Commentary

Testosterone in egg yolks: An ornithologist's perspective

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The past decade has seen a number of exciting discoveries regarding the passage of nutrients (1) and scarce secondary compounds (2) to mates and/or offspring through copulation and oogenesis. Until now, most of these discoveries have been in studies of insects, and biologists studying vertebrates may have been left, for no good *a priori* reason, with the impression that their organisms are simpler in this regard. Mammals, with their considerable postfertilization transfer of nutrients and hormones from mother to fetus during gestation, represent a fundamentally different pattern than is present in most insects. From this perspective, the reproductive patterns of birds are really much more insect-like: the bulk of the embryo's development takes place after the formation of the shell and its membranes, which effectively seal the chick in a capsule open only to gas exchange to develop with the material resources its mother included during oogenesis. The report by Schwabl (3) in this issue of the *Proceedings* opens up a new chapter in our understanding of just what those maternal resources can include. Suddenly, the developing chick's inheritance can include not only genes and the long-appreciated fat, protein, water, and minerals that every fertile egg is known to contain but also hormones. The presence of hormones in variable concentration suggests many possibilities for future work.

As an ornithologist with a decidedly nonendocrinological background, I will comment here on the fascinating possibilities that may lie ahead for a fusion of research in endocrinology and behavior, but all such comment is predicated on the completion of a good deal of supporting research verifying biological links that some will no doubt feel I too blithely assume. These biological links revolve around our rudimentary knowledge of the actual effects of testosterone (and other steroids) on the behavior of chicks all the way from hatching to adulthood. Much of what I will suggest assumes that variation in embryonic testosterone concentrations can lead to changes in aggressiveness and activity levels of chicks at hatching and that higher levels early on may transfer to higher levels of endogenously produced testosterone later in life. Schwabl cites one study (4) that

indicates androgen action on the spinal chord during embryonic development in the chick as well as a study with similar results in mice (5), but there is clearly a great deal to be learned about how testosterone affects the development of chick behavior at all ages. Schwabl's results show that the behavioral dominance of canary fledglings is positively and significantly related to the yolk testosterone concentrations of the eggs from which they hatched. But we need to know considerably more about how these changes develop in the chicks, how laying order might affect dominance independently of testosterone concentrations, how endogenously produced testosterone vs. yolk testosterone affect development, and how changing sensitivity of target tissues translates differences in yolk testosterone concentrations to differences in chick (and adult) behavior. In much of what follows, I will also assume that females can actually control levels of testosterone in the eggs they lay. The differences that Schwabl reports between patterns of hormones in the eggs of canaries and zebra finches, between different eggs laid by a given female canary, and between different females suggest considerable variation in this trait. But to establish parental control of yolk hormone levels, considerable work on the concentrations of hormones in the female's blood and the solubilities of the various hormones in yolk would have to be conducted to show that hormone concentrations in the eggs are not merely a direct reflection of circulating hormone levels in the female's blood stream at the time of yolk formation. Otherwise, females would not be able to change yolk hormone levels without also altering their own hormonal state, and the behavioral effects on the female are likely to serve as a strong selective deterrent to altering the levels of hormones in yolks. These considerable caveats aside, I will now venture out on some pretty long limbs in the hope that the sweetness of the fruits that appear at their tips justifies the risk!

Interactions between parent birds and their young have been of keen interest for almost 20 years. One aspect of this interaction was focused by Trivers (6) when he articulated the concept of parent-offspring conflict and explored its consequences for behavioral ecology. In brief,

since parent and offspring bear on average only half of their genes in common by descent, then the genetic interests of parent and offspring can be expected to diverge, especially in cases where the needs of offspring diminish and/or costs to parents increase. Much of the early work on this subject was dominated by a debate (reviewed in ref. 7) over whether the offspring could ever be expected to prevail in these conflict situations. If, as Schwabl suggests, testosterone in the yolk can increase the vigor and competitive ability of nestlings, his findings would suggest a tangible mechanism by which maternal control might be achieved. Many chicks will presumably benefit from having the elevated activity levels that result from higher testosterone concentrations, and conflict might then arise between parent and offspring over which chicks receive little or no testosterone. But it is easy to imagine that some embryos may not have sufficient reserves or sufficient inputs from their parents to sustain such elevated activity, and activity may rob resources from growth and maintenance and result in a net detriment to some chicks. In any event, if testosterone shows up in variable concentrations in the eggs of many wild species, and if chicks cannot counter variation in yolk testosterone by adjusting their endogenous production of the hormone or the sensitivity of their target tissues to it, there would be a strong indication that the mother has an effective means of gaining the upper hand in parent-offspring conflict. Other authors in the literature on parent-offspring conflict, most notably O'Connor (8), avoided the debate over who is in control and focused instead on the implications of the complicated web of relatedness and conflicts inherent not only between parent and offspring but also among the offspring within a brood as well. He predicted a spectrum of behaviors ranging from fratricide to infanticide and even suicide in response to decreasing food supply. Clearly, maternal manipulation of testosterone concentrations in eggs could either accentuate or ameliorate the conflicts of interest inherent in a brood of nestlings and its parents, and sampling testosterone concentrations of fresh eggs of known laying order in a variety of wild species should now be a high priority. Schwabl's findings also provide the blueprint for a valuable experi-

mental technique. Presumably, testosterone injections in the appropriate concentrations could be used to alter the status of some of the chicks in a brood to test the predictions made by O'Connor and others.

Against the background of parent-offspring conflict, the evolution of begging has also come to fascinate many ornithologists. If each parent and each of its offspring have divergent genetic interests, then, in the absence of significant costs of begging, the calls and postures used may give an overblown indication of offspring needs that reflects sibling competition in addition to communication of offspring hunger (9–12). The female parent, by shifting the stage in the laying cycle in which she initiates effective incubation, can affect the competitive environment among brood mates by altering the interval over which chicks hatch: those broods that hatch more asynchronously have larger asymmetries among brood mates from the very start. The degree of hatching asynchrony must respond, however, to other selective factors in addition to brood-mate competition (13), and one of the puzzles in begging studies has been how infrequently parents seem to ignore these asymmetries among their offspring (12, 14–17) in an effort to balance out the sizes of brood mates and thus increase the chances of a larger number of young fledglings. Schwabl seems justified in suggesting that the increasing testosterone concentrations in eggs laid later in the clutch could serve to even the playing field among chicks that may hatch asynchronously, since later-laid eggs tend to hatch later. So it may be that variation in testosterone concentrations reduces the need for parental discrimination of offspring requirements. It would be interesting in this vein to investigate further the two species (18–20) in which at least one parent (the female) is known to prefer smaller chicks during chick feeding. Is there no variation in yolk testosterone concentrations with laying order in these species? One of these species [the budgerigar *Melopsittacus undulatus* (18, 19)] is very asynchronous in hatching. Could the resulting nestling asymmetry in this species be too great to allow hormonal variation to compensate, thus encouraging the female to adjust parental feeding by discrimination of need instead? Such an explanation would presumably not apply to the second species [the pied flycatcher *Ficedula hypoleuca* (20)], in which hatching asynchrony is much less extreme.

Even if females are not controlling the competitive milieu for their chicks, they could be affecting the overall level of aggressiveness in their broods, and comparative work on testosterone concentrations in eggs could give valuable clues to the mechanism by which such diversity

has been attained. Any comparisons between species will only be possible if the considerable problems in comparing steroid assays from different species can be resolved. But assuming that these problems can be surmounted, there are many groups of species that suggest interesting comparisons. For instance, growth rates and the timing of fledging are quite variable in some groups [e.g., terns (*Sterninae*), auklets and murrelets (*Alcidae*)], and if females can control yolk testosterone levels in a manner independent of their own hormonal concentrations, variation in testosterone levels between species might provide a mechanism by which changes in growth patterns could be effected in an instant of geological time. Many brood parasitic species are uncommonly aggressive as chicks and have relatively high growth rates, and comparisons of testosterone concentrations in the eggs of parasitic and nonparasitic species might reveal a mechanism for rapid adjustment in this trait. The degree of territoriality also varies considerably in several families [e.g., bee-eaters (*Meropidae*), New World blackbirds (*Icteridae*), weavers (*Ploceidae*), swallows (*Hirundinidae*)] of closely related bird species, with some species defending large exclusive areas while others are densely colonial and defend only the immediate vicinity of the nest. If variation in testosterone levels affects the intensity of territorial behavior, it could lead to differences in the degree of coloniality in these and other groups (see also ref. 21). Alternative reproductive strategies among male fishes are often associated with differences in morphology, and the only clear analogue in the world of birds appears to be the distinctive courtship plumage patterns of alternative mating types in ruffs [*Philomachus pugnax* (22)]. If some of this variation is the result of modifying influences of yolk hormones, then theoretical models used to analyze the variation and evolution of plumage pattern in this species may need to be modified. Finally, it would be interesting to compare the testosterone concentrations in the eggs of sexually role-reversed species [e.g., jacanas (*Jacanidae*), phalaropes (*Phalaropodinae*), tinamous (*Tinamidae*), ostriches (*Struthionidae*)] with those in nonreversed relatives to see if part of this reversal is due to unusual concentrations of hormones in the eggs.

In a much more speculative vein, there have been occasional studies that have identified changes in the average sex ratios of young associated with laying order (reviewed in ref. 23), and covariation of testosterone concentrations with such a variation in sex ratio could have a powerful effect on the ontogeny of sexual dimorphism in size and behavior. If the sex of an egg could in some way be sensed or biased by the laying female, then the possibility of remarkable control

of the development of sexual roles in offspring could be achieved.

Now that the threshold has been past and ornithologists must consider the possibility that variation in hormone concentrations might be an important aspect of variability of the quality of eggs a female lays, it may be time to consider the possibility of the passage of other hormones as well. For instance, Schwabl detected estrogen, albeit in smaller concentrations, in some of the eggs he tested, and this, in conjunction with other evidence he cites (24, 25) for estrogen being passed on in the eggs of some species, raises the possibility that covariation of estrogen and testosterone could provide considerable flexibility in the control that the mother might exert over her offspring. In this case, comparative work on cooperatively breeding species [e.g., anis (*Crotophaginae*), jays (*Corvidae*), many Australian taxa] might be especially well advised.

The glimpse that Schwabl has provided into the possible influence that a mother can have on the behavior of her chicks provides a complement to the "ultimate" approaches and explanations favored by behavioral ecologists. Schwabl's findings mesh with the large body of work on connections between behavioral phenotypes and environmental selective factors (26–28), and further work in this area should enrich our understanding of how changes in behavior may come to be. Perhaps more importantly, if it turns out that variation in the testosterone concentrations in the eggs of wild birds is widespread and seems important to behavioral evolution, understanding this mechanistic link can provide field biologists with a powerful means to manipulate the message that is being sent from the mother to its offspring and to study the consequences of such manipulations for the subsequent development of the young.

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