing insightful comments on this paper.

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[Recibido: 10-10-03]

[Aceptado: 10-11-03]