Hormones in Avian Eggs: Physiology, Ecology and Behavior

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I. Introduction

Although the transfer of hormones in birds from mother to offspring via eggs had long been suspected (Riddle and Dunham, 1942), Schwabl's study was the first conclusive evidence showing that eggs contained important and variable amounts of maternal androgens, and that these androgens were important for offspring development (Schwabl, 1993). This discovery quickly bridged the gap from endocrinology to behavioral ecology, and led to a highly productive line of research. A good measure of the evolution of the field can be obtained by counting the number of papers dealing with egg androgens that have been covered in the different reviews published to date: 16 in 1997 (Schwabl, 1997b), 32 in 2003 (Gil, 2003), 51 in 2005 (Groothuis *et al.*, 2005b), and over 125 in the present review. This increase has widened the field considerably and new research has called into question initial assumptions, something that seems a good justification for the present review. Most research so far has centered on egg androgens, mainly testosterone (T), androstenedione (A4), and 5α-dihydrotestosterone (DHT), and therefore this review will follow this bias. However, recent studies have found an interesting role for other hormones, such as corticosterone (CORT) (Love *et al.*, 2005), and the door is not closed to further additions.

Why are avian egg hormones that interesting? In my opinion, there are three main characteristics that motivate their appeal to behavioral ecologists: (1) egg hormones are hidden female contributions to offspring, a sort of cryptic decision that challenges previous ideas about the impossibility of females tampering with a sealed developing embryo; (2) benefits of androgens are often balanced by costs, such as those related to the immune response (Folstad and Karter, 1992), and this trade-off has a strong appeal for evolutionary ecologists, used to the concepts of investment, costs, and fitness

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returns; and finally; and (3) yolk hormones have been shown to affect phenotypes in the long-term, and this makes them excellent candidates for the study of adaptive phenotypic plasticity in the context of evolutionary processes, a growing area of research in the last decade (West-Eberhard, 2003).

One particular problem for this area of research is that most studies are done by behavioral ecologists, eager to interpret patterns of variation in the light of evolutionary processes (Carere and Balthazart, 2007), but rather limited in understanding the physiological mechanisms. In contrast, the field has not enjoyed much attention from hard-core endocrinologists, thus leading to a situation where there is a plethora of ecological experiments and measurements, against a vacuum of basic studies on endocrine metabolism and mechanisms. There are practically no detailed studies of metabolic pathways, enzymatic reactions, regulation of receptor number or activity, gene expression, or the like. Unfortunately, these are badly needed to allow a proper interpretation of the patterns found by behavioral ecologists. It is hoped that this review could attract the attention of endocrinologists and embryologists about the possibilities of study of the transmission of maternal hormones in birds.

From an evolutionary perspective, hormones in eggs are a typical case of maternal effects. The concept of "maternal effect" refers to the particular source of environmental variance in studies of quantitative genetics that is explained by the fact of being raised by a particular mother (Falconer and Mackay, 1996). In other words, "maternal effects are any aspect of the mother's phenotype that affect her offspring phenotype" (Räsänen and Kruuk, 2007). Maternal effects are not purely environmental sources of variance because maternal behavior is also influenced by genetics; a recent revision concludes that there is widespread evidence for a genetic basis for a wide range of maternal effects (Räsänen and Kruuk, 2007). The current consideration of maternal effects is that they can be used as mechanisms of phenotypic plasticity, facilitating the survival of individuals facing new environments, and thus the establishment of colonizing populations (Mousseau and Fox, 1998). Furthermore, theoretical models have found that maternal effects can have much higher levels of genetic variance at equilibrium than ordinary genes, allowing an accelerated pace of adaptation to novel environments (Wade, 1998). However, maternal effects can also constrain or reverse evolutionary change, depending on the sign of the genetic covariance between maternal and offspring traits (Kirkpatrick and Lande, 1989).

II. PHYSIOLOGY

Maternal hormones can get into the egg either by a direct incorporation from the steroidegenic cells that surround the follicle while the yolk is being formed or through diffusion from blood vessels or the female's internal organs before the shell is formed.

In the case of androgens, female birds have two main organs for producing these steroids: the ovary and the adrenal caps (Nelson, 2000). Evidence shows that most of yolk androgen that is found in an egg is created locally (i.e., at the follicle), whereas blood circulating androgens have been considered to have a minute impact (Hackl *et al.*, 2003). However, since both systems likely influence each other (see Section II.B), and optimal androgen levels for females and eggs may not always coincide, this can lead to situations where egg hormone deposition may depart from an optimal allocation.

A. STEROIDOGENESIS

For obvious reasons, most research in this field has been conducted in the domestic chicken. It is difficult to know whether reproductive patterns found in that species are universal or particular to the Galliformes. In fact, recent work on the house finch shows that things can be dramatically different between species (Badyaev *et al.*, 2006a). Thus, the generality of the physiological patterns described here should be regarded with caution until comparative evidence is available.

Early female chick embryos possess two ovaries but, as development proceeds, the right ovary regresses so that, in adult birds, only the left ovary is functional [with the exception of the Falconiformes in which both ovaries are functional (Romanoff and Romanoff, 1949)]. The number of oocytes in the chick embryo also changes through development, reaching around 480,000 at hatching time (Johnson, 1999). However, in the adult hen only a few hundred of these reach maturity. The functionally mature ovary of a domestic hen is arranged with a clear hierarchy of follicles of different sizes that correspond to precise developmental stages (Johnson, 1999). This hierarchy is, however, not a universal pattern, and studies in budgerigars (*Melopsittacus undulatus*) and in house finch (*Carpodacus mexicanus*) show synchronous development of many oocytes (Badyaev *et al.*, 2005; Hutchison, 1977).

Bird species can be divided into two different groups, depending on whether clutch size is determined or undetermined (Kennedy, 1991). This difference can be observed externally because, in indeterminate layers, females will either reduce or increase their clutch size in response to the experimental addition or removal of eggs before the end of the clutch. On the contrary, in determinate layers, clutch size is not affected by manipulation and a fixed number of eggs is laid. These two patterns of behavior correspond to very different physiological mechanisms in females (van Tienhoven, 1961).

External factors such as day length, temperature, food, behavior, or social status provide the trigger for oogenesis and ovulation. Responsiveness to photoperiod is different between species and between populations of the same species (Lambrechts *et al.*, 1996; Silverin *et al.*, 1993). In passerine species of the temperate zone, ovarian growth can be induced by increasing photoperiod alone, but other cues such as presence of a nestbox (Hutchison, 1977) or male song (Morton *et al.*, 1985) greatly contribute to this development. It has been proposed that cascade-like effects of prolactin secretion may regulate most avian reproductive effort including clutch size, androgen deposition, and hatching asynchrony (Sockman *et al.*, 2001, 2006).

The central nervous system integrates the information from external factors and initiates the reproduction process by producing gonadotropin-releasing factor (GnRH) in the hypothalamus (Sockman *et al.*, 2001). This factor regulates the synthesis and sequential release of two gonadotropins: luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the hypophysis, which in turn stimulate gonadal growth and steroidogenesis. The final equilibrium between GnRH release and gonadal steroids is achieved through a series of positive and negative feedback loops (Harvey *et al.*, 1986). Ovulation of a mature follicle is preceded by an increase of LH and progesterone (P) concentrations.

A main process in the formation of the egg is the period known as rapid yolk deposition that allows a quick growth of the small follicles to the stage of large preovulatory follicles. The increase of yolk volume during this period follows a linear progression (Harvey *et al.*, 1986). The length of this process can be studied by looking at the number of concentric rings of yolk (Badyaev *et al.*, 2006a).

The main source of hormones found in avian eggs is the maturing follicles of the female ovary that contain specialized cells that create different hormones during their development (Huang *et al.*, 1979). However, the passage of hormones from the ovary to the egg is not well-understood because hormones found in the egg are not an even sample of concentrations found in the female (Williams *et al.*, 2004).

Follicles consist of concentric layers of tissue that surround the oocyte and yolk. From the outside toward the inside, we first find the *theca externa*, followed by the *theca interna*, the *granulosa* cells, the perivitelline membrane, and the oocyte plasma membrane (Johnson, 1999). Several types of cells in the follicle walls are specialized in the production of steroids (Johnson, 1999). *Granulosa* cells produce progesterone, *theca interna* cells produce androgens, and *theca externa* cells produce estradiol (E) (Huang *et al.*, 1979). Their activity is regulated by LH and FSH, but their response to these regulators depends on the degree of follicle development (Porter *et al.*, 1989).

The *granulosa* cells of the largest preovulatory follicles (F1) are the main source of circulating progesterone (Hernandez Vertiz *et al.*, 1993). *Theca* cells produce both estrogen and androgens, and the production decreases with increasing follicular maturation (Etches *et al.*, 1981). A comparison of T production in *theca* cells throughout the follicle hierarchy shows that production increases gradually during rapid yolk deposition, and then diminishes until becoming undetectable at the final preovulatory stage (Marrone and Hertelendy, 1983). The cells of the *theca externa* have an important steroid metabolizing activity, harboring enzymes that allow the conversion of A4 into T and E and of T into A4 (Hernandez Vertiz *et al.*, 1993). Postovulatory follicles continue to produce steroids before final atresia, and their low androgen production during incubation is brought about by a decrease in LH stimulation rather than by a reduction of the steroidogenic capacity of the *theca interna* cells at that stage (Rodriguez Maldonado *et al.*, 1996).

Although these processes are shared by all oocytes, there is variation for any given clutch in how many oocytes develop at the same time (Fig. 1), thus influencing the degree of independence of steroid deposition per oocyte (Badyaev *et al.*, 2005).

B. Female Control

Whether yolk androgen deposition is female-controlled or not is a recurrent question in the yolk androgen literature. Although this is obviously a highly relevant problem, it often leads to arguments in which proximal and evolutionary explanations are confounded.

What is actually meant by this question is whether modifications of androgen levels that serve a function in the female's own physiology affect yolk androgen deposition in maladaptive ways. Imagine an ovulating female who is challenged by a rival over a nest site. Androgen levels may increase to allow the female to answer this challenge, and this could be reflected in her yolk androgen levels, thus leading these eggs to contain excessive levels of androgens. This is problematic because the consequences of an "uncontrolled" yolk deposition may not be advantageous for the female's fitness.

It would be surprising if yolk androgen deposition was not buffered against the daily variations of plasma androgens that females may encounter, particularly because yolk androgen deposition does have important offspring fitness consequences. Selection is thus expected to have promoted the evolution of mechanisms to control the system, particularly so because the time of egg production is also characterized by high levels of intra- and intersexual aggression for resources or mates. What is the evidence for this?

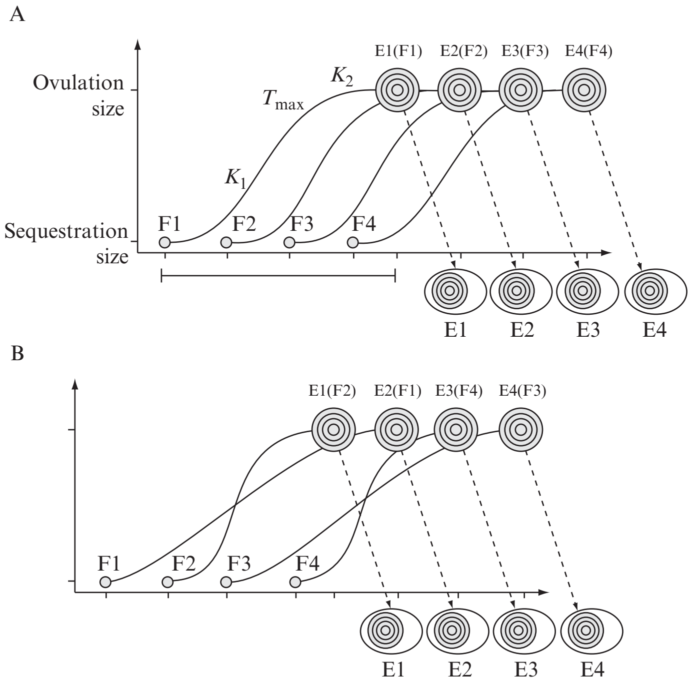

FIG. 1. Schematic illustration of the differences between the two possible patterns of rapid yolk deposition, ovulation, and egg laying: (A) traditional view in which there is a hierarchical order of oocyte sequestration (F1–F4) into the rapid yolk deposition phase that is maintained at ovulation and egg-laying (E1–E4), and in which all oocytes have similar growths; (B) hypothetical view in which there is not a concordance between the growth during rapid yolk deposition growth rate, the order of sequestration (F1–F4) and the order of ovulation (this example: F2, F1, F4, F3). Growing oocytes would differ in the duration of overlap with RYD phase of other oocytes, and variation in growth patterns could expose growing oocytes to distinct maternal hormonal profiles (Badyaev *et al.*, 2005). © 2007 The Royal Society.

We should begin by inquiring about the differential function of increased androgen for females and offspring. Unfortunately, almost the whole existing literature deals with effects in the offspring, and few studies consider the role of this high androgen production during oogenesis on female physiology (Staub and De Beer, 1997). In that respect, we do not know the function of this high androgen production for the female, apart from descriptive studies that relate ovarian androgen production to an internal signaling function within the reproductive cycle of the female (Staub and De Beer, 1997). However, no study so far that I know of has addressed the question of why there should be so much variation in yolk androgens levels, if the function of these is to provide an internal signal of ovulation.

The proportion of different steroids in the plasma levels of the ovulating female (estradiol, corticosterone, androgens) is not mirrored by egg deposition levels, showing that there is not a simple passive mechanism from plasma to egg (Williams *et al.*, 2004). That plasma levels do not have a major influence on follicle levels is further suggested by experiments involving direct bolus injections of T in laying females. These studies have detected only minute increases in yolk T levels with respect to the injected amount (Hackl *et al.*, 2003; Rutkowska *et al.*, 2005), although it is possible that these studies do not properly mirror the consequences of a continuously heightened plasma concentration. As things stand now, a positive correlation between plasma and yolk androgen levels as found in some studies (e.g., Schwabl, 1996b) could be due to either: (1) follicle steroidogenesis indirectly affecting plasma levels after local follicle production or (2) a higher-order steroid production control (hypothalamic GnRH secretion) affecting in the same direction both follicle and adrenal steroid production. The latter possibility is, however, not supported by a recent study that shows that GnRH injections only increase T levels in females in the week preceding oviposition, and that these levels are positively correlated with yolk T levels (Jawor *et al.*, 2007). This suggests that female androgenic responsiveness to external stimuli during oogenesis will be reflected in their yolk androgen concentration, but that androgen production outside this time is not controlled by GnRH.

The possibility for independent control (or even a negative feedback loop) between the two androgen production centers of the incubating female (follicle and adrenals) is not unexpected. For instance, a decoupling of plasma and yolk androgen levels has been shown in the canary, in which increases in plasma androgens did not result in a parallel increase in yolk levels (Marshall *et al.*, 2005). Similarly, a negative relationship between yolk T and plasma T levels after laying were found in the house sparrow (Mazuc *et al.*, 2003a), and an experimental increase of aggression in female lesser black-backed gulls failed to increase yolk androgen levels (Verboven *et al.*, 2005). In contrast, studies using permanent T implants have led to parallel increases in plasma and yolk T levels (Clotfelter *et al.*, 2004).

If aggressive behavior is modulated by androgen levels in females and females cannot decouple follicle androgen production from plasma levels, eggs laid after females experience aggressive situations would have excessively high levels of yolk androgens. A study showing a positive correlation between aggressive encounters and yolk androgen levels in tree swallows (*Tachycineta bicolor*) (Whittingham and Schwabl, 2002) suggested that females cannot wholly control what goes into their eggs. The problem with these correlative data is that females that take part in frequent aggressive encounters may be different in many respects from less aggressive females, and thus the relation between aggression and yolk T need not be a causal one. An experiment in which eastern bluebirds (*Sialia sialis*) were exposed to same sex intruders during laying brought about the unexpected finding that experimental females had lower levels of plasma androgens a day after the intrusion, whereas the yolks of the eggs laid in the interim contained higher levels of androgens (Fig. 2; Navara *et al.*, 2006c). The interpretation of this study is not straightforward. On the one hand, it could be said that it shows that females cannot control the levels of androgens that go inside their eggs. However, the authors suggest that females actively use yolk T deposition to regulate circulating levels, using the egg as a sink of excessive hormones. It is, however, strange to think of the generality of such a regulatory mechanism, because it could only be used for a very short period of the female's life. There are many ways to deal with high T levels, including receptor regulation or production of enzymes, but if high levels are not needed, the easiest solution would be not to produce them at all (Wingfield *et al.*, 1990).

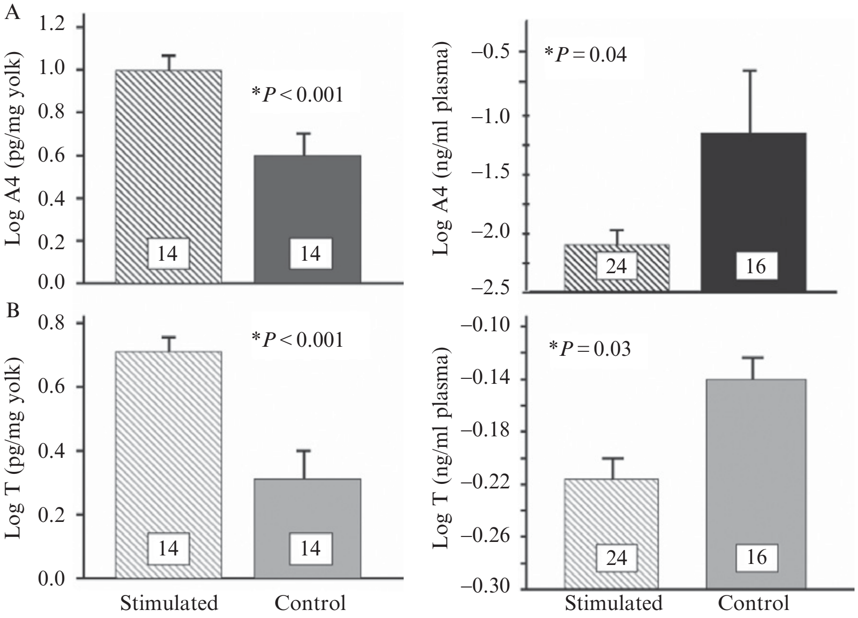

FIG. 2. Contrasting differences between levels of circulating androgens and yolk androgens in a study in bluebirds. Data in the first graph show yolk A4 (A) and T (B) values for eggs laid by stimulated and control females. Hatched bars, eggs in stimulated groups; solid bars, eggs in control groups. Data in the second graph show similar values for female hormone concentrations (Navara *et al.*, 2006c). © 2006 Blackwell Publishing.

Furthermore, the role of androgens in regulating female aggressive behavior is poorly understood (Ketterson *et al.*, 2005; Staub and De Beer, 1997). Some studies suggest a link between female T levels and intrasexual aggression (Cristol and Johnsen, 1994; Langmore *et al.*, 2002; Zysling *et al.*, 2006), but the evidence is by no means unequivocal (see for instance: Elekonich and Wingfield, 2000). On the other hand, evidence shows a link between T and coloniality: there are higher T levels in colonial species (Møller *et al.*, 2005) and a positive relationship between T levels and colony size in the cliff swallow has been found (Smith *et al.*, 2005). More studies such as that by Navara *et al.* (2006c) are badly needed to ascertain whether and how ovulating females can canalize hormones to the two possible targets (female and egg) and, if not, how do they deal with the consequences of such a lack of control.

III. Effects of Yolk Androgens

The study of how offspring characteristics are affected by yolk hormones is typically undertaken by comparing the effect of injections of androgens dissolved in some lipophylic solvent in the yolk with injections of the solvent as controls (Groothuis and von Engelhardt, 2005). This is by far the best methodology since using hormone modifications in the female to modify the egg's androgen content (Rutkowska and Cichon, 2006) may cause additional changes in egg composition that could confound the results. Because egg androgens covary with a series of other egg components (e.g., Groothuis *et al.*, 2006), correlative studies relating nestling characteristics to natural variation in yolk androgen deposition are not reliable tests of the function of yolk androgens (Gil *et al.*, 2006c; Schwabl, 1993).

However, the methodology used in manipulative studies varies widely, particularly in the dose and the androgens injected. Although these studies do not use pharmacological doses, these can still vary widely. A recent study in house finches has detected dose-dependent responses to injections (Navara *et al.*, 2005), reinforcing the need to carefully control this aspect, since most likely there will be dose-dependent responses in other species.

Little is known of the metabolic route followed by maternal androgens in the embryo. Although there are androgen receptors in embryos very early in development (Godsave *et al.*, 2002), we know very little of what happens with yolk androgens once incubation starts. Some authors have detected incredible fast reductions in androgen concentration after some hours of incubation (Elf and Fivizzani, 2002), suggesting strong metabolic changes in the yolk before the embryo was formed. However, a recent study in the zebra finch (*Taenyopigia guttata*) has found that this effect is likely due to dilution of yolk with albumin and that changes due to androgen metabolizing or production only start when embryos are 3–5 days old (the zebra finch has a 12-day incubation period) (Gilbert *et al.*, 2007). To my knowledge, no embryological study so far has followed radioactively labeled yolk androgen to identify target tissues or metabolic routes, and neither gene expression has been considered. These studies are badly needed if we wish to know the mechanisms of yolk androgen action. Furthermore, patterns of differential gene expression could direct future research into possible phenotypic long-term effects.

A. Short-Term Effects in Nestlings

1. *Development*

A large number of studies in a variety of species has found that nestlings hatching from androgen-injected eggs have shorter incubation periods, higher growth rates, or larger body masses than controls (Eising *et al.*, 2001; Navara *et al.*, 2006a; Pilz *et al.*, 2004; Schwabl, 1996a; Tschirren *et al.*, 2005). For instance, in the black-headed gull (*Larus ridibundus*), yolk androgens led experimental chicks to hatch a day earlier than controls and to outcompete their fellow mates in body mass and tarsus length (Eising *et al.*, 2001).

Most of the above studies used an experimental design nested within clutches, so that differences in nestling growth between treatments could be due to competition between siblings and to a direct effect of androgen in growth. However, a recent study in the spotless starling (*Sturnus unicolor*) has confirmed a similar trend in a between-clutches context, suggesting that growth benefits need not derive from competition between nestlings, but rather from direct growth effects or manipulation of parental feeding rates (Müller *et al.*, 2007a).

Development involves the coordinated growth of the whole body, but yolk androgens could affect this balance by prioritizing the growth of some structures over others. The development of the *musculus complexus*, the muscle that allows nestlings to break the shell at hatching, and later on to outcompete their siblings in stretching their necks while begging, is directly related to the levels of androgens to which the embryo has been exposed in the red-winged blackbird (*Agelaius phoniceus*) (Fig. 3; Lipar and Ketterson, 2000). Similarly, in the spotless starling, it has been shown that chicks hatching from androgen-injected eggs have wider gapes than controls (Müller *et al.*, 2004). This mechanism can be used to attract a disproportional share of parental feedings. For instance, a similar differential development of the gape flange has been found in runts in situations of strong sibling competition (Gil *et al.*, 2008a).

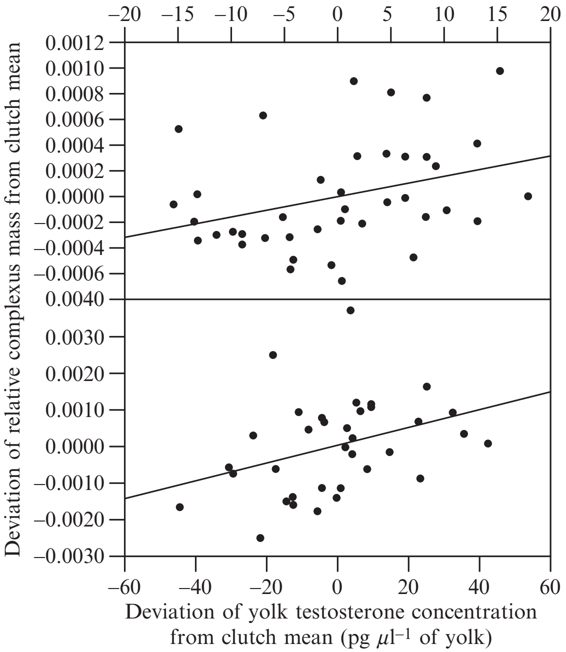

FIG. 3. Data showing how the growth of the "begging muscle" in red-winged blackbirds is influenced by yolk T. Graph shows the deviation of relative complexus mass from the mean of its clutch versus deviation of yolk T concentration from the mean of its clutch (Lipar and Ketterson, 2000). © 2000 The Royal Society.

Significant effects of yolk androgens on growth have, however, not always been found in nestlings (Rubolini *et al.*, 2006a; Tobler *et al.*, 2007b), and indeed some studies have identified negative effects on growth. For instance, in American kestrels (*Falco sparverius*), growth was severely impaired when eggs in early laying positions were injected with androgen levels that made them resemble late-laid eggs (Sockman and Schwabl, 2000). Similarly, posthatching body mass in yellow-legged gulls (*Larus chachinans*) was decreased in nestlings hatching from T-supplemented eggs (Rubolini *et al.*, 2006b).

2. *Begging*

Some studies have shown that begging (the probability of a nestling begging or begging intensity) is higher for nestlings hatching from androgen-injected than control eggs (Eising and Groothuis, 2003; Schwabl, 1996a). For instance, in the black-headed gull, chicks hatching from androgen-injected eggs have higher activity levels, react earlier and for longer to parental visits, and managed to obtain more food than their control siblings (Eising and Groothuis, 2003). Thus, in a within-nest situation, differences in androgen supplementation can affect nestling hierarchies caused by differential incubation patterns.

However, Pilz *et al.* (2004) found that, in the European starling (*Sturnus vulgaris*), none of a comprehensive list of measures of begging effort and intensity were affected by yolk T manipulation.

The young of some precocial birds produce embryonic vocalizations shortly before hatching, which are important for communicating the chick's developmental process to its parents and thus synchronizing parental behavior accordingly (Brua, 1996). A study in the yellow-legged gull has shown that embryos in T-injected eggs produce louder vocalizations than controls (Boncoraglio *et al.*, 2006), although no further differences in nestling begging were detected.

Further studies are needed on the effects of yolk androgens on begging effort because this type of effect would imply that the costs of parental care could be higher for parents feeding chicks hatching from eggs with high levels of androgens (Moreno-Rueda, 2007; Müller *et al.*, 2007b), either because of higher necessities of these nestlings, or because their behavior breaks the "golden rule" of honest begging (e.g., Rodríguez Gironés *et al.*, 1996).

3. *Metabolic Rate*

One possible source of costs of the increased growth promoted by yolk T could be higher energy expenditure in nestlings. An increased basal metabolic rates in nestlings hatching from T-injected eggs has been found in zebra finches (Tobler *et al.*, 2007b), but not in black-headed gulls (Eising *et al.*, 2003b). It is interesting to note that the detected basal metabolic rates increase in the zebra finch study was not linked to higher growth, suggesting that the costs of higher yolk T may depend on internal physiological processes not detected in external growth measurements.

4. *Immunocompetence*

The immunosuppressive effect of androgens is a well-known phenomenon (Folstad and Karter, 1992) that is applied in medicine to clinical situations in which it is necessary to either boost or depress the immune system (Bagatell and Bremmer, 2003). For instance, autoimmune diseases such as the rheumatoid arthritis can be controlled by androgen replacement therapies (Cutolo *et al.*, 1991). Numerous studies in behavioral ecology have addressed the question of whether androgen-mediated ornamentation is costly to bearers because of the immunosuppression caused by androgens (for a review see: Roberts *et al.*, 2004). Following the same logic, one of the first possible constraints on androgen–yolk allocation that was proposed was that of immunosuppression (Gil *et al.*, 1999).

In the black-headed gull, Müller *et al.* have shown immunosuppression of both the cell-mediated (Fig. 4) and the humoral components of the immune response in nestlings hatching from androgen-injected eggs (Groothuis *et al.*, 2005a; Müller *et al.*, 2005b). The presence of these negative effects together with possible growth benefits (see above) suggests that the final outcome of high yolk androgen exposure will depend on the posthatching environment of the developing offspring, including factors such as parasite exposure, degree of competition, or food availability. Differences in the strength of these factors might explain why immune costs could not be identified in a similar study in the great tit (*Parus major*) (Tschirren *et al.*, 2004). In another example, injection of high T levels in eggs did not result in a decrease in immunocompetence in the Chinese painted quail (*Coturnix chinensis*) (Andersson *et al.*, 2004). However, in this case, T treatment affected the positive covariance between cell immune response and growth, suggesting that T was affecting the normal developmental balance of the animals (Andersson *et al.*, 2004).

Finally, a study in house finches has provided the only evidence so far of positive effects of T egg treatment in nestling cellular immunocompetence (Navara *et al.*, 2006a). To explain these results, Navara *et al.* propose

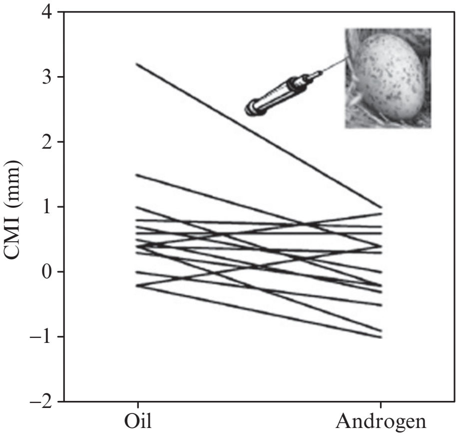

Fig. 4. Costs of high yolk T levels for offspring. Cell mediated immune responses of black headed gull chicks hatching from eggs injected with oil (oil) or androgens dissolved in oil (androgen). Lines connect matched pairs of chicks raised in the same foster nest (Groothuis *et al.*, 2005a). © 2005 The Royal Society.

that immunocompetence would only be a problem for chicks from T-supplemented eggs when resources are scarce. This hypothesis assumes that immunodepressive effects of T are caused by competition for resources and not because of direct modulation of the immune response via specific receptors (Ahmadi and McCruden, 2006).

5. *Survival*

Nestling survival has been shown to improve by egg androgen treatment in three studies, showing that T-chicks are less likely to die or die later than control chicks (Eising and Groothuis, 2003; Müller *et al.*, 2007a; Pilz *et al.*, 2004). In contrast to these results, a study in the American kestrel showed that, when eggs laid in the first-laying positions are injected with androgen doses designed to increase their levels to late-laying positions, experimental nestlings are less likely to survive than controls (Sockman and Schwabl, 2000).

6. *Hormone Levels*

Circulating androgens in nestlings have been linked to variation in sibling competition, begging, and development (Fargallo *et al.*, 2007; Gil *et al.*, 2008b; Goodship and Buchanan, 2006; Naguib *et al.*, 2004). Sometimes, it is happily assumed that differences in nestling plasma T are caused by differences in yolk androgens (Sasvari *et al.*, 1999). This link, however, has not been shown until recently in a study in the spotless starling (Müller *et al.*, 2007a), in which male and female nestlings hatching from androgen-injected eggs had higher T levels than control birds. This effect reinforces the notion that not only growth, but also nestling physiology is affected by yolk hormone variation.

7. *Sex-Specific Effects*

Three different studies have found sex-specific benefits of higher T allocation in eggs. Remarkably, the effects differ depending on the species: in the zebra finch, males seem to suffer in terms of growth by this higher allocation (Rutkowska and Cichon, 2006; Rutkowska *et al.*, 2007; von Engelhardt *et al.*, 2006), whereas in the barn swallow, the effect is inverted, and females suffer while males benefit (Fig. 5; Saino *et al.*, 2006). Interestingly, in the zebra finch study, the original sex differences in begging present in the control group were abolished in the experimental treatment (von Engelhardt *et al.*, 2006). This implies that sex-specific androgen effects can be used by females to balance their sex-specific investment. However, sex-specific effects are not always present (e.g., Eising *et al.*, 2006; Müller *et al.*, 2004). This variance

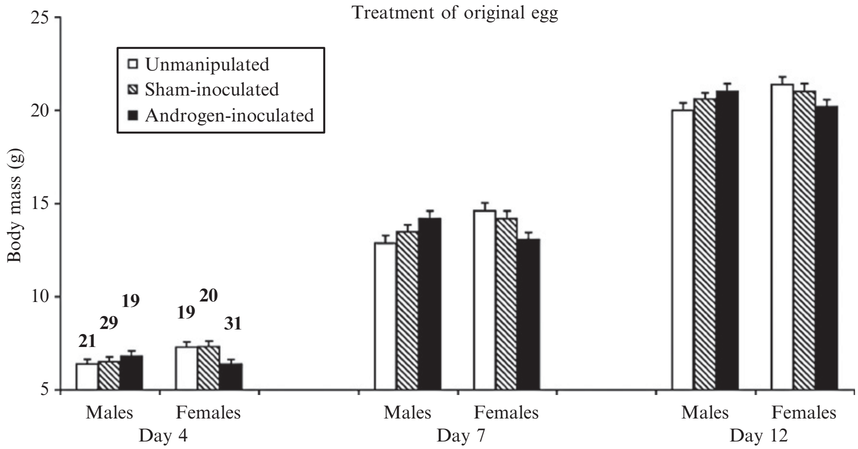

FIG. 5. Sex-specific yolk androgen effects in barn swallows: least square means of body mass at different ages (Saino *et al.*, 2006). © 2006 Oxford University Press.

could be due to differences in development of sexual dimorphism pathways, but too little data are available yet to conduct a comparative analysis of this aspect.

Few studies have examined the role of androgens in development through a reduction of the androgen effects rather than an enhancement. To do this, it is possible to block androgen receptors with an antiandrogen (for instance, flutamide), thus stopping receptor-mediated androgen action. Müller *et al.* (2005a) have used this method, uncovering an interesting sex×treatment interaction. In a study conducted in the black-headed gull, egg antiandrogen treatment resulted in a sexually distinct effect: an increase in male growth rate and a decrease in female growth and cell immune response (Müller *et al.*, 2005a). Although not easy to interpret, this study lends support to the notion that developmental pathways of the two sexes including trade-offs between immunity and growth are affected by androgens, thus setting the stage for a complex benefit/costs dynamics of androgen allocation.

This type of sexually antagonistic effects of yolk androgens has likely led to mechanisms of sex-specific androgen exposure, as shown in several studies in the house finch (e.g., Young and Badyaev, 2004). However, since within-clutch variation in yolk androgen levels is typically much lower than variation between-females, females are restricted in the range of possible sex-specific androgen quantity that they can deposit in their eggs (Saino *et al.*, 2006). Such a constraint would be expected to lead to the evolution of highly biased or unisexual sex-ratios, as found in some populations of the barn swallow *Hirundo rustica* (Saino *et al.*, 2006).

8. *Appraisal of Short-Term Effects*

There are several factors that could account for differences in the direction of effects (negative vs positive) between experiments.

1. Yolk androgen allocation is expected to respond to parent–offspring and parental conflicts (Müller *et al*., 2007b), and thus different species may find themselves at different evolutionary optima. Therefore, different effects would be gathered depending on whether, for instance, androgen deposition is already at a maximum or medium level. Since phylogeny can explain some patterns of androgen deposition (Gil *et al*., 2007), a way to control for this possibility would be to compare the effect of increased yolk androgens in similar species with low and high androgen levels for their taxonomic clade.
2. There are large differences between studies in the dose and type of androgens injected. Since dose-dependent interactions may shift the balance from benefits to costs (Navara *et al*., 2005), experiments should in the future work with several doses (preferably moving away from the population mean in small steps: 1 SD, 2 SD, . . .).
3. It is likely that yolk androgens may require a particular balance with other egg components (e.g., carotenoids, immunoglobulins). Because these components often vary with the laying order (Royle *et al*., 2001), it has been proposed that T injections using either the first or the last egg of a clutch may bring about completely different effects on growth (Sockman *et al*., 2006).
4. Differences in nestling growth due to yolk androgens may only be detected in situations when food is severely reduced (Pilz *et al*., 2004), and some studies may thus be suffering from a ceiling effect, in which a faster growth cannot be further induced when food is plentiful.
5. Finally, the fact that females increase androgen allocation to eggs fathered by attractive males may be interpreted in the sense that benefits of yolk androgens could be limited to offspring with certain phenotypic or genotypic quality (Gil *et al*., 1999). Sex-specific effects would be such a kind of genotype×environment interaction (Saino *et al*., 2006). These types of interactions are common in many maternal effects (Mitchell and Read, 2005), but to my knowledge no study so far has directly addressed this possibility as far as yolk androgens are concerned.

B. LONG-TERM EFFECTS ON THE OFFSPRING

Most research on the effects of yolk androgens has focused on the early stages of development of the young bird. However, the results of a handful of studies that have followed birds until adulthood have revealed an impressive persistence of effects of yolk androgens in adulthood. The mechanisms by which these effects are expressed in adulthood have not yet been studied. Two main possibilities are: either a priming effect in adult hormone secretion or a modification of androgen receptiveness in target tissues (Strasser and Schwabl, 2004). However, it has been recently suggested that this type of organizational effects of hormones may actually be indirect effects of early social experience (Carere and Balthazart, 2007). This means that the different social experiences that nestlings experience in the nest as the result of differential androgen exposure would be the cause of adult behavioral differences. Although this is certainly possible, in the case of behavioral effects, it is more difficult to envisage how indirect effects of maternal androgens could play a role in ornament determination, suggesting that the most parsimonious explanation is one of organizational effects on androgen-dependent physiology.

It is in the long-term context that the most exciting trade-offs of yolk androgen deposition are most likely to be detected and variation in this maternal effect is expected to relate to variation in life-history traits (Stearns, 1992). Long-term studies are badly needed to understand the functional significance of variation in yolk hormone deposition.

1. Behavior

The first effect of yolk androgens to be discovered was on adult dominance (Schwabl, 1993): canaries (*Serinus canaria*) hatching from eggs with high natural yolk levels had higher dominance ranks than birds hatching from low yolk T levels. Similarly, yolk T egg injections increased dominance in male and female house sparrows (*Passer domesticus*) defending a food source (Strasser and Schwabl, 2004). In the black-headed gull, a similar experiment led to an enhancement of aggressive and behavioral displays that are used in competition for food or mates or space (Fig. 6; Eising *et al.*, 2006).

Personalities, or behavioral syndromes, are suites of behaviors that tend to appear together in individuals, within or between environmental or social contexts (Sih *et al.*, 2004). Very often, the main axis of variation in personality is described by a bold-shy continuum, the animal equivalent of the human variation in extroversion (Wilson *et al.*, 1994). For instance, great tits can be classified while exploring a new environment into fast versus slow explorers (Verbeek *et al.*, 1994). These individual differences are heritable, and their maintenance in populations are preserved through antagonistic selection regimes in different years and in different sexes (Dingemanse *et al.*, 2004; Drent *et al.*, 2003).

Several studies have found significant effects of prenatal steroids in behavioral syndromes. For instance, high prenatal exposure to androgens

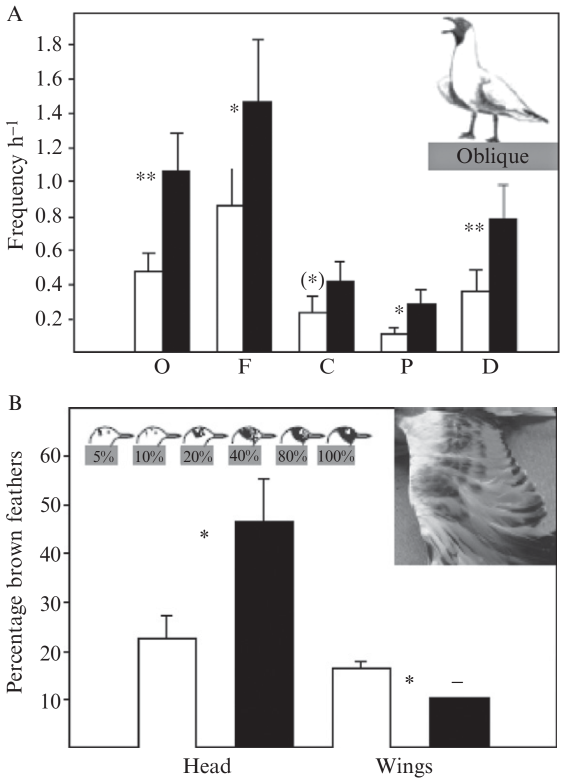

FIG. 6. Long-term effects of yolk androgen in behavior and ornamentation in the black-headed gull: (A) enhanced social behaviors (frequency) and dominance in birds injected with androgens (filled bars) compared with birds from eggs injected with oil (open bars). O, oblique display, F, forward display, C, Charge, P, aggressive peck, D, displacement; (B) stage of the nuptial brown head plumage and the juvenile characteristics of brown feathers on the wing in 10-month-old gulls (Eising *et al.*, 2006). © 2006 The Royal Society.

induces bold and proactive behaviors in quail, irrespective of sex (Daisley *et al.*, 2005). In this case, experimental birds took a shorter time while performing exploratory tasks, produced fewer distress calls, and were less likely to exhibit tonic immobility. Tobler and Sandell (2007) have shown that zebra finches that hatch from T-injected eggs have shorter latencies to approach and to eat when confronted with a novel food situation at 9 months of age. However, in this study, the introduction of a novel object in the set-up was found to elicit stronger neophobic responses in T-males than in controls. This experiment thus suggests an organizing role of yolk androgens on adult behavior (Tobler and Sandell, 2007). Since the behavior of experimental birds is reminiscent of the effects of T treatment in the behavior of adult birds, these authors suggest that the effects may be caused by similar mechanisms, that is increased androgen activation in adulthood.

The above evidence backs up suggestions for a physiological basis of individual differences in ways of coping with the environment (Koolhaas *et al.*, 1999). The enormous plasticity of yolk androgen levels suggests that females could use these modifiers in order to fine-tune their offspring phenotypes to match current selective regimes, to the extent that these can be predicted from environmental or social cues.

2. *Ornaments*

Although sexual differentiation takes place in the early embryo by means of processes based on estradiol (Carere and Balthazart, 2007), the development of sexually selected characters in birds is tightly linked to hormonal processes in adulthood, of both androgenic and estrogenic nature (Kimball and Ligon, 1999). Two studies in two different species have reported that individuals exposed to high androgen levels in the egg develop larger ornaments in adulthood. Male house sparrow hatched from T-injected eggs developed larger badges than those hatching from control eggs (Strasser and Schwabl, 2004). Similarly, in black-headed gulls, the same treatment led to a greater development of nuptial plumage at 10 months of age in both males and females, suggesting an acceleration of sexual maturation (Fig. 6: Eising *et al.*, 2006).

However, there is also negative evidence in this aspect of yolk androgen effects. A study in pheasant found negative effects of increased yolk T in spur length in both males and females (Rubolini *et al.*, 2006a), whereas no other male ornament was affected (ear tufts, wattle size, etc.). The remarkable results of this experiment are that, despite this lack of direct effect, the T treatment altered the covariance among these traits, suggesting an effect of prenatal hormones in the regulation of structural genes.

3. *Dispersal*

In a Swiss great tit population, the presence of nest ectoparasites (hen fleas: *Ceratophyllus gallinae*) selects for short dispersal distances (Tschirren *et al.*, 2007), possibly because of local adaptation of hosts to parasite pressure. In this population, it was found that experimentally parasitized females laid eggs with lower androgen concentrations than control females (Tschirren *et al.*, 2004). One possible interpretation of this study was that parasitized females were reducing the cost of androgen-induced immunosuppression in parasitized offspring, because their immune system would be more strongly compromised. However, a follow-up study, conducted on nestlings of the same population, found little evidence for immediate immune costs of increased yolk androgens (Tschirren *et al.*, 2005). The explanation to this paradox was provided in a subsequent study in which Tschirren *et al.* (2007) demonstrated that the dispersal distances of nestlings hatching from androgen-injected eggs were larger than those of control birds. Furthermore, it was shown that the lifetime reproductive success of nestlings hatching from unparasitized nestlings was higher, if nestlings dispersed longer distances. This suggests that females are adaptively manipulating the degree of philopatry of their offspring as a response to levels of parasite infestation.

4. *Other Traits*

No study so far has measured the long-term fitness effects of variation in yolk androgen levels in the wild, and such a study would probably require insurmountable sample sizes. However, there are a number of hypothesis based on life-history trade-offs that such an approach could answer. For instance, it has been proposed that fast growth caused by maternal androgens can have negative consequences for survival (Birkhead *et al.*, 2000). Studies in captivity have provided some clues toward this kind of trade-off, suggesting that yolk T may have an influence on the development of sex-specific male and female reproductive traits. A study in the Chinese quail (*C. chinensis*) shows that birds exposed to relatively high levels of yolk T developed smaller testis in the case of males, and laid smaller eggs in the case of females (Uller *et al.*, 2005). A transgenerational negative effect of yolk T in female breeding performance has also been detected in the form of reduced egg laying activity and egg fertility in female pheasants hatching from high-T eggs (Rubolini *et al.*, 2007).

The suspicion that yolk androgens may interfere with developmental processes is also supported by data showing that digit ratios (i.e., the ratio of the length of the second and fourth fingers) in female pheasant (but not in males) are affected by yolk androgen treatments. Although our knowledge of the genetic and developmental basis of digit ratios in birds is rather limited, this effect suggests that the expression of genes of wide morphological and organizational effects, such as homeobox genes, may be modified by embryonic exposure to androgens (Romano *et al.*, 2005).

C. CONSEQUENCES FOR PARENTS

1. *Physiology*

If we assume that increases in yolk androgen levels lead to increases in plasma androgen, it can be argued that females that deposit relatively higher amounts of yolk androgens in their eggs could be facing a series of costly consequences. Studies that have investigated the consequences of increased androgen levels in females have detected: lower immunocompetence (Duffy *et al.*, 2000), decreased choosiness at selecting mates (McGlothlin *et al.*, 2004), smaller likelihood of developing a brood patch (Clotfelter *et al.*, 2004), reduced clutch size (Fig. 7: Rutkowska *et al.*, 2005), etc. This body of evidence suggests that the female breeding optimum is likely to be one of relatively low T levels. Additionally, comparative analyses show that, although male and female T levels are correlated across species, female T levels are particularly reduced in species in which males have high T concentrations, suggesting antagonistic selection between sexes (Møller *et al.*, 2005; Rutkowska *et al.*, 2005). No study so far has attempted a manipulation of maternal yolk androgen deposition by means of a direct manipulation of follicle steroidogenesis, so all the evidence above is based on an assumed positive relationship between circulating and yolk androgen levels (see Section II.B, for a consideration of this topic).

2. *Behavior*

However, since yolk androgen levels can change nestling begging behavior, size, or activity levels, it is to be expected that parents may pay a higher cost when raising offspring hatching from eggs with high levels of yolk T (Müller *et al.*, 2007b; Winkler, 1993). In fact, it has been suggested that differential allocation of yolk androgens represents attempts at manipulating male parental care (Moreno-Rueda, 2007; Müller *et al.*, 2007b). Recent considerations about sexual conflict over parental care have proposed that females could manipulate paternal contributions by means of a self-imposed

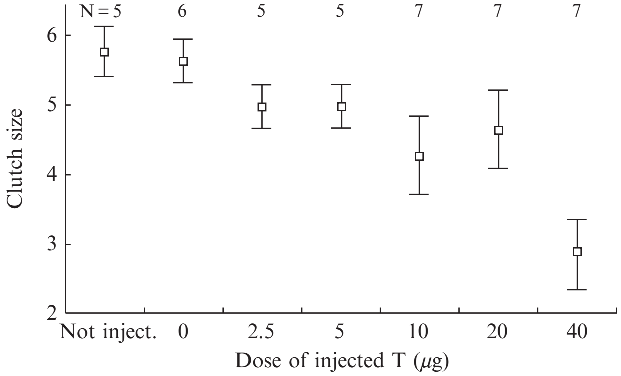

FIG. 7. Negative effects of T for breeding performance in zebra finches. Data are mean clutch sizes of females treated with increasing doses of testosterone (Rutkowska *et al.*, 2005). © 2005 Elsevier Inc.

handicap (Houston *et al.*, 2005), such as a large clutch or high levels of yolk T (Müller *et al.*, 2007b). It is to be expected that differences between sexes in provisioning rules would restrict the use of this manipulating mechanism, but it is also likely that these provisioning rules themselves are sex-specific counter-responses to this conflict (Müller *et al.*, 2007b). A recent study in great tits has addressed the response of parents to yolk androgen manipulation (Tschirren and Richner, 2008). The authors blocked the effects of yolk androgens by means of injections of the androgen-receptor blocker flutamide in experimental broods, and they further conducted a brood size manipulation. They found that both parents fed more enlarged broods, as expected from previous studies. However, paternal feeding rates were not affected by flutamide treatment, whereas females showed lower provisioning rates for enlarged flutamide-injected broods than from enlarged control broods (Tschirren and Richner, 2008). This result suggests that female great tits cannot manipulate male parental care by allocating high concentrations of yolk androgens into their eggs. The authors suggest that female yolk androgen deposition has evolved through a process of coadaptation that matches maternal food provisioning and offspring demand. These results are in line with previous studies that have identified a positive covariance between female-feeding rates and offspring-begging levels (Kölliker *et al.*, 2000) and may provide an explanation for the common finding that males are less sensitive than females to modifications in nestling begging levels (e.g., Kilner, 2002).

IV. Variation Within Clutches

A. Laying Order

One of the first levels of variation that attracted attention was that within-clutches; in canaries, Schwabl (1993) found consistent increases of yolk T levels with laying order (Fig. 8). Since many bird species show asynchronous hatching, in which chicks from late-hatching positions tend to hatch later than those from earlier laying positions, this distribution of androgens was thought to counterbalance the effects of asynchronous hatching (Schwabl, 1993). Indeed, later experiments in which within-clutch differences in yolk T were experimentally manipulated confirmed this explanation (e.g., Eising *et al.*, 2001).

In contrast with this pattern, other species such as the cattle egret (*Bubulcus ibis*) show decreasing levels of androgens with increasing laying order (Schwabl *et al.*, 1997). The function of this distribution would thus reinforce the effects of hatching asynchrony, possibly contributing to brood reduction and siblicide (Schwabl *et al.*, 1997).

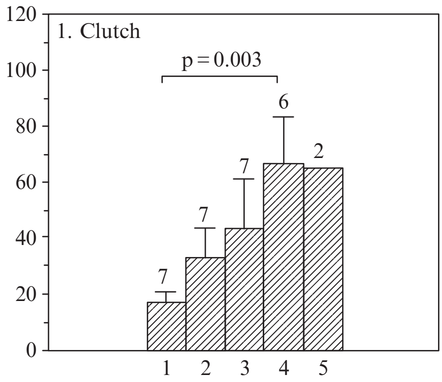

FIG. 8. Increasing concentrations of yolk testosterone with laying sequence in canaries (Schwabl, 1993). © 1993 National Academy of Sciences, U.S.A.

Consistent differences between species in the distribution of yolk androgens with laying order have been found for many species. For instance, increasing patterns have been found for red-winged blackbirds, canaries, or European starlings (Lipar *et al.*, 1999; Pilz *et al.*, 2003; Tanvez *et al.*, 2007), while decreasing trends are typical in zebra finches (Fig. 9), American coots (*Fulica americana*), and all gull species that have been studied (Gasparini *et al.*, 2007; Gil *et al.*, 1999; Groothuis and Schwabl, 2002; Reed and Vleck, 2001; Royle *et al.*, 2001). So far, a comparative study of how these differences in within-clutch distribution relate to patterns of hatching asynchrony or brood reduction has not been conducted.

Although within-brood distributions of yolk androgens can be highly species-specific, many differential patterns can be found within the same species. Given the effects of androgens in chick competitiveness, females could use within-clutch distributions of androgens to balance or reinforce the effects of hatching asynchrony. A study in the house wren (*Troglodytes aedon*) failed to find differences between asynchronous and synchronous hatching broods in the way that androgens vary with laying order (Ellis *et al.*, 2001). However, between-female differences have been found in relationship to a number of variables. For instance, in the pied flycatcher (*Ficedula hypoleuca*), differences are found when comparing first and second broods (Tobler *et al.*, 2007a) and in relationship to female ornamentation (Gil *et al.*, 2006a). In the European starling, A4 increases with laying order for clutches laid by monogamous females, whereas the contrary is true in the case of polygynous females (Gwinner and Schwabl, 2005).

However, it is in gulls where within-brood differences in yolk androgens are the most extreme (Groothuis and Schwabl, 2002), and where the best

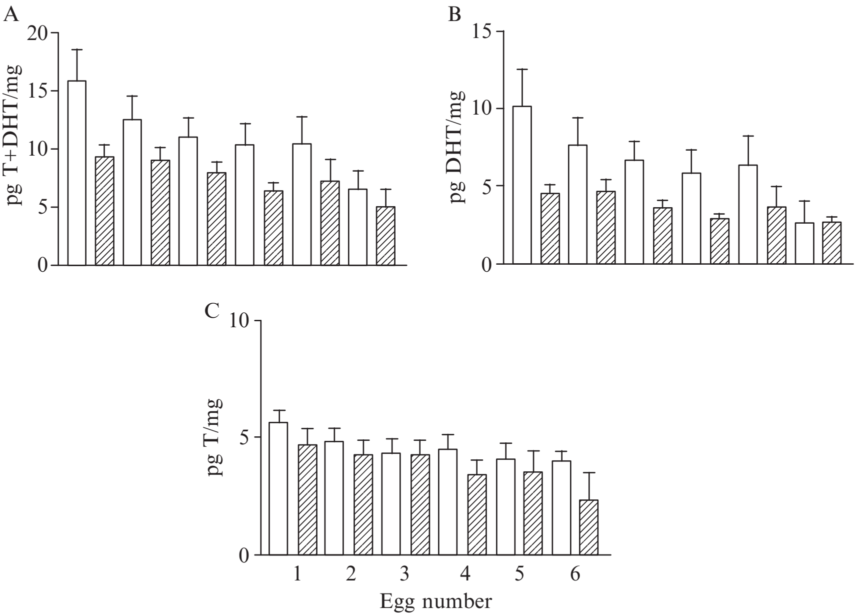

FIG. 9. Decreasing concentrations of yolk testosterone with laying sequence in zebra finches (Gil *et al.*, 1999). © 1999 The American Association for the Advancement of Science.

evidence for a functional role of these differences can be found. In the black-headed gull, clutches in which females induce a higher level of hatching asynchrony are precisely those in which a higher difference in yolk T between the first and last egg can be found (Müller *et al.*, 2004), supporting the idea that gulls use this differential allocation to compensate the effects of hatching asynchrony.

B. SEX

A possible overall difference in peahen (*Pavo cristatus*) yolk androgen levels between eggs bearing male and female embryos (Petrie *et al.*, 2001) was dismissed by further studies that showed that these differences were most likely due to differential use of androgens during incubation (Eising *et al.*, 2003a; Pilz *et al.*, 2005a; Loyau *et al.*, 2007).

However, female and male eggs have been shown to differ in yolk androgens under certain circumstances. For instance, in the zebra finch, male eggs have overall lower levels of T than female eggs, and this difference is largest in eggs laid in late-laying positions (Gilbert *et al.*, 2005). Sex differences are also affected by female fat condition and clutch size: in large clutches and in those laid by fat females, the female bias toward higher T disappears or is reversed in favor of males. An additional study from the same laboratory showed that sex differences in androgen levels were affected by female-feeding treatment (low vs high quality) (Rutstein *et al.*, 2005). Similarly, in the domestic hen, the difference in hormones between eggs-bearing male and female embryos depends on the rank order of the hens: high-ranking birds allocate more androgens to male than female eggs, whereas the pattern is the opposite in the low-ranking birds (Müller *et al.*, 2002).

The mechanism regulating these sex-specific differences in yolk androgen levels has been investigated by Badyaev *et al.* in a series of studies (Badyaev *et al.*, 2005, 2006a,b; Young and Badyaev, 2004). By using careful dissection of yolk rings, they have shown that oogenesis does not follow a strict follicle hierarchy as has been traditionally assumed (Johnson, 1999). In their study species, the house finch, several male and female oocytes develop in parallel (Fig. 1), and there are sex differences in the time that a given follicle overlaps with the rest of maturing oocytes (Young and Badyaev, 2004). By comparing two different populations, these authors showed that a temporal bias in the production of male and female oocytes allowed females to bestow distinct sex-specific hormone allocations in their eggs (Badyaev *et al.*, 2006b). The evolution of this mechanism makes sense in the light of the relatively strong sex-specific effects of androgens that have been found in some species (Saino *et al.*, 2006; von Engelhardt *et al.*, 2006), since females would be expected to adapt yolk androgen levels as a function of embryo sex [or probably vice versa: produce different sexes as a function of yolk androgen contents (Badyaev *et al.*, 2005)].

C. PATERNITY

Birkhead *et al.* (2000) rightly suggest that, in the case of mixed-paternity broods, females would not be able to deposit different amounts of hormones as a function of actual embryo paternity. This is because the effects of a given behavioral input over the physiology can only increase androgen levels in eggs that are laid 3–4 days after that moment. Thus, the time periods needed for an extra-pair copulation to fertilize an egg and to affect yolk hormone levels would not match. The only exception would be if females could predict that an extra-pair copulation is going to happen, but so far that possibility seems remote in birds (Birkhead *et al.*, 2000).

V. DIFFERENCES BETWEEN FEMALES

Although differences within a clutch provides the basis for fine-tuning mechanisms of female favoritism, one of the most impressive and suggestive levels of variation in yolk hormones is that between females.

Particularly, in Passeriformes, differences between females in mean yolk androgen levels can be extremely high; consider for instance the range between 8 and 66 pg/mg T in a given population of pied flycatchers (Tobler *et al.*, 2007a).

Individual differences have been found to be repeatable between clutches laid in the same year by the same female in three different species (Gil *et al.*, 2006b,c; Tobler *et al.*, 2007a). This repeatability, together with those differences found between quail selection lines (Gil and Faure, 2007), is consistent with a significant heritability of this trait, although a formal quantitative study of this aspect still needs to be done.

In this section, I shall deal with sources of individual differences in yolk androgens. Some of this variance has been shown to respond to plastic female deposition, showing that repeatability does not preclude important within-female variance.

A. Female Quality

Positive effects of androgens in nestling development (Schwabl, 1993) raised the question of why would such a deposition vary at all (Gil *et al.*, 1999) because selection would be expected to lead to an invariant optimal allocation of yolk hormones. A tentative first explanation to this question was that androgens could be costly for either the female or the offspring (Gil *et al.*, 1999). One way to answer this question is to look for patterns of condition-dependence, predicting that, if yolk androgens are costly, high-quality females should deposit higher amounts than low-quality females (Gil *et al.*, 2004a).

Several lines of evidence point toward a positive relationship between female quality and yolk androgen contents. In the great tit, experimentally parasitized females laid eggs with lower androgen levels than controls (Tschirren *et al.*, 2004). Levels of yolk T have been shown to increase with increasing dominance in female canaries (Tanvez *et al.*, 2007). In the European starling, higher yolk androgen levels were found for old females, and those laying early and large clutches (Pilz *et al.*, 2003). An immune challenge in house martins resulted in a reduction of yolk A4 and a similar trend for T levels (Fig. 10; Gil *et al.*, 2006b). Positive correlations between clutch size, arrival date, and yolk A4 levels in the barn swallow also suggest that high-quality females can afford to invest higher levels of yolk androgens (Gil *et al.*, 2006c). Furthermore, female zebra finches raised as nestlings in enlarged clutches lay eggs with lower T levels in adulthood (Gil *et al.*, 2004a), suggesting that yolk T allocation is sensitive to early developmental stress. Additionally, in kittiwakes *Rissa tridactyla*, a positive relationship between yolk A4 levels and immunoglobulin concentration is also suggestive of a positive relationship with female quality (Gasparini *et al.*, 2007).

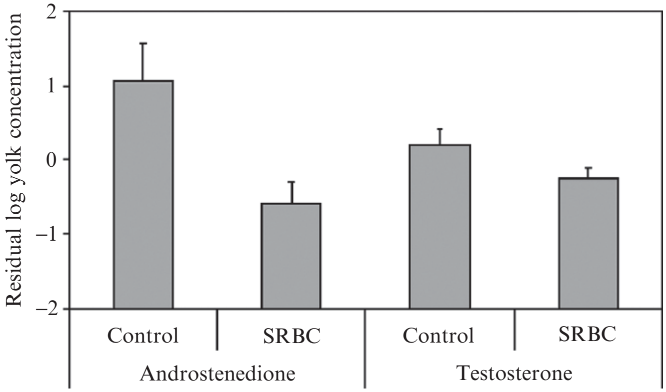

FIG. 10. Immune-challenged house martin females decrease androgen yolk levels with respect to controls. Graph shows means of residual concentrations corrected for laying order (Gil *et al.*, 2006b). © 2006 Springer Verlag.

However, although a positive relationship between female quality and yolk androgen levels is suggestive of costs of this deposition, it should be noted that this is not the unique explanation. If there is a relationship between female quality and alternative offspring phenotypes, differences in yolk levels may be due to females manipulating offspring and not to costs of androgen per se. For instance, in the case of the decrease in egg androgens observed in experimentally parasitized great tits, a follow-up study has shown that this decrease is used by females to manipulate offspring dispersal distance (Tschirren *et al.*, 2007). This is a remarkable example of a case where a straightforward inference about costs is shown to be incorrect.

On top of these difficulties of interpretation, additional experimental studies suggest that female quality and yolk androgens levels may not always be positively related. Two independent studies in two different gull species found that food-supplemented females transferred lower levels of androgens to their eggs (Gasparini *et al.*, 2007; Verboven *et al.*, 2003). Similarly, body condition and yolk androgen levels are negatively related in the pied flycatcher (Tobler *et al.*, 2007a).

The interpretation of these findings, which are completely opposed to the simplistic prediction of a cost assumption, is based on the idea that yolk androgens act as a short of compensation to resource deficits, and that therefore high-quality females would not need to resort to this compensation. Given that yolk androgen deposition can be costly for the offspring (e.g., Müller *et al.*, 2005b), selection would favor high-quality females to reduce yolk androgen levels (Gasparini *et al.*, 2007; Verboven *et al.*, 2003).

B. Coloniality

Several studies in a range of different species have found that females breeding at a natural high density lay eggs with higher levels of yolk androgens than females breeding at low density (Fig. 11; Mazuc *et al*., 2003a; Pilz and Smith, 2004; Reed and Vleck, 2001; Schwabl, 1997a). A similar pattern was found when comparing black-headed gulls living in the center and in the periphery of colonies (Groothuis and Schwabl, 2002). A possible interpretation of such a pattern is that increased female–female aggression in high-density colonies leads to these higher yolk T levels. However, Schwabl (1997a) proposed that such a mechanism could be used by females to provide their offspring with information about the conditions they would experience in adulthood, creating more competitive phenotypes. This interesting hypothesis is still waiting an experimental test, not just of the differences between colony sizes in yolk androgens (all based in correlations) but also of the adaptive consequences for the offspring. Studies that use intrusions to modify female behavior are not adequate tests of this hypothesis (e.g., Mazuc *et al*., 2003a) since what is required is large-scale colony size differences.

Schwabl's transgenerational plasticity hypothesis requires colony size to be heritable and differences in offspring phenotype to be adapted to the

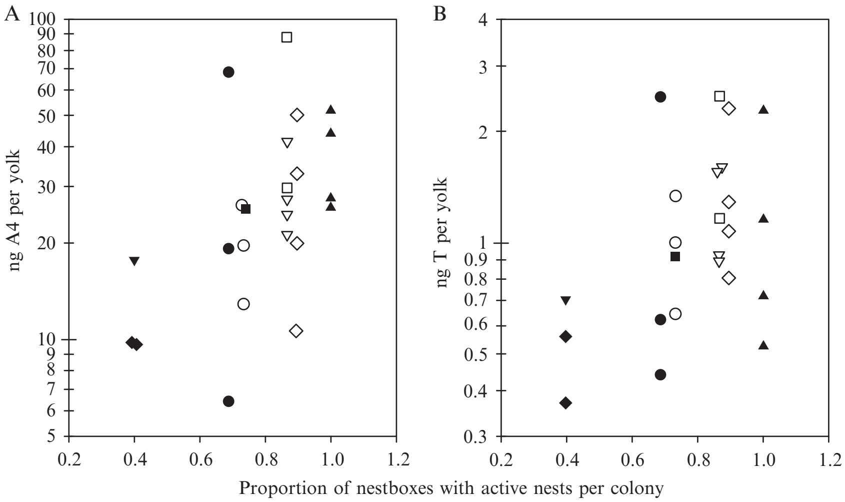

Fig. 11. Yolk androgen levels increase with breeding density in the European starling. Graph shows how the proportion of occupied nestboxes in a colony is positively associated with the total yolk content of both (A) A4 and (B) T. Plotted are the mean levels of total yolk androgen content per clutch (Pilz and Smith, 2004). © 2004 Blackwell Publishing.

different breeding densities (Schwabl, 1997a). The first requisite is met because significant heritability of colony size has been found in nature, suggesting that offspring tend to inhabit colonies of similar sizes to those of their parents (Møller, 2002). However, nothing is known about whether offspring phenotypes created by differential levels of yolk androgens match the selective regimes of the different densities. Although the original hypothesis referred to creation of competitive phenotypes (Schwabl, 1997a), an additional possibility is that differences in yolk androgen may induce differences in dispersal distance (Tschirren *et al.*, 2007). In this case, the basis of the adaptation may be linked to host-parasite coevolution. This possibility could work in principle because parasite pressure is closely linked to coloniality (Tella *et al.*, 2001). To the extent that increased yolk androgen leads to increased dispersal as shown for the great tit, differences in yolk androgen in relation to colony size would predict higher dispersal from large colonies. However, data for some species show dispersal to be reduced with increasing colony size (Serrano *et al.*, 2005), exactly the opposite pattern to what it would be predicted if yolk T was a mechanism regulating dispersal distances.

C. Differential Allocation

Costly ornamentation can provide organisms with reliable information on the genetic and phenotypic qualities or condition of the bearer (Jennions *et al.*, 2001). Current theory suggests that female preferences have partially evolved to take advantage of these correlates of quality because of indirect and direct benefits that they obtain from mating with males that have exaggerated ornamentation.

Life-history theory predicts that organisms should increase their parental investment when the value of a reproductive attempt is larger than average (Trivers, 1972). Thus, if male costly ornamentation indicates that offspring will be of higher phenotypic or genetic quality, it is expected that females should increase their investment in the offspring fathered by these males (Burley, 1988; Sheldon, 2000). This mechanism has been called differential allocation. Following this line of reasoning, and assuming that egg androgens are costly, several experiments have been conducted to investigate whether female birds show differential allocation of egg androgens in relation to male attractiveness. The first study to show this phenomenon used zebra finches, taking advantage of the fact that red-banded males are perceived as high attractive, whereas green-banded males are perceived as low attractive. In a cross-over experiment, where females from two different experimental groups encountered the two treatments in different sequence, female zebra finches were found to deposit higher levels of T and DHT in

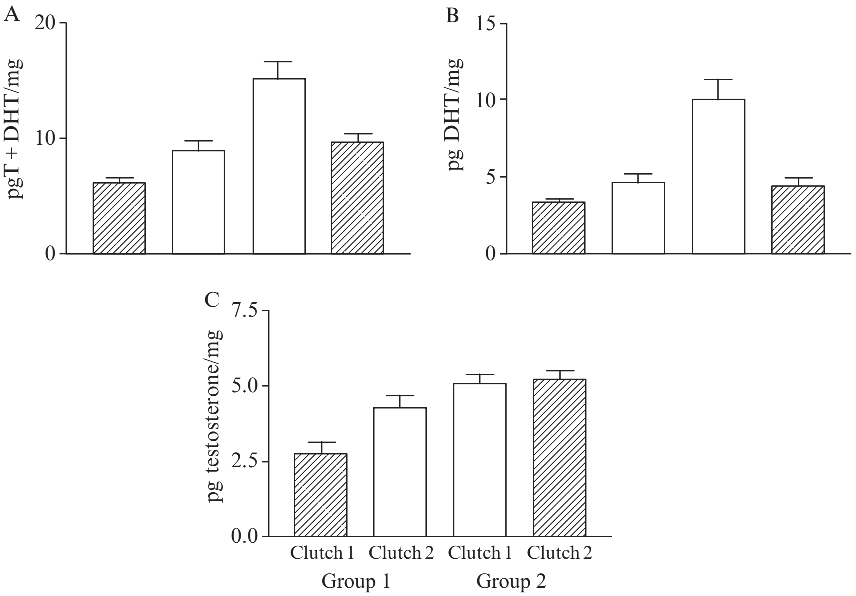

FIG. 12. Differential allocation between females mated to attractive and unattractive males in the zebra finch. Graphs show mean levels of yolk T+DHT (A), DHT (B), and T (C). Open bars represent the condition when the females were mated to red-banded males, and the shaded bars represent the condition when the females were mated to green-banded males. Group 1 females were mated to a green-banded male first and then to a red-banded male. Group 2 females were mated to a red-banded male first and then to a green-banded one (Gil *et al.*, 1999). © 1999 The American Association for the Advancement of Science.

their eggs when mated to red-banded males than green-banded males (Fig. 12). Exactly the same results were found in a similarly designed laboratory study, using canaries as experimental subjects and manipulating the attractiveness of song that was broadcasted to females (Gil *et al.*, 2004b; Tanvez *et al.*, 2004). Females in this case were found to increase their egg T levels by approximately 30%. A replication of this study by Marshall *et al.* (2005) resulted in a similar effect in plasma levels measured through fecal samples, but no differences in yolk androgen deposition.

The first test of the hypothesis in the wild was conducted in the barn swallow (*H. rustica*), a species in which male attractiveness can be modified by manipulating tail length; again in this case, females mated to males with elongated tails deposited higher levels of A4 (the most abundant androgen in the eggs of this species) than those mated to males with shortened tails (Gil *et al.*, 2006c).

The first interpretation of these results was in the sense that females invested larger quantities of a costly resource in the eggs fathered by attractive males, as the hypothesis of differential allocation would predict. The fact that yolk androgens can be costly further suggests that either the benefits that females derive from mating with attractive males are large enough to compensate these costs or the offspring of attractive males are better at withstanding these costs (Gil *et al*., 1999), for instance, if male ornamentation affects nestling immunocompetence (Johnsen *et al*., 2000).

However, alternative hypotheses have been proposed and these could also fit the data. A first possibility is that increased yolk androgen deposition was a mechanism that females could use to increase begging, and thus male parental care (Moreno-Rueda, 2007). This would make sense because in some studies, highly ornamented males have been found to provide poorer parental care than males with poor ornamentation (Burley, 1988; De Lope and Møller, 1993). However, the predictions of this hypothesis have not been put to the test in any study so far.

A second possibility is that the high androgen levels of females mated to attractive males are in fact an epiphenomenon of sex-ratio adjustments. Evidence confirms theoretical expectations that females bias sex-ratios toward males when mating with attractive males (West and Sheldon, 2002). Therefore, an increase in yolk androgen could be explained if either: (1) male-biased sex-ratios are caused by heightened androgen secretion by follicles or (2) male embryos require higher levels of androgens during incubation than females. Data so far are not conclusive enough to evaluate any of these possibilities (see Section VII).

However, the predicted outcome of differential allocation has not been substantiated in all studies. For instance, in the house sparrow, Mazuc *et al.* (2003b) manipulated T levels in experimental males to increase their attractiveness and found no differences in yolk T levels between females mated to experimental and control males (Mazuc *et al*., 2003b). The opposite pattern to that predicted by the differential allocation hypothesis was found in two nonexperimental studies. In the first of these, collared flycatcher females (*Ficedula albicollis*) laid eggs with higher androgen concentrations when mated to young males (Michl *et al*., 2005). Similarly, in the Eastern Bluebird (*S. sialis*), females mated to highly attractive males laid eggs with lower androgen levels that those mated to lowly attractive males (Navara *et al*., 2006b). These patterns were interpreted as compensatory mechanisms used by females to make up for poor quality or young males (Michl *et al*., 2005; Navara *et al*., 2006b); in that sense, the stimulatory effects of androgen on growth or begging may mitigate the costs of pairing with low-quality males. Although this mechanism could in principle work, the problem with these studies is that they are based on correlative data, and patterns of assortative mating can obscure the real effect of attractiveness. For instance, in the barn swallow study (Gil *et al*., 2006c), although the experiment showed that females laid eggs with higher androgen levels for males with elongated tails, there was no relationship between natural variation in tail length and yolk androgen. This discrepancy may stem from the fact that females paired to long-tailed males are not a random sample of birds (Møller, 1991).

Prelaying differential allocation has important implications for studies of good-genes sexual selection because it shows that viability effects related to male ornamentation can be confounded by female differential investment (Gil and Graves, 2001; Gil *et al.*, 1999; but see: Sheldon, 2000). The empirical measurement of good-gene effects had got round the problem of postlaying differential effects (Burley, 1988) by means of cross-fostering experiments (Johnsen *et al.*, 2000), but now that prelaying effects have been found, experiments using artificial insemination are required (Welch *et al.*, 1998).

D. BROOD PARASITISM

Conspecific brood parasitism is a strategy by which females lay eggs in the nests of other females of their own species (Rothstein and Robinson, 1998). Although the prevalence of this strategy is rather low, in some species, it can represent an important addition to female fitness (Ahlund and Andersson, 2001). It is to be expected that, if egg androgen deposition is used by females to manipulate male parental care (Moreno-Rueda, 2007; Müller *et al.*, 2007b), parasitic eggs should contain higher amounts of androgens that nests laid in the own nests. Two studies addressing this question in the European starling (Pilz *et al.*, 2005b) and in the blue tit (*Cyanistes caeruleus*) (Vedder *et al.*, 2007) have shown that parasite eggs do not contain higher levels of egg androgens than normal eggs. However, since in these two species the occurrence of conspecific brood parasitism is relatively low, it is perhaps not surprising that selection has not resulted in a fine-tuning of hormone deposition. Furthermore, since at least in the case of starlings, conspecific parasitism is typically performed by low-quality females (Sandell and Diemer, 1999), it might not be possible for these females to lay eggs with high androgen levels.

VI. COMPARATIVE STUDIES

Differences between species in yolk androgen contents are huge and raise important questions about their physiological significance (Williams *et al.*, 2004). As an example, consider two closely related sparrow-like passerine species, such as the house sparrow (*P. domesticus*) and the African masked weaver (*Ploceus velatus*), in which average yolk T levels are, respectively, 40 and 4 pg/mg (Gil *et al.*, 2007). It is difficult to find a key developmental difference between these species that would explain this tenfold difference in yolk androgen concentration. Allometry, for instance, is not an explanation: size differences between species do not explain variation in yolk T and only weakly so in the case of A4 (Gil *et al.*, 2007; Schwabl *et al.*, 2007).

Data from the largest comparative study conducted so far (Gil *et al.*, 2007) show that in the case of T, the taxonomic level at which most of the variation is explained is that of the family and the genus, whereas in the case of A4 is that of order (Fig. 13; Gil and Biard, unpublished data). However,

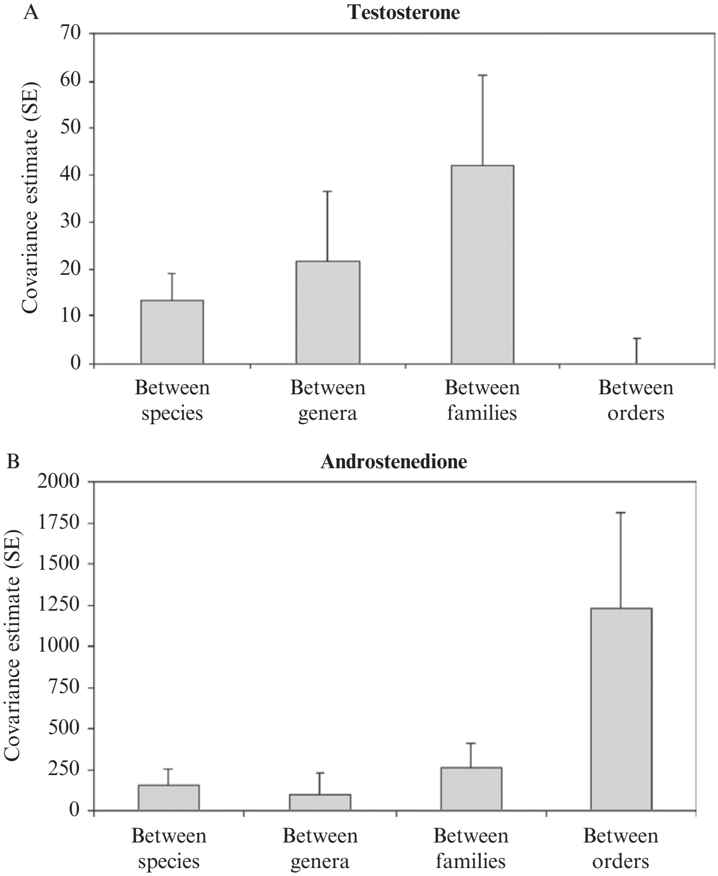

FIG. 13. Estimates of variance due to different taxonomic levels for yolk T (A) and A4 (B). Unpublished analysis from data for 101 species presented in Gil *et al.* (2007) (Biard and Gil, unpublished data).

despite this phylogenetic signal that could induce some evolutionary constraints, there was still significant variation explained at the level of the species, suggesting that ecological and social factors could play an important role in shaping yolk androgen levels.

Comparative studies are necessary in evolutionary ecology to confirm that species-specific traits are adaptations to the environment (Harvey and Pagel, 1991). Since adaptation is the result of historical processes, experiments in extant species cannot prove that a given character has evolved as an adaptation to a given environment. Comparative analyses are then used to examine whether evolutionary transitions of a character are associated with transitions in the proposed adaptation (e.g., Tella, 2002). Following this logic, most comparative studies so far have studied the suite of possible adaptations that studies conducted in individual species have previously identified, namely development, sexual selection, coloniality, and brood parasitism.

Further studies have addressed possible less-obvious across-species correlates of higher yolk androgen, uncovering suggestive patterns that merit further study. Garamszegi *et al.* (2007b) have found a positive but curvilinear relationship between yolk T and brain size that the authors interpret as backing up the role of yolk T in the development of the brain during embryogenesis. The same authors have discovered a negative pattern between repertoire size and yolk T that could be explained as consequence of the accelerating effects that high levels T have for song crystallization during early development (Garamszegi *et al.*, 2007a).

A. Development

The main axis of variation in bird development is described by the altricial–precocial continuum (Starck and Ricklefs, 1998). Whereas some birds hatch naked, unable to thermoregulate and depend completely on their parents to be fed, other species start to move and feed by themselves some hours after hatching. Because yolk hormones have been shown to affect development, a first question regards the link of these egg components with the mode of development (Schwabl, 1999). Data show that there is not a direct relationship between T or A4 yolk levels and developmental mode (Gil *et al.*, 2007). When we correct for body mass, there is no relationship between mode of development (altricial–precocial spectrum) and yolk hormone levels.

The positive relationship between body or egg mass and yolk A4 levels is consistent in two different studies (Gil *et al.*, 2007, Schwabl *et al.*, 2007), suggesting that embryos or nestlings require a concentration of A4 proportional to their body size. This effect is reminiscent of other mass-related functional correlates of embryonic development, such as functional maturity of organs (Starck and Ricklefs, 1998).

By gathering yolk hormone data from the literature, Gorman and Williams (2005) showed for the first time that there was a negative relationship between species-specific yolk T levels and duration of the incubation period in Passeriformes (Fig. 14), thus suggesting that yolk hormones could have evolved as an indirect genetic effect (Wolf *et al.*, 1998). However, they found no effect of yolk T levels on the duration of the nestling period. These patterns were consistent after correcting for the common phylogeny.

Two additional studies that followed have brought conflicting evidence in this respect. The study with the largest sample so far (Gil *et al.*, 2007) also found a similar negative relationship between developmental period and yolk T levels in the Passeriformes, but this relationship disappeared when similarity by common descent was controlled for (Fig. 15). In contrast, Schwabl *et al.* (2007) replicated the same results of Gorman and Williams, observing a strong negative relationship between embryonic incubation

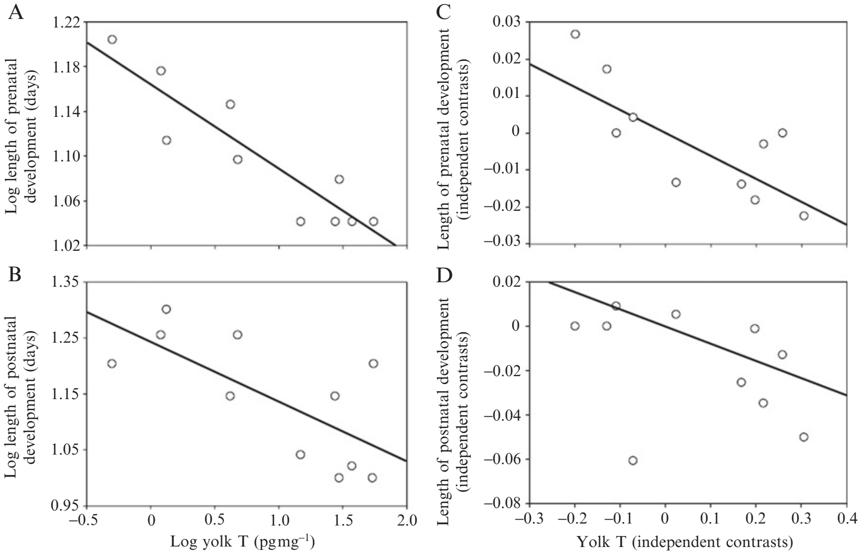

FIG. 14. Species correlations between log yolk T concentration and log (A) length of the prenatal and (B) postnatal developmental periods, and independent contrasts relationships for yolk T concentration and (C) length of the prenatal and (D) postnatal developmental periods (Gorman and Williams, 2005). © 2005 The Royal Society.

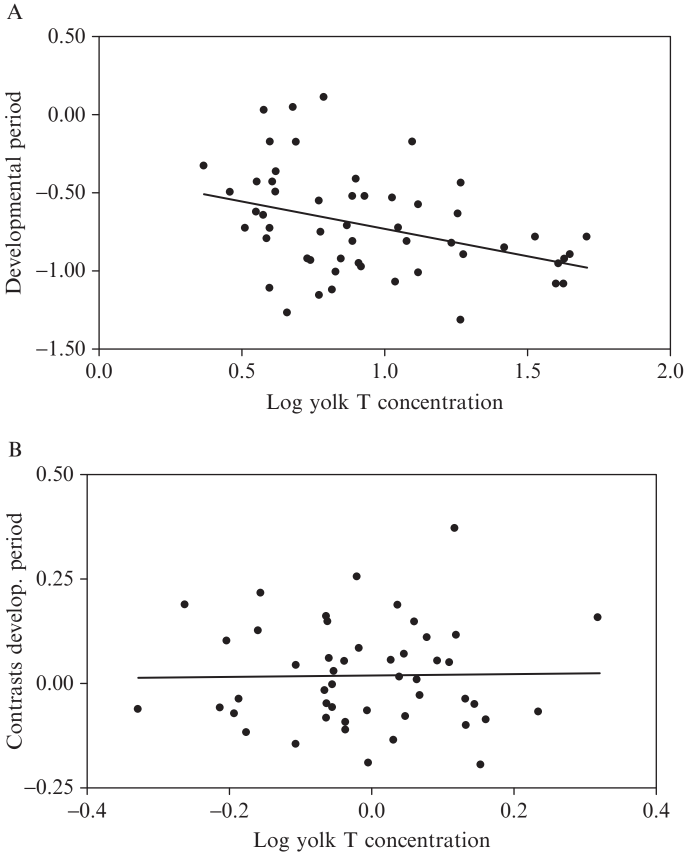

FIG. 15. Relationship between yolk T concentration and residual developmental period (PC1) in Passeriformes, using species-specific data (A) or independent contrasts (B) (Gil *et al.*, 2007). © 2007 University of Chicago Press.

period and yolk T in a sample of Passeriformes, also when a phylogenetic correction was applied (Schwabl *et al.*, 2007). An even stronger relationship was found with DHT, which is the most powerful androgen in bird yolk, suggesting a strong role of this hormone in development. The same study identified a positive relationship between nestling predation rate and yolk T concentration, suggesting that predator selection for faster development in birds (Bosque and Bosque, 1995; Martin, 2002) may have acted through this maternal effect in Passerines.

These studies, while presenting some degree of conflicting evidence, suggest that selection for modifications of the length of the developmental periods is somehow linked to modifications of the embryo's exposure to maternal steroids in the order Passeriformes.

In contrast to the Passeriformes, a general analysis covering the whole Aves class has found little evidence for a direct effect of yolk androgens in reducing overall developmental periods (Gil *et al.*, 2007). In a sample of 101 bird species, there was no relationship between developmental period (as measured by the joint covariance of incubation and nestling periods) and either yolk androgen (A4 or T). However, it was found that A4 was positively related to the relative duration of the incubation period over the nestling period, and negatively to the relative duration of the nestling period (Fig. 16). In other words, after controlling for the overall duration of development, species with developmental periods characterized by a relatively long incubation period and a relatively short nestling period had high A4 yolk concentrations (Gil *et al.*, 2007).

Previous comparative studies have found that relatively short incubation periods in birds are associated with higher parasitism-driven mortality and higher prevalence of blood parasites (Møller, 2005; Ricklefs, 1992). It is difficult to understand how A4 may mediate this effect because we do not have enough data on the distinctive effects of A4 over other androgens (see Section VIII.C). However, two nonmutually exclusive mechanisms could be envisaged: a reduction in the nestling period through increased begging or an increase in the incubation period through a retarding effect on embryo development. Although specific experiments comparing the effects of A4 versus T have not been conducted, we could assume that those experiments in which a high A4/T ratio has been injected most likely provide us with information on the specific effects of A4. In that sense, a reduction of the nestling period through begging is consistent with data from the black-headed gull in which chicks hatching from androgen-injected eggs begged more strongly than control chicks (Eising and Groothuis, 2003). The second possibility however is less clear since different studies provide conflicting evidence (Eising *et al.*, 2001; Sockman and Schwabl, 2000).

The large differences in longevity that exist between temperate and tropical species are considered to be a major cause of variation in life-history traits between bird species living in these areas (Martin, 2002). For instance, incubation periods are longer, and clutch sizes smaller in the tropics than in temperate zones, and experimental and comparative data suggest that this variation is explained by reduced parental investment and risk-taking behavior in tropical species (Ghalambor and Martin, 2001; Martin, 2002). Thus, time-dependent selection pressure in the tropics has

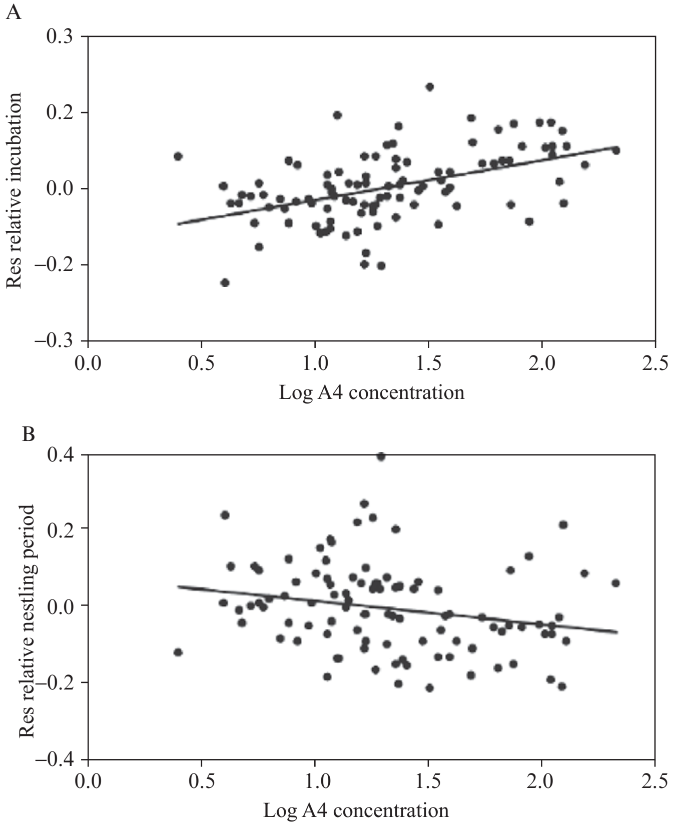

FIG. 16. Relationship between yolk A4 concentration and residual incubation period (A) and residual nestling period (B) using species data (Gil *et al.*, 2007). © 2007 University of Chicago Press.

led to low nest attentiveness, cooler average egg temperatures, and slower development. What could be expected in terms of yolk androgens? Assuming that yolk androgens decrease developmental periods, the logical prediction would be one of higher androgen levels in the tropics. However, recent data show exactly the opposite pattern: lower yolk T and DHT levels in the eggs of tropical birds (Martin and Schwabl, 2008). A possible explanation for this pattern is based on a negative trade-off between growth rate and developmental quality (e.g., cellular maturity, immune function). (Arendt, 1997, 2000; Billerbeck *et al.*, 2001; Case, 1978; Ricklefs, 1992). Long-lived tropical species may require the developmental benefits of slow growth to build high-quality offspring, and this could trade off with yolk

T-mediated rapid growth (Martin and Schwabl, 2008). Additionally, there might be some kind of physiological incompatibility between fast development caused by high androgen levels and incubation conditions characterized by low nest attentiveness and cooler average egg temperatures. Regardless of the reasons for the selective advantage of slow growth rates in tropical nestlings, the match of slow development with low levels of egg androgens in these species reinforces the evidence for a role of maternal hormones in shaping developmental periods across species.

B. Coloniality

Although there can be considerable variation in colony size within a given species (Brown and Brown, 2000), different species can be classified by their degree of breeding colonial habit from strictly solitary to those always reproducing in large colonies. It is to be expected that, if high yolk androgen levels is an adaptive trait for individuals nesting in high densities (see Section V.B, e.g., Pilz and Smith, 2004), a positive relationship between yolk androgen levels and coloniality habit should also be found across species. Indeed, a comparative study of a wide range of avian species has shown a positive relationship between yolk A4 levels (but not T) and breeding coloniality (Gil *et al.*, 2007). This result was confirmed by analysis of independent contrasts, which revealed that transitions to coloniality have coevolved with increases in yolk A4 concentration over evolutionary time (Fig. 17).

Positive relationships between group size and yolk androgens have been hypothesized to be an adaptation because of the particular ‘‘competitive’’ phenotype that is assumed to be produced this way (Schwabl, 1997a). An alternative explanation to these adaptative views is that, increased androgen levels in the eggs of colonial species or individuals are an unselected consequence of increased androgen levels in female plasma (see Section II.B, for a lengthy consideration of the ‘‘female control’’ issue). In this respect, there is evidence linking T female levels and coloniality; for instance, colonial species have higher T levels than solitary species (Møller *et al.*, 2005), and colony size and female T levels are positively correlated in the cliff swallow *T. bicolor* (Smith *et al.*, 2005). However, if increased yolk androgen in colonial birds is a simple epiphenomenon of increased androgens in females, one would also expect a positive relationship between yolk T and coloniality. This lack of relationship with T levels, in the face of an increase in yolk A4 levels (Gil *et al.*, 2007), suggests some kind of physiological filter in the female and that A4 may indeed be a mechanism used to modify the phenotype of colonial species.

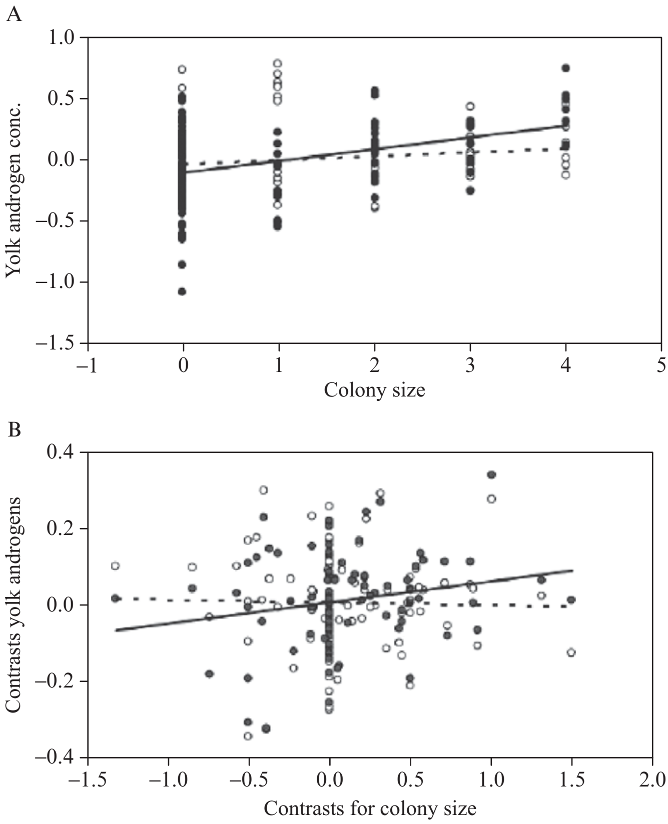

FIG. 17. Relationship between yolk androgen concentrations and coloniality, using species-specific data (A) and independent contrasts (B). The A4 concentration data is shown by solid circles and solid lines and T data by open circles and dashed lines (Gil *et al*., 2007). © 2007 University of Chicago Press.

C. SEXUAL SELECTION

A link between sexual selection and yolk androgens is suggested by two different lines of research: (1) studies that have shown that females modify yolk androgen levels in response to male attractiveness (see Section V.B, or e.g., Gil *et al*., 1999) and (2) studies that have found accelerated development of ornaments in birds that have been exposed to high levels of androgens *in ovo* (see Section III.B.2 or e.g., Eising *et al*., 2006). This joint evidence leads to the prediction that species subject to higher levels of sexual selection should present higher levels of yolk androgens.

A comparative study using two different correlates of intensity of sexual selection (sexual dichromatism and mating system) found, however, little support for this prediction (Gil *et al.*, 2007). No differences were found between monogamous and polygynous species in either yolk T or A4. In the case of sexual dichromatism, although monochromatic species had lower T levels than dichromatic species, this difference was no longer significant after controlling for common descent.

However, sexual dichromatism and mating system are only useful surrogates for the intensity of sexual selection, and it is desirable that future studies would address this prediction using more direct estimates of the intensity of sexual selection.

D. Brood Parasitism

Since obligate brood parasites do not suffer from the costs of raising highly demanding offspring, it is to be expected that these species would benefit by laying eggs with high levels of androgens (Hauber and Pilz, 2003). However, studies conducted so far have found evidence at odds with this prediction.

For instance, no systematic difference between brown-headed cowbird (*Molothrus ater*) T levels and a suite of usual hosts could be found in two different studies (Fig 18; Hahn *et al.*, 2005; Hauber and Pilz, 2003): in some hosts, it was higher, and in other hosts much lower. Similarly, common cuckoos (*Cuculus canorus*) have lower T levels than their great reed warbler hosts (*Acrocephalus arundinaceus*) (Török *et al.*, 2004); and great spotted

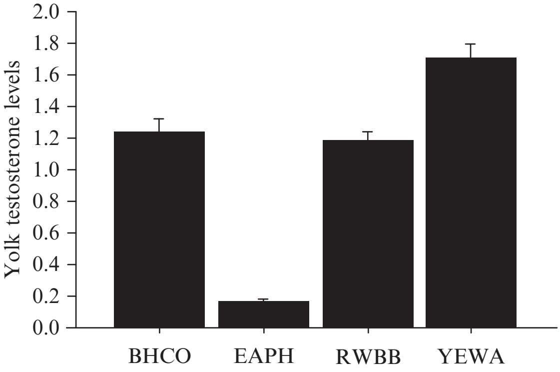

Fig. 18. Yolk testosterone concentrations of brown-headed cowbirds and three host species. Abbreviations: BHCO, brown-headed cowbird; EAPH, eastern phoebe; RWBL, red-winged blackbird; YEWA, yellow warbler (Hauber and Pilz, 2003). © 2003 University of Notre Dame.

cuckoos (*Clamator glandarius*) have lower androgen levels (T and A4) that magpies (*Pica pica*) (Gil and Soler, unpublished data). Communally breeding guira cuckoos (*Guira guira*) have also relatively low concentrations of yolk hormones when compared to similarly sized species (Cariello *et al.*, 2006).

However, Hahn *et al.* (2005) found that egg T levels of brown-headed cowbirds varied between different populations in a remarkable way; recently, expanding populations showed a trend for higher yolk-T levels than ancestral populations. This difference, if confirmed by further analysis, suggests the possibility of adaptive phenotypic plasticity for establishment in novel areas and differential dispersal (Hahn *et al.*, 2005; Tschirren *et al.*, 2007).

E. Correlated Selection

Artificial selection is an excellent tool to explore the genetic architecture of traits. Some studies have taken advantage of artificial selection programs to analyze the correlated response in yolk hormones that has followed these selection programs. A comparison of Japanese quail lines selected for high and low social reinstatement responses (a measure of the strength of sociality) has showed that yolk T levels have diverged in a correlated way with this selected behavior (Gil and Faure, 2007). Female quails of the high line (high social motivation) laid eggs with higher T levels than quails from the low line (low social motivation), while the nonselected line had intermediate values between these two (Fig. 19).

Since high yolk T levels induce a proactive phenotype in this species (Daisley *et al.*, 2005), the match between yolk T levels and the behavior of the selected lines suggests that a causal link between yolk T and proactive behavioral phenotype, following a series of selection episodes, rather than pleiotropy or linkage disequilibrium (Price and Langen, 1992). Detailed genetic analyses would be needed to examine the genetic architecture of these traits, which suggest the presence of an indirect genetic effect (Wolf *et al.*, 1998).

VII. A Mechanism for Sex-Ratio Adjustment?

In the last decade, many examples of adaptive sex-ratio allocation in birds have been reported (e.g., Komdeur *et al.*, 1997), suggesting that females can bias the overall sex ratio of their broods and even the position of different sexes along the hatching order (Badyaev *et al.*, 2002). Since sex-determination (i.e., chromosome segregation) happens a few hours before ovulation, when the follicle is already mature, it is possible that differences between eggs in hormone concentration might affect sex determination (Krackow, 1995).

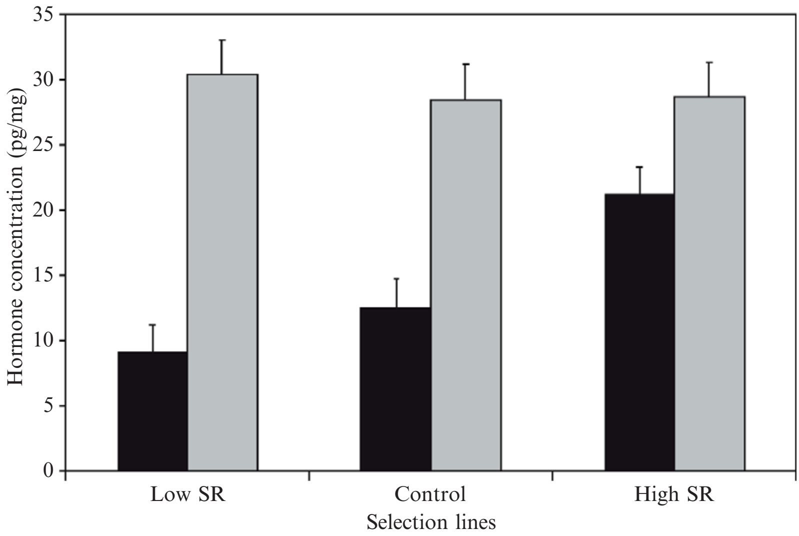

FIG. 19. Yolk androgen in eggs coming from quails of different selection lines. Graph shows T concentration (black bars) and A4 concentration (shaded bars) in the yolks of eggs laid by females belonging to the low social reinstatement, control, and high social reinstatement lines (Gil and Faure, 2007). © 2007 John Wiley & Sons.

Different hormones have been proposed to act as sex-ratio distorters, including P (Correa *et al.*, 2005), CORT (Pike and Petrie, 2006), and androgens (Rutkowska and Cichon, 2006). However, evidence so far is only based on suggestive correlations, and a proper physiological model has not been proposed or tested.

Suggestive evidence for a link between T and sex ratio comes from a study by Veiga *et al.* (2004) that showed that female spotless starlings that have been fitted with T-implants-produced male-biased sex ratios. Remarkably, this effect was detected not only on the year of implantation, but also years later, when the implants should have been effectively empty (Veiga *et al.*, 2004). Similarly, in the zebra finch, injecting T in laying females resulted in a modification of sex-ratio variation along the laying order (Rutkowska and Cichon, 2006), with a tendency to overproduce sons over daughters. However, between-female differences in mean yolk androgen levels were not related to brood sex ratio in the barn swallow (Gil *et al.*, 2006c).

The differences in growth of oocytes that become male and female in the house sparrow found by Badyaev *et al.* (2006a) also suggest a link between sex and hormone levels. However, the direction of the relationship is not easy to disentangle: is sex determined by hormone levels in the oocyte? or do females modify oocyte content as a function of the sex that is going to be produced? To sum up, although certain links between maternal hormones and sex ratio have been found, data so far do not show a clear endocrine mechanism by which sex ratios could be modified.

VIII. Egg Cocktails

The vast majority of studies on egg hormones have concentrated on androgens, but there are several other hormones that are found in eggs and that have consequences for the offspring.

A. Estradiol

Estradiol is a powerful estrogen involved in processes of sexual differentiation in birds (Adkins-Regan *et al.*, 1994; Balthazart and Ball, 1995), although its effects vary between species. Several studies have found that female birds injected with estradiol lay eggs with high estradiol content, producing strong modifications of the sexual differentiation of the embryo (Adkins-Regan *et al.*, 1995; Riddle and Dunham, 1942). For instance, female Japanase quail hatching from these eggs presented a atypically high incidence of right oviducts (Adkins-Regan *et al.*, 1995).

Avian yolks contain small amounts of estradiol (Adkins-Regan *et al.*, 1995; Schwabl, 1993), but these are lower than those needed to modify embryonic sex differentiation (Carere and Balthazart, 2007). Studies so far have found surprisingly little variation between eggs in yolk estradiol levels despite huge plasma variation in females through oviposition (Williams *et al.*, 2004), although differences along the laying order have also been described (Williams *et al.*, 2005). However, and unlike in reptiles, where natural variation in egg estradiol can have a dramatic effect on phenotypes (Lancaster *et al.*, 2007), no study so far has identified substantial relationship between estrogen variation and offspring morphology or behavior.

B. Corticosterone

CORT is the main stress hormone in birds, and is secreted by the adrenal glands after stimulation of the hypothalamo-pituitary-adrenocortical (HPA) axis (Sapolsky, 1992). The production of this hormone in adults is strongly linked to the stress response that involves a fast mobilization of energy, a shut down of reproductive and immunity activities and in general a preparation of the individuals to face the emergency situation (Wingfield and Kitaysky, 2002).

Levels of this glucosteroid have been found in eggs, with large variation between species [from 2 pg/mg in quails to 30 pg/mg in peacocks (Love *et al.*, 2008)] and is found in both yolk and albumen of avian eggs. In contrast to androgens, CORT does not arrive inside the egg by means of specific follicle cells. Rather, it is assumed that it gets there by passive diffusion from blood vessels, although evidence is equivocal. For instance, Rettenbacher *et al.* (2005) injected hens with CORT and found a rather reduced transfer of the hormone to the yolk. High amounts of CORT fed to hens did result in higher levels in yolk, but ACTH injections did not lead to significant increases in yolk CORT. The higher concentrations were found in the albumen and the outer layers of yolk, suggesting that this hormone reaches the egg through passive diffusion from the blood stream.

There is some evidence that stressful events experienced by the laying female result in increases in egg CORT levels. For instance, Saino *et al.* exposed laying barn swallows to either a predator (cat) or a nonpredator (rabbit). They subsequently found higher CORT concentrations in the eggs of the females that had been exposed to the cat than in those exposed the rabbit (Saino *et al.*, 2005). A comparison of quail selected for low- and high-stress responses has shown correlated responses in egg CORT levels, suggesting that either birds with high plasma CORT levels lay eggs with higher CORT concentrations, or else that artificial selection has acted on an partially correlated epigenetic mechanism, by which maternal CORT levels induce phenotypes characterized by an HPA activity upregulation (Hayward *et al.*, 2005).

Studies so far seem to coincide in that the consequences of higher CORT exposure are largely detrimental. Thus, in the barn swallow, CORT-injected eggs were less likely to hatch than control eggs, and those that did hatch were lighter and showed a poorer growth of tarsi and wings (Saino *et al.*, 2005). CORT-implanted Japanese quail laid eggs with higher CORT contents can control birds, and the chicks hatching from those eggs showed reduced growth and increased HPA activity in response to a stressful situation (Hayward and Wingfield, 2004). A direct injection of CORT into eggs of the same species resulted in similar negative effects in the case of male offspring, but not for females (Hayward *et al.*, 2006). CORT has been shown to increase incubation time, reduce begging displays, and depress cell-mediated immunity in yellow-legged gulls (Rubolini *et al.*, 2005).

Since many of these effects on morphology and physiology can negatively affect survival probabilities, the most likely conclusion is that females cannot buffer eggs against detrimental increases in circulating CORT levels. Alternatively, other authors have suggested that this transfer may be adaptative (Hayward and Wingfield, 2004), in the sense that an upregulation of HPA responsiveness could be advantageous under certain circumstances.

Another possibility is that the adaptative role of CORT may be based on its sex-specific effects. For instance, a study in the European starling shows that CORT implants in females result in eggs containing increased levels and yolk CORT, leading to increasing male embryonic mortality and lower male nestling growth (Love *et al.*, 2005). This evidence is backed up by an additional experiment linking CORT levels to female-biased broods in peahens (Pike and Petrie, 2005). It has been suggested that such a mechanism could be advantageous since it would allow adaptive sex-specific brood reductions, thus leading to a better match between female condition and reproductive investment (Fig. 20; Love *et al.*, 2005, 2008). Further research is needed to examine this exciting possibility.

C. Three Androgens, One Effect?

Three main androgens have been found in avian yolks: testosterone (T), androstenedione (A4), and 5α-dihydrotestosterone (5α-DHT). No published study so far has examined the differential effects of each of these hormones in development.

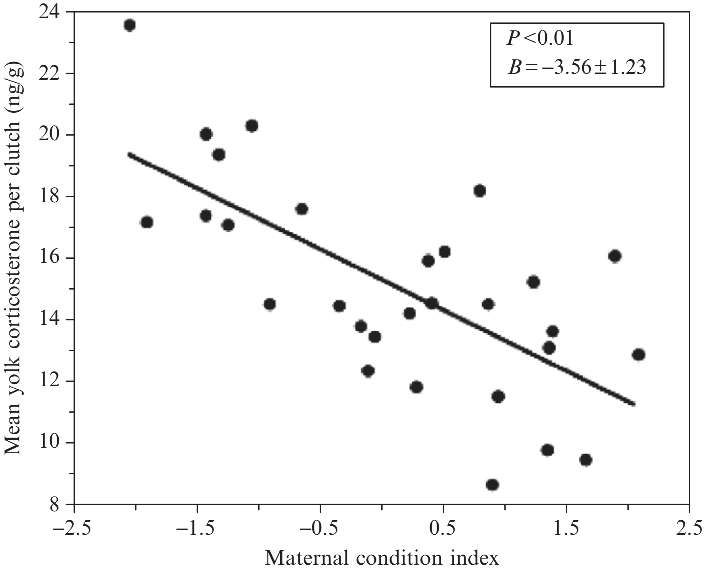

Fig. 20. Relationship between maternal condition and mean yolk CORT levels per clutch for individual female European starlings (Love *et al.*, 2008). © 2008 Elsevier Inc.

Schwabl *et al.* (2007) make the point that yolk androgens can be ranked in terms of their androgenic effect, stating that A4 is much weaker than T and T is much weaker than DHT as showed by *in vitro* studies that measure affinity for the androgen receptor (Sonneveld *et al.*, 2006). However, DHT has only a stronger androgenic effect than T when measured *in vitro*, in the absence of enzymes that increase their clearance *in vivo* (e.g., hydroxylases, sulfatases, reductases) (Sonneveld *et al.*, 2006). In fact, depending on the enzymatic milieu, DHT can be even less potent than T (Kumar *et al.*, 1999). Therefore, it seems rather meaningless to judge the potential androgenic effect of the suite of possible yolk androgens without knowing the precise chemical matrix in which these androgens act in the embryo. Furthermore, since species-specific concentrations of DHT and T are strongly correlated in Passeriformes ($r = 0.9$) (Schwabl *et al.*, 2007), it becomes rather difficult to ascertain the specific roles of each of these two hormones.

However, the case of A4 is a different story. First, relationships between A4 and T are not very strong, although they are also positive (Gil *et al.*, 2007; Schwabl *et al.*, 2007). Second, some relationships between androgen concentration levels and ecological or behavioral variables are only found for either T or A4, suggesting that these two androgens are not identical (e.g., Gil and Faure, 2007; Gil *et al.*, 2006b). In fact, evidence from other organisms shows that A4 could even inhibit grow (Dlugonski and Wilmanska, 1998; McGivern *et al.*, 1996). Since the action of A4 depends on the conversion to T by the enzyme 17β-hydroxysteroid dehydrogenase (17HSD) (Horton and Tate, 1966), its effects very likely will depend on the availability of this enzyme (Dlugonski and Wilmanska, 1998). 17HSD is present and functional in the developing avian embryo (Bruggeman *et al.*, 2002), but it is not known whether species differ in 17HSD availability. Additionally, A4 can follow different conversions to both estrogen and DHT, if the required enzymes are present. As in the case of DHT, we are back to a situation in which we need hard data on metabolic conversions *in vivo* to know the precise roles of each hormone and be able to interpret pattern of variation in nature.

D. Other Components

Apart from basic nutritional components of eggs, such as lipids, protein, or water, a series of substances known to alter the immune system have been found in varying quantities in eggs. For instance, differences between eggs in lysozyme (Saino *et al.*, 2002), carotenoids (Royle *et al.*, 2001), or immunoglobulin content (Morales *et al.*, 2006) can result in differences in nestling growth or fitness.

Several studies have suggested that these substances may interact with T levels, and that adjustment of T levels within-clutches may be specifically allocated in balance with these components (Royle *et al.*, 2001). In gulls, where within-clutch differences in egg size and composition are of paramount importance for nestling survival (Nager *et al.*, 2000), it has been found that both carotenoid and immunoglobulin concentrations decrease with laying order whereas T increases (Groothuis *et al.*, 2006; Royle *et al.*, 2001). The interpretation of this pattern could be that egg production constraints eggs that are laid late in the sequence to contain less basic resources, but that females increase T in these eggs to increase the competitiveness of nestlings in situations in which abundant food would not be a limit to chick growth (Royle *et al.*, 2001).

It is worth mentioning that surprisingly very little research has been devoted to the implications of variation in thyroid hormone deposition, a hormone directly involved with differentiation and embryonic processes, and that is known to pass from mother to offspring via yolk (McNabb and Wilson, 1997; Wilson and McNabb, 1997).

IX. CONCLUSIONS AND FUTURE DIRECTIONS

Since the existence of androgens in eggs was discovered by avian ecologists (Schwabl, 1993), this field of research has known a tremendous surge. Research so far has been shaped by the assumption that yolk androgens are something of a magic component of eggs, an added extra that boosts nestling growth and increases reproductive success later on in life (e.g., Eising *et al.*, 2001; Schwabl, 1996a). According to this premise, research has sought adaptive patterns of yolk deposition, such as differential allocation in relation to male quality (Gil *et al.*, 1999, 2006c), breeding density (Pilz and Smith, 2004; Schwabl, 1997a), or brood parasitism (Hauber and Pilz, 2003; Pilz *et al.*, 2005b). Consequently, large levels of variation in egg androgen levels between females have been explained by costs in either females (Gil *et al.*, 2006b) or nestlings (Müller *et al.*, 2005b). Although this scenario may still be true to some extent, some recent developments reviewed above have added several layers of complexity to it. Further research would greatly benefit by considering these implications to their full extent if we wish to avoid an erroneous interpretation of this maternal effect. Succinctly, these are the main points that in my view call for a revision of our initial views on egg androgens.

- *Growth benefits not so large*: First reports on the effects of yolk androgens in nestlings suggested that development could be substantially accelerated and asymptotic size increased by increasing the amount of yolk androgen (e.g., Schwabl, 1996a). Since then, several studies have reported negative effects for nestling growth and survival (Navara *et al.*, 2005; Sockman and Schwabl, 2000), or benefits accrued only under some particular conditions (Pilz *et al.*, 2004). All this evidence suggests that yolk androgens are not a universal mechanism that mothers can use to modify developmental speed and that this might not be their main selected effect.

- *Sex-specific optima*: Two different studies show that the sex of the embryo is of paramount importance in determining whether high yolk androgen will be beneficial or not (Saino *et al.*, 2006; von Engelhardt *et al.*, 2006). This suggests patterns of ontogenetic sexual conflict that may constrain adaptive sex-ratio allocation.
- *Neither beneficial nor detrimental*: A recent study in the pheasant (*Phasianus colchicus*) has shown that whereas birds hatching from androgen-injected eggs are not bigger, heavier, or have larger ornaments, the covariance among different traits in these birds is modified with respect to controls (Rubolini *et al.*, 2006a). This can be interpreted as androgens somehow interfering with main developmental processes or organizational gene expression, modifying the general development of the phenotype in a complex way, rather than boosting development quantitatively. Other authors have previously suggested that yolk androgens may not be costly resources in themselves, but rather modulators used to alter nestling phenotypes (Navara *et al.*, 2006b).
- *Effects on behavioral phenotypes and adult development*: The few studies so far that have investigated the consequences of yolk androgen exposure in adulthood are finding long-lasting effects in sensitivity to steroids, affecting sexual maturation patterns (Eising *et al.*, 2006; Strasser and Schwabl, 2004). Specially interesting are modifications of behavioral phenotypes or dominance that underline the role of yolk androgens in engineering alternative behavioral strategies (Daisley *et al.*, 2005; Schwabl, 1993).
- *Who benefits*? The important final question of whether females or offspring are benefited in the long run by differential androgen deposition (Müller *et al.*, 2007b) cannot be answered unless we know in detail the short- and long-term effects of yolk androgens in parents and young. These effects are largely unknown and therefore it is a rather frustrating exercise to try to examine the implications of yolk androgens for staging evolutionary conflicts.

To summarize, recent research shows that the original view of increased yolk androgens as something intrinsically beneficial for the nestlings is misguided. Rather than boosting or improving development quantitatively, the effects of yolk androgens probably pertain more to the modification of phenotypes within a framework of alternative strategies in the development of morphology and behavior. These strategies are known to respond to density-dependent cycles, and imply different payoffs depending on phenotype sex or individual quality (McNamara and Houston, 1996). In that respect, differential deposition of egg androgens by females in relationship to male attractiveness or breeding coloniality may be better interpreted as an active modification of the behavioral and phenotypic characteristics of these offspring. To sum up, as in some many other things in biology, the simple rule of more is better is likely not the case as far as yolk androgens are concerned.

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