

Hormones and the development of sex differences in behavior

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Abstract Birds exhibit striking diversity in behavioral sex differences. A necessary complement to the study of the ecology and evolution of these sex differences is discovering the proximate physiological mechanisms for their development (sexual differentiation) and adult expression. Experiments with Japanese quail (*Coturnix japonica*) have shown that sex differences in crowing, strutting, and sexual receptivity are produced partly or largely by hormonal dimorphism in adulthood (activational effects of sex steroid hormones), whereas the sex difference in copulatory mounting is produced by permanent actions of sex steroids occurring early in development, during the embryonic period in this precocial species (organizational effects). Experiments with zebra finches (*Taeniopygia guttata*), an altricial species, have revealed organizational effects on singing and mating that occur after hatching. In both species, sex differences in whether birds are interested in females vs. males are produced by organizational rather than activational effects. Results of experimental manipulations of sex steroid actions in juvenile zebra finches suggest hormonal regulation of the onset of interest in the opposite sex as sexual maturity is reached. Zebra finches are socially monogamous and permanently paired across breeding attempts. Experimental reduction of sex steroid actions had no effect on pairing success in either sex. The regulation of adult pair formation by sex hormones is more likely to occur in species that pair seasonally. The concepts of organization and activation and the results of these experiments raise a number of questions and are a potential source of hypotheses about

developmental changes responsible for the evolution of species diversity in sex differences.

Keywords Japanese quail · Pair formation · Sex steroid · Sexual differentiation · Zebra finch

Introduction

The roughly 9000 species of living birds exhibit an impressive diversity with respect to behavioral differences between the sexes. Such differences are quite marked in species such as turkeys, manakins, and ruffs, in which males but not females engage in elaborate courtship displays, whereas females but not males perform all of the parental care. In buttonquail, the roles are reversed, and females but not males produce advertisement calls (Madge and McGowan 2002). In albatrosses and many other seabirds, on the other hand, there are few obvious sex differences in behavior, and displays tend to be performed by both sexes together.

Understanding both the fact of sex differences and the diversity in their nature and magnitude can be achieved by addressing all four of Tinbergen's (1963) aims for ethology: function, phylogeny, development, and physiology. These last two have been the focus of this author's research program. The progress that has been made by the author and other researchers and the many unanswered questions that remain form the subject of this review.

Sexual differentiation of behavior: conceptual background

Sexual differentiation is the developmental process that produces sex differences in morphology and behavior.

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Although birds have sex chromosomes, most of the genome is in the autosomes. The fundamental question then is, how are two sexual phenotypes created from a largely monomorphic genome? There are several possibilities (reviewed in Adkins-Regan 2005). The small portion of the genome that is dimorphic (the genes on the sex chromosomes) could be directly responsible. Alternatively, sex differences in behavior could be learned through experience or by observing others. They could also be situational, arising from local demography or social context (for example, group sex ratio), and could reflect decisions about the appropriate tactics to adopt in that context. They could arise through hormone action. In 1959, Phoenix, Goy, Gerall, and Young proposed that organization very early in development by an animal's endogenous sex hormones was the mechanism producing the sex difference in the mating behavior of guinea pigs. Their theory remains an essential conceptual starting point for an understanding of how hormones contribute to different behavior in males and females. They distinguished between two kinds of effects of sex steroid hormones: activational and organizational. Activational effects occur at puberty and in adulthood and serve to regulate behavior, allowing it to be expressed when that is appropriate. Typical examples would be the cyclic estrous behavior of female mammals, which is activated by ovulatory levels of estrogens or estrogens plus progesterone, and seasonally occurring breeding behavior (territory establishment, nest building, mating) of birds living in the temperate zone, which is activated by increases in gonadal androgens and estrogens (Wingfield and Farner 1993). Organizational hormone effects, on the other hand, occur during a limited critical period early in development, the fetal or neonatal period in the case of mammals. During this period sex steroids produced by the gonads permanently establish the future behavioral phenotype of the animal (masculine or feminine).

According to this theory, sex differences in the behavior of adults can result from either activational or organizational effects. If the adult sex hormone levels are sexually dimorphic, and if sex reversal of those levels results in sex reversal of behavior, one would conclude that the sex difference was activational. For example, in a number of species of birds, normally only males sing, but the administration of testosterone to adult females causes them to begin singing (reviewed in Balthazart and Adkins-Regan 2002). The females are capable of singing but do not normally sing because they lack sufficient activational testosterone. If sex reversal of adult hormone levels does not alter the behavior, however, then an organizational hormone effect that occurred much earlier in development is an alternative hypothesis. As the Phoenix et al. (1959) experiment and numerous others have shown, many behavioral sex differences in mammals are produced by hormonal activation, organization, or a

combination of both, and organization follows a pattern in which the presence of testicular hormones in early development masculinizes the nervous system of genetic males and the absence of testicular hormones produces a feminized nervous system (Nelson 2005). In this manner, two sexual phenotypes are created from an initially undifferentiated embryo and a largely monomorphic genome, and the role of the sex chromosomes is mainly to establish the sex of the gonads. Although there are several other routes to adult sex differences in behavior, it is remarkable how many of those studied in mammals turn out to be produced through hormonal organization and/or activation.

With hormonal organization, sex differences can be generated that do not risk the appearance of unwanted traits in the "other" sex because the trait can be permanently suppressed or prevented from developing in one sex. This is important for understanding the evolution of traits that make a positive contribution to fitness in one sex but have a negative effect in the other. With activation alone as a mechanism, there would always be the possibility of the trait appearing to a slight degree in the "wrong" sex, because both sexes produce the same hormones, only in different quantities.

Sexual differentiation in Japanese quail (*Coturnix japonica*)

Does the organizational hormone theory of sexual differentiation apply to the behavior of birds? The domestic Japanese quail has been the subject of a number of experiments to answer this question. These birds have significant advantages for such research, including high fecundity, rapid development, and some clear sex differences in hormonally regulated behavior. As is typical of some other galliform birds, males emit frequent loud crows, perform a strutting display, and engage in copulatory mounting with females. Females do not show any of these behaviors and instead may adopt a receptive posture in response to male's mating attempts. All of these behaviors are dependent on gonadal hormones for their expression. They are absent in birds that are gonadectomized or housed on short days to produce gonadal regression, and they are restored by treating males with testosterone or females with estradiol (Beach and Inman 1965; Adkins and Adler 1972; Balthazart et al. 1986).

Testosterone and estradiol levels of adult birds are sexually dimorphic, with higher testosterone in males and higher estradiol in females (Balthazart et al. 1986). Giving females testosterone elicits some crowing and strutting, and giving males estradiol causes them to adopt the receptive posture to other males' mating attempts (Adkins and Adler 1972; Adkins 1975; Schumacher and Balthazart 1983; see

also Yazaki et al. 1999). Thus, the sex differences in crowing, strutting, and receptive behavior are partly or largely (receptivity) activational in origin.

Testosterone-treated females do not engage in copulatory mounting, however, as if this sex difference is organizational (Adkins and Adler 1972; Balthazart et al. 1986). This hypothesis was first tested by injecting eggs with estradiol during incubation, in the expectation that the critical period for an organizational effect would likely fall during the embryonic period in this highly precocial species (Adkins 1975). A single egg injection of 50 µg of estradiol benzoate (EB) given on day 10 of the 17- to 18-day incubation period eliminated copulatory mounting behavior from the males that hatched from these eggs, even though they were given testosterone as adults prior to testing to ensure that their activational hormone levels were adequate for the expression of mounting. The males now had a female phenotype with respect to copulatory mounting. A subsequent experiment examined the critical period for this effect (Adkins 1979). Copulatory mounting was again completely eliminated from males by injecting EB on day 10, and nearly completely eliminated by injection on day 11; however, there was no effect if injection occurred after day 11 (Fig. 1a). With injection on day 10, copulatory mounting was significantly reduced or eliminated with as little as 1 µg EB (Fig. 1b), indicating that the male brain is evidently very sensitive to estrogen at this time. Females hatched from eggs injected with an anti-estrogen (the estrogen receptor antagonist CI-628) on day 9 of incubation and given activational testosterone as adults mounted other females, showing a male phenotype for this behavior, whereas there was no effect of this treatment on males (Fig. 2) (Adkins 1976).

Both of these dramatic sex-reversing effects (elimination of mounting in males by embryonic treatment with estradiol and the appearance of mounting in females by embryonic treatment to prevent estrogen action) have also been obtained in the laboratory of Jacques Balthazart, where impressive progress has been made in exploring the neural bases for such effects (Balthazart and Adkins-Regan 2002). Embryonic exposure to estrogen has also been shown to reduce mounting in male chickens (Wilson and Glick 1970; Sayag et al. 1989). Taken together, these results indicate that the sex difference in these galliform birds in copulatory mounting is hormonally organized and suggest a pattern of sexual differentiation in which embryos develop as males unless exposed to estrogen before hatching, and embryos exposed to estrogen develop as females. In other words, the ovary establishes the female phenotype through an estrogenic signal, and the absence of this ovarian signal results in males. This is the opposite pattern from mammalian sexual differentiation (in which the presence of testes imposes maleness), but it is entirely consistent with findings that female embryos produce more

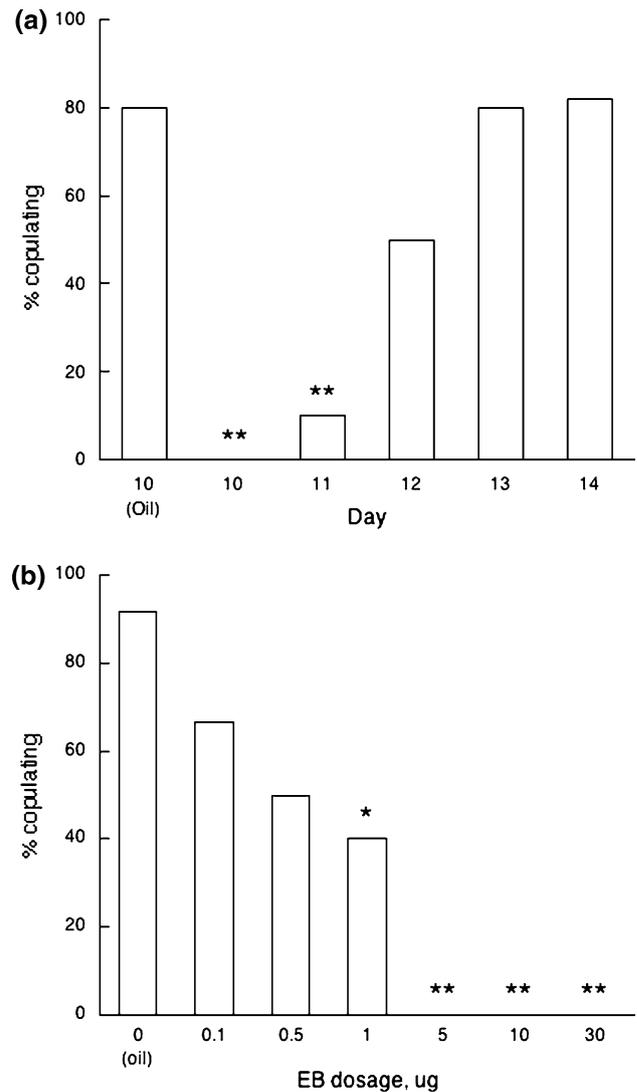


Fig. 1 Critical period (a) and dose–response function (b) for the reduction of male Japanese quail copulatory behavior by embryonic treatment with estradiol benzoate (adapted from Adkins 1979). Eggs were injected on different days of incubation with 50 µg (a) or injected on day 10 with different doses (b). All males were given testosterone propionate as adults prior to testing. ** $P < 0.01$ compared with oil injected controls, * $P < 0.05$

estradiol than male embryos and have higher estradiol to testosterone ratios (Tanabe et al. 1979; Schumacher et al. 1988; Ottinger et al. 2001). Although the pattern of differentiation is reversed from mammals, the fundamental importance of hormonal organization for generating a sex difference in mating behavior is shared with mammals.

Sexual differentiation in zebra finches (*Taeniopygia guttata*)

The other avian species that has figured prominently in studies of how sex differences in behavior are produced is

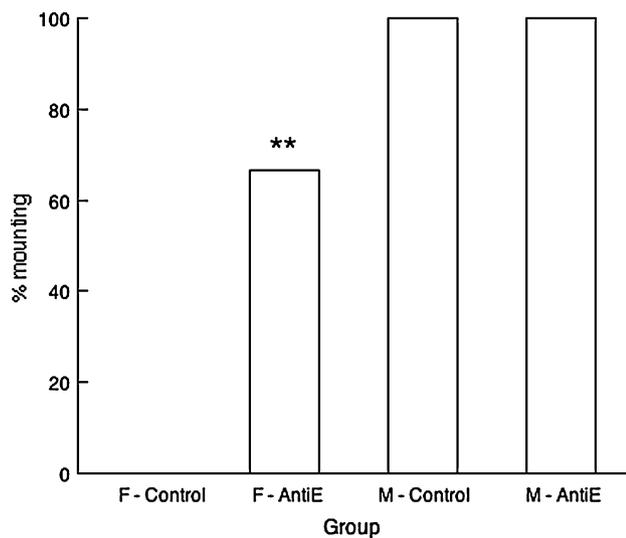


Fig. 2 Masculinization of the copulatory behavior of female Japanese quail produced by embryonic exposure to an anti-estrogen (*AntiE*) (adapted from Adkins 1976). *F* females, *M* males. ** $P < 0.01$ compared with female controls (none of which mounted)

an Australian passeriform, the zebra finch, in the family Estrildidae. Zebra finches breed well in captivity or under domestication and share with Japanese quail the advantage of rapid development. Developmental mode is altricial, however, making the early posthatching period a likely time for any organizational critical period. Although zebra finches are socially monogamous in their mating system, nonetheless there are pronounced sex differences in singing (only males sing) and copulatory mounting (seldom seen in females). Adult males have higher testosterone levels than females, but there is little sex difference in estradiol levels (Adkins-Regan et al. 1990). Adult females given testosterone do not sing or show copulatory mounting, as if these sex differences have been organized earlier in development (Arnold 1980; Adkins-Regan and Ascenzi 1987).

Initial tests of this hypothesis treated nestlings with estradiol or EB. Injections of EB during the first two posthatching weeks were found to eliminate copulatory mounting in males, the same result obtained in Japanese quail except for the species difference in timing of the critical period (Fig. 3) (Adkins-Regan and Ascenzi 1987). However, as first discovered by Gurney and Konishi (1980), estradiol treatment during this same period, especially during the first week, masculinizes singing in females instead of eliminating it in males (Fig. 3) (Adkins-Regan and Ascenzi 1987; Adkins-Regan et al. 1994). Females treated with testosterone or other androgens as nestlings are masculinized only slightly or not at all (Adkins-Regan and Ascenzi 1990; Grisham and Arnold 1995).

These nestling hormone effects on adult copulatory mounting and singing support the hypothesis that the sex differences in these behaviors are hormonally organized

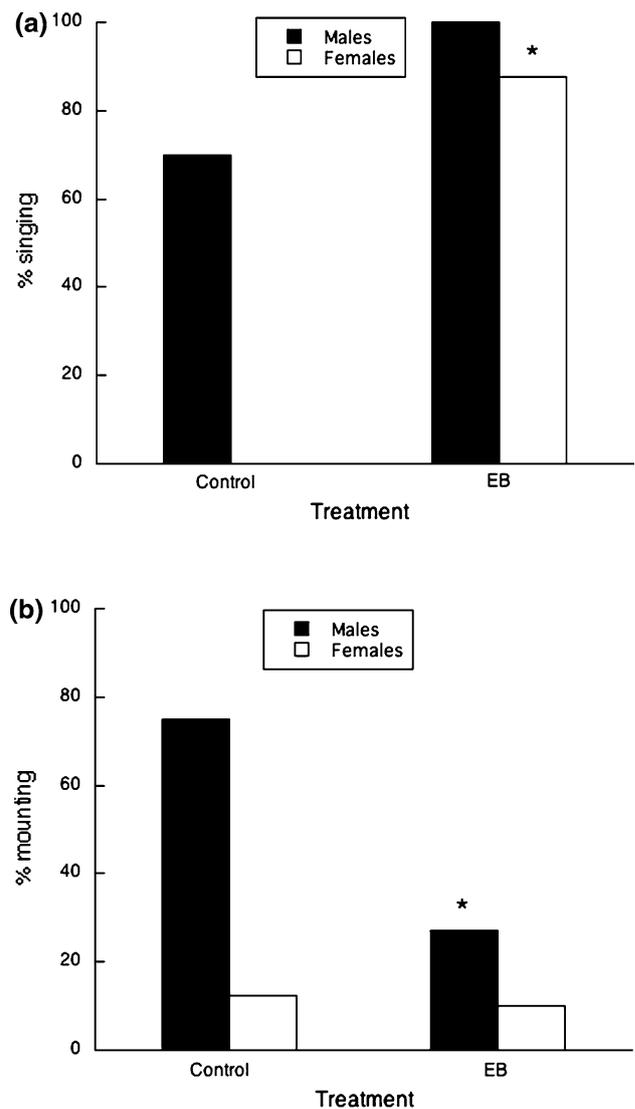


Fig. 3 Treatment of nestling zebra finches with estradiol benzoate (20 $\mu\text{g}/\text{day}$ for the first two posthatching weeks) masculinized singing in females (a) but demasculinized mounting in males (b) (adapted from Adkins-Regan and Ascenzi 1987). * $P < 0.05$ compared with controls of the same sex

and point to an estrogenic signal as the key for sexual differentiation in this species. However, the fact that the same treatment feminizes (demasculinizes) one behavior (mounting) while masculinizing another (singing) is quite odd and difficult to reconcile with any simple pattern of sexual differentiation. Subsequent research produced additional results that are incompatible with either a mammalian or a quail model of sexual differentiation (reviewed by Arnold and Schlinger 1993; Wade 1999; and Balthazart and Adkins-Regan 2002). The measurement of sex steroid levels during zebra finch development did not reveal any replicable sexual dimorphism in hormone levels (any dimorphic endocrine signal for sexual differentiation). In addition, the treatment of nestlings with estrogen receptor

antagonists or estrogen synthesis inhibitors did not produce the effects that would be predicted; they neither masculinized copulatory mounting in females nor eliminated singing in males. Sex reversal of the gonads by treatments of embryos failed to sex reverse singing, which instead was always consistent with the bird's genetic sex. This would seem to rule out organization by gonadal hormones altogether. And yet treatment of the nestlings with estradiol does produce singing females. What is the real signal for sexual differentiation that estradiol is perturbing, and where is it produced? The problem of explaining zebra finch sexual differentiation has not yet been solved, but alternative hypotheses are being actively pursued. One possibility is that the signal is a neurosteroid – that is, a steroid produced *de novo* in the brain itself in greater amounts in one sex (Schlinger and London 2006). Another is that brain sex is produced by cell-autonomous products of genes on the sex chromosomes (Arnold 2002; Wade et al. 2005). These genes could be either on the Z chromosome, with a double dose producing a male brain, or on the W chromosome, preventing development of a male brain.

Development of mate preferences

Behavioral sex differences in birds include not only motor patterns like singing and mounting, but also mate preferences. For example, females may prefer males performing elaborate displays or singing a large repertoire of songs, whereas males of these species cannot have this as a mate choice criterion if females do not display or sing. Female zebra finches prefer males with higher song rates and songs learned correctly from an adult male tutor, whereas males prefer fecund females, those that have been eating a diet likely to lead to more eggs (Williams et al. 1993; Collins et al. 1994; Jones et al. 2001). Males of many species tend to be less discriminating (choosy) in their choice of mating partners. Treatment of adult female dark-eyed juncos (*Junco hyemalis*) with testosterone lowers (masculinizes) their choosiness scores, suggesting the fascinating possibility that this sex difference might have an activational hormone basis (McGlothlin et al. 2004). The most obvious sex difference in mate preference is that females prefer males for mating and pairing and males prefer females. In sexual partner preference (as this phenomenon is called), it is the targets for the animals' behavior (their approach and attraction) that differ according to the sex of the subjects and define the phenomenon – and not the behavior itself. Is sexual partner preference in birds an organizational or an activational phenomenon? In mammals, organizational hormone effects are key (Adkins-Regan 1988; Baum 2006).

An interesting study on Japanese quail by Balthazart et al. (1997) can be interpreted as supporting an

organizational hypothesis for sexual partner preference in a galliform species. Male, but not female, quail are motivated to look at a female through a window, and giving females testosterone does not make them interested in other females. Females hatched from eggs injected with an estrogen synthesis inhibitor, however, are fully masculinized with respect to interest in looking at females, just as they are quite masculinized in their copulatory mounting.

A series of experiments with zebra finches has investigated the hormonal basis of sexual partner preference in this socially monogamous species that pairs for life. When gonadectomized adults were given implants of EB or testosterone propionate, females paired with males and males paired with females regardless of which kind of hormone implant they were given (Adkins-Regan and Ascenzi 1987). This suggests that the activational hormone environment (whether it is dominated by estradiol or testosterone) may not be very important for determining the direction of the preference. It is possible, however, that the expression of the birds' preferences for the opposite sex requires adult levels of sex steroids and that hormonal changes as sexual maturity is reached activate such expression. This hypothesis was tested by manipulating hormones of juveniles during the period from 25 to 90 days posthatching (Adkins-Regan and Leung 2006). By 90 days, birds are sexually mature. The birds' social preferences were tested weekly during this study period, with the choice between unpaired males, unpaired females, and their family members (parents and siblings), and the group of stimuli they spent the most time near was measured. Males with implants of testosterone showed a premature interest in unpaired females (Fig. 4a), and females with implants designed to reduce sex steroid actions [the androgen receptor antagonist flutamide plus the estrogen synthesis inhibitor 1,4,6-androstatriene-3,17-dione (ATD)] failed to show any increased interest in unpaired males as they reached 90 days of age, unlike control females (Fig. 4b). These results suggest that the expression of a bird's sexual partner preference, an important component of the developmental transition to adulthood, is regulated in part by activational effects of sex hormones.

What then determines the direction of the sexual partner preference? Several experiments show that early hormonal manipulations can alter the direction of the preference, thereby supporting an organizational hypothesis. Females injected with EB during the first two posthatching weeks paired with females instead of males as adults (Fig. 5) (Mansukhani et al. 1996). This effect only occurred, however, if they had also spent their juvenile lives in an all-female aviary, and it requires tactile contact with other females to occur (Adkins-Regan 2005). Genetic females hatched from eggs injected on day 5 of incubation with an estrogen synthesis inhibitor (fadrozole), who had testes

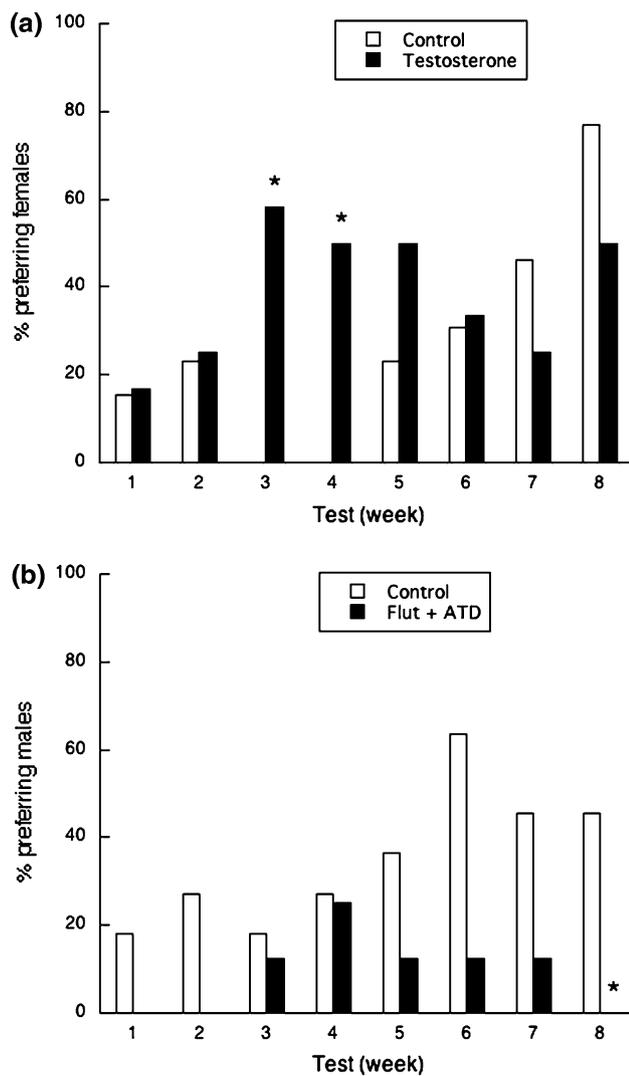


Fig. 4 Juvenile male zebra finches implanted with testosterone propionate showed a premature preference for unpaired females in weekly choice tests (a), whereas juvenile females implanted with flutamide (*Flut*) plus 1,4,6-androstatriene-3,17-dione (*ATD*) to lower sex steroid action failed to show an increased preference for males (b) (adapted from Adkins-Regan and Leung 2006). * $P < 0.05$ compared with controls

instead of ovaries, also preferred to pair with other females (Fig. 6) (Adkins-Regan and Wade 2001).

To date, sexual partner preference is the only sex difference in mate preference for which an organizational hypothesis has been tested. In both Japanese quail and zebra finches, early hormone treatments can produce permanent sex reversal of this preference. In the highly social zebra finch, there seem to be contributions of some kind of social experience to this developmental process as well. In fact, manipulation of social experience alone can eliminate sexual partner preference, producing males and females that are equally interested in pairing with both sexes (Adkins-Regan and Krakauer 2000).

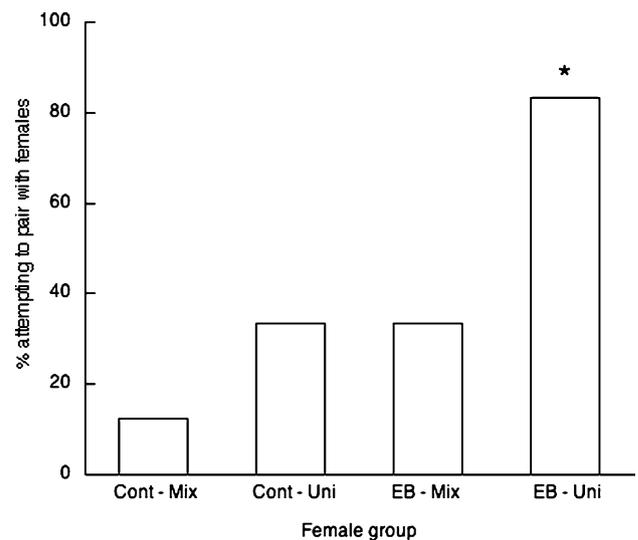


Fig. 5 Female zebra finches treated with estradiol benzoate (*EB*, 20 $\mu\text{g}/\text{day}$ for the first two posthatching weeks) preferred to pair with other females as adults if they had been housed in an all-female aviary as juveniles (adapted from Mansukhani et al. 1996). *Cont* Control, *Mix* mixed-sex housing, *Uni* unisex (all-female) housing. * $P < 0.05$ compared with Cont-Mix

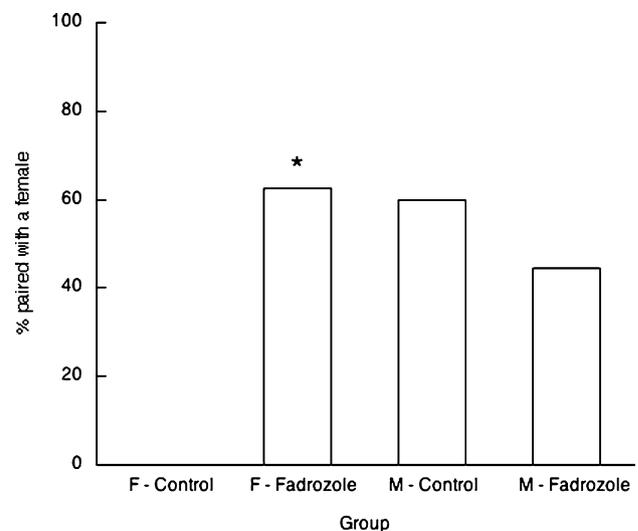


Fig. 6 Female (*F*) zebra finches hatched from eggs injected with the estrogen synthesis inhibitor fadrozole on embryonic day 5 preferred to pair with females as adults, whereas males (*M*) were unaffected (adapted from Adkins-Regan and Wade 2000). * $P < 0.05$ compared with *F*-control

This conclusion about the organizational hypothesis has to be qualified, however. As with the studies of copulatory mounting and singing, there are still some unsolved puzzles. How can both estrogen and an estrogen synthesis inhibitor produce the same effect (females that prefer other females)? And if estrogen is the signal for masculinization, why does early treatment with an estrogen synthesis

inhibitor fail to produce males that prefer other males (Adkins-Regan et al. 1996)?

Formation of pair relationships

The vast majority of avian species are socially monogamous, and male-female pairs are a conspicuous feature of bird life. Much research has been devoted to understanding the function and ecology of monogamy and deviations from genetic monogamy in the form of extra-pair matings. The development and physiology of pairing has been comparatively neglected. With respect to sex differences, pairbond behavior is relatively monomorphic. Zebra finches are a representative example: paired birds clump together (sit in direct physical contact), preen each other (allopreen), and spend time in the nest together, particularly during the nest building period. Nor is there any obvious sex difference in the motivation to pair. Is such monomorphic behavior even hormone-dependent? Are high (reproductive) levels of sex hormones required for pair formation and maintenance? The answers to these questions are far from obvious because this species is continually paired even when not actively breeding, and the behaviors themselves can be shown by young juveniles with their family members (Zann 1996).

In order to see if reproductive levels of sex hormones are required for pair formation, young adult zebra finches that had never been paired were given a combination of treatments to lower sex steroid action: the androgen receptor antagonist flutamide plus the estrogen synthesis inhibitor ATD (Tomaszycki et al. 2006). Treated birds and control implanted birds (those with empty implants) were introduced into aviaries with untreated birds of the opposite sex. Sex ratios in these aviaries were 2:1 males to females for male subjects and 1:2 males to females for female subjects, so that the treated birds would have to be motivated to pair and successful in competing with the controls to succeed. Thus, the probability of subjects successfully pairing with an opposite-sex bird was 50%. Although the flutamide plus ATD treatment was effective in causing regression of the vasa deferentia in males and cessation of egg laying in females, it had no effect on the percentage of birds that successfully paired; percentages were not lower than for the controls (Fig. 7a, b). The only effect seen on pairing was that treated females that failed to pair with a male were more likely than controls to pair with another female rather than remain unpaired. Whether this reflects greater motivation to be paired or some kind of hitherto undetected activational hormone effect on the direction of sexual partner preference is unclear. In any event, this experiment argues against an important role for breeding

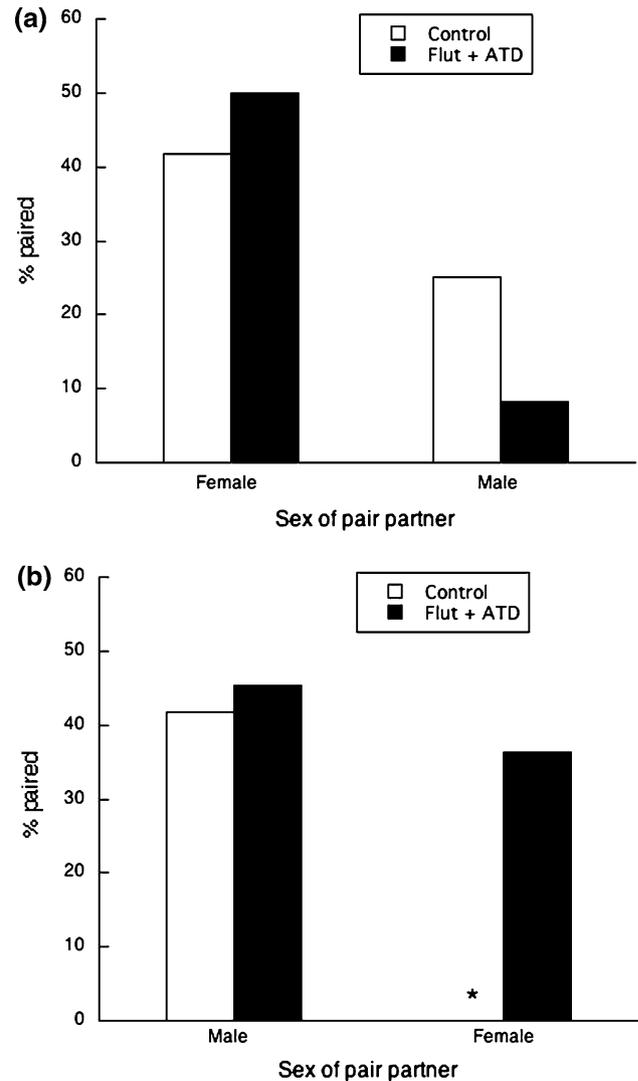


Fig. 7 Adult male (a) and female (b) zebra finches treated with flutamide (*Flut*) plus 1,4,6-androstatriene-3,17-dione (*ATD*) to lower sex steroid action were just as likely as controls to succeed in pairing with an opposite-sex bird (adapted from Tomaszycki et al. 2006). Among females that failed to pair with a male, treated females were more likely to pair with another female than controls were. * $P < 0.05$

levels of sex steroid action in the formation of male-female pairs in zebra finches.

This species is relatively aseasonal, breeding in response to unpredictable rainfall (Zann 1996). Such a lack of any significant involvement of sex hormones in adult pair formation may generalize to other species that are permanently paired even when not actively breeding. It is quite possible that sex hormones have a greater role in birds that form pairs on a seasonal basis, a hypothesis that needs to be tested with an experimental manipulation. Even in seasonally pairing birds, however, high levels of sex hormones do not seem to be required for continued maintenance of the existing pair relationship through the breeding cycle

because sex steroids often fall dramatically at the onset of incubation, even when the pair remains together to share the incubation and feeding of the young (Wingfield and Farner 1993).

Implications and conclusions

These experiments with Japanese quail and zebra finches show that some of the elements of the theory of hormonal organization can account for the development of sex differences in the behavior of birds. In Japanese quail, it is clear that actions of estrogen during the embryonic period produce those components of the sexual phenotype that cannot be sex reversed by adult hormone manipulations. The signal that sends males and females down different developmental paths is ovarian estrogen and the estrogen to androgen ratio. In zebra finches, it is less clear what the signal is (whether it is hormones, neurosteroids, or cell-autonomous gene products), but its actions are occurring early in development and are permanent.

Many questions have not yet been answered. There are, of course, the puzzles of zebra finch sexual differentiation as well as a lack of knowledge of how sexual differentiation of behavior occurs in birds other than quail, chickens, and zebra finches. What other clades use the quail/chicken rule? Does the sister clade to the galliforms, the anseriforms, use the same rule? Whatever the zebra finch rule is, will it apply only to estrildid finches, or will it explain sexual differentiation in oscine passerines more generally? With respect to pair formation, what is the physiology of pairing in zebra finches, if it does not lie in sex steroids? What, if any, is the involvement of other molecules known to be important for affiliative relationships in mammals, particularly neuromodulators and neurotransmitters such as oxytocin family peptides, endogenous opiates, and dopamine? With respect to sexual partner preference, is the developmental process that produces preferences for the opposite sex for mating and pairing purposes the same process that produces “preferences” for the same sex for aggressive purposes? Birds often show sex-specific aggression in defending a territory or a mate, with females targeting other females and males targeting other males, a phenomenon that can be captured with laboratory testing paradigms (e.g., Adkins-Regan and Robinson 1993). Like sexual partner preference, this must involve sex recognition, and could be an organizational phenomenon.

The progress that has been made in understanding sexual differentiation opens up the possibility of understanding species differences in dimorphism and social systems with respect to the developmental changes that underlie evolutionary divergence and changes in state. Three examples illustrate some of this promise of this

knowledge. First, as progress is made in figuring out zebra finch song differentiation (i.e., how male and female brains for singing are produced), testable hypotheses can be proposed to explain the developmental mechanisms for evolutionary changes from singing by males only to singing by both sexes or vice versa. Such changes have occurred a number of times in oscine passerines (Garamszegi et al. 2006; MacDougall-Shackleton and Ball 1999); for example, a change from singing by males alone to duetting has occurred in the wrens of the *Thryothorus* genus (Farabaugh 1982; Brenowitz and Arnold 1986; Levin 1996; Nealen and Perkel 2000; Mann et al. 2006). Second, evolutionary changes have occurred in sex roles in birds such as phalaropes, with females competing for males and males carrying out all the incubation. The hypothesis that these “reversed” sex roles are based on adult (activational) hormone sex reversal has been tested and rejected, leaving hormonal organization as one – yet untested – alternative hypothesis (Schlinger et al. 1989; Fivizzani et al. 1990; Eens and Pinxten 2000). Third, many galliform birds are polygynous with female-only parental care, but several of the species in the family Cracidae are socially monogamous with biparental care (del Hoyo 1994). The quail experiments offer the promise of accounting for reductions or increases in sex differences in parental behavior in galliform birds.

The quail experiments have also established this species as the leading model for testing the effects of endocrine-disrupting chemicals on the developing brains and behavior of birds (Halldin 2005; Ottinger et al. 2005). The sensitivity of the male embryo’s brain to estrogens means that even weakly estrogenic chemicals could seriously interfere with future copulatory behavior. Without copulation, there is no fitness. It is urgently important to know how widely this deleterious effect generalizes among birds.

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References

- Adkins EK (1975) Hormonal basis of sexual differentiation in the Japanese quail. *J Comp Physiol Psychol* 89:61–71
- Adkins EK (1976) Embryonic exposure to an antiestrogen masculinizes behavior of female quail. *Physiol Behav* 17:357–359
- Adkins EK (1979) Effect of embryonic treatment with estradiol or testosterone on sexual differentiation of the quail brain: critical period and dose-response relationships. *Neuroendocrinology* 29:178–185

- Adkins EK, Adler NT (1972) Hormonal control of behavior in the Japanese quail. *J Comp Physiol Psychol* 81:27–36
- Adkins-Regan E (1988) Sex hormones and sexual orientation in animals. *Psychobiol* 16:335–347
- Adkins-Regan E (2005a) Hormones and animal social behavior. Princeton University Press, Princeton
- Adkins-Regan E (2005b) Tactile contact is required for early estrogen treatment to alter the sexual partner preference of female zebra finches. *Horm Behav* 48:180–186
- Adkins-Regan E, Ascenzi M (1987) Social and sexual behaviour of male and female zebra finches treated with oestradiol during the nestling period. *Anim Behav* 35:1100–1112
- Adkins-Regan E, Ascenzi M (1990) Sexual differentiation of behavior in the zebra finch: effect of early gonadectomy or androgen treatment. *Horm Behav* 24:114–127
- Adkins-Regan E, Krakauer A (2000) Removal of adult males from the rearing environment increases preference for same sex partners in the zebra finch (*Taeniopygia guttata*). *Anim Behav* 60:47–53
- Adkins-Regan E, Leung CH (2006) Sex steroids modulate changes in social and sexual preference during juvenile development in zebra finches. *Horm Behav* 50:772–778
- Adkins-Regan E, Robinson TM (1993) Sex differences in aggressive behavior in zebra finches (*Poephila guttata*). *J Comp Psychol* 107:223–229
- Adkins-Regan E, Wade J (2001) Masculinized sexual partner preference in female zebra finches with sex-reversed gonads. *Horm Behav* 39:22–28
- Adkins-Regan E, Abdelnabi M, Mobarak M, Ottinger M A (1990) Sex steroid levels in developing and adult male and female zebra finches (*Poephila guttata*). *Gen Comp Endocrinol* 78:93–109
- Adkins-Regan E, Mansukhani V, Seiwert C, Thompson R (1994) Sexual differentiation of brain and behavior in the zebra finch: critical periods for effects of early estrogen treatment. *J Neurobiol* 25:865–877
- Adkins-Regan E, Yang S, Mansukhani V (1996) Behavior of male and female zebra finches treated with an estrogen synthesis inhibitor as nestlings. *Behaviour* 133:847–862
- Arnold AP (1980) Effects of androgens on volumes of sexually dimorphic brain regions in the zebra finch. *Brain Res* 185:441–444
- Arnold AP (2002) Concepts of genetic and hormonal induction of vertebrate sexual differentiation in the twentieth century, with special reference to the brain. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT (eds) *Hormones, brain and behavior*, vol 4. Academic Elsevier, Amsterdam, pp 105–136
- Arnold AP, Schlinger BA (1993) Sexual differentiation of brain and behavior: the zebra finch is not just a flying rat. *Brain Behav Evol* 42:231–241
- Balthazart J, Adkins-Regan E (2002) Sexual differentiation of brain and behavior in birds. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT (eds) *Hormones, brain and behavior*, vol 4. Academic Elsevier, Amsterdam, pp 223–301
- Balthazart J, Delville Y, Sulon J, Hendrick J C (1986) Plasma levels of luteinizing hormone and of five steroids in photostimulated, castrated and testosterone-treated male and female Japanese quail (*Coturnix coturnix japonica*). *Gen Endocrinol* 5:31–36
- Balthazart J, Castagna C, Ball GF (1997) Aromatase inhibition blocks the activation and sexual differentiation of appetitive male sexual behavior in Japanese quail. *Behav Neurosci* 111:381–397
- Baum MJ (2006) Mammalian animal models of psychosexual differentiation: when is ‘translation’ to the human situation possible? *Horm Behav* 50:579–588
- Beach FA, Inman NG (1965) Effects of castration and androgen replacement on mating in male quail. *Proc Natl Acad Sci USA* 54:1426–1431
- Brenowitz EA, Arnold AP (1986) Interspecific comparisons of the size of neural song control regions and song complexity in duetting birds: evolutionary implications. *J Neurosci* 6:2875–2879
- Collins SA, Hubbard C, Houtman AM (1994) Female mate choice in the zebra finch – the effect of male beak colour and male song. *Behav Ecol Sociobiol* 35:21–25
- del Hoyo J (1994) Family Cracidae. In: del Hoyo J, Elliott A, Sargatal J et al. (eds) *Handbook of the birds of the world*, vol 2. Lynx Edicions, Barcelona, pp 310–341
- Eens M, Pinxten R (2000) Sex-role reversal in vertebrates: behavioural and endocrinological accounts. *Behav Proc* 51:135–147
- Farabaugh SM (1982) The ecological and social significance of duetting. In: Kroodsma DE, Miller EH (eds) *Acoustic communication in birds*, vol 2. Academic, New York, pp 85–124
- Fivizzani AJ, Oring LW, El Halawani ME, Schlinger BA (1990) Hormonal basis of male parental care and female intersexual competition in sex-role reversed birds. In: Wada M, Ishii S, Scanes CG (eds) *Endocrinology of birds: molecular to behavioral*. Japan Scientific Societies Press/Springer, Tokyo/Berlin, pp 273–286
- Garamszegi LZ, Pavlova DZ, Eens M, Møller AP (2006) The evolution of song in female birds in Europe. *Behav Ecol* 18:86–96
- Grisham W, Arnold AP (1995) A direct comparison of the masculinizing effects of testosterone, androstenedione, estrogen, and progesterone on the development of the zebra finch song system. *J Neurobiol* 26:163–170
- Gurney ME, Konishi M (1980) Hormone-induced sexual differentiation of brain and behavior in zebra finches. *Science* 208:1380–1383
- Halldin K (2005) Impact of endocrine disrupting chemicals on sexual differentiation in Japanese quail. Neuroendocrine and behavioral consequences of embryonic exposure to endocrine disrupting chemicals. In: Dawson A, Sharp PJ (eds) *Functional avian endocrinology*. Narosa Publishing House, New Delhi, pp 299–309
- Jones KM, Monaghan P, Nager RG (2001) Male mate choice and female fecundity in zebra finches. *Anim Behav* 62:1021–1026
- Levin R (1996) Song behaviour and reproductive strategies in a duetting wren, *Thryothorus nigricapillus*: I. Removal experiments. *Anim Behav* 52:1093–1106
- MacDougall-Shackleton SA, Ball GF (1999) Comparative studies of sex differences in the song-control system of songbirds. *Trends Neurosci* 22:432–436
- Madge S, McGowan P (2002) Pheasants, partridges, and grouse. Princeton University Press, Princeton
- Mann NI, Barker FK, Graves JA, Dingess-Mann KA, Slater PJB (2006) Molecular data delineate four genera of “*Thryothorus*” wrens. *Mol Phylogenet Evol* 40:750–759
- Mansukhani V, Adkins-Regan E, Yang S (1996) Sexual partner preference in female zebra finches: the role of early hormones and social environment. *Horm Behav* 30:506–513
- McGlothlin JW, Neudorf DL, Casto JM, Nolan V, Ketterson ED (2004) Elevated testosterone reduces choosiness in female dark-eyed juncos (*Junco hyemalis*): evidence for a hormonal constraint on sexual selection? *Proc R Soc Lond B* 271:1377–1384
- Nealen PM, Perkel DJ (2000) Sexual dimorphism in the song system of the Carolina wren *Thryothorus ludovicianus*. *J Comp Neurol* 418:346–360
- Nelson RJ (2005) An introduction to behavioral endocrinology, 3rd edn. Sinauer, Sunderland
- Ottinger MA, Pitts S, Abdelnabi MA (2001) Steroid hormones during embryonic development in Japanese quail: plasma, gonadal, and adrenal levels. *Poultry Sci* 80:795–799

- Ottinger MA, Quinn MJ, Lavoie E, Abdelnabi MA, Thompson N, Hazelton J, McKernan M, Wu JM, Henry PFP, Viglietti-Panzica C, Panzica GC (2005) Neuroendocrine and behavioral consequences of embryonic exposure to endocrine disrupting chemicals. In: Dawson A, Sharp PJ (eds) Functional avian endocrinology. Narosa Publishing House, New Delhi, pp 271–284
- Phoenix CH, Goy RW, Gerall AA, Young WC (1959) Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinol* 65:369–382
- Sayag N, Snapir N, Robinzon B, Arnon E, el Halawani ME, Grimm VE (1989) Embryonic sex steroids affect mating behavior and plasma LH in adult chickens. *Physiol Behav* 45:1107–1112
- Schlinger BA, London SE (2006) Neurosteroids and the songbird model system. *J Exp Zool* 305:743–748
- Schlinger BA, Fivizzani AJ, Callard GV (1989) Aromatase, 5 α - and 5 β -reductase in brain, pituitary and skin of the sex-role reversed Wilson's phalarope. *J Endocrinol* 122:573–581
- Schumacher M, Balthazart J (1983) The effects of testosterone and its metabolites on sexual behavior and morphology in male and female Japanese quail. *Physiol Behav* 30:335–339
- Schumacher M, Sulon J, Balthazart J (1988) Changes in serum concentrations of steroids during embryonic and post-hatching development of male and female Japanese quail (*Coturnix coturnix japonica*). *J Endocrinol* 118:127–134
- Tanabe Y, Nakamura T, Fujioka K, Doi O (1979) Production and secretion of sex steroid hormones by the testes, the ovary, and the adrenal glands of embryonic and young chickens (*Gallus domesticus*). *Gen Comp Endocrinol* 39:26–33
- Tinbergen N (1963) On aims and methods of ethology. *Z Tierpsychol* 20:410–433
- Tomaszycki ML, Banerjee SB, Adkins-Regan E (2006) The role of sex steroids in courtship, pairing and pairing behaviors in the socially monogamous zebra finch. *Horm Behav* 50:141–147
- Wade J (1999) Sexual dimorphisms in avian and reptilian courtship: two systems that do not play by mammalian rules. *Brain Behav Evol* 54:15–27
- Wade J, Tang YP, Peabody C, Tempelman RJ (2005) Enhanced gene expression in the forebrain of hatchling and juvenile male zebra finches. *J Neurobiol* 64:224–238
- Williams H, Kilander K, Sotanski ML (1993) Untutored song, reproductive success and song learning. *Anim Behav* 45:695–705
- Wilson JA, Glick B (1970) Ontogeny of mating behavior in the chicken. *Am J Physiol* 218:951–955
- Wingfield JC, Farner DS (1993) Endocrinology of reproduction in wild species. In: Farner DS, King JR, Parkes KC (eds) Avian biology, vol 9. Academic, London, pp 164–328
- Yazaki Y, Matsushima T, Aoki K (1999) Testosterone modulates stimulation-induced calling behavior in Japanese quails. *J Comp Physiol A* 184:13–19
- Zann RA (1996) The zebra finch: a synthesis of field and laboratory studies. Oxford University Press, Oxford