

S14-3 Hormones, sexual dimorphism, and mate choice

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Abstract Sexual selection through mate choice is a major force in the evolution of ecologically relevant behavior in birds. Sexual selection favors behavior, including aspects of mate choice itself, that is different in males and females. How are these differences created during development? What processes produce the adult male and female phenotypes? Many experiments have shown that the organizing actions of sex hormones during critical periods early in development, even before hatching, are an important mechanism for generating adult sex differences in copulatory behavior. Sexually selected behavior such as courtship and mate choice occurs prior to copulation and is not the same behavior as mating itself. Are sex differences in these sexually selected behaviors also produced through organizational hormone actions? Experiments with zebra finches (*Taeniopygia guttata*) have addressed this question by manipulating sex hormone levels in embryonic and nestling birds and then testing them for singing or pairing preference when adult. Female zebra finches treated with estrogen as nestlings, or treated with an estrogen synthesis inhibitor as embryos (so that they develop testes instead of ovaries), prefer to pair with other females instead of males. These results confirm the potential importance of organizational hormone effects on adult mate preference, thereby integrating physiological mechanisms, development, and behavior of known adaptive significance. The organizational concept may also prove valuable in understanding the phylogeny of sexually dimorphic behavior.

Key words Organizational hormones, Sexual selection, Mate choice, Sexual dimorphism

1 Introduction

Many features of birds have evolved through the process of sexual selection, in which conspecifics are the agents of selection. Preference for potential mates on the basis of behavior or appearance is one form of such selection. Mate choice results in the evolution of sexual differences, also called sexual dimorphism. Many elaborate male behaviors such as singing or crowing and courtship displays have evolved because they make the males more attractive to females (Andersson, 1994). Because males and females have different genetic interests and are looking for different qualities in mating partners, mate choice itself is sexually dimorphic. That is, mate choice can act as a selective agent, shaping preferences by selection. In some species, for example those in which males lek, females choose mates but males do not, trying instead to mate with any female that comes near. In other species in which males sing and females do not, females may choose singers as mates while males do choose females, but on a different basis. The most fundamentally dimorphic aspect of mate choice is the preference itself for the opposite sex of the partner: females choose males as partners and males choose females.

These preferences are adaptive; but does understanding their function tell us everything we need to know? We also need to know how they originate and develop, that is, how males and females develop dimorphic mate choice as

they mature. Most of the genome within species, except for the small fraction that determines whether testes or ovaries develop, is much the same in both sexes; yet different behavior develops. How? Sex steroid hormones such as estradiol and testosterone are important mechanisms for expressing sex-limited traits. They regulate gene expression and thus have potential to create sex differences in behavior within a common genome. But both sexes produce both hormones, and in some birds the hormone levels in the blood overlap between males and females, so the answer to the question is still far from complete.

2 Hormone organization and its role in dimorphic sexual behavior

During the twentieth century, research with laboratory mammals led to important discoveries about the sources of sex differences (Arnold, 2002). It showed that gonadal sex steroid hormones are produced during early development, even before birth, and that these hormones organized future sex differences in behavior. Sex hormones are considered to organize behavior when: (1) their action occurs early in development, (2) their action is limited to a critical period, (3) their effect of their action is permanent, and (4) their action establishes the sexual phenotype of an animal's behavior.

Beginning in the 1970's, a number of experiments conducted in two laboratories showed that the mating be-

havior of Japanese quail, *Coturnix japonica*, is hormonally organized (Balthazart and Adkins-Regan, 2002). Quail mating behavior is sexually dimorphic: males mount females, but females never mount another bird. This is not just because males have more testosterone as adults than do females. Even if females are injected with high doses of testosterone they will not mount other females. But if eggs are injected with estradiol before hatching, none of the males will mount when they become adult. They have been turned into females in this behavior. Such a dramatic sex reversal in behavior occurs even at a very low dose, and with a single injection (Adkins, 1979). And if eggs are injected with an estrogen antagonist (estrogen receptor blocker) or estrogen synthesis inhibitor, the females will themselves mount when they are adults; they have been turned behaviorally into males (Balthazart and Adkins-Regan, 2002).

Such effects are permanent and limited to a critical period. A parallel effect has been reported in the zebra finch, *Taeniopygia guttata* (Adkins-Regan and Ascenzi, 1987): young males treated with estradiol failed to mount as adults. Because zebra finches hatch at a less developed stage, the critical period for effecting this treatment is the first week or two after hatching, not pre-hatching. These experiments in two unrelated birds confirm that sexual differences in a behavior closely linked to reproductive success arise from hormonal organization.

Does hormonal organization also create sexual dimorphism in mate choice and other sexually selected behavior such as courtship display and singing? These behaviors are also important for fitness. In Japanese quail, much of the sexual difference in crowing and strutting is due to dimorphism in adult hormone levels. In contrast, difference in interest between the sexes in looking at a female — males are much more interested — is hormonally organized in the same manner as mating itself (Balthazart and Adkins-Regan, 2002). Most other research addressing hormonal organization of mate choice and related behavior has been conducted with zebra finches.

Zebra finches choose their partners at an early age and pair for the life of the partner. Paired males and females spend much time preening each other, sitting in direct physical contact and occupying a nest box together. There are no sexual differences in these behaviors. Instead, the real difference in zebra finches (and other birds) lies in the choice of partner, with males usually choosing females, not males, and females usually choosing males, not females. Pairing preference can be measured both in free-flight aviaries containing a group of birds and in two-choice tests where a subject bird chooses

between a male and a female. A further sex difference related to mate choice in zebra finches is song: males sing, a behavior that is attractive to females, but females never sing. Are such differences in mate choice and singing produced by organizational actions of sex hormones?

Beginning with Gurney and Konishi (1980), several laboratories have shown that female zebra finches injected with estradiol during the nestling period grow up to sing remarkably well and to have male-sized song regions in the brain (Balthazart and Adkins-Regan, 2002). It might seem paradoxical that estradiol, an estrogen, should turn females into males with respect to singing. Such masculinization is not uncommon in studies of hormones and behavior; and, moreover, normal male zebra finches have as much circulating estradiol as females, both as nestlings and as adults (Adkins-Regan et al., 1990).

Can partner preference also be sex reversed by manipulations of hormones at early stages? Adkins-Regan and Wade (2001) carried out an experiment in which finch eggs were injected with a drug (fadrozole) that prevents the formation of estrogens such as estradiol. Females hatching from these eggs had testes or ovotestes rather than the normal single ovary, confirming that, in normal development, estrogen acts to produce the ovary, much as estrogen organizes female mating behavior in normal development in quail (lack of mounting). Fadrozole treated female finches were also sex-reversed in their pairing preference. Here there is another apparent paradox, because estrogen masculinizes singing whereas its inhibiting masculinizes partner preference. Singing and partner preference are likely to have different neural bases, however. Thus hormone manipulations at early stages of growth caused partial or complete sex reversal of behaviors related to mate choice. This supports the hypothesis that hormonal organization creates sex differences in these behaviors in birds.

3 Issues for phylogeny and biology

The hormone organizational concept may be used to address some important comparative and phylogenetic issues in biology. First, among the nearly 10 000 species of birds in the world, there is an enormous range of sexual dimorphism in behavior and appearance. In some species, the sexes look identical to our eyes, and in others very different. The same is true of sexually selected behavior, such as singing. In some species, such as those that duet, both sexes sing equally well. In others such as zebra finches, females never normally sing. Such diversity is thought to

Table 1 Comparison of effects of early treatments on zebra finch behaviors

Treatment	Behavior		
	Singing	Mounting	Partner preference
Estradiol	F→M	M→F	F→M
Estrogen synthesis inhibitor	0	0	F→M

F→M = masculinization; M→F = femininization/demasculinization; 0 = no effect.

be the consequence of the degree and type of sexual selection that has occurred in the evolution of the species. When confronted with it in closely related groups of birds, we can ask whether the hormone organizational concept can help us understand the developmental mechanisms responsible for its evolution.

Wrens of the genus *Thryothorus* wrens are a case in point. Within this genus there is marked variation in singing between the sexes: in some species only males sing whereas in others both sexes do, some duetting (Farabaugh, 1982; Levin, 1996). Males of species in which males alone sing have greater sexual dimorphism in the brain song nuclei (Brenowitz and Arnold, 1986; Nealen and Perkel, 2000). Enough is known about how song system dimorphism develops to be able to envisage, as these researchers have, how a small developmental change, such as in the timing or amount of estrogen action, might result in a shift from sexual monomorphism to dimorphism in singing or vice versa. This example also serves as a reminder that the nervous system and its developmental rules are critical to understanding evolutionary change in behavior.

A second issue is raised by research on zebra finches which suggests that the rules for the hormonal organization of mounting, singing, and partner choice might be somewhat different (Table 1). All three behaviors seem to be organized by sex steroids, but a different pair of results is seen for each behavior. This helps us to understand how such behaviors evolve somewhat independently of one another. Given that females prefer singing males, it would be disastrous for the species if the evolution of singing required a loss of mating (e.g., mounting) behavior in males. Divergent hormonal pathways (developmental cascades) provide the necessary disconnect for changing one behavior without disrupting others. They allow the evolution of diverse degrees and kinds of singing and mate choice without perturbing mating, which must occur. Divergent developmental cascades could also be the mechanistic basis for sex role reversal in birds, as in buttonquail, Turnicidae, in which females sing and are more brightly colored yet still produce eggs that need to be fertilized.

A third issue concerns the evolution of elaborate behaviors in male birds, far more than in male mammals, to attract females, especially potentially costly courtship signals such as the display of the peacock or song of the skylark. Reeve and Shellman-Reeve (1997) have proposed that the potential for evolving to a novel adaptive peak of this kind derives in part from the genetic architecture of the lineage, which differs in birds and mammals. Birds have Z and W sex chromosomes, and males are the homogametic sex; mammals have X and Y chromosomes, and females are the homogametic sex. According to Reeve and Shellman-Reeve's (1997) protected invasion theory, a novel trait will

be less susceptible to extinction in early evolutionary stages if it appears in the ZZ or XX sex, that is, the sex with the two similar sex chromosomes. Sexual selection is usually stronger for males; and in birds, it is the male that has the two similar sex chromosomes. Thus protected invasion theory helps us understand why elaborate males seem to have evolved more often in birds. What does this have to do with hormones? Because they regulate the expression of genes on autosomes to produce sex-limited trait expression, steroids in effect bring these genes under the protected invasion principle.

Hormonal organization is an especially powerful trait expression mechanism because it occurs early in the life of the animal and is permanent. By integrating its study with research and theory on development and behavior, we can achieve greater understanding of the evolution of sexually selected behavior, for which birds are justly famous.

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