Review

Maternal effects in quail and zebra finches: Behavior and hormones

Elizabeth Adkins-Regan [a,b,*], Sunayana B. Banerjee [c], Stephanie M. Correa [d], Cécile Schweitzer [e]

[a] Department of Psychology, Cornell University, Ithaca, NY 14853, USA
[b] Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA
[c] Department of Biology, Emory University, Atlanta, GA 30322, USA
[d] Department of Cellular and Molecular Pharmacology, University of California-San Francisco, San Francisco, CA 94143, USA
[e] Biogéosciences, Université de Bourgogne, Dijon, France

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ABSTRACT

Maternal effects are influences of parents on offspring phenotype occurring through pathways other than inherited DNA. In birds, two important routes for such transmission are parental behavior and non-DNA egg constituents such as yolk hormones. Offspring traits subject to parental effects include behavior and endocrine function. Research from the Adkins-Regan lab has used three avian species to investigate maternal effects related to hormones and behavior. Experiments with chickens and Japanese quail have shown that maternal sex steroids can influence sex determination to produce biased offspring sex ratios. Because all birds have a ZZ/ZW chromosomal sex determining system in which the female parent determines the sex of the offspring, these results raise the possibility that maternal steroids can influence the outcome of sex chromosome meiosis. Learning has been shown to influence egg investment by female quail in ways that are likely to alter offspring phenotype. In quail, embryonic and exogenous sex steroids have well established and long-lasting effects on sexual differentiation of behavior during a critical period *in ovo*, but elevated yolk testosterone has long-term effects on behavior that do not seem to be occurring through an alteration in sexual differentiation. In biparental zebra finches, removal of mothers alters not only later behavior, but also the adult response of the hypothalamic–pituitary–adrenal (HPA) axis to an environmental stressor, as indicated by plasma corticosterone. Birds raised only by fathers have lower levels of mRNA for both glucocorticoid receptors in several brain regions as adults. These studies add to the evidence that one generation influences the behavioral or endocrine phenotype of the next through routes other than transmission of DNA. Additional research will be required to understand the adaptive significance of these effects.

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1. Introduction

1.1. Parental effects

Parental effects are influences of parents on offspring phenotype occurring through pathways other than inherited DNA. Such effects are widespread taxonomically and are thought to have significant long-term evolutionary implications in addition to their impact on the offspring generation (Bonduriansky and Day, 2009; Mousseau and Fox, 1998). The routes of transmission for such effects range from constituents of the gametes such as mitochondria to the behavior of the parents and the opportunities for social learning they provide. Because eggs contain more non-DNA constituents that could affect offspring than sperm do, and because maternal behavior is more common in mammals than paternal

or biparental behavior, parental effects are often referred to as "maternal effects" or the two names are used interchangeably.

Characteristics of offspring phenotypes subject to parental effects include behavior and endocrine function. Parental effects on avian behavior have long been known to be significant and to have important consequences for reproduction. A classic example is sexual imprinting, in which the future choice of a bird's mate is guided by learned morphological features of its parents and siblings such as color (Immelmann, 1972). The behavior of the parents is also a major source of parental effects, as when birds learn their songs from the parents (Marler and Slabbekoorn, 2004). Because avian eggs are large, a second important route of transmission for parental effects is the non-DNA egg constituents. The eggs are formed over a period of a few days and therefore reflect the mother's condition during egg formation along with other aspects of her phenotype. The discovery by Hubert Schwabl that canary and zebra finch egg yolks contain sex steroid hormones from the mother (among many other things) that vary with laying order opened up an exciting new research field "yolk hormone mediated maternal effects"

* Corresponding author. Address: 218 Uris Hall, Cornell University, Ithaca, NY 14853-7601, USA. Fax: +1 607 255 8433.
  E-mail address: er12@cornell.edu (E. Adkins-Regan).

that is now a focus of attention by behavioral ecologists as well as avian endocrinologists (Schwabl, 1993). A multitude of questions are being asked, ranging from the endocrinology of yolk hormone deposition to the fitness consequences of yolk hormones for mothers and offspring. The current state of the field has been well summarized in several recent reviews Gil (2008) and Groothuis et al. (2005) [see, for example, 20,23,24].

This review will focus on research from the Adkins-Regan lab that has used Japanese quail and zebra finches to explore parental effects, either those mediated by maternal hormones or long-term effects on offspring endocrinology and behavior produced by parental behavior. This research program represents a continuing interest in long-term effects of early hormone exposure on later behavior (especially what are called organizational hormone effects) and in the role of early social experience in later reproductive behavior.

1.2. The research species

Japanese quail (*Coturnix japonica*) and zebra finches (*Taeniopygia guttata*) are very different kinds of birds in many ways, so that research with both is complementary and provides a wide view. Japanese quail are in the same family (Phasianidae) as chickens, whereas zebra finches are in the family Estrildidae, a part of the large clade of oscine passerines ("songbirds"). Japanese quail chicks are highly precocial at hatching, whereas zebra finch chicks are altricial in their development. Wild Japanese quail chicks are usually cared for by the mothers only, whereas zebra finches have biparental care, with male and female parents sharing the duties roughly equally (Gilby et al., 2011; Madge et al., 2002; Zann, 1996).

The two species also have three important characteristics in common. First, compared to other birds they reach reproductive maturity unusually rapidly (six weeks from hatching in Japanese quail, about 12 weeks in zebra finches), a major advantage for developmental research. Second, genomic resources are available. The zebra finch has a sequenced genome and the Japanese quail has a taxonomic relative with one (the chicken) (Hillier and et al., 2004; Warren et al., 2010). Third, both species are common in avian laboratory research, with rich bodies of information about behavior, hormones, and neuroendocrinology (Balthazart et al., 2009; Buchanan et al., 2010; Mills et al., 1997; West, 2009).

2. Do maternal sex steroid hormones influence sex determination?

Females of species with chromosomal sex determination, such as birds, are expected to produce equal (unbiased, 1:1 M:F) sex ratios. Sex allocation theory predicts biased sex ratios under certain circumstances, however (West, 2009). For example, one specific prediction of the more general Trivers–Willard theory of sex allocation is that high quality females (such as those in good body condition) will achieve greater fitness by producing (and therefore are selected to produce) more male than female offspring whenever males are larger than females, larger males outcompete other males to achieve greater reproductive success, and high quality females can better produce larger males (Trivers and Willard, 1973). A large literature has tested this and other hypotheses of sex allocation theory in wild and captive birds, with numerous reports of biased sex ratios (West, 2009). How are females able to do that? The question is particularly interesting in birds, because the avian female, with ZW sex chromosomes, is the genetic sex determining parent, raising the possibility of maternal influence over the process of meiosis that determines which sex chromosome will be in the eggs. That makes it important to know whether primary sex ratio (sex at fertilization) is biased or only the secondary sex ratio (sex at hatching).

Maternal effects mediated by the mother's sex steroids inspired two new hypotheses to help solve the mystery of how female birds are able to bias offspring sex ratios. The discovery of substantial maternal androgens in the yolks of freshly laid eggs suggested the possibility that maternal yolk hormones could be involved in sex determination, with females biasing eggs toward males by allocating more yolk androgens (hypothesis 1) (Petrie et al., 2001). Do male egg yolks actually contain more testosterone? The first attempt to find out relied on steroid measurements in peahen eggs after 10 days of incubation (Petrie et al., 2001). This is problematic because the embryonic gonads have developed and begun producing their own sex steroids before then, so that the measures could reflect embryonic hormones as much or more than maternal hormones.

To better test this hypothesis in a galliform bird, Kevin Pilz, in collaboration with Hubert Schwabl, measured yolk steroids in fertilized quail eggs when they were freshly laid and unincubated (day 0) or had been incubated for 10 days, in order to see how yolk hormones, especially maternal androgens, changed during incubation as a function of embryo sex (Pilz et al., 2005). A small sample of yolk was withdrawn on day 0 and then the eggs were incubated for later yolk sampling and genetic sexing of the embryos. On day 0 there was no sex difference in any of the three androgens measured (Fig. 1). By day 10 levels of all three androgens had dropped substantially. This presumably reflects metabolism of maternal androgens by the embryos together with low androgen production
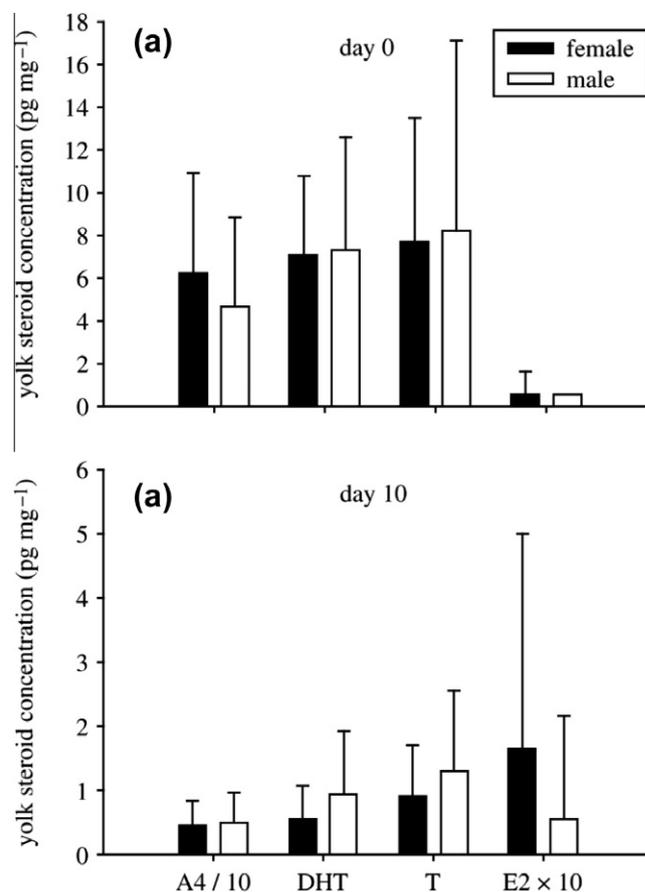

Fig. 1. Sex steroid concentrations (means ± standard deviations) in yolks of male and female quail eggs. There was no sex difference in yolk concentrations in freshly laid eggs (a). After 10 days of incubation (b), all the androgens, but not the estradiol, had declined markedly (note difference in y-axis scale) ($P < 0.0001$ for each androgen), overall concentrations across steroids differed significantly by sex ($P < 0.05$), estradiol concentrations tended to be higher in female eggs ($P < 0.056$) and ratios of estradiol to total androgens were much higher in female eggs ($P < 0.0094$). Reproduced from (Pilz et al., 2005).
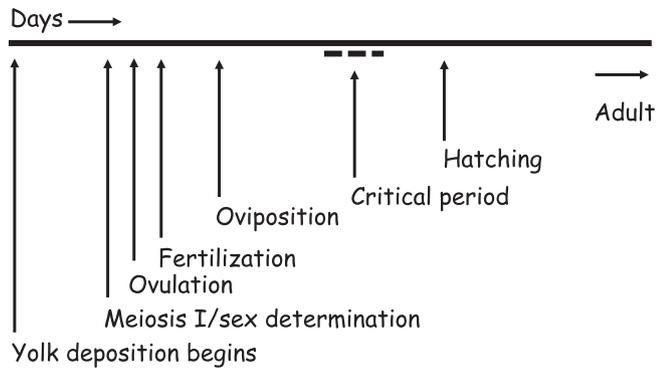
Fig. 2. Schematic avian developmental timeline from the beginning of yolk deposition through oviposition and hatching to adulthood. Note that genetic sex is determined shortly before ovulation and prior to fertilization. "Critical period" refers to the period of hormonally organized sexual differentiation that has been established in Japanese quail (Balthazart et al., 2009).

by the embryos (Ottinger et al., 2001). As expected based on the published literature with this species concerning embryonic steroid production, estradiol levels were now higher than on day 0, and estradiol to androgen ratios were significantly higher in female than male yolks, reflecting sexual differentiation of gonadal hormone production (Ottinger et al., 2001; Schumacher et al., 1988).

Thus there was no support from this study for hypothesis 1 (more maternal yolk androgens in male eggs).

While this rules out a simple causal role for yolk testosterone in quail sex determination, it remains possible that yolk sex steroids are associated with ovum sex in interaction with other factors, including laying order (Badyaev et al., 2005; Goerlich et al., 2010; Muller et al., 2002).

The second hypothesis tested in the lab (hypothesis 2) focused on maternal hormones not in the yolk at laying but instead produced by the follicular cells around the yolk at the time of meiosis, when it is determined which maternal sex chromosome, Z or W, an ovum will receive. Meiosis occurs shortly before ovulation (Fig. 2). Because the follicular cells are producing mainly progesterone at that time (Etches and Duke, 1984), Stephanie Correa hypothesized that maternal progesterone might influence the course of meiosis to bias offspring primary sex ratio (Correa et al., 2005). She tested this hypothesis in collaboration with Patricia Johnson in two ways. First, inseminated chickens were given single injections of one of two dosages of progesterone shortly before ovulation, at the time of meiosis. Each hen's plasma progesterone level was measured, and the first egg laid after injection was genetically sexed following a period of incubation. The higher dose produced elevated plasma progesterone and a female biased sex ratio, and across all groups maternal plasma progesterone levels were higher when eggs were female (Fig. 3a and b). This effect was not due to an alteration in
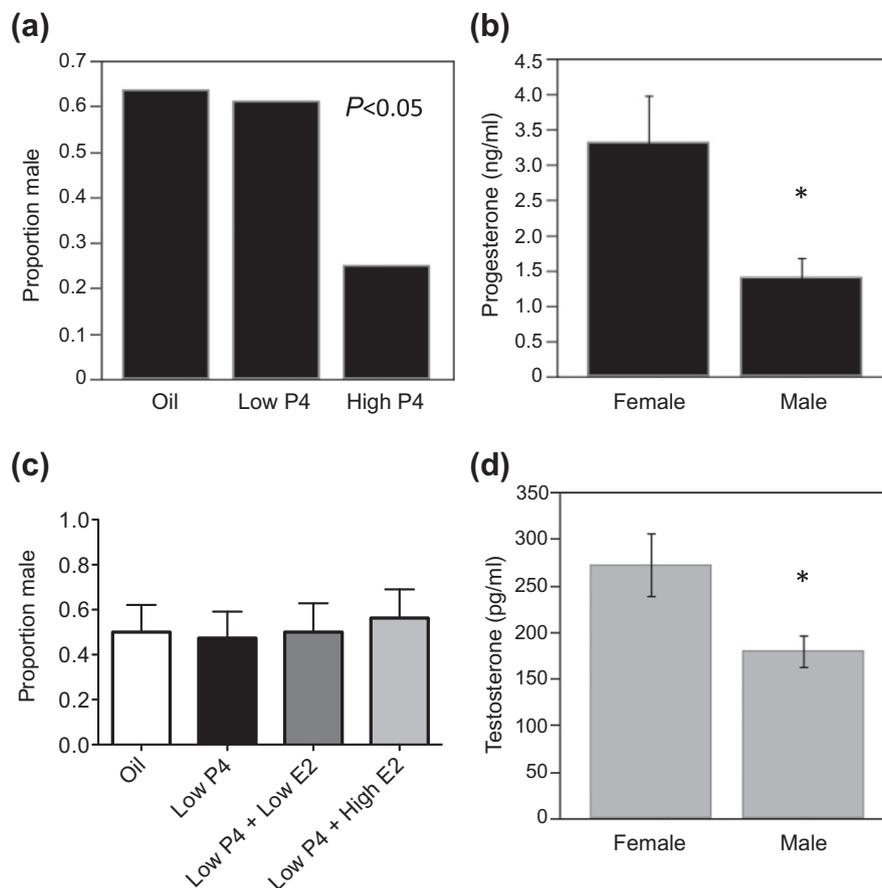

Fig. 3. Manipulation of maternal progesterone (P4) but not estradiol (E2) in chickens alters offspring sex. (a) The sex ratio produced in each of three treatment groups: Oil, Low P4 (0.25 mg P4), and High P4 (2 mg P4). The effect of progesterone on sex is significant (Likelihood ratio test, $P < 0.036$). (b) Plasma progesterone levels were higher in hens that laid female eggs than in hens that laid male eggs ($P < 0.006$) in samples taken two hours after injection, the approximate time of meiosis I for progesterone-induced ovulations. (c) The sex ratio produced in each of four treatment groups: Oil, Low P4 (0.25 mg P4), Low P4 + Low E2 (0.25 mg P4 + 0.05 mg E2), and Low P4 + High E2 (0.25 mg P4 + 0.10 mg E2). Estradiol manipulation during progesterone-induced ovulations did not affect offspring sex ($P = 0.978$). (d) Plasma testosterone levels were significantly higher in hens that produced females than in hens that produced males ($P = 0.011$). Testosterone was measured in P4-manipulated hens (Oil, Low P4, and High P4) two hours after injection, the approximate time of meiosis I for progesterone-induced ovulations. Reproduced from (Correa van Veen, 2007; Correa et al., 2005). *$P < 0.05$.

the hens' estradiol levels, for the following reasons. Although progesterone injection did raise estradiol as well, an estradiol injection combined with the lower progesterone dose had no effect on egg sex ratio (Fig. 3c), and maternal plasma estradiol did not differ for female and male eggs. In this study the progesterone injections also increased the hens' plasma testosterone. Furthermore, maternal testosterone was higher for female eggs (Fig. 3d). This raises the possibility that it is testosterone rather than progesterone that is responsible for the effect of the progesterone injections. In the statistical model of the data, however, progesterone was a better predictor than testosterone of egg sex (Correa van Veen, 2007). This again supports hypothesis 2, although further research, for example, in which endogenous progesterone is lowered, will be needed to clarify the role of progesterone in sex ratio biasing. In addition, the testosterone results suggest that it should not be ruled out as a potential influence on sex ratios.

A subsequent study looked at changes in endogenous sex steroids in response to mating and see if the changes predicted the sex of the fertilized eggs that were laid. Japanese quail were the subjects because of their advantages for mating behavior trials. In addition to mating behavior, the condition of the females
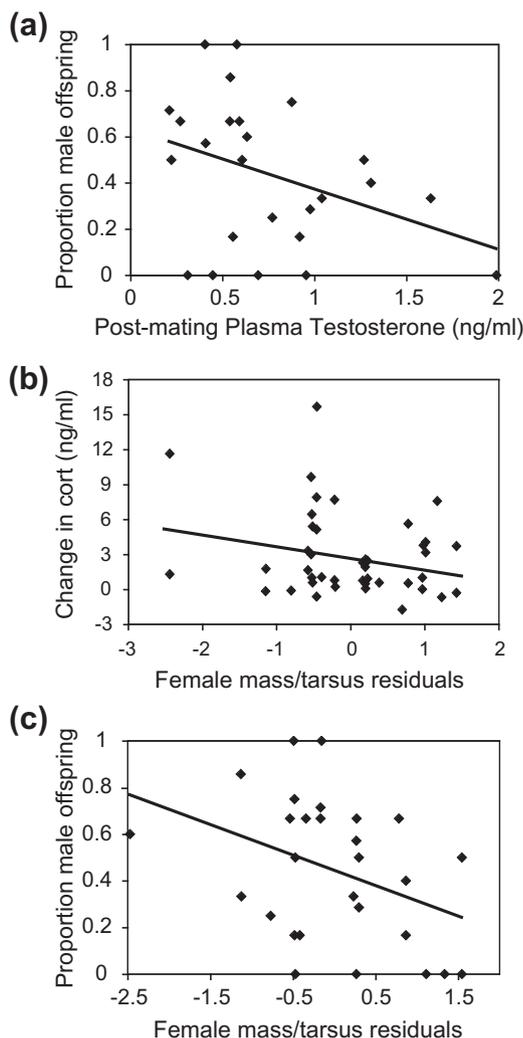

(a)

(b)

(c)

Fig. 4. Female testosterone levels and body condition predict offspring sex ratios in Japanese quail. (a) Plasma testosterone concentration after a mating trial was a significant predictor of the primary sex ratio (the proportion of fertilized eggs that were male) after a single insemination ($P < 0.01$). (b, c) Female mass/tarsus residuals were a significant predictor of both the post-mating change in female corticosterone from baseline and the primary sex ratio after a single insemination ($P < 0.01$). Reproduced from Correa et al. (2011).

(residuals of the regression of mass on tarsus length, where higher mass to tarsus length indicates better condition) was also measured, because female condition is an important predictor of offspring sex ratio in the Trivers–Willard theory of sex allocation, as described above. The results of the study showed that: (a) most females showed some degree of post-mating increase in corticosterone, (b) female quail in better condition had smaller post-mating increases in corticosterone, and (c) females in better condition also produced fewer sons than females in relatively poor condition when male mating behavior was taken into account in the statistical analysis (Fig. 4b and c) (Correa et al., 2011). Corticosterone concentrations themselves did not predict offspring sex ratio, however, nor did progesterone concentrations. Instead, the best predictor of offspring sex ratio was testosterone, with higher absolute testosterone concentration after a mating trial predicting fewer sons (Fig. 4a). Thus the results of this quail study also support the hypothesis that maternal steroids can influence the sex of eggs, and add to the evidence that testosterone may play a role. As in the chicken study, testosterone was associated with fewer, not more, sons, further evidence against hypothesis 1. In work from another lab with a non-galliform species, the zebra finch, experimentally elevated maternal testosterone did produce a male-biased sex ratio, but with reduced hatching success for those male embryos (Rutkowska and Cichon, 2006).

With respect to corticosterone, Pike and Petrie did find that experimentally elevating corticosterone in laying female quail led to more female offspring (Pike and Petrie, 2006). It is possible that a brief elevation in corticosterone, as in the mating trial study above, does not affect sex ratio, but a more sustained elevation, as in the Pike and Petrie study, does, perhaps by altering the level of another hormone.

It is not clear why progesterone was associated with a sex ratio bias in chickens but not in quail. A possible evolutionary explanation will be discussed below. Because the female bird is the heterogametic (ZW) sex, it is possible that sex ratio biasing in chickens occurs because progesterone biases the course of meiosis. It is not known, however, what the mechanism is for such meiosis biasing, and how it could differ in the two species.

3. Do females engage in strategic egg investment to influence offspring phenotype?

Avian females can also affect offspring phenotype through egg characteristics such as size. Egg size depends on multiple factors such as individual female identity, age, laying order, etc. (Christians, 2002). An interesting possibility is whether female birds can strategically vary egg size adaptively (in ways that enhance offspring fitness) depending on characteristics of the male mate or the sex of the ovum (Horváthová et al., 2012). For female Japanese quail, whether the male's arrival can be predicted in advance is an important determinant of how many fertilized eggs she will lay following mating. Females lay more than twice as many fertilized eggs when mating in a context (an environment as represented by a room and a cage) in which they have mated previously (Adkins-Regan and MacKillop, 2003). The females have learned that the context predicts male arrival. The type of learning is classical or Pavlovian conditioning, a highly adaptive (fitness enhancing) process (Domjan, 2005). Are maternal effects mediated by egg size also subject to this kind of learning? Joanna Rutkowska found that female condition (mass in relation to tarsus length) was positively related to egg mass only if the females had mated in a context predicting male arrival (Rutkowska and Adkins-Regan, 2009). Furthermore, those females laid heavier eggs for daughters than for sons (means 12.2 g and 11.9 g, respectively). Thus learning influenced egg mass and, if greater egg mass enhances offspring
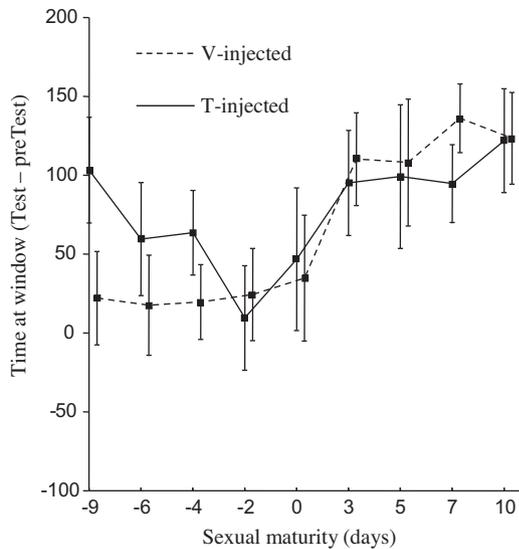
Fig. 5. Elevated yolk testosterone does not affect the time male quail spend looking at females. Shown is the mean ± SEM time (in sec) spent at the window (test–pretest) measured in males from testosterone-injected (T) and vehicle-injected (V, control) eggs from 9 days before sexual maturity to 10 days after sexual maturity. Reproduced from Schweitzer et al. (2013).

size or quality, should be included among the many factors that influence maternal investment.

This result raises two questions. First, which egg components are contributing to the egg mass effect, and through what mechanisms (Chin et al., 2012)? Second, when the females have a learned opportunity to strategically invest in eggs, why is it the female eggs that are heavier? Adult female Japanese quail are larger and heavier than males, and large size may be more of an advantage for females than for males. This might also help explain why the sex ratio bias associated with progesterone occurs in chickens but

not in quail. Male chickens are larger than females. The Trivers–Willard hypothesis of sex allocation would predict a different offspring sex bias by females in good condition in these two species, all other things being equal.

4. Do maternal yolk hormones have long-lasting organizational-like effects on later behavior?

The discovery of maternal steroids in egg yolks raised important questions about the consequences of these hormones for the behavior of the offspring. Most research has focused on offspring behavior during the early post-hatching period, but more recently there have been several reports of effects of elevating testosterone in freshly laid egg yolks on later adult behavior (Partecke and Schwabl, 2008; Ruuskanen and Laaksonen, 2010; Strasser and Schwabl, 2004). These reports suggest that endogenous maternal yolk steroids might be capable of organizing future behavior, that is, permanently affecting the behavioral phenotype by acting on the nervous system during early development. Such organizational hormone effects could contribute to within-sex individual differences in behavior. It is conceivable that they could even affect the sexual differentiation process that is also going on as a result of hormone exposure during pre-hatching development (Carere and Balthazart, 2007).

The Japanese quail is an ideal species to examine the relationship between maternal yolk steroid effects and sexual differentiation processes. Sexual differentiation has been extensively investigated and it has long been known that the sex steroids produced by quail embryos determine the adult sexual phenotype for behavior (Balthazart et al., 2009). Several key sex differences (for example, copulatory mounting, a male-specific behavior) are hormonally organized by embryonic hormones during a critical period prior to hatching (see Fig. 2). Relatively high levels of estrogens (as would normally occur in genetic female embryos) lead to absence of male typical behavior like mounting in adulthood (i. e., a female behavioral phenotype), whereas relatively low levels of estrogens
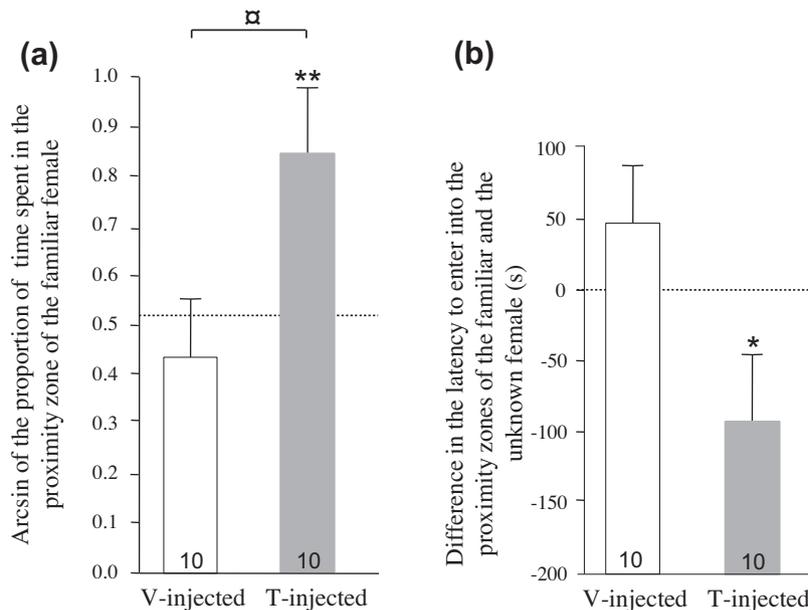

Fig. 6. Elevated yolk testosterone produces males (T-injected group) that prefer to be near familiar females both as juveniles and adults, whereas control males (vehicle[V]-injected group) do not show such a preference. (a) Arcsine transformed mean ± SEM proportion of time spent in the proximity zone of the familiar female across all two-choice tests given both before and after sexual maturity. (b) Mean ± SEM difference in the latency to enter into the proximity zones of the familiar and the unfamiliar (unknown) females. **, *: significant preference (**$P \leqslant 0.01$; *$P \leqslant 0.05$). ¤: significant difference between treatments ($P \leqslant 0.05$). The dotted line indicates the preference threshold (spending more than 50% of the time in the proximity zone of the familiar stimulus or entering earlier into the proximity zone of the familiar stimulus). Reproduced from Schweitzer et al. (2013).

(as in genetic male embryos) lead to the presence of male typical behavior. Genetic male eggs injected during the critical period with either estradiol or testosterone (which can be aromatized to estrogens) are demasculinized, that is, have reduced male traits. Yet in discussions of behavioral consequences of maternal yolk hormones, it is often assumed that yolk testosterone will enhance male traits. That could only happen in quail if the maternal effect pathway were different from the sexual differentiation pathway.

In order to see whether yolk testosterone typical of a maternal allocation (a) has any long-term behavioral effects in quail or (b) affects sexual differentiation of behavior, Cécile Schweitzer injected yolks of freshly laid quail eggs with a high but physiological amount of testosterone (50 ng, in order to elevate concentrations to 16 ng/g yolk, where the mean yolk testosterone of uninjected eggs in other studies is 13 ± 8 ng/g) (Schweitzer et al., 2013). Several interesting results emerged. First, a behavior that shows a pronounced sex difference in this species was not affected in the way that would be predicted if there were interference with the organizational period of the sexual differentiation process. The behavior is time spent looking at females. Among adult quail, males look at female stimuli much more than females do. This is a learned response based on experience mating with females. Because of the dependence on mating experience, the sex difference in looking time might be indirectly affected by hormone exposure during the organizational period along with (because of) an effect on copulatory mounting. Such a hypothesis is supported by a study in which females hatched from eggs treated with an aromatization inhibitor, a treatment known to produce copulatory mounting in females, also showed interest in looking at female stimuli (Balthazart et al., 1997, 2009). Thus if maternal yolk testosterone alters sexual differentiation, it would be predicted to lower looking time in males. In the Schweitzer et al. study, however, testosterone treatment of eggs did not reduce male looking time (Fig. 5). Instead, both control and testosterone exposed males showed the same increase in looking as they reached sexual maturity. The second result was seen when the males were tested both as juveniles and as adults for their preference for affiliating with a familiar vs. unfamiliar female. Control males do not show any preference (Fig. 6). Males from testosterone treated eggs, however, preferred familiar females. Because this effect occurred regardless of the males' ages, even prior to sexual maturity, it is unlikely to be a sexually motivated preference and cannot be readily interpreted as an alteration in sexual differentiation. The third result concerned a non-behavioral trait, body weight. Females of this species weigh more, but this sex difference is not a result of hormonal organization from embryonic hormones (Koba et al., 2008). Yet testosterone affected female weight, and females from testosterone treated eggs weighed more, not less, than control females.

Thus physiologically realistic elevated yolk testosterone did have long-term organizational-like effects on quail that cannot be explained by positing interference with sexual differentiation. There seem to be multiple pathways for organization by yolk testosterone depending on the amount, the source (mother or embryo) and when it is first present in biologically significant amounts in the yolk (at laying or later). Furthermore, no adult male behavior was demasculinized in the study (treated males copulated and crowed at normal rates) and in no case was a male typical trait enhanced.

5. Are there long-term effects of parental behavior on offspring hormones?

In species with parental care, parental behavior is a major source of parental effects on offspring phenotype. Most obviously, how well the young are provisioned determines their size, condition and survival. Short-term effects of parental behavior on the

endocrine systems of offspring are also well established, as when failure to provision adequately results in elevated glucocorticoids (de la Mora et al., 1996; Kitaysky et al., 2003; Quillfeldt et al., 2006).

Among mammalian researchers, a great deal of attention has been paid recently to the possibility that parenting might have a long-term, even permanent, effect on the functioning of the hypothalamic-pituitary-adrenocortical (HPA) axis of the offspring. It has been known for over 50 years that rat pups of mothers handled in infancy (a mild stressor) show a less pronounced HPA response (lower corticosterone levels) to a novel stressor (Levine, 1967), a maternal effect. Separations from the mother or naturally occurring variations in maternal behavior also result in altered HPA function (Liu et al., 1997). Such maternal effects are permanent, and the adult HPA seems to be programmed earlier in development for its response profile to adult stressors. In the biomedical literature this phenomenon is often described as early adversity leading to poorer adult phenotypes (as pathological). Ecologists and animal behaviorists are more inclined to consider that these maternal effects might be predictive adaptive responses in which mothers
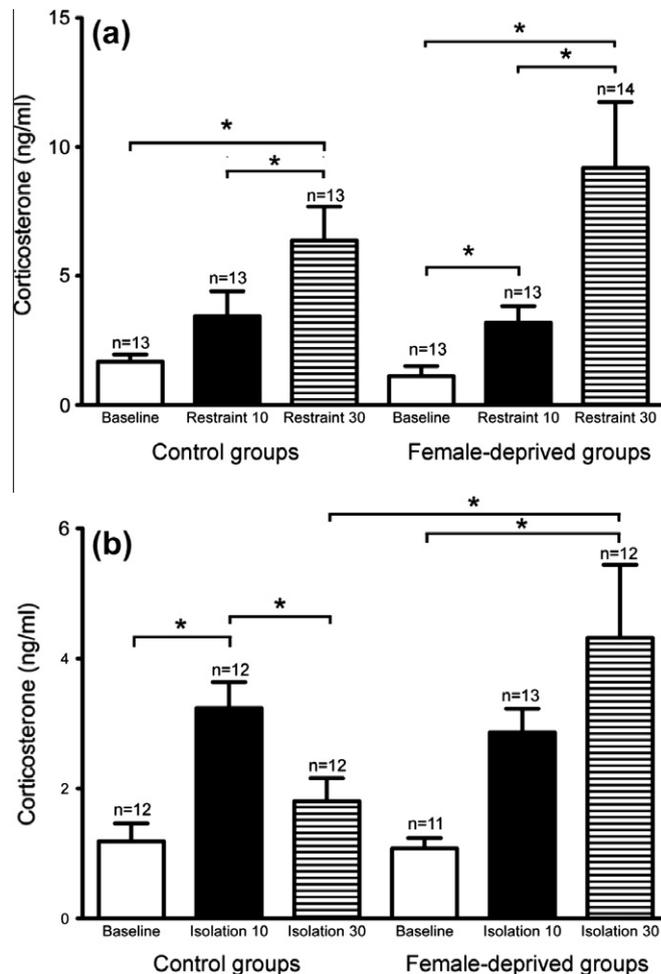

Fig. 7. Early absence of the female parent has long-term consequences for HPA axis function in response to a social stressor in zebra finches. Shown are mean ± SEM corticosterone concentrations in control and female deprived birds of both sexes of offspring after they were adults. In (a) groups were sampled at baseline or after 10 or 30 min of restraint. The control and maternally deprived groups did not differ, with both showing a similar response. In (b) groups were sampled at baseline or after 10 or 30 min of social isolation in a novel environment. The corticosterone concentrations of the maternally deprived birds were still elevated at 30 min, whereas those of the control group had returned to baseline. $^*P < 0.05$. Reproduced from Banerjee et al. (2012).

transmit information about environmental conditions to their offspring that will better prepare them to deal successfully with similar environmental conditions (Monaghan, 2008; Sachser et al., 2011). Both views could be correct depending on the severity of the early conditions, as when brief maternal separations lead to a less reactive and probably "healthier" HPA but longer separations lead to a more reactive HPA that has negative health consequences (Levine, 2005).

Most birds are biparental, and such species provide excellent opportunities to increase understanding of parental effects of these kinds in two ways. First, one can see whether separation from maternal behavior has any long-term effect on the offspring HPA when there are two parents instead of mothers only. Will the fathers compensate for maternal behavior absence so that the HPA is unaffected? Second, one can compare outcomes of maternal vs. paternal separation, a more interesting comparison in birds, where both sexes feed the young, than in biparental mammals, where only the lactating mother feeds the young.

Zebra finches, with biparental care and rapid development, are ideal for such research. Long-term effects of parental behavior and other characteristics on later offspring behavior, including singing and mate choice, are already well established (Zann, 1996). For example, chicks raised without fathers do not show the normal preference for pairing with the opposite sex as adults, and males raised without mothers show a strong preference for pairing with other males (Adkins-Regan and Krakauer, 2000; Banerjee, 2010). In a recent study, Sunayana Banerjee removed female parents from

breeding aviaries when chicks were still very young and measured the corticosterone responses of the adult offspring to a stressor (social isolation in a novel room) (Banerjee et al., 2012). The female-deprived offspring survived and gained weight just like the controls with two parents, so that any long-term effects on endocrine function would be due to the loss of the mothers' behavior and not to nutritional stress. As adults the corticosterone responses of the female-deprived birds to 30 min of restraint (a standard stressor) were no different from those of the controls: a significant elevation after 10 min and an even greater elevation at 30 min (Fig. 7). Yet they were hyper-responsive to the social stressor. After 30 min of isolation in the novel room, corticosterone concentrations of controls were similar to baseline (were no longer elevated), whereas those of the female-deprived birds were still quite elevated (Fig. 7). Furthermore, female-deprived birds had lower levels of the mRNAs for both adrenal glucocorticoid receptors in the hypothalamus, as determined by qPCR (Fig. 8). These altered mRNA levels might account for the dampened negative feedback on corticosterone at 30 min. Interestingly, maternal separation in rats also leads to lower forebrain glucocorticoid receptor mRNA levels (Liu et al., 1997). This suggests that a permanent re-programming of the brain's regulation of the glucocorticoid response by maternal disruption might be a widespread phenomenon wherever there is parental care, not just something that only happens in mammals or where there is female-only care. In rats naturally occurring variations in maternal behavior lead to different levels of forebrain glucocorticoid receptor gene expression through an epigenetic mechanism (DNA methylation) (Weaver et al., 2004). It is not yet known whether a similar epigenetic mechanism is responsible for the effect of maternal behavior absence in zebra finches. Additional research is also required to know whether any of the effects of mother removal on zebra finch offspring are predictive adaptive responses, that is, whether the altered HPA response would enable birds to be more successful in difficult social environments.

6. Conclusions

Based on our studies along with those of many other research groups, it seems likely that parental effects are ubiquitous in birds. Some are hormonally mediated or have hormonal consequences. Some are behaviorally mediated or have behavioral consequences. Some are influenced by social factors or learning. Their existence and impact on phenotype are clear. Many questions about their underlying mechanisms and evolution remain to be answered, however (Groothuis and Schwabl, 2008). How do they occur? How can steroids affect meiosis (if they do) to bias sex ratios (Pike and Petrie, 2003; Rutkowska and Badyaev, 2008)? What are the selective pressures that lead to tailoring of offspring phenotype through parental effects rather than DNA? Who benefits from parental effects – the parents, the individual offspring, or both? What are their long-term consequences for the evolutionary process (Danchin et al., 2011)? Field as well as laboratory studies will be required to answer these questions.

Acknowledgments

Fig. 8. Early absence of the female parent has long-term consequences for glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) mRNA levels in zebra finches. Shown are mean ± SEM GR mRNA (a) and MR mRNA (b) levels in control (CTRL) and female deprived (FD) adult zebra finch offspring in the hippocampus (HIPPO), hypothalamus (HYPO) and cerebellum (CERE). $^*P < 0.05$. Reproduced from Banerjee et al. (2012).

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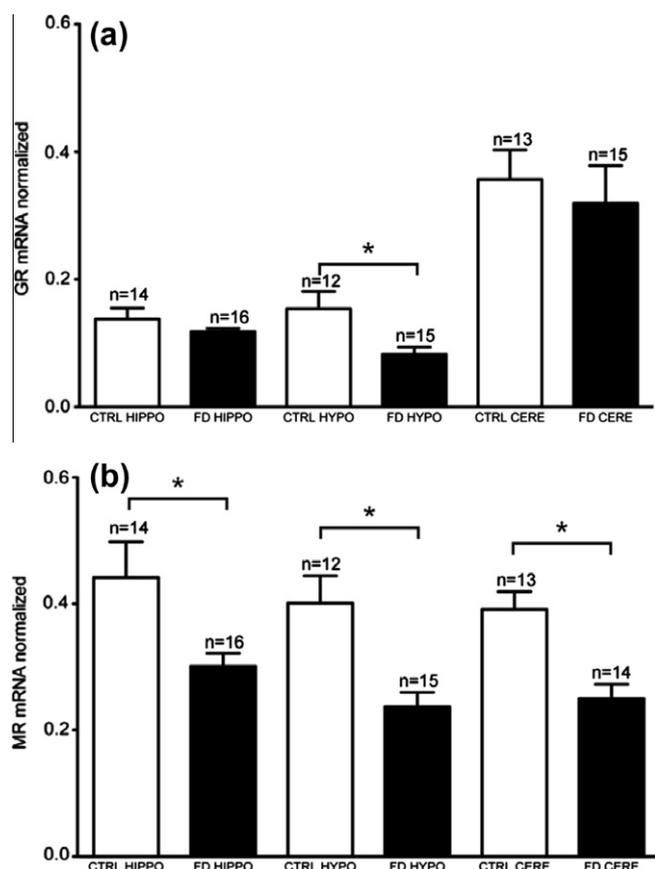
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