7 Diversity and Evolution of Hormone-Behavior Relations in Reproductive Behavior

David Crews

As we learned in chapter 3, each individual is bipotential. Even though adult males and females display behaviors typical of their sex, both are capable of displaying those behaviors characteristic of the other sex. To what extent are the differences observed between adult males and females due to chromosomal constitution, to differences in nongenomic yet heritable sex-typical experiences, and to evolutionary forces? It is unfortunate, but true, that we know only what we study, and we tend to study only what we know. Recent studies of species that lack sex chromosomes indicate that the organizational-activational concept does not apply to all organisms.

Because we are mammals, many people find other mammals more interesting than fishes, insects, or snakes. But some of the most fascinating variations in reproduction have arisen among the reptiles and fishes, where relations between hormones and mating behavior can be very different from those described in the previous chapters. These alternative solutions to common life problems force us to look again at mammals, including ourselves, and ask questions about the evolutionary forces that have produced the hormone-behavior relations described in the previous chapters. We also see that in spite of the variation in the ways in which hormones affect reproduction and sexual behavior, in those species of vertebrates that have been investigated, the same brain regions are important. By taking advantage of the great variety in the ways species have solved common problems, we gain insights that may not be obvious from studies of more conventional laboratory species.

Without due consideration of the neural and behavioral correlates of differences between higher taxa and between closely related families, species, sexes, and stages, we cannot expect to understand our nervous systems or ourselves.
—T. H. Bullock (1984)

We have evolved a nervous system that acts in the interest of our gonads, and one attuned to the demands of reproductive competition.
—M. Ghiselin (1974)

Introduction

Through televised documentaries we have been to the poles and everywhere in between observing the lives of animals. The episodes captured on film usually focus on sexual or aggressive behaviors and adaptations to life's perils. What stands out is the incredible diversity of the behaviors exhibited. Each species engages in some behaviors that are characteristic of and unique to tha species. These are referred to as species-typical behaviors. From reading th first few chapters, you have probably already realized that there is also grea diversity in the ways in which hormones can affect an animal's nervous system and behavior. To the student, this diversity might seem like an unwante complexity, something else to complicate the story and your studying. It i the object of this chapter to convince you that as you gain an appreciation o the diversity in hormone-behavior relations, you will begin to understan more completely the basic mechanisms mediating these processes. In addition, you will gain a greater understanding of and appreciation for the evolution of hormone-brain-behavior relations.

At the most fundamental level, the study of diversity provides us with different perspective from which to view hormone-brain-behavior relation (Bartholomew 1982; Bern 1972; Bullock 1984; Crews and Moore 1986; Diamond 1983; Prosser 1988). There has been a tradition in modern times t specialize: the study of the causes and development of behavior is usuall separated from the evolution and ecological patterns of behavior. There ha also been a trend toward reductionistic investigations of behavior. In othe words, scientists have been inclined to investigate the molecular changes i the brain that are correlated with a behavior rather than to study the actua behavior itself. However, it is important to keep in mind that there are man levels of biological organization and that molecules always function withi cells, cells within organs, organs within animals and, ultimately, animal within environments. The cellular and molecular aspects of hormonal actio (that is, proximate causation) are important for our understanding of thes processes. But they tell us very little about the behavior itself, especially abou why the behavior is exhibited. For this, we must turn to studies of the whol animal and questions of ultimate causation. To learn about the evolution o behavior, we must turn to ecological and evolutionary analyses.

Those scientists interested in immediate causes of behavior tend to b unaware of the great advances that have been made in evolutionary biology Similarly, most scientists interested in ecological and evolutionary question ignore advances in neuroendocrinology and molecular biology. Quite simply "reductionists see little to be gained from holistic studies, and whole organism biologists do not recognize the value of molecular analysis" (Prosser 1986). This philosophical gap and lack of communication between these two approaches to the study of brain-behavior relations make it difficult to be a generalist. Yet to understand behavior, we must attempt to integrate the different levels of biological organization. If done with insight, it can lead to new discoveries in evolution, ecology, physiology, and molecular biology.

This chapter emphasizes an interdisciplinary approach to behavioral analysis, including an evolutionary approach. Obviously, a scientist can apply Darwinian thinking to behavioral questions without being an evolutionary biologist, as is evident in the now-vibrant field of human evolutionary psychology. For readers who might be encountering an evolutionary or ecological approach for the first time, it is important *not* to assume that evolutionary thinking is confined to a particular discipline. Indeed, modern Darwinian theory can be a powerful tool for molecular, anatomical, physiological, and behavioral research regardless of your formal disciplinary home.

The general principles that should guide you in considering behavioral endocrinology from an evolutionary and ecological perspective will be discussed next. These principles will be illustrated by describing some of the modes and patterns of reproduction in animals. A variety of evolutionary forces have led to various behavior-controlling mechanisms. These will be discussed first and will be followed by examples of the diversity in hormone-behavior relations that have been observed to date. These and other studies are at the interface of ecological physiology, evolutionary biology, and behavioral endocrinology. Next, several nonconventional animal model systems will be described in detail to illustrate how these three fields might be integrated. Finally, some thoughts are offered about why complementarity is a fundamental aspect of biological organization, whether a principle tenet of behavioral endocrinology encompass all vertebrates, and how hormone-brain-behavior relations might have evolved. We will conclude with some thoughts about the evolution of sex versus the evolution of sexual behavior.

Overview of General Principles

Modes of Reproduction

In the preceding chapters, the discussion of sexual behaviors has focused on species with two sexes. In the animal kingdom, however, this is not the only way species propagate. We tend to focus on mammals because their mode of reproduction is like ours and employs internal fertilization of the egg in a female by a male, with the female giving birth to live young. But this is only one mode of reproduction, and a remarkable array of reproductive modes has evolved.

Animals can be classified according to (1) the division across individuals according to egg or sperm production (or both), (2) the method of production of the young, and (3) the method of fertilization (table 7.1). Most vertebrates have two sexes, male and female. As you have already learned, this means that during development, the fetal gonads become testes in males and ovaries in females. This separation of the gonads into separate-sexed individuals is called gonochorism. On the other hand, in some species the same individual has both types of gonad and can produce both eggs and sperm. These individuals are called hermaphrodites. In some hermaphroditic species (e.g., earthworms and sea bass), the production of eggs and sperm occurs simultaneously. In others (e.g., coral reef fish), the production of eggs and sperm occurs sequentially. It is important to keep in mind that these two forms of hermaphroditism are fundamentally different. In the former instance, individuals are two sexes at the same time, whereas in the latter instance, individuals are only one sex at a time.

Table 7.1 Different Modes of Reproduction

PLACEMENT OF TESTES AND OVARIES	DEVELOPMENT OF FETUS	FERTILIZATION	EXAMPLE
Gonochoristic (separate male and female individuals)	Viviparity	Internal	Most mammals; many reptiles and some amphibians; flies
		External	Marsupial frog; gastric brooding frog; seahorse
	Oviparity	Internal	All birds; monotreme mammals; most reptiles; some amphibians; some fish; many sharks; most insects
		External	Most amphibians and fish
Hermaphroditic (individuals have both eggs and sperm)	Viviparity	Internal	Colonial tunicates
		External	None apparent
	Oviparity	Internal	Most land snails and slugs
		External	Solitary tunicates; corals
Parthenogenetic (all individuals have ovaries)	Viviparity	Internal	Thrips, Amazon molly
		External	None apparent
	Oviparity	Internal	Mole salamander
		External	Top minnow

Note: The reader should recognize that the scheme presented is simplified. Each category represents extremes on what is really a continuum, and intermediate forms exist. Furthermore, some animals may actually exhibit different reproductive modes at different stages of their life cycle. The interested reader should consult Blackwelder and Shepard (1981) for a more complete survey of the diversity of animal reproduction.

Finally, there are some species where all individuals have only ovaries and produce only eggs. This is called parthenogenetic reproduction. (The word comes from the Greek words *parthenos*, meaning virgin, and *genesis*, meaning birth.) In some of these all-female species, such as the Amazon molly, the popular aquarium fish, sperm from the male of another species is required to activate development, although the sperm's genome is not incorporated into the genome of the offspring. In other parthenogenetic forms (e.g., aphids and whiptail lizards), sperm are not required for complete and normal development.

Still another important distinction in modes of reproduction is the method by which the young are reproduced—viviparity or oviparity. Basically, viviparity ("live-bearing") exists when the young develop within the body of the mother, as in mammals. An important component of viviparity is that nutrients and waste products are exchanged between the mother and the fetus. Oviparous animals are egg-layers. Eggs may be ovulated and laid singly or by the thousands and may or may not have protective shell coverings. Still another reproductive mode, which is believed to have led to viviparity, is called ovoviviparity. In this instance, the fertilized eggs are retained within the body, but there is a reduction in the placental membranes, compared with viviparous animals, and no nutrients are exchanged.

The last distinction to be made is whether fertilization occurs internally or externally. Mammals, birds, and reptiles all practice internal fertilization. The mode of fertilization in amphibians, fish, and sharks and rays varies from species to species. This distinction is important because hormones can have opposite effects in these two modes of fertilization. It is also important because male and female reproductive behaviors, including mating behaviors, can vary dramatically depending whether fertilization is internal or external.

Patterns of Reproduction Among Seasonal Breeders

Multiple patterns of seasonal reproduction exist even within species that have two sexes. As discussed in chapters 4, 5, and 12, many species of animals are seasonal breeders. One important distinction among reproductive patterns depends on whether the animal mates in relation to its seasonal gonadal cycle. If the animal produces and releases its sperm or eggs during the mating season, this is known as an associated reproductive pattern (figure 7.1). If a species produces sperm and eggs at some time other than during the mating season and then stores them until mating occurs, this is known as a dissociated reproductive pattern. A third possibility is when the gonads are maintained continually at or near maximum development, which is known as a constant reproductive pattern; these are the opportunistic breeders referred to in later chapters.

Humans have developed ways to modify the environment and so have become emancipated from many of the factors that have led to seasonal reproduction in other animals. There is evidence, though, of some vestiges of seasonality in humans, which were imposed by periodic variations in food availability and high temperatures (Bronson 1995). For example, there is a seasonal variation in birth rates, and hence in the rate of conception, in Hong Kong, where the low temperatures in January are correlated with a peak in conceptions.

The animal model systems most widely used in behavioral endocrinology are those of the rat, guinea pig, mouse, and hamster. These species, as well as many other mammalian and avian species, exhibit associated reproductive patterns. That is, the periods of sexual behavior are restricted to the periods when the gonads are active. In species exhibiting an associated reproductive pattern, sexual behavior in the male is activated by elevated serum concentrations of testicular steroid hormones (see chapter 5). In the female, it is the pattern and amount of estrogens and progestins produced by the ovary that regulate the periods of sexual receptivity (see chapter 4). In species with dissociated reproductive patterns, breeding activity occurs when the gonads are small and not producing gametes or steroid hormones. In these species, sexual behavior is independent of steroid hormone control but is activated by some other stimulus. Species exhibiting a constant reproductive pattern are always ready to breed, waiting only for a specific environmental cue. In the

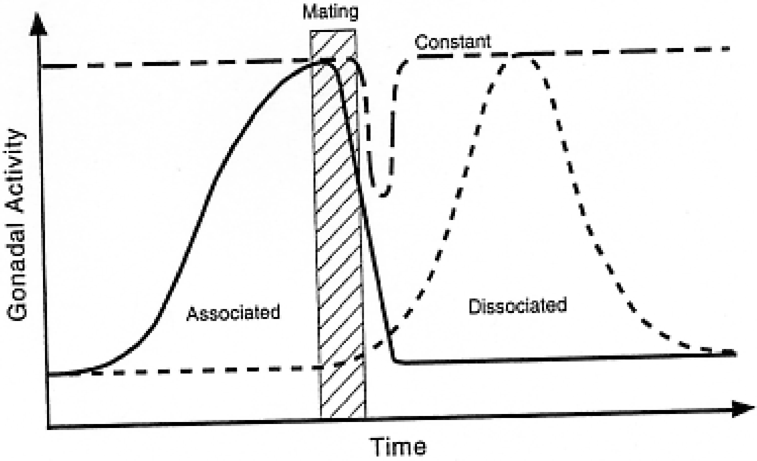


Figure 7.1 Vertebrates display a variety of reproductive patterns. Here gonadal activity is defined as the development of eggs and sperm and/or increased sex steroid hormone secretion. Individuals exhibiting the associated reproductive pattern (solid line) live in temperate regions where seasonal cycles are regular and prolonged; in such species, the gonads are fully developed at the time of mating and circulating levels of sex hormones are maximal. Individuals exhibiting the dissociated reproductive pattern (dashed line) live in extreme environments in which seasonal changes are regular, but the length of time available for breeding is limited; in such species, the gonads are small and sex steroid hormone levels are low at the time of mating. Individuals exhibiting a constant reproductive pattern (hatched line) live in harsh environments where breeding conditions are completely unpredictable; in such species, the gonads are maintained at nearly maximal development so that when breeding conditions do arise, breeding can occur immediately. Just as the reproductive cycles have adapted to the environment, so too have the neuroendocrine mechanisms subserving breeding behavior. The temporal uncoupling of sexual behavior and gonadal recrudescence in vertebrates exhibiting these different reproductive patterns is reflected in the dynamics of their hormone-brain-behavior relationship. *NB*: The dimension of reproductive pattern is depicted as mutually exclusive extremes only for the sake of argument; intermediate forms are known to exist. (Adapted from Crews 1984.)

Australian outback, the zebra finch will begin to court and build nests within minutes of the first drops of rain.

Another pattern of reproduction is determined by whether ovulation is spontaneous or induced (conaway 1971). In many animals, gonadal activity is initiated by environmental changes that occurred many weeks or months earlier (see later discussion). Once initiated, the eggs develop and are released spontaneously at a particular time regardless of whether or not mating occurs. This is known as spontaneous ovulation. In other animals, however, mating activity actually induces ovulation or, in some, gonadal growth. Cats and rabbits are examples of animals that are behaviorally receptive during the breeding season but do not ovulate unless mated. As discussed in chapter 4, experiments have shown that the act of copulation in cats and rabbits enables a neuroendocrine reflex that results in luteinizing hormone (LH) release by the pituitary and consequently ovulation. Other animals show even more extreme variations. In the ferret and the mink, the eggs do not undergo final maturation nor does ovulation ever occur unless the female experiences the violent precopulatory behavior of the male in which he energetically throws her about. In the musk shrew and the red-sided garter snake, ovarian activity is not even initiated unless the female is first courted and mated.

Some of the Evolutionary Forces Leading to the Reproductive Modes and Patterns Displayed by Animals Today

A successful approach to investigating and making sense of diversity in the neuroendocrinology of reproduction is to look for generalities across species. The comparative method is the traditional approach in biological investigations. It has been shown repeatedly that a comparison of various organisms leads to discoveries of themes or traits that recur throughout all animals. Traits are any property of the organism that a scientist is interested in measuring. When closely related species are compared, it becomes possible to trace the progressive specialization of traits and thus illuminate the course of evolution.

But simply comparing various species, populations, individuals, and even genes without concern for their shared history is not scientifically or statistically valid. New statistical methods known as evolutionary or phylogenetic analyses make it possible to estimate these historical relationships among any set of morphological, behavioral, or molecular data, and then depict these relationships in the form of a branching diagram, known as a phylogenetic tree (Hillis et al. 1996; Ryan 1996; Thorton 2001). For example, DeVoogd et al. (1993) compared the song and the structure of certain brain areas involved in song production (see chapter 8) among 41 species of songbirds. They found a significant correlation between the relative volume of a song-control nucleus known as the high vocal center (HVC) and the size of the species' song repertoire. Further, their analysis suggested that behavioral changes preceded brain changes; that is, selection for an enhanced song repertoire augmented the volume of the HVC.

While it is important to know what is present, it is equally important to know what might occur but is not present. For example, in table 7.1, we see that there apparently are no examples of hermaphroditic or parthenogenetic viviparous species practicing external fertilization. What is absent usually reflects a basic and sometimes insurmountable conflict among constraints or limitations inherent in the environment, development, or evolutionary history of an organism. When we understand such constraints, we can account for observed differences and, whatever the cause, predict what might be found in certain circumstances. Such an understanding can also suggest what is unlikely or has not evolved in our world.

Four concepts can guide you as you learn about these evolutionary forces. The first considers the evolutionary underpinnings of sexual behavior: did they emerge from forces that facilitated reproduction between individuals or from forces that prevented individuals from breeding? The second concerns the distinction between natural selection and sexual selection. The third describes the nature of the constraints that have shaped the evolution of reproductive processes. The fourth is the importance of individual variation and the concept of phenotypic plasticity.

REPRODUCTIVE SYNERGISM VERSUS REPRODUCTIVE ISOLATION

In an evolutionary sense, reproduction is the single most important element in an individual's life. It is more important even than the length of an individual's survival. Simply put, and with rare exceptions, if an individual does not reproduce, its genes will not be represented in future generations.

Reproduction has been host to the evolution of many specialized behaviors. Detailed analyses indicate that in many species, behaviors associated with reproduction tend to be highly ritualized, stereotyped, and characteristic of a species. We assume that individuals exhibiting these behaviors left more offspring. However, although they have both focused on sexual behaviors, behavioral endocrinologists and evolutionary biologists have traditionally viewed the function of sexual behaviors differently. Traditionally, behavioral endocrinologists have emphasized the fact that reproductive behaviors serve to coordinate the events that lead to successful reproduction, and evolutionary biologists have emphasized the fact that reproductive behaviors serve to isolate a species. This gulf has narrowed recently as we have learned more about mating and its consequences.

The position that reproductive behaviors have evolved to coordinate hormonal, gonadal, and behavioral events is known as *reproductive synergism*. Proponents of this position point out that reproduction is a carefully regulated process. Each successive phase of reproduction is dependent upon preceding events. At the same time, each phase sets the stage for what follows (figure 7.2). Reproduction occurs only when the participants send and receive appropriate visual, auditory, chemical, seismic, ultrasonic, or electrical signals (see chapter 8). This results in the appropriate coordination of the maturation and release of each individual's gametes (eggs and sperm) in an effort to increase their reproductive success.

The position that reproductive behaviors evolved to keep species from interbreeding is known as *reproductive isolation*. Proponents of this position hold that species-typical sexual behaviors serve as reproductive isolating mechanisms to maintain species boundaries. If individuals from different species do mate, fertilization will not occur. If fertilization does occur, viable offspring often will not result because the egg lacks the necessary sets of compatible chromosomes required for normal growth and development. Even when the species are closely related, indicating a recent (in an evolutionary time scale) divergence, the young produced tend to be sterile.

Recently there has been a shift in evolutionary biology away from the isolationist perspective (Carson 1987). The argument is that if evolution favors reproductive success, then it is more in the individual's interest to focus on selecting the best mate than to avoid mating with the wrong species. Further, the act of mating is only the beginning of a complex process in which the

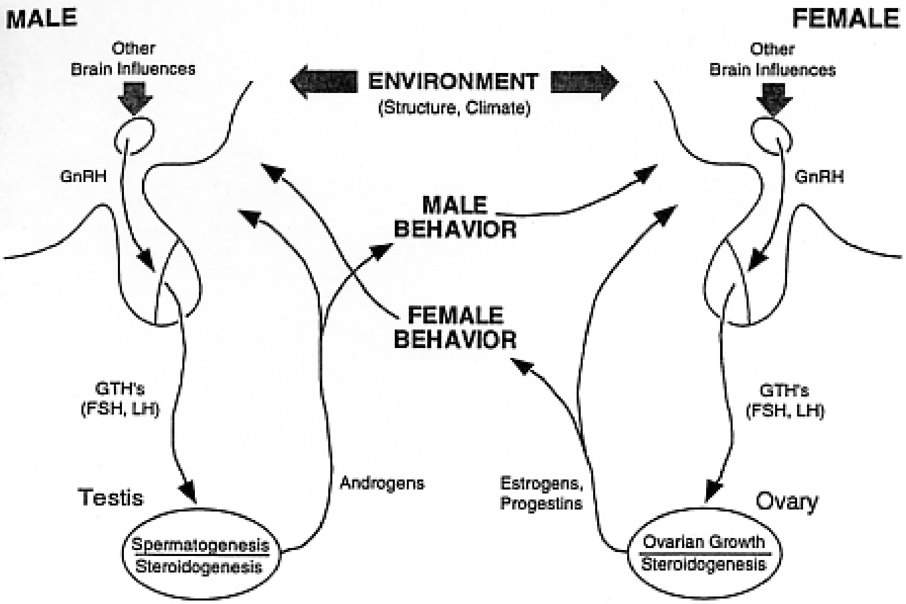


Figure 7.2 Dynamic relationship between the internal and external environments in the control of mating behavior in vertebrates. The complementary behavior of the male and the female help to synchronize the maturation and release of the sperm and eggs so that fertilization occurs. Changes in climate, ecology, or behavior of other members of the species initiate and modulate gonadal and hormonal changes during reproduction. Thus, hormones regulate behavior in the individual animal and are themselves affected by other stimuli, including the behavior and, indirectly, the physiology of its mate. In such reciprocal systems, each successive phase of reproduction depends upon preceding events and, at the same time, sets the stage for the following phase. (See figures 1.9 and 1.10 for details of hypothalamus-pituitary-gonadal axis.)

male's intromission and insemination induce behavioral and physiological responses in the female that increase the likelihood that his sperm will fertilize her eggs (Eberhard 1996).

NATURAL SELECTION VERSUS SEXUAL SELECTION

Darwin considered natural selection and sexual selection as different processes. Both are primary forces driving the evolution of traits. For our purposes, natural selection results in traits that are adaptational responses to changes in the environment. The resulting variation in traits between and within species is shaped by differential survivorship. In other words, animals that survive are those with traits that are adaptive to their environment (Williams 1966).

Sexual selection, in its simplest form, states that males compete for females and females choose between them. Most of the research in this field today focuses on how females choose males on the basis of the morphological and/or behavioral characteristics they display. Female choice continues after intromission with a host of physiological, morphological, and behavioral hurdles that continue to evaluate male quality, enabling the female to maximize the genetic contribution of desirable mates. It is, of course, even more complicated in that females compete among themselves for males as well as for resources. Natural selection and sexual selection often act in opposite directions on male traits, favoring, for instance, drabber plumage (natural selection) on the one hand and showier plumage (sexual selection) on the other.

Sexual selection has led to the evolution of extravagant sex-linked coloration, ornamentation, and behaviors with no apparent function in the struggle for survival. Such traits are often sexually dimorphic. Males tend to develop specific traits that are involved in courtship or mating and occur during the breeding season. In this instance, individual variation in these traits is sculpted by differential reproductive success (Clutton-Brock 1988). Indeed, we will see how this variation can reflect different neuroendocrine mechanisms or even serve as the substrate for the evolution of novel mechanisms. The number of offspring an individual produces during its lifetime will determine whether this trait is continued in the species. Thus, "Sexual selection is responsible for most of the morphological, physiological, and behavioral characters that are observed as subserving the efficiency of the reproductive act as an important monitor of fitness" (Carson 1987, 598).

Darwin conceived of sexual selection as arising from aggressive interactions between males (male-male competition) and the female's selection of a mate (mate choice). Males compete among themselves for access to females. A variety of traits, such as antlers, horns, and tusks, have evolved as a result of male-male antagonism as they compete for access to a female's eggs.

Aggression between males can have a direct effect on female reproduction. It will prevent other breeding males from having access to a female or from harming the female. Aggression among males can also have an indirect effect. It can inhibit or suppress the normal reproductive physiology of the female (figure 7.3) or even terminate a pregnancy. Mate selection may also be based on the availability of a particular resource such as food. Thus, in many species, females choose real estate and males compete to control access to that real estate.

At least three competing variants of the natural selection hypothesis have been offered for the evolution of female preference (Kirkpatrick and Ryan 1991), each of which emphasizes that females choosing optimally will produce young whose viability and survivorship are enhanced by the choice. The "good genes" explanation suggests that a female benefits by mating with a particular male that has traits that enhance survivorship, thereby imparting these benefits to her young. In this case, the quality of the male's displays is assumed to reflect the individual male's genetic constitution. A second variant of the natural selection hypothesis is the "handicap principle," which postulates that females favor males with "expensive" traits. These are traits that put the male at greater risk of predation or have greater energetic cost. It is reasoned that males that can exhibit elaborate traits have a greater handicap and therefore must be more fit than males with less developed traits.

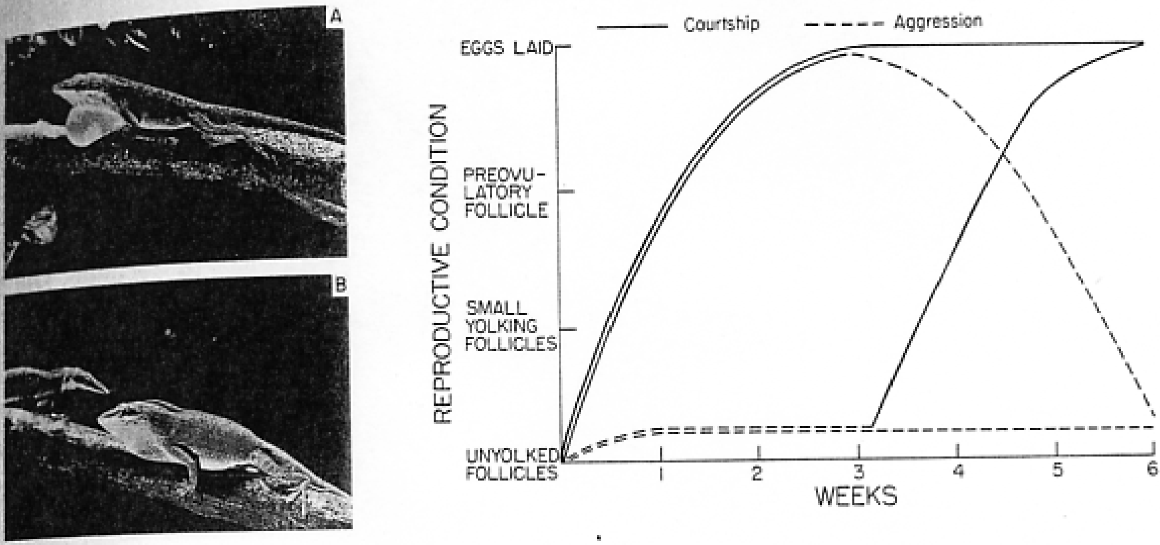


Figure 7.3 Different behaviors can stimulate or inhibit reproduction. In the left panel are pictures of male green anole lizards performing a courtship display (*A*) and an aggressive display (*B*). Note the differences in body posture. In the right panel, we see how the courtship display facilitates ovarian growth in female green anoles, whereas the aggressive posture inhibits ovarian growth. In this experiment, females were exposed to the courtship or aggression stimulus for the entire 6 weeks, or for 3 weeks and then the stimulus switched for the next 3 weeks.

Finally, a relatively recent variant is the "parasite load" idea. If a male is parasitized, the breeding plumage is dull rather than brilliant. This idea suggests that brighter coloration or some other elaborate trait is associated with a genetic resistance to deleterious parasites. Thus, females choose brighter, more colorful males because this coloration indicates heritable disease resistance that can be passed on to their young. In all three forms of the natural selection hypothesis, male traits are regarded as being indicative of high genetic quality. Female preference is thought to evolve because of advantages her offspring acquire as a result of the female mating with these males.

The sexual selection hypothesis has two main variants. One is Fisher's runaway sexual selection hypothesis. In this paradigm, male traits that influence mate choice become genetically correlated with female preference by virtue of the mating system; that is, the gene(s) for the trait is linked to the gene(s) for the preference. As the traits increase in frequency, the preference for the trait passively increases in the female. Thus, females do not gain directly from choosing particular males. Rather, the preference evolves as a correlated response to selection of the male trait.

A more recent variant of the sexual selection hypothesis that attempts to account for the evolution of female preferences is that of sensory exploitation. Ryan (Ryan 1990; Ryan et al. 1990) has pointed out that sensory systems are predisposed to specific stimuli. For example, as you will see in chapter 8, the frog auditory system is tuned to certain frequencies and female frogs prefer certain qualities in the call of males. The sensory exploitation hypothesis postulates that males have evolved calls to exploit preexisting sensory biases in the female that evolved for reasons independent of female mate choice.

The latter two hypotheses present different scenarios for the evolution of behavior-controlling mechanisms. Fisher's runaway selection hypothesis presumes a genetic linkage between male traits and female preferences, so that changes in the female are a passive function of changes in the male. The sensory exploitation hypothesis predicts that preference in the female is determined by various constraints and that male behavior has changed to maximize stimulation of the female's sensory systems. Whatever the evolutionary reason, the act of choosing the correct mate is of utmost importance. We will see how in birds the compatibility of mates has a dramatic effect on whether or not young are produced.

CONSTRAINTS ON REPRODUCTION

You have learned that sexual behavior in vertebrates is dependent on gonadal steroid hormones for its activation. However, as we will see, this conclusion stems primarily from the species that have been studied. It does not reflect a universal truth. Indeed, species differences in hormone-behavior relations can often be traced to adaptational responses to the physical and social environments. This is because reproduction is constrained by (1) the immediate environment, (2) limitations inherent in developmental and physiological processes, (3) the social and behavioral context, and (4) evolutionary history.

Environmental Constraints

The evolution of reproductive seasons is determined by factors selecting against those individuals bearing young during times of food scarcity or other adverse environmental conditions. Individuals that produce young during optimum conditions have been favorably selected. In other words, these individuals were most likely to reproduce and pass on their genes to the next generation. This has been termed the *ultimate* cause of breeding seasons. Ultimate factors that determine the timing of breeding seasons include adequate food, availability of nesting materials, and predation pressure. *Proximate* causation refers to those stimuli used by the organism to actually initiate and terminate breeding. Well-known examples of proximate factors are seasonal fluctuations in day length, temperature, moisture, and so on. The responsiveness of the neuroendocrine system to proximate cues may vary seasonally. This seasonal variation probably reflects endogenous circadian and circannual rhythms (see chapter 12).

Environmental constraints on reproduction are especially severe when the environment is harsh. For animals living in a harsh environment there is only a brief favorable period for reproduction. Under these conditions, animals must respond rapidly and directly to physical changes in order to mate. The female must respond rapidly in order to reproduce. The young must grow sufficiently to survive the upcoming harsh conditions. These species exhibit an explosive or opportunistic pattern of reproduction in which all breeding activity is compressed into a few days or weeks.

Developmental and Physiological Constraints

Developmental and physiological processes may also dictate when animals breed. These processes can shape the mechanisms controlling reproduction. For example, mature sperm in most species cannot be produced in less than 6 weeks. Although some mice and ground squirrels can produce mature sperm in as little as 31 days, they appear to be the only exceptions to this rule. A similar time constraint applies to the production of eggs, although some small rodents are capable of generating eggs in less than a week. In cold-blooded vertebrates, it is common for egg maturation to take many months or even years.

A second constraint is the temperature-dependence of gonadal activity. Gamete production and steroid hormone secretion will not occur at cold temperatures. This presents a problem for many animals that live at high altitudes or latitudes. For these animals, mating must occur as early in the spring as possible so that young can be born and grow enough to survive the next winter. Some species produce sperm during the summer and then store them through the winter. In this way, the male is able to inseminate females immediately on emergence from hibernation. In many bats, sperm storage lasts for several months, but in some reptiles it can be as long as 17 years. Seasonally high temperatures can also inhibit reproduction, which occurs in humans living in equatorial regions where spermatogenesis is sufficiently suppressed to influence the incidence of fertilization (Bronson 1995).

In many mammals, including some species of bears, the western spotted skunk, and kangaroos, implantation of the embryo is delayed. The embryo goes into a kind of suspended animation called embryonic diapause that can last for as little as one week or as long as several years. At the end of diapause, the embryo implants and development resumes. In other species, particularly cold-blooded vertebrates, there simply is not enough time available in one season for gametes to grow, adults to mate, and the young to develop. Cold-blooded animals, or ectotherms, rely more on the external environment for temperature regulation, whereas mammals and birds, often called warm-blooded animals or endotherms, rely on metabolic energy to produce and maintain an elevated body temperature. This can be an arbitrary distinction, however, as many ectotherms, such as honeybees and lizards, can maintain elevated body temperatures by behavioral means.

Warm-blooded animals living at high altitudes or latitudes typically migrate to warmer areas or hibernate for the duration of the cold months. In those species that hibernate, such as small rodents, it was long a puzzle how males could enter hibernation with small testes but emerge with large testes. Recently, it has been discovered that animals such as Turkish hamsters periodically arouse from hibernation, warming up for a few hours or days. It is during these arousals that the gonads grow. Cold-blooded animals living in extreme environments, however, do not exhibit periodic arousals, nor do they show any signs of gonadal growth during the cold winter months. In these animals, it is common for gamete growth and steroid secretion to be temporally dissociated from breeding activities.

The embryonic environment may also represent a constraint, shaping an individual's physiology, body, and even intellect. In some mammals, the position of the fetus relative to siblings in utero or its intrauterine position can mold morphology, reproductive physiology, and sociosexual behavior in adulthood (Clark and Galef 1995). In gerbils, mice, and rats, female fetuses located between two males (2M females) are exposed to higher levels of androgens produced by the neighboring males than female fetuses located between two females (2F females) (see chapter 13). As adults, 2M females have lower estradiol and higher testosterone levels in the circulation, have a masculinized phenotype, and are less attractive to males and more aggressive to females. 2M females also produce litters with more male-biased sex ratios relative to 2F females. Additionally, 2M females have later onsets of estrous cycles, longer estrous cycles, shorter reproductive lives, and fewer litters. Differences in phenotype due to intrauterine position are also evident in males. Males positioned next to two males (2M males) are more aggressive and more sexually active than are males positioned next to two females (2F males). It should be noted, however, that the pattern of intrauterine effects is not necessarily consistent across species. For example, in gerbils 2M males outcompete their 2F counterparts in reproductive success, whereas in mice 2F males are more sexually active than are their 2M brothers.

A mechanism by which intrauterine position may affect or constrain reproduction is by altering brain metabolism in specific brain areas. A convenient measure of metabolic activity of brain nuclei is cytochrome oxidase histochemistry.[1] In gerbils, the metabolic capacity in the sexually dimorphic area of the preoptic area (SDA-POA) and the posterior portion of the anterior hypothalamus is greater in 2M than 2F females. In gerbils, the SDA-POA is responsible for copulatory behavior in males, and the posterior portion of the anterior hypothalamus is an area replete with neurons containing gonadotropin-releasing hormone. These differences in metabolic capacity may explain, respectively, the behavioral and hormonal masculinization of 2M females relative to 2F females.

First noted by Aristotle, the female spotted hyena in many ways resembles the male; in fact, it is larger, more aggressive, and more dominant. Most remarkable, however, is that the female's clitoris is so hypertrophied that it resembles the male's penis. This unique pattern of female urogenital development results from the increased production of androstenedione from the mother's ovaries during pregnancy, which in turn is transformed to testosterone in the placenta and subsequently transported to the fetus (Glickman et al. 1997).

There is no doubt that the early environment plays a profound role in health in humans. It has long been recognized that malnutrition during pregnancy influences the subsequent growth, cognitive ability, and immune system of offspring, dictating even the length of life. For example, the circulating levels of androgen and sex hormone binding globulin during the mother's pregnancy may also have an organizing effect on both the hormone profiles and psychosocial test scores of young adult women (Udry et al. 1995). In this remarkable study, a substantial portion of the variance in the women's "gendered" behavior was accounted for by measurements of androgen exposure during only the mid-trimester of development and of the circulating levels of androgen as adults. Then there is the curious property of the cochlea whereby it produces spontaneous otoacoustic emissions. In general, human females produce more emissions than males (McFadden 1997). The prenatal environment influences the frequency of these emissions. The sexual dimorphism is not evident in the female of opposite-sex fraternal twins, suggesting that the hormonal environment created by the male fetus decreases these emissions in the female co-twin. Finally, through meta-analysis of published twin studies, it was demonstrated that the environment in utero might play a significant role in the heritability of IQ (Devlin et al. 1997).

What about animals that lay eggs rather than gestate the young internally? The yolk produced by the female that supports embryonic development is full of hormones (Bern 1990). In birds, the hormonal profile of the female is reflected in the yolk; hence, during the early stages of follicular growth, when androgens and estrogens are the dominant steroid hormones in the systemic circulation of the female, these hormones occur in the highest concentrations in the initial layers of yolk deposition. During the final stages of follicular growth and immediately after ovulation as the egg enters into the oviduct to get its shell coat, progesterone levels are highest, and this hormone is in higher concentrations in the outer layers. Thus, how the yolk is utilized by the growing embryo—from the outside in, or from the inside out—becomes an important question.

Hormone content can also change through egg laying. In canaries and zebra finches, eggs laid later have higher testosterone levels than eggs laid earlier; this, in turn, correlates positively with the subsequent growth and social rank of the individual. This is believed to be a way in which the younger, weaker hatchlings can compete with their older nestlings. In the cattle egret, however, it is the earliest egg that has the highest level of testosterone. In this instance, the oldest chick will evict the younger, weaker chick from the nest when resources are limited.

Females also appear to be able to control the amount of hormones in their eggs. Female zebra finches prefer to mate with males that have red leg bands (as discussed earlier, this appears to be related to the fact that the intensity of the male's red beak is related to his circulating concentration of androgens). Females that chose males with red leg bands had greater amounts of androgens in their eggs. Since more androgens in the yolk result in more aggressive offspring, a female's mate choice may indirectly (via hormones) influence the reproductive success of her offspring.

Finally, the experiences of the female while pregnant or even the behavior of the mother toward the neonate can influence how the individual grows and behaves. Indeed, 40 years ago it was discovered that the adverse effects of crowding mice during pregnancy can continue to be detected in the physiology and behavior of the following two generations of progeny. Handling pregnant females or housing them in socially unstable conditions can induce similar stress effects. The attention paid by the mother to her young is also important in behavioral development. For example, mother rats behave differently toward male and female pups; furthermore these differences reinforce and accentuate subsequent sex differences when the pup reaches adulthood (Moore 1995).

Social and Behavioral Constraints

As described in chapter 11, stress can have a profound negative effect on reproduction. Crowding, social domination, and captivity can effectively inhibit reproduction. On the other hand, specific social conditions or behaviors can also stimulate reproduction. Research with species representing every vertebrate class has shown that certain behaviors are necessary for the proper stimulation of the physiological changes that must occur in reproduction. For example, in ring doves and in green anole lizards (figure 7.3), the courtship behavior of the male is required if the ovaries of the female are to grow. Indeed, the rate of ovarian growth is determined by how much male courtship behavior the female sees. The male's aggressive behavior has the opposite effect and inhibits ovarian growth (Crews 1975).

Evolutionary Constraints

The evolutionary history, or phylogeny, of the species is another constraint predisposing the evolution of certain mechanisms and not others. Simply put, what has come before determines to a large extent what will follow. We might predict, therefore, that closely related species sharing a similar reproductive pattern but living in different environments will exhibit similarities in the neuroendocrine mechanisms underlying their modes of reproduction.

An example of this can be found in the garter snake (*Thamnophis*), a large genus believed to have radiated into the New World after crossing the Bering Strait. All garter snake species that have been examined to date, even those living in Mexico, exhibit a dissociated reproductive pattern and probably exhibit the same neuroendocrine mechanisms controlling mating behavior. We might also predict that distantly related species living together and facing similar challenges would have similarities in the neuroendocrine mechanisms controlling mating behavior. For example, most vertebrates that originated in the tropics but presently live in temperate regions exhibit an associated reproductive pattern. Thus, constraints imposed by both the external and internal environments of a species influence its social displays and their physiological consequences.

GENETIC RECOMBINATION AND PHENOTYPIC PLASTICITY

We are all individuals and, ultimately, evolution is the consequence of the behavior of individuals. Individuality is a product of sexual recombination; in all instances, with the single exception of the blue-green algae or cyanobacteria, present-day asexual organisms arose from sexual organisms. But sex is costly compared to asexual reproduction. There are a number of reasons for this (Maynard Smith 1978), but in terms of the growth of a population, meiotic recombination can disrupt co-adapted combinations and, in organisms where eggs and sperm combine, *halves* the rate of reproduction. Why then is sex so common? Recent studies on sexual and asexual populations of yeast reveal that the sexual yeast are more efficient at removing deleterious mutations than asexual yeast (Zeyl and Bell 997).

The "selfish gene" hypothesis posits that the fundamental struggle of evolution takes place not among individuals but at the level of the gene, and that behavior, physiology, morphology, and indeed every somatic cell exist only to promote the successful maintenance and dispersal of an individual's genes (Dawkins 1976). Genes, however, require a vehicle, the organism, to go through time. Genetic replication is accomplished in virtually all organisms (but see below for instructive exceptions) by sexual reproduction. Reproductive success can be defined as the successful reproduction of offspring, or grandchildren. Thus, it is not so much survival of the individual organism as the continuity of its genes that matters in evolution.

Phenotype is a concept that describes the constellation of morphological, physiological, and behavioral traits that, when considered together, characterize the individual. Thus, the phenotype refers to aspects of an individual's behavior, brain, body parts, physiology, or organs at any given time. We have come to appreciate that the phenotype can change throughout different stages of an individual's life history and that every genotype can yield a variety of phenotypes.

Phenotypic plasticity refers to the process by which the internal and external environments induce different phenotypes from a given genotype (Piglucci 1996). An example of phenotypic plasticity can be found in amphibians (Denver 1997). Probably the most important environmental variable for a tadpole is water availability, and frogs living in deserts accelerate metamorphosis as the ponds created by seasonal rains dry up. This is an adaptive response to the risk of mortality. As the water level falls, the brain produces corticotropin-releasing hormone, the primary vertebrate stress neurohormone. This in turn activates the thyroid and interrenal endocrine systems that control metamorphosis.

Survey of Diversity in Hormone-Behavior Relations

The Importance of Mate Compatibility

Making the correct choice of a mate can have a definite impact on a female's reproductive success. This is seen particularly well in long-lived species where it has been possible to monitor an individual's lifetime reproductive success. For example, for the past 35 years, Coulson has studied a colony of kittiwake gulls. These gulls nest on the windowsills of a riverside warehouse in Northumberland, England. In many instances, they pair for life. Other kittiwakes, however, choose a different mate the next breeding season. In about half of the pairs that break up, mates are changed because the original partner has died. In the other half, pairs of kittywakes "divorce." The cause of divorce can be traced to the failure of the pair to hatch at least one egg the preceding year. Not only do successful pairs fledge more young, but they also produce eggs faster, indicating that females in these pairs reach breeding condition earlier (Thomas and Coulson 1988).

Bluhm (1985) has studied the reproductive consequences of pair incompatibility using canvasback ducks. The reproductive success of females that were allowed to stay with their self-chosen partner was compared with the reproductive success of females that were separated from their self-chosen partner and paired with another male chosen at random. The results were clear-cut. Only females from pairs with self-selected males laid eggs. A similar situation was found in the cockatiel where reproductive success was enhanced if females were allowed to choose a mate as opposed to being forcibly paired with a male (Yamamoto et al. 1989).

Bluhm also pioneered an exciting new area of research in behavioral endocrinology that includes the endocrine correlates of mate compatibility. She has found that the female canvasbacks from forced pairings lack specific hormone changes (Bluhm et al. 1984). We know from research with other vertebrates, including humans, that copulation can trigger changes in circulating hormone levels, which can promote sperm transport and implantation. Courtship stimulation can result in more rapid ovarian growth in animals such as ring doves and green anole lizards. Thus, behavior-physiology interactions are an important aspect of neuroendocrine relations. You will learn more about this subject in chapter 18.

Mixed Reproductive Strategies

If environmental, physiological, social, and phylogenetic constraints can influence reproductive processes (like when the gametes are produced), it is likely that the neuroendocrine mechanisms controlling each of these processes must have undergone corresponding adaptations. What evidence is there for this?

First, recall the associated and dissociated reproductive patterns described earlier. Although we know a good deal about the neuroendocrinology of sexual behavior in species exhibiting associated reproductive patterns, we know next to nothing about these mechanisms in species that exhibit dissociated reproductive patterns. This deficit has occurred because scientists have concentrated almost exclusively on species which have associated reproductive patterns. Indeed, even in those species in which we know that gonadal growth is associated with mating, we often do not know whether it is the cause or the

Table 7.2 Reproductive Strategies in Gonochoristic Vertebrates

		MALE	
		Associated	*Dissociated*
FEMALE	*Associated*	Many laboratory and domesticated mammals; most birds; many temperate and tropical lizards, crocodilians	Most temperate turtles and tortoises, Indian lizard, timber rattlesnake, rough earth snake; tiger salamander; pike
	Dissociated	Musk shrew; Arctic fulmar; Mexican spiny lizard, Australian skink, mole skink, leaf-toed gecko, European viper, eastern coral snake; shiner perch, catfish	Hibernating bats; several rattlesnake spp., cobra, and harmless North American snakes; plaice, common carp

Gonadal activity may be temporally associated or dissociated from mating behavior in each sex. The result is a reproductive strategy in which the sexes have the same or different reproductive patterns.

consequence of the mating behavior. The importance of this point should not be underestimated. As you have already learned, in a variety of species, mating can initiate gonadal activity in the female or in the male. In other species, copulatory stimuli are known to be responsible for the final stages of gamete development or for successful fertilization or implantation of the fertilized embryo.

If we consider just these two patterns alone, it is possible to discern four basic reproductive patterns in vertebrates (table 7.2). In most domesticated species, both the male and the female have an associated reproductive pattern. There also are a number of species in which both sexes exhibit a dissociated reproductive pattern. However, there are some species in which the sexes exhibit a mixed reproductive pattern. In these species, one of two things can happen. The male can produce sperm before breeding and store them until mating occurs. Alternatively, mating occurs and the female stores the sperm in her reproductive tract for later use. In this instance, the act of mating initiates gonadal growth in the female.

Those species that have mixed reproductive patterns hold great promise for untangling ecological and evolutionary forces on reproductive behavior. That is, in species exhibiting mixed reproductive patterns, different selection forces must have caused the sexes to differ in fundamental ways in the organization and activation of behavior-controlling mechanisms. For example, Rissman (1995) discovered that in the Asian musk shrew, mating behavior in the male coincides with testicular growth and is activated by increasing testicular androgen secretion. In the female, however, receptivity precedes ovarian growth and is independent of estrogen from the ovary. Instead of a behavioral estrus cycle such as occurs in rodents, female musk shrews become receptive and allow mating within minutes of their encounter with a male. The female's receptivity is regulated by testosterone secreted by the adrenal glands and the ovaries and which, in turn, is converted in the POA to estrogen in the neurons that produce the enzyme aromatase.

Different reproductive strategies can also be found within the same sex of a single species. A recent discovery suggests that individuals within a species may differ fundamentally in neuroendocrine-controlling mechanisms. For example, in several rodent species, researchers have found that individuals within a population may utilize different proximate cues for regulating gonadal activity. Photoperiod may be important in some individuals; in others, it may be temperature or food (Bronson 1989; Prendergast 2001). Individual differences in the required proximate cue can be adaptive, resulting in a fine-tuning of reproduction in the population in response to its environment.

A recent discovery in behavioral ecology is that individuals within a given population may adopt distinctly different physiologies, morphologies, or behaviors. These are termed alternative life-history strategies. In some species these alternative life history strategies are heritable, whereas in others they appear to arise from social or environmental conditions. For example, there are three types of male bluegill sunfish: a large colorful male that defends territories and solicits females, a small male that sneaks matings when the territorial male is otherwise occupied, and a large but drab male that mimics females in appearance (Gross 1984). The sneaker steals fertilizations by streaking in to release sperm as the female releases her eggs. The female mimics effectively insert themselves between a courting territorial male and the female he is courting; in this manner the female is courted by the male but it is the mimic that fertilizes the eggs. Developmental and genetic studies indicate that sneakers grow up into female mimics, whereas territorial males produce male young that are large and brightly colored. Circulating concentrations of androgens differ in the different types of male bluegill sunfish, with androgen levels being higher in territorial males than in sneakers or in female-mimics (Kindier et al. 1989).

Many fish are hermaphroditic. Some have testicular and ovarian tissue simultaneously and trade behavioral roles during the spawning act (simultaneous hermaphrodite). Others undergo a sex change during life (sequential hermaphrodites). There are two basic types of sequential hermaphrodites. Protogynous individuals mature and reproduce first as females and then, as they age, turn into males. Protandrous individuals develop and reproduce first as males, and then later turn into females. A third type, continual sex change, was recently described in the Okinawan goby. In this species the individual functions first as a female, changes into a functional male, and then reverts to a female, repeating this process over and over again. This latter pattern is functionally equivalent to simultaneously hermaphroditic species in that individuals alternate their behavior and the type of gamete that is shed in successive matings. How they differ is that in continual sex change, the gonads undergo a complete morphological change, producing exclusively the gonad-typical gamete during each successive phase, whereas in simultaneous hermaphroditism, the gonads are ovotestes.

Behavioral endocrinologists have begun to study these unusual reproductive systems. It was believed originally that the behavioral changes observed in sex-changing fish were a consequence of the changes in the gonads or their hormonal products. Yet experiments with a variety of protandric and protogynic coral reef fish consistently yielded negative results. That is, while administration of steroid hormones will cause a morphological sex change, it takes weeks for this transformation to be completed. Yet in nature, sex change is primarily under social control and can be induced experimentally by removing the dominant TP males from small reef populations. Within minutes of removal of the dominant male, the largest female will begin to behave as a male, aggressively defending the coral head and soliciting other females. It is only many days later that the morphological changes in the gonad are observed and some days after that, the physiological changes. In other words, approximately two weeks after a female takes over the male role, the ovaries are transformed into testes.

Recently this assumption that the gonads drive sex change has been turned on its head. The protogynous bluehead wrasse has two alternative male mating morphs. Females and one adult male morph are light yellow with a dark dorsal stripe (initial phase or IP coloration) and are not territorial or aggressive. Terminal phase males (TP males) are brightly colored with blue heads, green over most of the body, and a vertical white bar bordered by black bars just posterior to pectoral fin insertion. TP males exhibit territorial aggression in defense of spawning sites, as well as distinctive courtship behaviors. During an afternoon spawning period, females travel to spawning sites, usually coral heads. If the site is defended, the TP male will court the female and the pair will mate. If the spawning site is undefended, the female will mate with a group of IP males. Mating consists of a rapid ascent called a "spawning rush"; eggs are released at the apex of this rush, and the fish quickly return to the bottom. Females spawn approximately two out of every three days, while successful TP males spawn 30 to 50 times per day on average.

Godwin et al. (1996) conducted a study in which the largest bluehead female in a group was ovariectomized and the dominant male was removed all in the same morning. Surprisingly, the female would still exhibit all of the behavioral and color changes that typically follow the removal of the dominant male. Indeed, even though these neutered females do not shed gametes, they entice other females to spawn with them. In almost all instances, this occurred immediately, just as they would occur when an intact female assumed the dominant role and defended a coral head. Further studies indicate that the transcript of the gene coding for arginine vasotocin changes in parallel with these behavioral changes. When considered together with the experiments with the unisexual whiptail lizard that will be detailed later, it is clear that sexuality resides in the brain and not in the gonad.

If the social or environmental stimuli are transduced in the brain, how then is the information transferred to the gonads? There are afferent and efferent neural connections between the gonad and the brain in vertebrates, including hermaphroditic fish, that are important to reproduction. Electrical stimulation of these circuits in a simultaneous hermaphroditic sea bass results in sperm or egg release, whereas in some other species, denervation leads to atrophy of the gonads. Further, the steroidogenic cells in the gonad are themselves directly innervated, and electrical stimulation of appropriate brain regions can either increase or decrease gonadal steroid secretion. That sex steroid hormones will functionally sex-reverse fish (albeit over a prolonged time period) and that the steroidogenic cells are in close proximity with the gonial cells in the gonad suggest that an individual's perception or psychology, combined with a fundamental requirement of behavioral complementarity (see below), mediate the behavioral role an individual adopts (in a simultaneous hermaphrodite) or drives the process of sex-change (in a sequential hermaphrodite) (Crews 1993).

Proximate Stimuli Activating Sexual Behavior

We know that for a number of vertebrates the steroid hormones secreted by the active gonads are necessary for sexual behavior. This elevated concentration of gonadal steroid hormones is required if proximate stimuli such as the behavior or scent of another individual or some change in the environment are to release sexual activity. In these animals, the sex hormones are altering the perceptions of the individual and stimuli take on new meanings (Beach 1983). Other organisms, however, have circumvented this reliance on gonadal steroid hormones. Instead, they rely solely on various proximate cues to activate sexual behavior. Here we will mention some examples of this by considering first the environment as a source of critical stimuli. We will then consider animals in which the behavior or tactile stimuli provided by members of the opposite sex initiate sexual behavior. Finally, we will learn how chemical signals, or pheromones, can trigger complex sexual behavior in vertebrates.

ENVIRONMENTAL STIMULI

If mating behavior can occur when the circulating concentrations of sex steroid hormones are low, does another stimulus activate sexual behavior? In species inhabiting extreme environments and that are opportunistic breeders, the use of other stimuli is apparent. Physiological constraints have led to specific stimuli being co-opted as proximate triggers for sexual behavior. We have mentioned the desert-dwelling zebra finch in which the male maintains mature sperm and high androgen levels throughout the year. Similarly, the female's ovaries contain developed ova at all times. In the deserts of western Australia, rain may occur only once in a three-year period. The falling rain initiates reproductive behavior in the zebra finch. Within 10 minutes after rain begins falling, the zebra finch begins copulating. Nest are built within 4 hours. Eggs are laid within a week. Thus, although the androgen-dependence of sexual behavior in adult male zebra finches is well established, androgens may play only a permissive role. That is, the ability of rainfall to activate sexual behavior depends upon the priming effect of androgen. While considerable research has been conducted on the reproductive behavior and biology of zebra finches, in all the studies on their mating behavior, water has been freely available. Thus, although it is likely that the specialized adaptation to water in the zebra finch will be reflected in the neuroendocrine-controlling mechanisms, this water hypothesis remains to be tested experimentally.

SOCIAL STIMULI

Wingfield has shown that endocrine profiles associated with mating are specific to the social system of a number of species of birds (Wingfield et al. 1990). This probably reflects important differences in the neuroendocrinology of these birds. For example, in species of birds in which one male pairs with one female to help to feed its young (monogamy), the male exhibits only a brief period of high levels of testosterone when territories are being established in the spring. In species in which males pair with two or more females and do not help feed the offspring (polygyny), the male maintains high testosterone levels and remains aggressive throughout the breeding season. When testosterone was administered to males so that high testosterone levels were maintained, males from monogamous species became polygynous in their behavior.

Social context is also important in sexual behavior and its consequences. Behaviorists interested in mating systems focused initially on male sexual and aggressive behaviors. This was supplanted by investigations of female choice of males and, most recently, has developed into studies of how a female's social rank relates to her reproductive success. For example, dominant female chimpanzees have a disproportionate share of quality resources and, in turn, tend to reproduce more frequently and wean their offspring faster (Pusey et al. 1997).

CHEMICAL STIMULI

Another example of diversity in the hormone-behavior relations concerns the relationship between hormones and chemical signals or pheromones. The original definition of hormones emphasized internal communication and the definition of pheromones emphasized external communication. However, this distinction is blurring as scientists discover that some hormones may also function as pheromones. A well-known example is found in pigs (Signoret 1976). Two metabolites of androgen play an integral part in the courtship and copulatory sequence. When the boar faces the sow, he positions himself in front of her and forcefully breathes into her face. The air current picks up the molecules of sex attractant secreted by submaxillary salivary glands in the male's mouth, which has a distinctive odor. If the female is receptive, one whiff of the male will cause her to stand immobile, arching her back in lordosis, and allow the boar to mount and copulate. Application of this discovery to animal husbandry has resulted in the manufacture of an aerosol preparation containing these androgen metabolites called Boar Mate, which is used to immobilize sows for artificial insemination.

Another example of hormones serving as pheromones can be found in goldfish (figure 7.4). Research by Sorenson and Stacey (1991) has revealed that the female produces two hormones that have specific, separate actions on the physiology and behavior of the male. One hormone is a progestin produced by the preovulatory follicle. This hormone is involved in the final maturation and ovulation of the oocyte. It is also excreted into the water in which the fish are swimming and has a profound influence on the male's endocrine physiology (figure 7.4). The presence of this hormone in the water leads to increased circulating concentrations of pituitary gonadotropins and testicular progestins, which in turn stimulate sperm production.

Another group of hormones found to activate species-typical reproductive behaviors are the prostaglandins. Administration of prostaglandins elicits female-typical spawning behavior in goldfish and other fishes with external fertilization. During ovulation, prostaglandins are released and receptive behavior is stimulated in the female. In the water, prostaglandins then trigger sexual behavior in males.

It is of interest that in species with internal fertilization, prostaglandins have very different effects. For example, in crickets, lizards, red-sided garter snakes, and guinea pigs, prostaglandins stimulate rejection behavior and the loss of sexual receptivity. If prostaglandins are given to female or to male Tamara wallabies (small kangaroos), they will crouch and behave as if they were giving birth.

Studies of fish hormonal pheromones may force us to reconsider some basic concepts of pheromonal function. For example, it is doubtful that the hormonal pheromone system of goldfish functions in true chemical "communication." There is no evidence that the female chemical signal is either specialized or released in such a way as to increase female reproductive success directly. Rather, it appears more likely that goldfish hormonal pheromones function in "chemical spying" in a manner consistent with the sensory exploitation interpretation of sexual selection. In other words, males have evolved the ability to detect these chemicals in response to intense competition among males for access to ovulated females. Such chemical spying almost certainly represents an early stage of pheromonal evolution. This association would be expected to lead to chemical communication when increasing the efficiency of signaling could increase the reproductive success of the signaler.

We have sampled just a portion of the diversity in hormone-behavior relations found in animals. Regardless of the mechanics of reproduction, the coordination of individuals leads to a synchronization of reproductive processes. This is true even in species that do not reproduce sexually. As men-

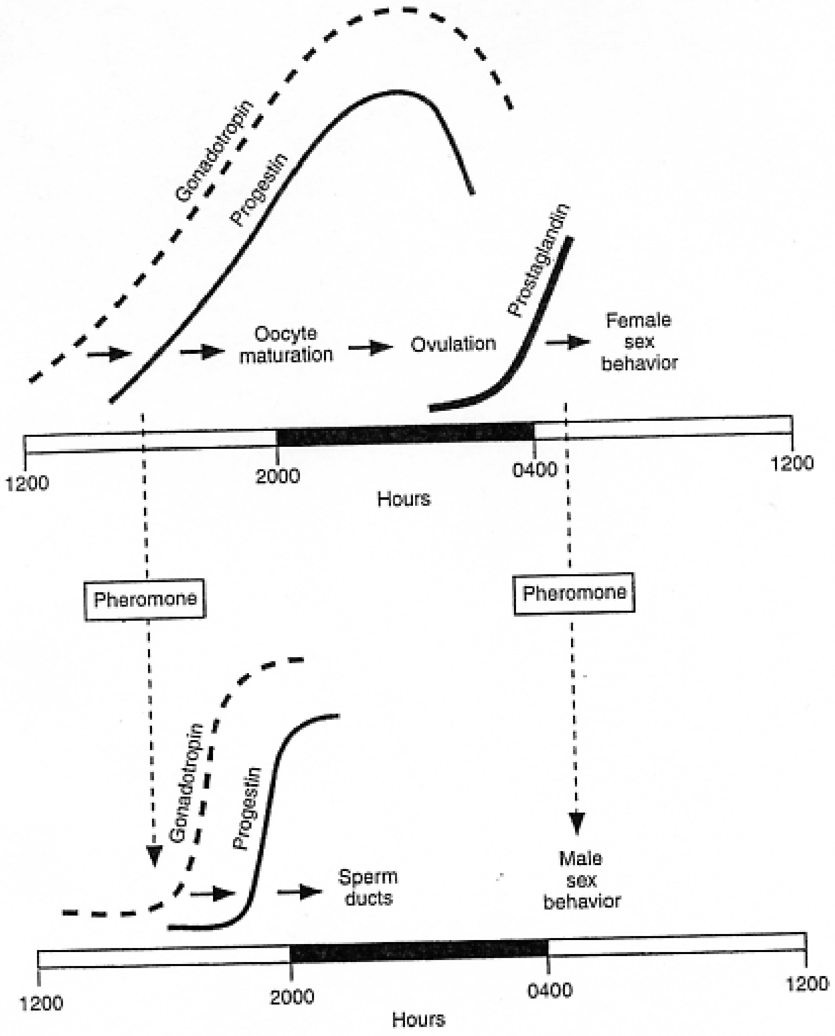


Figure 7.4 Example of how released hormones can function as pheromones. In the goldfish, the ovulating female sequentially releases two hormones with distinct pheromonal effects on male physiology and behavior. This dual pheromone system is set in motion when environmental cues (warm water temperature and appropriate spawning substrate) trigger a preovulatory surge of gonadotropin release from the pituitary. The gonadotropin surge rapidly stimulates ovarian follicle cells to synthesize a progestin, 17α,20β-dihydroxy-4-pregnen-3-one, which acts hormonally on the oocyte to promote maturation (resumption and completion of meiosis). When released to the water, this progestin acts via sensitive and specific male olfactory receptors to rapidly increase plasma gonadotropin, which in turn increases the numbers of sperm in the sperm ducts by stimulating testicular synthesis of this same progestin. Males that detect the progestin pheromone are believed to benefit by being able to release more sperm during the highly competitive spawning. When ovulation occurs, the presence of the oocytes in the reproductive tract stimulates synthesis of a prostaglandin, $PGF_2\alpha$, which triggers female spawning behavior. At the same time, the prostaglandin is released to the water and stimulates males to court the ovulated female.

tioned in the beginning of this chapter, some species consist only of female individuals that reproduce parthenogenetically; no males exist. There are two forms of parthenogenesis in vertebrates. In gynogenetic reproduction, the female mates with males of another species and his sperm induce the development of the embryo. In obligate parthenogenesis, sperm are not required to induce embryogenesis but, as we will see, the presence of male-like behavior is still important. Thus, individuals of even all-female species must coordinate their reproductive states to insure successful reproduction.

Particularly in birds and mammals, the freedom to choose a mate is an essential element in successful reproduction. It is evident from this survey that males and females can vary in ways other than by producing sperm or eggs. When the gametes are produced relative to when mating occurs and whether the gametes are stored or used immediately varies according to the constraints outlined above. Males and females need not respond to these pressures in the same way. This conclusion is clearly evident in species in which one sex exhibits an associated reproductive pattern and the other sex has a dissociated reproductive pattern. Comparative studies reveal that the "exceptional cases" are less rare and more widespread than is generally realized. Finally, the trigger for sexual behavior can vary from species to species. In some it is a change in a particular physical factor in the environment such as rainfall or even the color green, which signify new growth of vegetation. In other species it can be the sight, sound, or smell of other individuals. In these instances, hormones are affecting the perceptions of the individual.

Insights into the Evolution of the Process and Mechanisms of Reproduction

Nongenetic Transmission of Traits

We all learned in introductory biology that traits are inherited by the transmission of genes. Some may also have heard about Lamarck, a contemporary of Darwin. A keen observer of animal behavior, he suggested that traits might also be transmitted to the next generation if the organism was exposed to, or engaged in, certain activities. Lamarkianism, or the inheritance of acquired characteristics, was discredited early this century. However, like many things, there was an element of truth that only in recent years has been recognized. We now know that traits can be transmitted from parent to offspring both by nongenomic means as well as by the inheritance of genes. As we have seen, social context, the architecture of pregnancy, and the physiological state of the female as she deposits yolk prior to laying can all play a role in the nongenetic transmission of traits across generations. For example, one of the more interesting aspects of the effects of intrauterine position is *how* phenotypes can be transmitted across generations. As stated above, 2M females tend to have litters with more male-biased sex ratios. This means that the female offspring of such females are more likely to be 2M females than are the female offspring of 2F females. The same is true for male offspring (i.e., males are more likely to have male fetuses as neighbors). Therefore, through hormonal effects in utero, the life histories of females may be transmitted nongenomically.

TEMPERATURE-DEPENDENT SEX DETERMINATION

In species with genotypic sex determination, such as mammals, the inheritance of specific chromosomes, whether in type or in number, fixes the sex of the individual at the moment of fertilization (figure 7.5). Scientists have known for many years that in certain plants and invertebrates, the sex ratio can be skewed by environmental conditions, so much so that only female or male young may result. However, it has only been in the last two decades that we have come to realize that many vertebrates also exhibit environmental sex determination. You just read about one type of environmental sex determination, behavior-dependent sex determination, in which the individual's perception of its social environment establishes gonad type. Another type is temperature-dependent sex determination (TSD), in which the temperature experienced by the embryo determines whether it becomes a male or a female.

Temperature-dependent sex determination occurs in all alligators and crocodiles, most turtles, and many lizards (figure 7.6); in snakes and in the tuatara (spiny-backed reptiles), gonadal sex is determined by sex chromosomes as in mammals and in birds. Species with TSD lack sex chromosomes and have little or no genetic predisposition to respond to temperature in particular ways. Research with the red-eared slider turtle indicates that the physical stimulus of temperature is transduced in the midtrimester of development to modulate expression of the genes coding for steroidogenic enzymes and sex steroid hormone receptors (Crews 1996).

Unlike the fish just discussed, reptiles are gonochoristic. Thus, in TSD species each individual has an equal ability to become a male or a female. Temperature serves as the trigger, activating one sex-determining cascade while suppressing the complementary sex-determining cascade; that is, being a male also means not being a female. Environmental sex-determining mechanisms, of which TSD is one, are believed to be the evolutionary precursor to the genotypic sex-determining mechanisms characteristic of birds and mammals (figure 7.7) (Crews 1994). A still unanswered question is whether females select a particular ground temperature when excavating the nest. That is, gravid females may use temperature in choosing the place or depth to place their eggs. Given what we know already, both the sex ratio and the phenotype of the offspring would be altered by such a choice. If such nest-site selection occurs, is it influenced by the temperature which the female herself experienced as an embryo?

What do we know about the sexual differentiation process in TSD species? To date only one species, the leopard gecko, has been studied (Crews 1997; Crews et al. 1998). In the leopard gecko, only females result if eggs are incubated at 26 °C (= low-temperature females); above 34 °C (= high-temperature

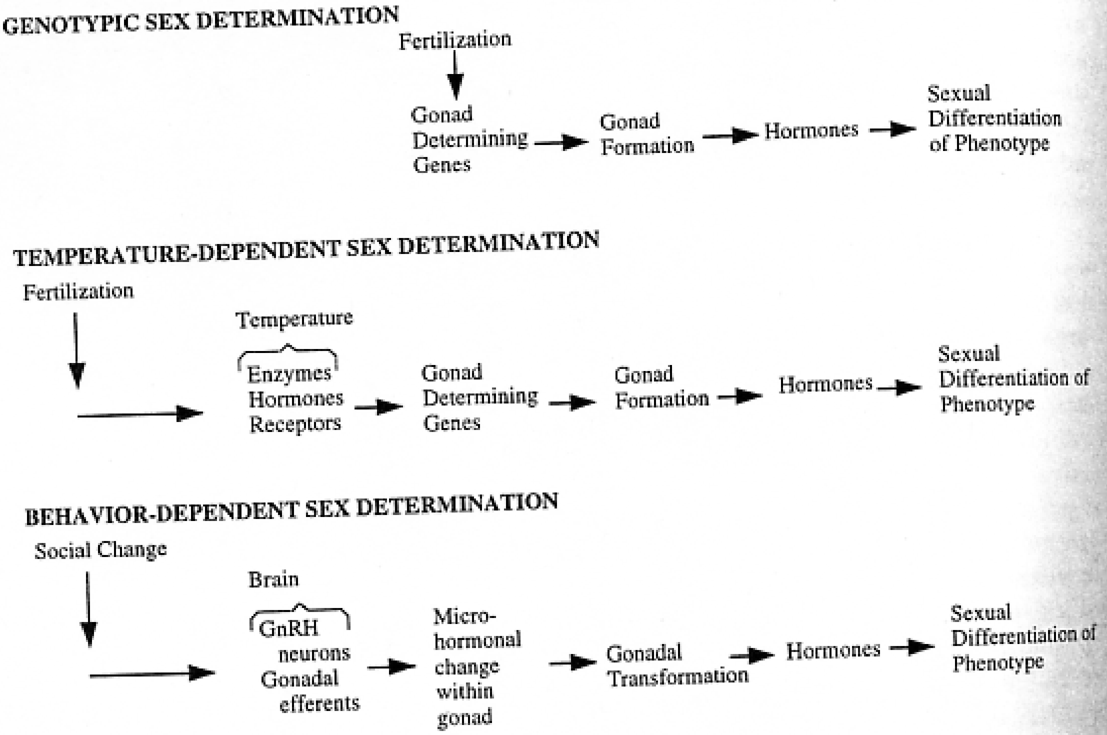


Figure 7.5 Different mechanisms of sex determination in vertebrates. In vertebrates with sex chromosomes (male or female heterogamety), gonadal sex is fixed at fertilization by the union of specific chromosomes. Only after the gonad is formed do hormones begin to exert an influence, sculpting specific structures that eventually will differ between the sexes. Research on fish with behavior-dependent sex determination suggests that social stimuli encountered by the adult leads to sex change via the brain, acting first on hypothalamic neurons that secrete arginine vasotocin and gonadotropin-releasing hormone which, in turn, act on neurons that project to the gonads. The activity of these neurons modifies the endocrine environment within the gonad, bringing about gonadal transformation. Research on reptiles with temperature-dependent sex determination indicates that sex determination in such species is fundamentally different in at least one way. Gonadal sex is not irrevocably set by the genetic composition inherited at fertilization, but rather depends on which enzymatic and hormone receptor genes are activated during development by temperature. Incubation temperature modifies both the temporal and spatial sequence of steroidogenic enzymes and hormone receptors so that sex-specific hormone milieus created in the urogenital system of the developing embryo determine gonad type. In such species, an alternative form of sexual differentiation may exist. (From Crews 1993.)

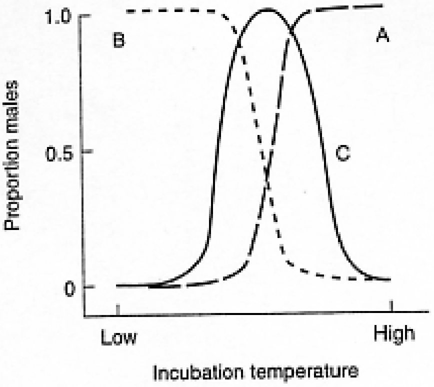


Figure 7.6 Response of hatchling sex ratio to incubation temperature in various egg-laying reptiles. These graphs represent only the approximate pattern of the response and are not drawn according to any single species. The three patterns recognized presently are (*A*) only females produced from low incubation temperatures, males at high temperatures, (*B*) only males produced from low incubation temperatures, females at high temperatures, and (*C*) only females produced at the temperature extremes, with male production at the intermediate incubation temperatures. Genotypic sex determination also occurs in other reptiles with the result that the hatchling sex ratio is fixed at 1:1 despite incubation conditions. (From Crews 1994.)

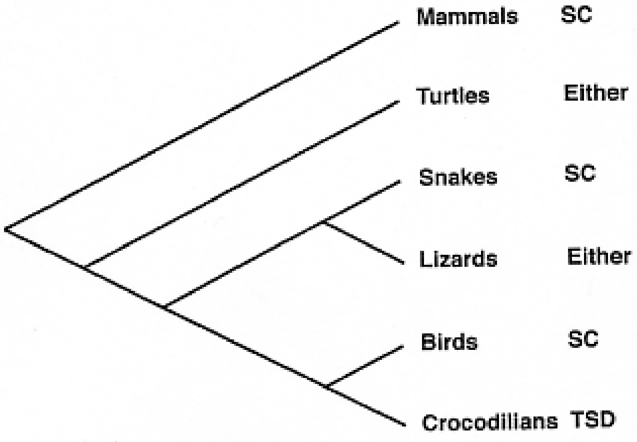


Figure 7.7 Phylogeny of sex-determining mechanisms in amniote vertebrates. In lizards and turtles both SC and TSD have been documented. SC, sex chromosomes determine sex; TSD, temperature-dependent sex determination. (From Crews 1994.)

females) only about 5% of the hatchlings are male. Incubation at 30 °C produces a female-biased sex ratio, and 32.5 °C produces a male-biased sex ratio. This becomes important because we have found that by incubating eggs at various temperatures and then following individuals as they age, much of the phenotypic variation seen among adults, both between and within the sexes, can be accounted for by the individual's incubation temperature. For example, adult leopard geckos are sexually dimorphic, with males having open secretory pores anterior to the cloaca. In low-temperature females these pores are closed, whereas in females from a male-biased temperature they are open. Head size is also sexually dimorphic, with males having wider heads than females, yet within females, those from a male-biased temperature have wider heads than do those from a low temperature. Similarly, although males are the larger sex, incubation temperature has a marked effect on growth within a sex. Females from a male-biased temperature grow faster and larger than females from a female-biased temperature, and become as large as males from a female-biased temperature.

TEMPERATURE DEPENDENCE OF OTHER TRAITS

Circulating concentrations of testosterone in adult males are approximately 100 times higher than in adult females. However, the endocrine physiology of the adult varies in part due to the temperature experienced during incubation (figure 7.8). For example, plasma estrogen levels are significantly higher in males from a female-biased temperature than in males from a male-biased temperature. Among females, circulating estrogen levels are significantly higher, and androgen levels significantly lower, in low-temperature females than in females from a male-biased temperature.

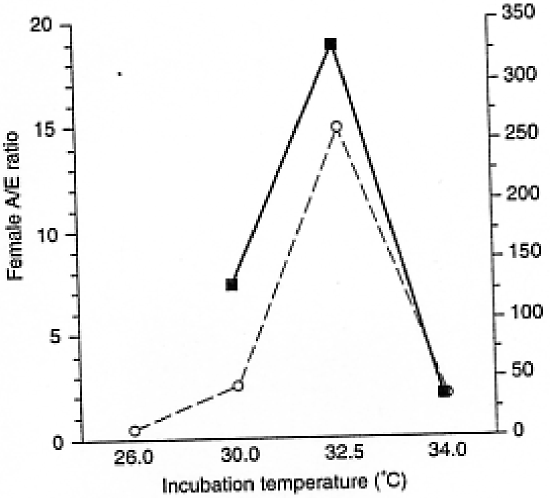


Figure 7.8 Ratio of the plasma levels of total androgens (A) and estrogens (E) in adult female (dashed line) and male (solid line) leopard geckos, *Eublepharis macularius*, from different incubation temperatures. (From Crews et al. 1997.)

Aggression

Incubation temperature also has a major influence on the nature and frequency of the behavior displayed by the adult leopard gecko. For example, females usually respond aggressively only if attacked, whereas males will posture and then attack other males but rarely females. However, males from a female-biased temperature are less aggressive than males from the higher, male-biased temperature and, although not as aggressive as males from that same incubation temperature, females from a male-biased temperature are significantly more aggressive toward males than are females from a low or female-biased temperature. These same females show the male-typical pattern of offensive aggression. Incubation temperature also influences the ability of exogenous testosterone to restore aggression. Following ovariectomy and testosterone treatment, low-temperature females do not exhibit increased levels of aggression toward male stimulus animals, whereas females from male-biased temperatures return to the high levels exhibited while gonadally intact. This suggests that incubation temperature influences responsiveness to steroid hormones in adulthood in males.

Courtship

This is a male-typical behavior. In a sexual encounter, the male will slowly approach the female, touching the substrate or licking the air with his tongue. Males also have a characteristic tail vibration, creating a buzzing sound, when they detect a female. Intact females have never been observed to exhibit this tail-vibration behavior, regardless of their incubation temperature. However, if ovariectomized females are treated with testosterone, they will begin to tail-vibrate toward female, but not male, stimulus animals; males appear to regard such females as male because they attack them.

Attractiveness

This is a female-typical trait and is measured by the intensity of a sexually active male's courtship behavior toward the female. Females from a male-biased temperature are less attractive than females from lower incubation temperatures. Long-term castrated males are attractive and initially courted by intact males, presumably because of their odor, but on further chemosensory inspection they are attacked. This suggests that both sexes can produce both a female-typical attractiveness pheromone and a male-typical recognition pheromone, as the red-sided garter snake does (see below).

Brain Morphology

It stands to reason, therefore, that the morphological, physiological, and behavioral phenotypes we have discovered in the leopard gecko might be reflected in neural phenotypes. The first (and surprising) discovery was that at temperatures that produce both sexes, the volume of the POA and the VMH do not significantly differ between males and females (figure 7.9). There are, however, consistent differences across incubation temperatures. The volume of the POA is larger both in males and in females from the male-biased

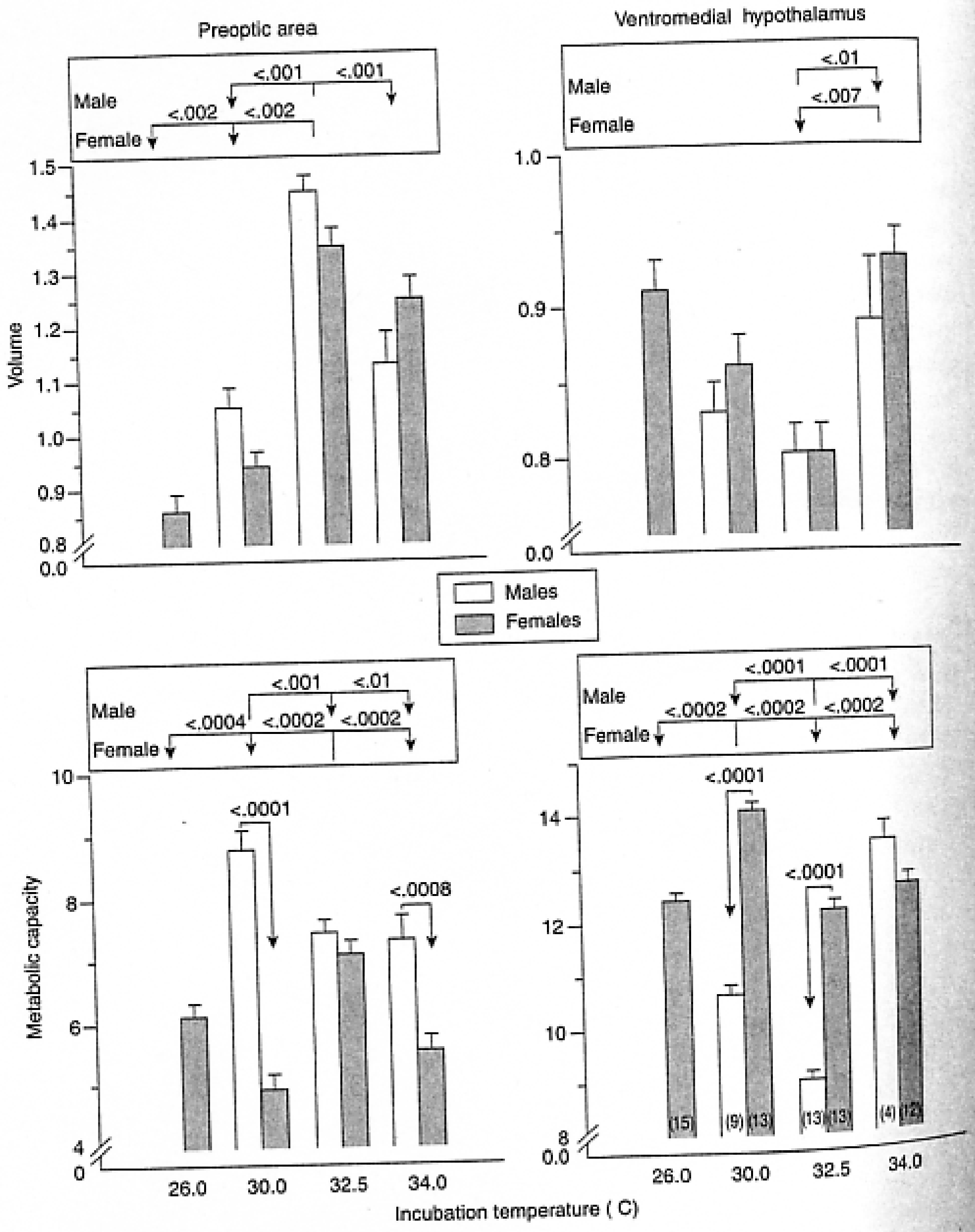


Figure 7.9 Effect of incubation temperature and gonadal sex on the volume (*top panels*) and cytochrome oxidase (COX) activity (*bottom panels*) of the preoptic area (POA) (*left panels*) and ventromedial hypothalamus (VMH) (*right panels*) in the leopard gecko, *Eublepharis macularius*. Volumes are normalized by entire forebrain volume. Significant differences (entries are p values) within each sex are illustrated in box above each panel, indicating the effect of incubation temperature. Significant differences between the sexes are illustrated *above bars*, indicating the effect of gonadal sex. Sample sizes in parentheses. Means depicted with vertical bars representing standard error. (From Crews et al. 1997.)

temperature than in animals from the female-biased temperature. Similarly, the volume of the VMH is larger in low-temperature females than in females from the male-biased temperature. This suggests that the incubation temperature of the embryo directly organizes the size of these brain areas independent of its effect on gonadal sex.

Thus, experiences before birth arising out of the hormonal and physiological milieu during embryogenesis can have a profound effect on the adult phenotype. Experiences accumulated over a lifetime as well as during a reproductive season set the stage for how individuals respond to these immediate stimuli. Indeed, experience needs to be viewed broadly to include the genetic history of the individual, the population, and the species, as well as the environment in which the individual develops.

Hormone Independence of Courtship Behavior in the Red-Sided Garter Snake

As discussed in chapter 1, Berthold demonstrated in 1849 that the testes of the rooster produced a chemical that dramatically changed the animal's behavior. Since that experiment, there have been thousands of studies on a variety of vertebrate species that have yielded similar results. Yet with rare exceptions, all the species studied exhibited an associated reproductive pattern (Crews and Moore 1986) (figure 7.1 and table 7.2). The consistency of the data has led many to presume that gonadal steroid hormones activate mating behavior in all vertebrates. Here the dissociated reproductive pattern provides us with a natural experiment. In the Candian red-sided garter snake, a basic conflict between environmental and physiological constraints has created novel neuroendocrine mechanisms (Crews 1990). As we will see, the neuroendocrine mechanisms controlling courtship behavior in adult male red-sided garter snakes does not rely on the activational effects of sex steroid hormones.

The red-sided garter snake is found in the northern United States and in Manitoba, Canada. In these regions winters are usually severe, and the snakes may spend as long as 9 months in hibernation in subterranean limestone caverns (figure 7.10). With the spring thaw, the animals emerge, and there is a brief 3- to 4-week breeding season. After this there is a summer feeding phase that can last 2 to 3 months. During this time the females also gestate and give birth. Beginning in late August, animals start returning to the caverns from which they emerged the previous spring.

Increasing ambient temperatures stimulates the emergence from winter dormancy in the spring. Males emerge en masse while the females emerge singly beginning about 1 week later (figure 7.11). As each female emerges, males in the immediate vicinity approach her. A pheromone on the female's back elicits a vigorous response in the male called "chin-rubbing." Chin-rubbing consists of the male coursing up and down the back of the female rapidly and repeatedly with his chin, all the time rapidly flicking his tongue on the female's back. The male next aligns his body along the female's body and attempts to intromit a hemipenis. (All male lizards and snakes have two

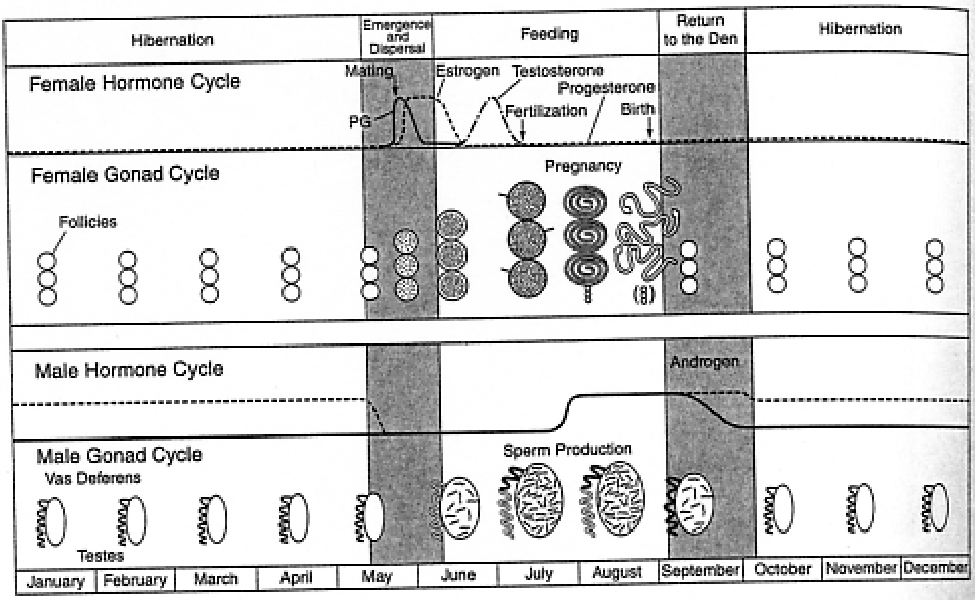


Figure 7.10 The major physiological and behavioral events in the annual reproductive cycle of the red-sided garter snake in Canada. Animals spend most of the year underground. In the spring, they emerge and mate before dispersing to summer feeding grounds. In the female, mating initiates gonadal growth as well as changes in the hormone profile. Young are born in late summer. Since all metabolic processes slow down during the cold months, androgen levels in the male will be elevated in the spring if he entered hibernation with elevated levels (dotted lines); however, androgen levels are usually basal on emergence (solid line). Sperm are produced during the summer after mating and are stored in the vas deferens (heavy squiggle line next to testis) over winter.

penises rather than one and alternate their use in successive matings.) Since one hundred or more males may be attracted to a single female, a writhing mass of snakes results, called a "mating ball" (figure 7.11). After the female mates, usually with only one of the males, she immediately leaves the den area. Approximately 3 weeks after the first emergence, virtually all the females have emerged from hibernation. Males then leave the area and disperse to their feeding grounds, not to return until the fall when the weather cools.

All these events can be duplicated in the laboratory simply by cooling animals, during the fall, to a body temperature of 2° to 4 °C and maintaining animals at this temperature for 17 weeks. In the laboratory, males typically do not court the first few days after emergence, but then show intense courtship which wanes 3 to 4 weeks following emergence. Only prolonged periods of cold temperatures can restimulate male behavior. Indeed, captive males will never again exhibit this same intense and synchronous courtship behavior unless they are hibernated.

SPERM IS STORED OVER WINTER IN MALES

Males emerge from hibernation with regressed testes and epididymides enlarged with stored sperm (figure 7.10). The sperm that a male uses in mating are produced during a testicular growth cycle the previous summer. In the adult male, spermatogenesis begins about 6 weeks after emergence in the spring and continues for 10 to 12 weeks. Associated with spermatogenesis is an increase in the circulating concentrations of androgens. Testicular collapse occurs when males return to begin hibernation. If the winter is early, they may enter hibernation while androgen levels are still elevated. If the androgen titer is high in the fall, it will be elevated initially on emergence in the spring due to the slow metabolic clearance rates during hibernation.

FEMALES DO NOT STORE MATURE OVA

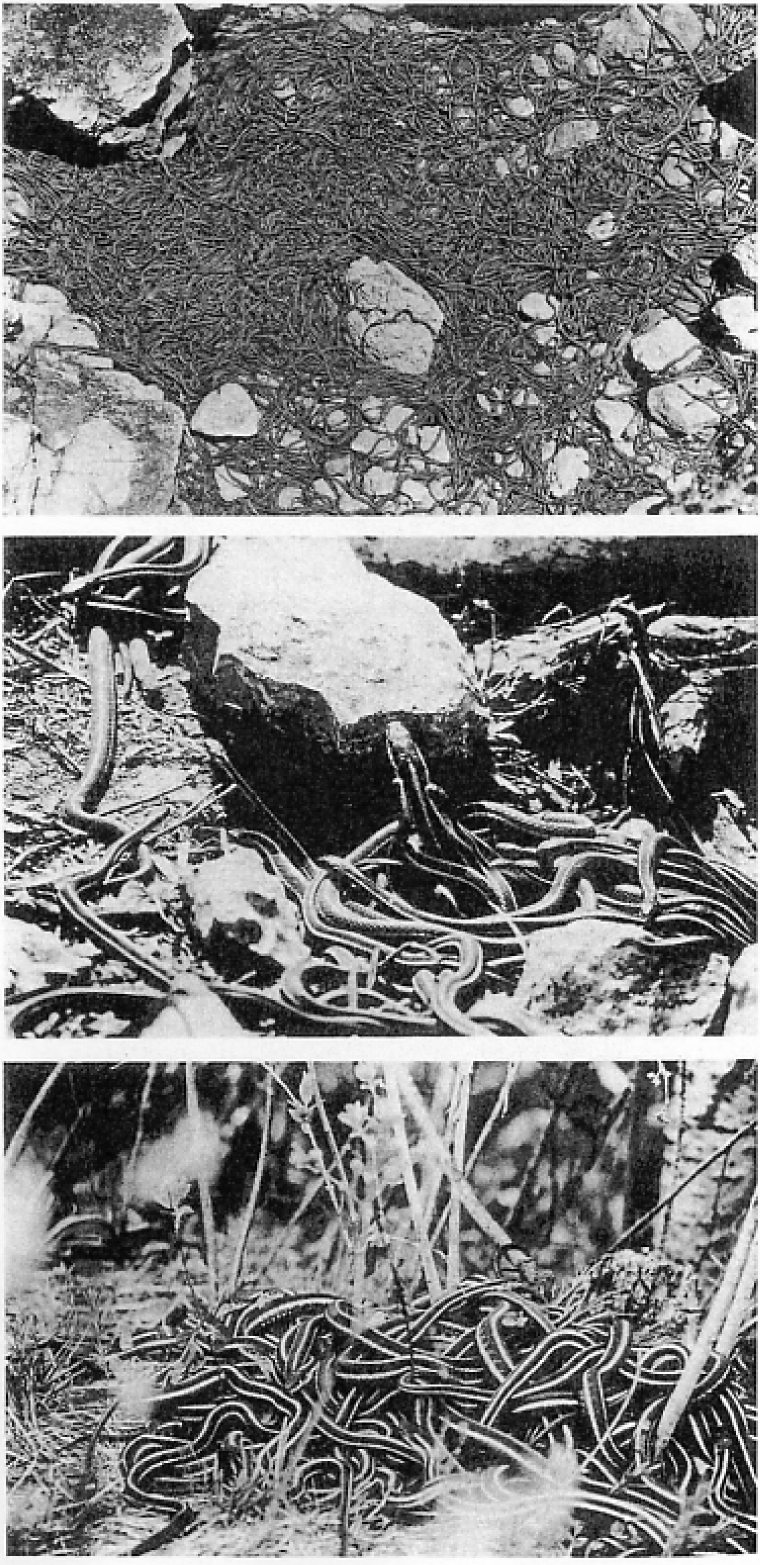
Females also emerge with regressed ovaries and low serum estrogen. Unlike sperm, mature ova cannot be stored successfully over a winter. However, females are attractive and receptive on emergence and quickly mate. As soon as the female mates, she becomes unattractive and unreceptive to males. Mating stimulates a neuroendocrine reflex that culminates in ovarian growth and ovulation 6 weeks later. Immediately after mating, or perhaps initiated during copulation, there is a significant rise in circulating concentrations of prostaglandins. This is followed shortly by a surge in the circulating concentrations of estrogen, which remains elevated for several days before declining to basal level. Estrogen remains low until 3 to 6 weeks later, when the ovarian follicles enlarge.

This mating-induced neuroendocrine reflex is essential for ovulation. Specific sensory stimulation received during mating initiates this neuroendocrine reflex. It begins with the male's intromission into the female's cloaca. (The word *cloaca* in Latin means "sewer"; it refers to the fact that in all vertebrates except mammals there is a single urogenital opening.) Anesthetization of the cloaca or spinal cord prior to copulation prevents the transmission of sensory information essential for the reflex (Mendonça and Crews 1990, 2001). Females so manipulated do not show the surge in hormones associated with mating and their follicles do not grow.

A question that immediately comes to mind is how can red-sided garter snakes mate when their gonads are regressed and sex steroid hormone concentrations are low? As we might expect, the neuroendocrine mechanisms controlling reproduction in the red-sided garter snake are different from those of more commonly studied species that mate when their gonads are fully active. In this species the circulating concentrations of androgen are low during courtship, and testicular steroidogenesis does not occur until after all courtship behavior has ended.

COURTSHIP BEHAVIOR

Chin-rubbing behavior in the male red-sided garter snake is elicited by the tongue-flick delivery of the female attractiveness pheromones to the vomeronasal organ (VNO). The VNO projects to the nucleus sphericus, which in turn projects to the POA. As in other vertebrates, this brain area is involved in the display of the male's courtship behavior. 2-deoxyglucose (2DG) uptake in the POA is increased in males that are courting females. If the POA is



destroyed in males shortly after they have emerged, they will immediately stop courting. Even if the lesions are produced before entering hibernation, males with damage in the POA will not court. The deficits in courtship behavior that result following lesions of the POA are not due to the destruction of hormone-concentrating neurons in the POA. We know this because courtship behavior is not activated by increased concentrations of testicular androgens. It probably has something to do with the ability of lesioned animals to detect temperature, since in addition to abolishing male mating behavior, POA lesions also disrupt thermoregulatory behavior. Research with mammals indicates that this brain area is the final common pathway in temperature regulation.

Temperature then appears to be the key requirement for courtship in male red-sided garter snakes. As long as males have undergone a period of low-temperature dormancy, they will display courtship behavior on emergence into warm temperatures and in the presence of an attractive female. Photoperiod plays no role in the activation of courtship, which is unlike most homeotherms; males housed in complete darkness for a year will court as long as they have been through hibernation. Nor do testicular androgens play a role in the activation of courtship behavior. Adult males castrated on emergence from hibernation will still court females with the same intensity and duration as intact males; indeed, such castrates will again court the following spring if they undergo hibernation. Significantly, androgen treatment neither maintains courtship behavior in castrated males in the spring nor will it stimulate courtship in males in the summer (when courtship does not occur). Even if androgen is implanted directly into the POA, males fail to court. Administration of other sex hormones also fails to elicit courtship behavior.

It is not the case that male red-sided garter snakes are insensitive to androgens. Androgen is concentrated in the same brain areas as observed in other vertebrates, including the POA. Further, if males are castrated and then maintained in the laboratory and hibernated at the normal time for several years, then the frequency and intensity of their courtship behavior gradually declines (Crews 1991). After the third emergence, castrates respond to females at very low levels. If they then receive a testosterone implant during the summer when their testes would normally recrudesce and are hibernated the following fall, they will once again exhibit vigorous courtship behavior the following spring. Thus, testicular androgens organize the nervous system during the summer in such a way that the male will respond to the appropriate stimuli the following spring rather than activating male courtship behav-

Figure 7.11 Emergence of male red-sided garter snakes at hibernaculum entrance during the spring. (*Top*) Males emerge first and en masse. (*Middle*) Females then emerge singly over a 3-week period, resulting in the formation of mating balls. The female is the snake with the large head in the center of the figure; the rest are courting males. In garter snakes, the females are about three times larger than males. Unlike in most mammals, the testes inhibit body growth in male garter snakes. (*Bottom*) A mating ball of red-sided garter snakes, there is only one female.

ior in the spring. The long latency (8 months) between the androgen exposure in the summer and the consequent behavioral response the following spring suggests that the hormone-mediated organization of the brain is occurring on a seasonal basis, a process that can be regarded as seasonal organization. But if brain organization occurs on a seasonal basis in red-sided garter snakes, then it is of a fundamentally different nature than that for early organization effects described for species exhibiting an associated reproductive pattern. In this respect it is not surprising that unlike in other species, there are no statistical differences between male and female red-sided garter snakes nor are there differences across seasons in the size of those limbic nuclei involved in the control of sexual behavior (Crews et al. 1993).

Chapter 12 will describe how the pineal gland, via its hormone melatonin, is a major neuroendocrine transducer of photoperiod in birds and mammals, affecting the circadian rhythmicity of various locomotor, feeding, and drinking activities, as well as seasonal reproductive cycles. In cold-blooded vertebrates, such as reptiles, the pineal transduces changes in temperature as well as photoperiod. Since the only manipulation known to stimulate courtship in male red-sided garter snakes is exposure to prolonged periods of low temperatures followed by subsequent warming, we decided to investigate to see if the pineal is somehow involved in the control of male sexual behavior. Male garter snakes will not exhibit courtship upon emergence if their pineal is removed in the fall before they enter hibernation, yet the same operation will have no effect on courtship behavior if performed in the spring when they emerge from hibernation (figure 7.12, top panel) (Crews et al. 1988; Mendonça et al. 1996a, b). Thus, it appears an intact pineal is necessary for proper interpretation of the temperature cue but once the cue is transduced, the pineal gland per se is unnecessary in maintaining the behavior.

The day/night, or diel, cycle of melatonin secretion varies seasonally (Mendonça et al. 1995). Melatonin levels are approximately 5-fold higher in the night than in the day during the summer and fall, but during hibernation, melatonin cannot be detected in the circulation. On emergence, melatonin levels rise rapidly and precipitously and, within an hour of emergence, are at least 20-fold higher than levels seen in the fall. This surge in melatonin secretion lasts only about 24 hours, and thereafter the typical day/night difference is evident. Removal of the pineal disrupts the diel cycle of melatonin secretion, suggesting that the reestablishment of a normal diel cycle may function to synchronize and modulate courtship behavior in male red-sided garter snakes.

Some male red-sided garter snakes never court when they emerge from hibernation. What was surprising was the effect of pinealectomy on these noncourting males. In each of the three years of our experiments, pinealectomy of noncourting males increased their courtship activity; that is, they suddenly begin to court frequently and vigorously (figure 7.12, bottom panel) (Mendonça et al. 1996b). This is remarkable because it is the first experimental manipulation besides hibernation that has been found to stimulate a non-

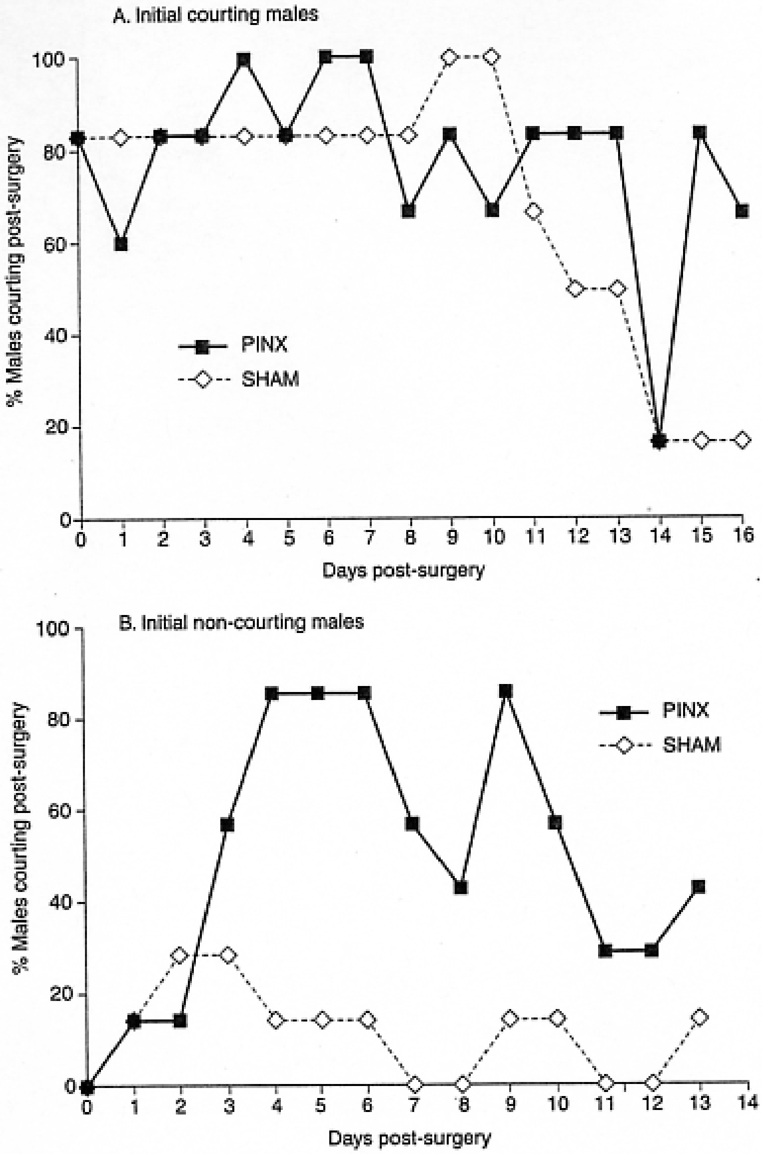


Figure 7.12 The effect of pinealectomy on courtship behavior in courting and noncourting male red-sided garter snakes, *Thamnophis sirtalis parietalis*. (*Top*) The percentage of male snakes that courted vigorously in the spring on emergence from hibernation. (*Bottom*) The percentage of male snakes that initially failed to court in the spring on emergence from hibernation. PINX, pinealectomized; SHAM, sham operation. (From Mendonca et al. 1996.)

courting male garter snake to exhibit courtship behavior. It is possible that males that court in the spring have a different neuroendocrine mechanism controlling courtship behavior than noncourting males. Intact noncourting males lack a normal diel pattern of melatonin secretion when sampled either just a few days or 10 days after emergence (when the mating period is about half over). It may be that these animals are not able to process the temperature change that occurs at emergence and stimulates courtship.

TERMINATION OF MATING BEHAVIOR

Why do male garter snakes stop courting females about 1 month after emergence from hibernation? This decline in courtship behavior is not due to the males becoming used to females. Males that have had daily exposure to females exhibit courtship behavior equal to that of males kept isolated after emergence and only tested later with attractive females. The answer may lie in the natural sequence of events. Even after spending many months without food, recently emerged male red-sided garter snakes do not feed. This is not due to a lack of food, because males will refuse food if it is offered. Females, on the other hand, begin to feed soon after they emerge and leave the den areas. Males leave the den to forage for food 3 to 4 weeks after emergence, at the same time courtship begins to decline. The remainder of the summer is spent feeding. Males do not court attractive females during the summer feeding phase. This voluntary abstinence from food is common in species inhabiting marginal habitats. In ground squirrels, pinnipeds, Emperor pigeons, the arctic fulmar, and the red-sided garter snake, feeding and mating behaviors are mutually exclusive events in at least one of the sexes. The hormone neuropeptide Y (NPY) is known to play a role in the regulation of both feeding and sexual behavior and, as in mammals, infusion of NPY into the brain of male red-sided garter snakes will cause them to temporarily cease courtship of females and begin to eat.

We have seen how environmental and endocrine factors influence specific reproductive functions and behaviors in the red-sided garter snake. Some conclusions that can be drawn include the following: (1) gonadal growth and sexual behavior are not necessarily temporally associated, (2) sexual behavior can be independent of gonadal steroid hormones, (3) the initiation, maintenance, and termination of sexual behavior are independent of each other and are controlled by different cues, and (4) behavioral differences between the sexes can occur without corresponding sex differences in the size of the brain areas that regulate these behaviors.

Evolution of the Neuroendocrine Control of Sex-Typical Behaviors

Most whiptail lizard species are gonochoristic, having both male and female individuals that reproduce sexually. However, one-third of the 45 species of whiptail lizards are unisexual, consisting only of individuals that reproduce by true parthenogenesis. We know that the parthenogenetic species arose from the hybrid mating of two sexual whiptail species and, in many instances, we know which species were involved. For example, the parthenogen *C. uniparens* arose from the hybrid union of *C. inornatus* and *C. burti*. Furthermore, we know that *C. inornatus* is the maternal ancestor of *C. uniparens* and *C. burti* is the paternal ancestor. Thus, two-thirds of the triploid genome of *C. uniparens* is derived from *C. inornatus*.

Just as the research on the red-sided garter snake has led to new information about neuroendocrine adaptations, the study of unisexual vertebrates has proved useful for understanding how these neuroendocrine adaptations may have evolved. This is because these species allow the investigator to study brain-behavior evolution in a manner impossible with conventional species. That is, parthenogenetic whiptail lizards and their related sexual species present a "snapshot" of evolution. Here we have an opportunity to compare the neuroendocrine mechanisms that control reproductive behaviors in the descendant species with those of the ancestral species. This ability in turn allows us to address two fundamental issues from a new perspective. First, do the neural circuits that subserve male-typical and female-typical sexual behaviors differ or are they similar? Second, how might the cellular mechanisms that control sexual behaviors have evolved?

PSEUDOSEXUAL BEHAVIOR IN THE PARTHENOGENIC LIZARD

As pointed out earlier, reproductive behaviors tend to be unambiguous, characteristic of the species, and critical to the individual's reproductive and evolutionary success. Twenty years ago I made the serendipitous observation that unisexual whiptail lizards exhibit behaviors remarkably similar to the courtship and copulatory behavior of related sexual species (figure 7.13). In gonochoristic whiptail lizards (e.g., *C. inornatus*), the male approaches and investigates the female with his bifid (split) tongue, an action that presumably indicates involvement of chemical senses. If the female is sexually receptive, she stands still for the male, allowing him to mount her back. Usually just before the male mounts the female, he grips with his jaws either a portion of the skin on the female's neck or her foreleg. As the male rides the female, he scratches her sides and presses her body against the substrate. The male then begins to maneuver his tail beneath the female's tail, attempting to appose their cloacal regions. During mating, one of two hemipenes is intromitted into the female's cloaca. With intromission, the male shifts his jaw-grip from the female's neck to her pelvic region, thereby assuming a contorted copulatory posture I have termed the *doughnut*. This posture is maintained for 5 to 10 minutes, after which the male rapidly dismounts and leaves the female.[2]

This same sequence of events is observed in at least five species of unisexual whiptail lizards (figure 7.13). That is, one individual will approach and mount another individual and, after riding for a few minutes, the mounting (male-like) individual will swing its tail beneath that of the mounted (female-like) individual, apposing the cloacal regions. At the same time, the mounting individual will shift its jawgrip from the neck to the pelvic region of the

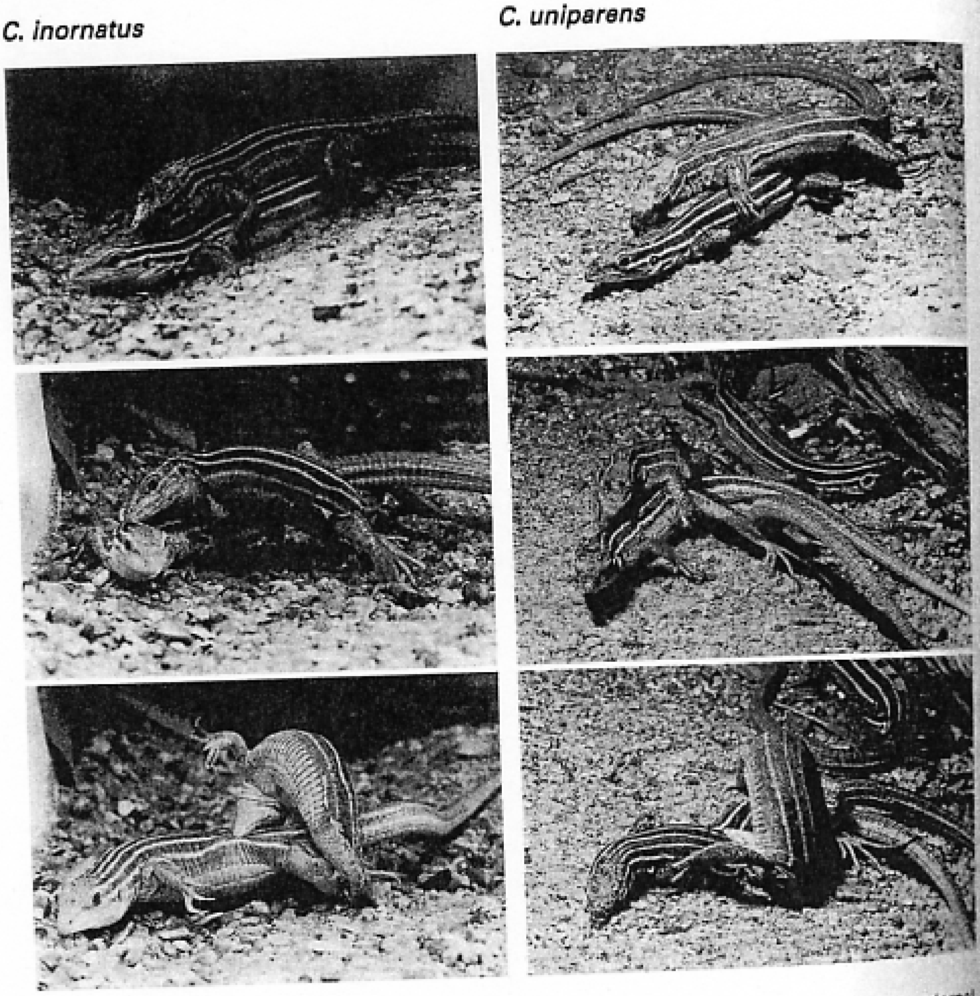


Figure 7.13 Mating sequence (*left*) in the gonochoristic whiptail lizard, *Cnemidophorus inornatus*, the maternal ancestor of the parthenogenetic whiptail, *C. uniparens*. Pseudosexual behavior (*right*) in the all-female parthenogenetic whiptail lizard, *C. uniparens*. Note the similarity in the behavioral sequence to that of its gonochoristic ancestor. (From Crews 1987.)

mounted individual, forming the doughnut posture. Since parthenogens are morphologically female, there are no hemipenes, and intromission does not occur. Two weeks later these roles reverse and the mounting individual is now mounted.

How do we know whether pseudosexual behavior in a unisexual lizard is not just a trivial discovery? (Every scientist should ask himself or herself this kind of question whenever an unusual behavior or phenomenon is discovered.) First, we know that pseudosexual behavior is not peculiar to *C. uniparens*. Pseudosexual behavior has been observed in at least five other species of parthenogenetic whiptail lizards as well as in the parthenogenetic morning gecko. Furthermore, behaviors similar to pseudosexual behavior have been described in other unisexual or asexual vertebrate and invertebrate species. So this is not an isolated occurrence of the behavior. Second, pseudosexual behavior facilitates ovarian development. Studies show that parthenogens are more likely to lay eggs and will lay more clutches if they engage in pseudosexual behavior (Crews et al. 1986). This indicates that the behavior has an adaptive function.

Is pseudosexual behavior simply the result of the development of parthenogenesis? If this were true, one would not expect females of sexually reproducing species to exhibit male-typical behaviors or males to exhibit female-typical behaviors. This is clearly not the case. Females mounting and males allowing mounting are a normal part of sexual activity in many species. Importantly, females of the ancestral species *C. inornatus* have occasionally been observed to mount other females. Thus, pseudocopulatory behavior is not a newly evolved trait in unisexual whiptails. Rather, the neural circuits underlying these behaviors have been retained from their sexual ancestral species. This reflects, in part, the brain organization of the sexual behavior in vertebrates.

Could pseudosexual behavior be a consequence of captivity? Is this an unusual behavior induced by the stress of the laboratory? If this were the case, we would not expect *C. uniparens* to reproduce at normal levels in the laboratory. Reproduction is an extremely complex process that is sensitive to the organism's environment. Even slight perturbations in certain variables can result in captive animals failing to reproduce, a fact evident in many zoos. In my laboratory, however, both unisexual and sexual whiptail lizards reproduce as frequently as they do in nature. That is, they typically lay three clutches of eggs, each containing three-to-four eggs, during the course of a reproductive season. Also, it is clear that stress does occur in these animals, as evidenced by the fact that in cages containing three or four individuals, there is always one dominant individual who lays many clutches and one subordinate individual who does not lay eggs.

Since our original observations, pseudocopulation has been observed in nature in *C. uniparens* and other parthenogenetic whiptail lizards as well as in other all-female species of fish, fruit flies, and geckos. This behavior in whiptails has been quantified using indirect evidence, namely the incidence of copulatory bite marks on the back (Crews and Young 1991). These are caused by the jaw-grip of the mounting animal, which leaves a distinct black V-shaped scar on the lateral abdominal area of the mounted animal. We found copulation marks in over three-quarters of adult females of the sexual ancestral species, *C. inornatus*, but only on about half of the descendant parthenogen, *C. uniparens*. Since in our study the animals were collected at the beginning of the reproductive season, we predicted that only half of the parthenogens would bear copulation marks initially since these animals assume complementary roles and the mounting animal would not be mounted until it was about to ovulate.

HORMONE DEPENDENCE OF PEUDOSEXUAL BEHAVIOR

Do parthenogenetic whiptails have high levels of male-typical androgens when displaying male-like behavior? No. Radioimmunoassay of the circulating androgens revealed uniformly undetectable plasma concentrations at all stages of the reproductive cycle. Not only is there no evidence of transient surges in these androgens during the course of the ovarian cycle, but postovulatory animals exhibiting male-like pseudosexual behavior are no more likely to have detectable androgens than are preovulatory parthenogens exhibiting female-like pseudosexual behavior. Also, the nature and pattern of sex steroid hormone secretions in females of the sexual species and in the parthenogens are virtually identical. Taken together, this indicates that the evolution of parthenogenesis has not been accompanied by an alteration of the usual pattern of endocrine changes.

The reproductive cycles of both female sexual whiptails and unisexual whiptails are very similar. Both consist of a series of 3 to 4 discrete ovarian cycles that are 3 to 4 weeks long. The ovaries simultaneously ovulate from 1 to 3 eggs each. After ovulation the ova pass into the oviducts, where shell deposition occurs. Shelled eggs are usually laid 7 to 10 days after ovulation. Production of the next clutch may be initiated within several days of egg laying.

The temporal patterns of ovarian hormone secretion are also remarkably similar in the ancestral and descendant species. In both, the circulating concentrations of estradiol increase as the follicle grows, peaking around the time of ovulation (figure 7.14). Progesterone levels begin to increase during the latter stages of follicular maturation and are at their peak after the time of ovulation. In both female whiptails and parthenogenetic whiptails, circulating concentrations of androgens are uniformly low and not detectable by radioimmunoassays.

Although the unisexual whiptail descended directly from a sexual whiptail, the two species differ in an important aspect of their reproductive biology; namely, circulating concentrations of estradiol in reproductively active parthenogenetic whiptails are approximately five-fold lower than in reproductively active female sexual whiptails (Young and Crews 1995) (figure 7.15). Since changes in the circulating concentrations of sex steroid hormones can have dramatic effects on endocrine physiology and behavior, one might expect that the several-fold difference in estradiol between the parthenogenetic whiptail and the sexual whiptail would be accompanied by species differences in estrogen-dependent phenomena. Comparisons of estrogen receptor (ER)-mRNA content in the brains of whiptail lizards indicate that parthenogenetic whiptails have higher concentrations of ER-mRNA expression in the POA than do females of the ancestral sexual species. These species differences in circulating concentrations of estradiol and ER-mRNA are accompanied by differences in sensitivity to estradiol. Dose-response studies reveal that lower dosages of estradiol benzoate are required to induce receptive behavior as well as changes in gene expression in the POA and VMH of parthenogenetic

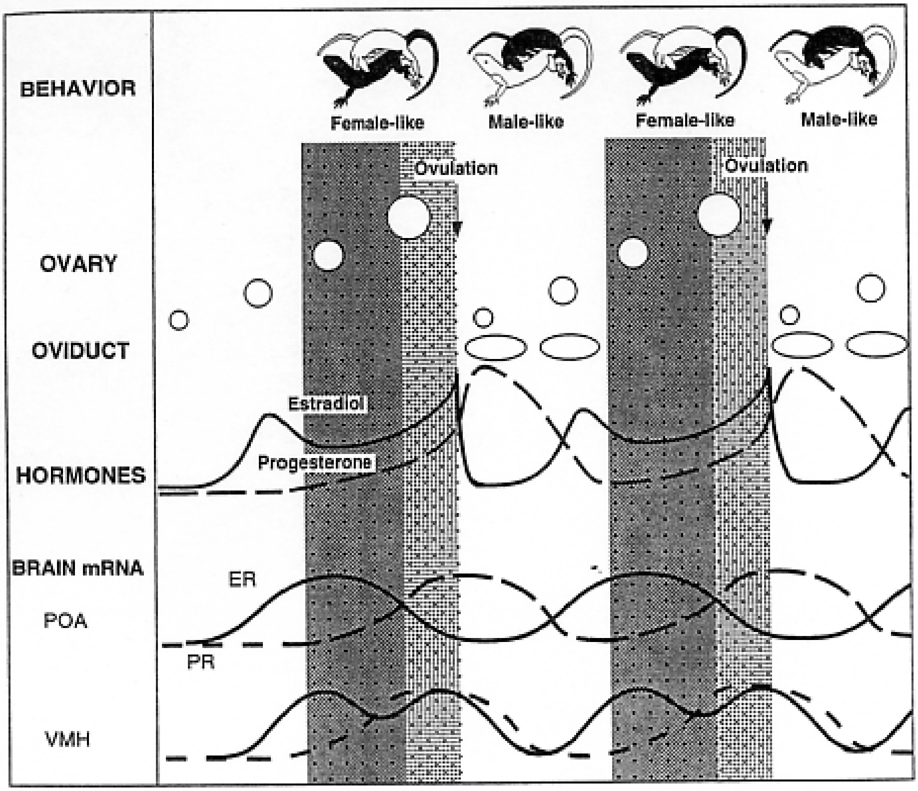


Figure 7.14 Relation among malelike and femalelike pseudosexual behavior, ovarian state, and circulating levels of estradiol and progesterone during different stages of the reproductive cycle of the parthenogenetic whiptail lizard. The transition from receptive to mounting behavior occurs at the time of ovulation (arrow). Also shown are the changes in the expression of genes coding for estrogen receptor (ER)-mRNA and progesterone receptor (PR)-mRNA in the preoptic area (POA) and the ventromedial hypothalamus (VMH), two brain areas involved in the regulation of pseudosexual behavior. (Redrawn from Crews 1996.)

whiptails than in sexual whiptails (figure 7.15). As in other vertebrates, the VMH is involved in the hormonal induction of receptive behavior in whiptail lizards. Thus, species differences in reproductive physiology (e.g., brief follicular phases, as in the rat and mouse, rather than extended follicular phases, as in whiptail lizards and rabbits) may explain species differences in neuroendocrine-controlling mechanisms.

Female *C. inornatus* are receptive to the courtship displays of the male only in the vitellogenic or yolking stage. Females are highly aggressive to courting males prior to ovulation or during pregnancy. Removal of the ovaries abolishes sexual receptivity, whereas estradiol replacement therapy will restore receptivity. Interestingly, postovulatory female *C. inornatus* have been observed to exhibit the heterotypical or male-typical mounting behavior, and there is some evidence that progesterone will stimulate female-female mounting in the sexual whiptail.

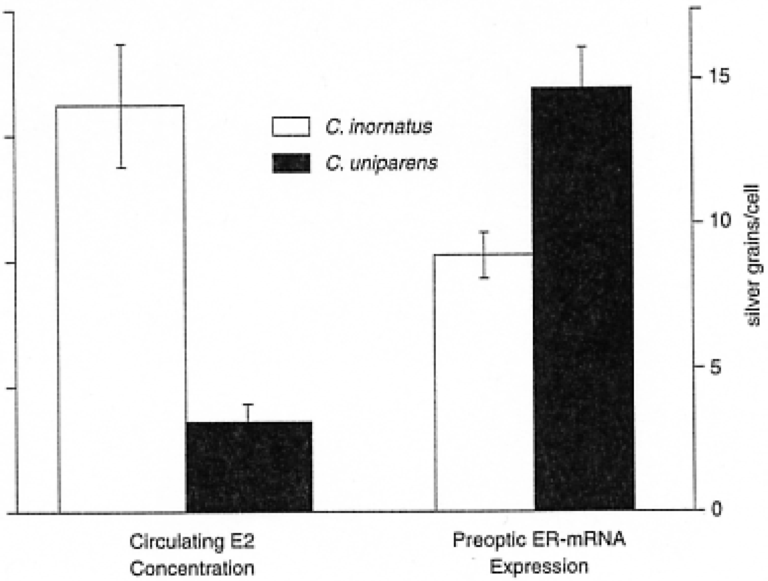


Figure 7.15 Circulating estradiol concentrations and estrogen receptor ER-mRNA expression in the preoptic area of the vitellogenic female whiptail (*Cnemidophorus inornatus*) and the parthenogenetic whiptail, (*C. uniparens*). (From Young and Crews 1995.)

Pseudosexual behavior in parthenogenetic whiptails is also related to the ovarian state. Female-like receptive behavior is limited to the preovulatory stage of the follicular cycle, whereas expression of male-like mounting behavior occurs most frequently during the postovulatory stages of the cycle (figure 7.14). These behavioral roles during pseudocopulations in the unisexual species are paralleled by differences in the circulating levels of sex steroid hormones. That is, individuals show primarily female-like behavior during the preovulatory stage when estradiol concentrations are relatively high and progesterone concentrations relatively low. Just the opposite is seen during the display of male-like behavior in the postovulatory phase, when concentrations of estradiol are low and concentrations of progesterone have increased.

It appears that ovarian hormones trigger pseudocopulatory behaviors in unisexual whiptails. In support of this idea, pseudocopulatory behaviors have never been observed in reproductively inactive or ovariectomized individuals. This means that in the absence of hormones, pseudocopulation does not occur. In addition, female-like receptivity is almost completely restricted to the yolk deposition phase. Male-like pseudocopulatory behavior, on the other hand, is most frequent in postovulatory or pregnant animals. Also, individuals alternate female-like and male-like pseudosexual behaviors as they progress through the ovarian cycles during the course of the breeding season.

When investigating the causal basis of any behavior, the place to begin is with those physiological events that are in transition at the onset of the behav-

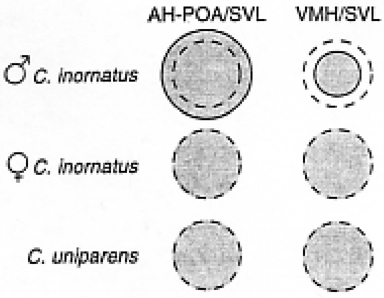


Figure 7.16 Schematic representations of the volumes of the sexually dimorphic areas in the brain relative to body size in sexual and parthenogenetic whiptails. To aid in comparison, the volume of the AH-POA and the VMH of a female *C. inornatus* is represented as a dashed outline in other drawings.

ior. It is clear that the transition from female-like to male-like pseudosexual behavior occurs when hormone concentrations in the blood are changing. At ovulation, estradiol concentrations decrease and progesterone concentrations increase (figure 7.14). Is it possible that this shift in hormone concentrations plays a crucial role in controlling the expression of pseudosexual behavior? To test this, animals were ovariectomized and then treated with progesterone, estradiol, or with a sham (control) operation. The results were clear-cut. Pseudocopulations occurred only in pairs in which both individuals were hormone-treated, but in a complementary fashion. In other words, animals treated with estradiol exhibited the female-like role and the progesterone-treated parthenogen assumed the male-like role.

NEURAL BASIS OF PSEUDOSEXUAL BEHAVIOR

The greatest advantage of unisexual vertebrates, in my opinion, is that they enable us to address the fundamental question of the neural basis of sex-typical behaviors from a new perspective. The idea that there are dual neural circuits in the brain of all vertebrates, one mediating mounting and intromission behavior and the other mediating receptive behavior, is not new. Researchers have long commented on males that exhibited female-typical sexual behaviors or, conversely, females that exhibited male-typical sexual behaviors. The bulk of modern research, however, has focused on the neuroendocrine mechanisms controlling homotypical behaviors, namely mounting behavior in gonadal males and receptive behaviors in gonadal females. In other words, each neural circuit has been studied extensively, but almost always in isolation from its complement. The parthenogenetic whiptails allow us to study these circuits operating together. Comparison studies will allow us to say what is common to both and what is specific to each.

An opportunity to study sexually dimorphic dual neural circuits together in the same individual is provided in the whiptail lizard. We find complementary sexual dimorphisms in two hypothalamic regions of the ancestral species, *C. inornatus* (figure 7.16). The POA is larger in males than in females whereas the VMH is larger in females than in males. Other research has shown that the POA is involved in mounting behavior whereas the VMH controls sexual receptivity, as in rodents and other mammals (see chapters 4 and 5). During hibernation or following castration, the POA shrinks and the VMH enlarges (that is, these brain areas become femalelike); in the female *C. inornatus* these areas show no change across seasons or after the female is treated with exogenous androgen. These results clearly indicate that in the ancestral sexual species structural dimorphisms develop in the adult and, further, that testicular androgens control the seasonal growth of these areas.

Given that the parthenogen exhibits both male-like and female-like pseudosexual behaviors, it would seem reasonable to expect the brain of the parthenogen to be bisexual, resembling both the male and the female of the ancestral sexual species. After all, the same brain areas are involved in the regulation of these behaviors. Implantation of androgens into the POA elicits, and lesioning this area abolishes, mounting behavior in both the sexual and unisexual whiptails. Implants of androgens into the VMH fail to elicit mounting behavior and have no effect on receptive behavior. Conversely, implantation of estrogen in the VMH activates receptivity in both species, but estrogen implants in the POA have no effect on receptive or mounting behavior. Lesion damage in the dorsolateral VMH, the area containing ER, abolishes estrogen-dependent receptivity. Finally, using 2DG uptake as a measure of metabolic activity, researchers found that parthenogenetic whiptails displaying male-like pseudosexual behavior had a 6-fold greater uptake in the medial POA than parthenogens displaying female-like receptivity (Rand and Crews 1994). In contrast, the VMH showed significantly greater 2DG accumulation in receptive parthenogens than in courting parthenogens.

To my great surprise, we found that the size of the POA and VMH of the parthenogenetic whiptail did not resemble both males and females of the sexual ancestral species (Crews et al. 1990; Wade et al. 1993). Even in individuals exhibiting male-like or femalelike pseudosexual behavior naturally or under hormone treatment, the POA and the VMH were similar in size, as is characteristic of female whiptails. A similar relationship exists at the level of individual neurons located in these brain regions. Further, there was no difference in neuron somata size in those individuals exhibiting malelike pseudosexual behavior than in those exhibiting femalelike pseudosexual behavior. Even if the parthenogen is treated with androgen so that it exhibits only male-like behavior and coloration, the brain remains unchanged.

Does this reflect the genetic sex or the gonadal sex of the individual? There is some evidence that the genetic basis for male-typical sexual behavior may be distinct and separate from that for female-typical sexual behavior, and further, that the genetic mechanisms of sex determination may influence the brain directly (reviewed in Arnold et al. 1995; Crews et al. 1998). But this important question cannot be addressed with conventional animal models. As outlined in chapter 3, the Y chromosome-linked gene initiates the genetic cascade leading to testicular development, thereby causing individuals to develop a male-typical phenotype. Female development is generally believed to be a default mode, resulting from the absence of this gene. Thus, in species with sex chromosomes, the sexes differ in genetic constitution and hence, genetic sex and gonadal sex are inextricably linked.

The whiptail lizards provide a unique opportunity to circumvent this problem. The male is the heterogametic sex (XY) in the ancestral sexual species, and the descendant parthenogenetic species is triploid (XXX). We have found that administration of the aromatase inhibitor Fadrazole early in embryogenesis results in hatchlings being gonadally male with fully developed hemipenes and vasa deferentia; on achieving sexual maturity, their testes even produce motile sperm. This ability to create normal-appearing males in an otherwise all-female species is further evidence that each individual possesses all of the genetic machinery to produce the male phenotype and lacks only a hormone-mediated trigger for activating the male-determining cascade and suppressing the female-determining cascade. Thus, we now have an animal model that allows us to distinguish the effects of genetic sex from gonadal sex on sexually dimorphic traits. That is, we can ask if the sexual dimorphisms evident in brain and behavior in the ancestral species are also evident when we compare female parthenogens and "created male" parthenogens.

We have discussed how the parthenogens and females of the sexual species have a smaller AH-POA volume and a larger VMH volume as well as a greater increase in PR-mRNA in the VMH in response to estrogen treatment than males of the sexual species, all of which can serve as a marker for the female-like brain. Developmental studies indicate that this latter sex difference, namely the ability of estrogen to increase abundance of PR-mRNA in the VMH, is observed in hatchlings of the sexual species, but not in the parthenogenetic species. This is consistent with other research and indicates that there can be a significant genetic sex component in sexually dimorphic traits.

EVOLUTION OF PSEUDOSEXUAL BEHAVIOR

How have the cellular mechanisms subserving pseudosexual behaviors evolved? Again, unisexual organisms provide an unusual opportunity for study of this question because we can compare the ancestral with the descendant species to discover how the neuroendocrine mechanisms regulating behavior might have changed. Specifically, we want to know how the progesterone activation of male-like pseudosexual behavior in the parthenogen evolved from the ancestral sexual species in which male-typical sexual behavior is androgen-dependent.

Whiptail lizards are seasonal breeders, with male *C. inornatus* courting and copulating only during the spring and early summer months. At this time, the circulating concentrations of androgens in males are elevated. The circulating concentrations of progesterone during this period are low and unchanging. Estradiol is undetectable. As in other vertebrates exhibiting an associated reproductive pattern, the courtship and copulatory behaviors of the male *C.*

inornatus depend upon testicular steroid hormones. Castrated males court females significantly less often than do intact, sexually active males and, as expected, treatment of castrates with exogenous androgens reinstates courtship and copulatory behaviors. However, administration of exogenous progesterone also restores the complete repertoire of sexual behavior in about one-third of castrated males. Indeed, these progesterone-sensitive males actively court and copulate with females with an intensity equal to that shown by castrates treated with androgen. While not all exogenous androgen-sensitive males are sensitive to progesterone, all progesterone-sensitive individuals are sensitive to both progesterone and exogenous androgens, suggesting that progesterone-sensitive males are a subset of exogenous androgen-sensitive males.

It is always possible that progesterone is being converted to another hormone and its metabolites produce the results described above. To test whether progesterone was acting directly to activate mounting behavior, we used various ligands, or synthetic agonists and antagonists that bind to the hormone receptors. In mammals, R5020 acts as an agonist and simulates the effect of progesterone at its receptor, whereas RU486 acts as an antagonist and prevents receptor activation. In castrated male *C. inornatus*, administration of R5020 stimulates sexual behavior in a similar proportion of castrated males as does progesterone, while RU486 prevents sexual behavior in castrated, progesterone-treated males. Other work revealed that (1) progesterone synergizes with testosterone as well as dihydrotestosterone to stimulate sexual behavior in males (Lindzey and Crews 1992), much like estradiol and progesterone synergize to elicit sexual receptivity in female rodents, and (2) that the androgen receptor (AR) of the sexual species is similar to that of mammals in its specificity and kinetics (Lindzey and Crews 1993). Taken together, these data suggest that progesterone is probably exerting its stimulatory action as a progestagen and not via conversion to other sex steroid hormones. These data also suggest that progesterone acts via the progesterone receptor (PR) and not via AR. This conclusion is further supported by the finding that implantation of progesterone directly into the POA induces courtship and copulatory behavior in progesterone-sensitive, but not in progesterone-insensitive, males; the pattern of regulation of AR- and PR-mRNA in progesterone-sensitive and progesterone-insensitive males is also different (Crews et al. 1996).

Is this behavioral responsiveness to progestin in males specific only to reptiles? Initially this was an odd question, as progesterone has long been believed to be a "female hormone." Recent studies, though, on the physiology of progesterone in males points to a functional role. For example, dihydroxyprogesterone stimulates spawning behavior in castrated rainbow trout. In male rats there is a pronounced diurnal rhythm in progesterone secretion, with the peak in progesterone levels coinciding with the period of greatest copulatory activity. When administered in physiological dosages (rather than the pharmacological dosages usually used), progesterone causes some castrated male rats to mate with receptive females; when combined with subthreshold dosages of testosterone, all the males respond (Witt et al. 1995).

Further, this progesterone response is blocked if the progesterone antagonist RU486 is administered either systemically or directly into the brain. Thus, although progesterone has long been known to be involved in the control of female-typical sexual behavior, new evidence points to a previously unsuspected role of progesterone in the control of male sexual behavior. Indeed, progesterone and androgen appear to synergize in the control of male courtship behavior much as progesterone and estrogen synergize in the control of female sexual receptivity.

As discussed in chapters 2 and 4, progesterone need not be present for the PR to be activated. How might this occur? Sex hormone receptors and neurotransmitters are found in limbic nuclei such as the POA and the VMH and can even be located in the same neuron, suggesting that there are various signaling pathways in behaviorally relevant neurons. We have already learned in chapters 4 and 5 that sex steroid hormones can act directly or indirectly by altering neurotransmitter biosynthesis. Neurotransmitters and neuropeptides such as norepinephrine, GnRH, oxytocin, and GABA facilitate, whereas CRF, ACTH, and NPY inhibit, sexual behavior. Serotonin and dopamine are interesting in that depending upon the receptor type occupied, they can either stimulate or suppress sexual behavior. For example, dopamine can induce receptivity in estrogen-primed female mice as effectively as progesterone. This ligand-independent activation of PR by dopamine and its agonists occurs through the D_1 subtype receptor. Other studies indicate that dopamine is released in the POA in male rats during sexual interactions, and that the amount of dopamine produced is positively correlated with the intensity of sexual behavior an individual male displays (Hull et al. 1997).

We have used the PRKO male mouse as a model to study the role of PR in androgen-dependent male sexual behavior (Phelps et al. 1998). We first found that PRKO males were much more sensitive to the effects of castration than were wild-type males. They were also less responsive to androgen-replacement therapy. This effect may be mediated by dopamine, because administration of a selective dopamine agonist elicits sexual behavior in wild-type male mice, but not in their PRKO counterparts (Woolley et al. 1999). Taken together, these results indicate that progesterone and its receptor are as much a part of the hormonal mediators of sexual behavior in males as in females. In other words, progesterone is as much a "male" hormone as it is a "female" hormone.

How might this evolution from an androgen-dependent, male-typical behavior in the sexual ancestral species to a progesterone-dependent, male-like behavior in the unisexual descendant species have occurred? Gould and Vrba (1982) have pointed out that two distinct historical processes can produce existing features. One of these is adaptation, or the gradual selection of traits that results in improved functions. Some traits, however, evolved from features that served other roles or had no function at all, and only later were co-opted for their current role because they enhance current fitness. The latter process, in which a useful feature is not built by selection for its current role, may be termed exaptation. The difference between the two is that in adaptation, traits are constructed by selection for their present functions. Exaptations are co-opted for their present use. In the present case, the variation in sensitivity to progesterone in the sexual ancestral species may be the substrate on which selection operated. This resulted in the novel hormone-brain-behavior relationship observed in the parthenogen. That is, because reproduction requires reciprocal behavioral stimulation, the elevation of progesterone following ovulation presented a reliable and appropriately timed stimulus that, given the low circulating concentrations of androgens, was coopted to trigger mounting behavior in the parthenogen. Evidence that this, indeed, has occurred is indicated by the finding that exogenous estradiol upregulates PR-mRNA in the POA of the parthenogen, but not in females of the ancestral sexual species (figure 7.17).

Thus, in the sexual ancestral species, individual variation in the sensitivity to progesterone appears to have served as the substrate for the evolution of the novel hormone-brain-behavior relationship observed in the parthenogen. That is, the elevation of progesterone following ovulation presented a reliable stimulus that, given the low circulating concentrations of androgens, was coopted to trigger mounting behavior in the parthenogenetic whiptail. Further, the ability of the parthenogen to express malelike pseudocopulatory behaviors is not because it has developed a morphologically masculinized POA, but because it has coopted a naturally occurring progesterone surge to

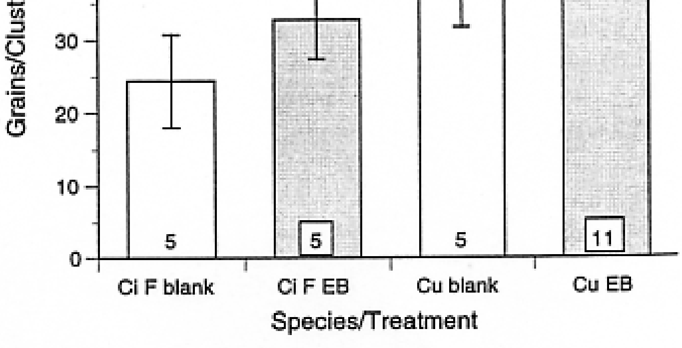


Figure 7.17 Evolution of a novel neuroendocrine mechanism controlling male-typical mounting and copulatory behavior. Depicted is the abundance of progesterone receptor-mRNA measured as average number of silver grains per cluster in the preoptic area (POA) of the ancestral sexual female (F), *Cnemidophorus inornatus*, (Ci), and descendant parthenogenetic, *C. uniparens*, Cu, whiptail lizards receiving no hormones (blank) or estradiol benzoate (EB). (Redrawn from Crews 1996.)

trigger the masculine behavioral potential remaining in a feminized brain. This is an important point because it shows again how behavioral differences need not be paralleled by structural differences in the brain.

We have seen how species can be compared to dissect the neuroendocrine mechanisms underlying sexual behavior and how the study of individual variation can be used to trace the evolutionary sequence that gave rise to alternative forms. This comparative approach can in turn lead to new concepts that may help to explain species differences in endocrine physiology and behavior. In this regard at least three factors appear to be important: (1) sensitivity to sex steroid hormones, (2) hormone-dependent regulation of sex steroid hormone receptor gene expression, and (3) neuroanatomical distribution of steroid receptor gene expression, especially in nonlimbic structures.

How Study of Diversity Raises New Questions About Old Answers

The Principle of Complementarity

Evolution of a species, a behavior, or a hormone does not occur in a vacuum. Complementarity, or the combination of parts required for completion, is evident at all levels of biological organization (table 7.3). Animals and plants have evolved in the presence of other plants and animals and their survival has become dependent upon, or is modulated by, the presence or behavior of others within the environment. For example, figs depend on fig wasps for pollination; the fig wasps oviposit in the fig and the resulting young feed on the fruit. Herbivores depend on plants for sustenance. Plants depend upon animals to disperse seeds. Sexual reproduction requires two kinds of complementary gametes, eggs and sperm. During mating, two individuals must interact in a complementary fashion if fertilization is to occur. In reproductive physiology, there are complementary feedback relationships among hormones and the complementary interaction of hormones and their receptors such as those discussed in chapter 1. Thus, complementarity is fundamental to biological systems.

Table 7.3 Examples of the Principle of Complementarity in Behavioral Biology

LEVEL OF BIOLOGICAL ORGANIZATION	COMPLEMENTARY PROCESS	EXAMPLE
Species	Coevolution of plants and animals	Figs and fig wasps
Organism	Sexual behaviors	Intromission and receptive behaviors
Physiology	Signal-receiver	Feedback control in neuroendocrinology
Molecule	Hormone-receptor	Steroid hormones and their receptors

At every level of biological organization there are fundamental complementary processes.

This perspective is useful when considering complex systems such as reproduction. Anyone who has ever watched carefully how animals mate will be struck by the complementary nature of copulation and of the actions that lead up to it. Normally, copulation is a behavior made possible only by mutual consent. This consent is a reflection not only of the satisfactory nature of the external stimuli provided, but also of the internal milieu that motivates each individual to seek a partner. Beach (1979) referred to this as "the principle of stimulus-response complementarity." According to this principle, successful mating will occur only if both partners are in the appropriate physiological and behavioral condition and if the behaviors of each partner elicit the appropriate behavioral response from the other. This can be restated as two simple sentences: Mounting will not be successful if the mounted animal is not receptive. A receptive animal will not be mounted unless there is a willing partner.

The principle of stimulus-response complementarity has broad implications. For example, it can be applied to all animals, regardless of their mode of reproduction and of the genetic or gonadal sex of the participants. Female-associated responses tend to evoke masculine responses in both males and females. Similarly, male-associated responses tend to evoke feminine responses in both females and males.

This principle may also have an impact on our understanding of sexual selection. Just as there is variance in male displays, there is variance in female preference. Indeed, if we examine any morphological, physiological, or behavioral trait, we will find that females are more variable than males—an important phenomenon waiting to be investigated. As Bluhm (1985) has documented, female canvasback ducks forced into pairs with males they did not select fail to lay eggs. Could this phenomenon be generalized to other species? If we refer to a successful pairing as a "resonance" between the preference behaviors of the partners, may this reflect a similar "resonance" at a physiological level? That is, when females choose their mating partners, are they selecting males whose physiology will stimulate maximum reproductive success? As Bluhm has shown, such a hypothesis is relatively easy to test. The answer should reveal itself best in those species exhibiting strong mate preference or giving evidence of assortative mating.

The concept of complementarity in biological systems is also useful because it encourages investigators to think of units rather than single elements. In biology it is often said that the whole is more than the sum of its parts. This is readily seen in mating behavior where two individuals must coordinate and synchronize reproductive processes. Only from such interactions will reproduction be successful. This logic can be extended to all levels of biological organization. Still, the trend in behavioral endocrinology is to focus on a single sex, a single behavior, or a single hormone. This is evident in studies where the investigator may spend years determining the reproductive cycle in the male (or female) while ignoring the potential contribution of the partner.

On the other hand, at the genetic level, individuals are far from being complementary. Differences in reproductive interests lead to something very different from harmony and mutual coordination in courtship and mating behavior. In fact, another descriptor for male-female relations is the conflict of reproductive interests. Put simply, no two sexually reproducing individuals, other than monozygotic twins, share identical genetic (reproductive) interests. In this framework, courtship behavior by males and mate choice by females may represent "salesmanship" and "consumerism," respectively.

Can the Organizational Concept Apply to Vertebrates Lacking Sex Chromosomes?

The organizational concept has had great heuristic value and aided our understanding of the fundamental elements of sexual differentiation in mammals, birds, and other vertebrates. But can it be applied to vertebrates that exhibit other modes of reproduction? In its present formulation the organizational concept appears to be restricted to gonochoristic vertebrates that have genotypic sex determination (Crews 1993). In reptiles with TSD, such as leopard geckos, the environment during development plays a major role in shaping the adult phenotype. In hermaphroditic fish where sex changes in the adult organism, can there even be an organizing or neutral sex? In parthenogenetic lizards only female individuals exist, yet each individual exhibits both male-like and female-like pseudosexual behaviors. Can the organizational concept be redefined and still account for the evidence at hand (figure 7.18)?

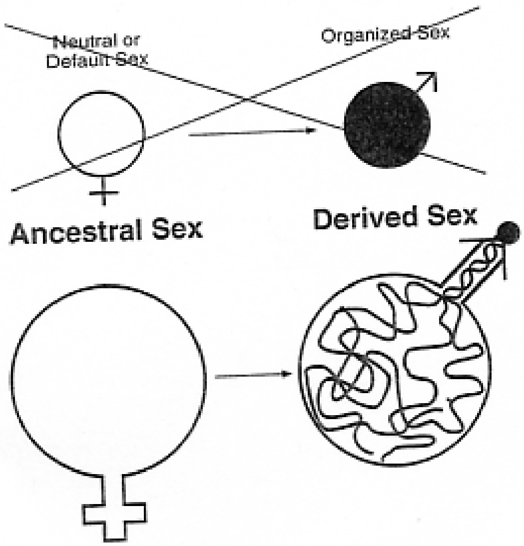


Figure 7.18 The organizational concept (*top*) postulates that the female is the neutral or the default sex, whereas the male is the organized sex. This paradigm emphasizes how males and females differ. Yet the organizational concept does not apply to vertebrates lacking sex chromosomes. An alternative paradigm (*bottom*) called the Evolutionary Concept, focuses more on the common origin of females and males, namely a single fertilized egg. Since males evolved only after the evolution of self-replicating (i.e., female) organisms, the female can regarded as the ancestral sex, whereas the male is regarded as the derived sex. (From Crews 1993.)

There can be little doubt that males evolved only after the evolution of replicating (i.e., female) organisms. Males have repeatedly been gained lost, but females have remained. Speaking in terms of mechanisms, and outcomes such as the presence of male and female individuals, the fen may be considered as the ancestral sex and the male as the derived sex. is not inconsistent with some of the original interpretations of the evidenc organization in mammalian sexual development. Such a perspective wh I call the Evolutionary Concept accounts for the observation that malen appears to be imposed upon a female phenotype, not vice versa. This for lation is independent of which sex is the heterogamete (XY or ZW) in th species with genetic sex determining mechanisms. This in turn suggests intriguing possibility that males may be more like females than females like males. In mammals the relative ease of masculinizing individuals c pared to the difficulty of defeminizing individuals would be a case in po Thus, Professor Higgins may have posed the wrong question in *My Fair L* when he asked rhetorically "Why can't a woman be more like a man?" more interesting (and perhaps more accurate) question may be "Why can male be more like a female?"

The Evolutionary Concept perspective affords several problems worthy study. First, at a basic level, the above question can only be answered a study of the *similarities* of the neuroendocrine mechanisms subserv female-typical and male-typical sexual behavior. This would be a depart from the present focus on sex *differences* and the emphasis on study mounting in gonadal males and receptivity in gonadal females. Is there evidence that the Evolutionary Concept would yield new insights? One w documented instance concerns the role of estrogen in the control of behavior. In most species of female vertebrates, estrogen activates sexual ceptivity. Circulating testosterone is aromatized to estrogen in the brain sexually active males and, in some species, estrogen is the active molecule males as well as in females. Another example already discussed is role progesterone in facilitating hormone-induced sexual behaviors in both sex

Hypothetical Steps in the Evolution of Behavioral Controling Mechanisms

Evolution is the cumulation of successful outcomes. We have already se how a steroid hormone–dependent neuroendocrine mechanism might chan But how could such a complex and delicate system have evolved (table 7. There appears to be an ancient functional relationship between gamete (i sperm and or eggs) production and gonadal steroid secretion (Kanatani a Nagahama 1980). Gametes are never produced without a concomitant rise gonadal steroid production. It is often the case, however, that gonadal stero hormones are secreted without the production of sperm or eggs. It is cle that the sex steroid hormones have come to serve as activators of sexual havior in a wide variety of vertebrate species.

How might hormone-brain-behavior relations have evolved in vertebrate As a first step, the nervous system might have become responsive to h

Table 7.4 Hypothetical Scenarios in the Evolution of Hormone-Brain-Behavior Relationships in Vertebrates

1. Functional association between gametogenesis and steroid hormone production.
2. Functional association between steroid hormones and nervous system responsiveness.
3. Functional association between higher neural centers, the pituitary, and gonadal activity.
4. Functional association of gonadal steroid production and the development and later activation of gamete delivery systems.
5. Recruitment of integrative (limbic) areas influencing behavior via expansion of steroid-sensitive hypothalamic areas involved in the control of pituitary gonadotropin secretion.
6. Functional association of specific sexual signals and the secretion of gonadal steroids.

The first four major steps are probably shared by all vertebrates and hence are more ancient than the last two, which probably vary between species.

mones during development. This could have been followed by the development of a structure to regulate gonadal activity—in vertebrates, the anterior pituitary gland. Because the anterior pituitary is situated directly beneath the hypothalamus, a second step would involve the hypothalamic modulation of pituitary activity. As the animal perceives changes in its environment, this information could have been integrated at the level of the hypothalamus.

Development of a feedback control system involving the hypothalamus, the pituitary, and the gonads is the foundation of the functional association between gonadal hormone secretion and reproductive behavior. An expansion of the brain regions involved in the feedback control of pituitary function, including adjacent behavioral and integrative areas, could have mediated the development of these mechanisms. A further step in the evolution of hormone dependency of sexual behavior is seen in some species in which specific sexual signals elicit the secretion of reproductive hormones.

However, it should be emphasized that the presence of sex steroid hormone–concentrating neurons in behavioral integrative areas is not evidence a priori of a functional association between sex steroid hormones and sexual behavior. As pointed out earlier, neither female red-sided garter snakes nor female Asian musk shrews exhibit hormone-dependent sexual receptivity, yet sex steroid hormone–concentrating neurons have been identified in both species. As described earlier, species exhibiting dissociated reproductive patterns appear to have evolved alternative neuroendocrine mechanisms.

Another chicken-and-egg puzzle concerns the evolution of hormones and their receptors. For example, what came first, hormones or their receptors? How can a hormone evolve unless there is a receptor present to recognize it? Alternatively, how could a receptor evolve without hormones being already present? One popular scenario is that the common biochemical pathway in steroid metabolism, with progesterone being the precursor to androgens, which, in turn, give rise to the estrogens, reflects the evolutionary sequence for steroid hormones and their receptors. In this paradigm new receptors evolved with the emergence of each new class of steroid hormone. Hence, progesterone receptor (PR) would be the most ancient steroid hormone receptor and estrogen receptor (ER) the most recently evolved steroid hormone receptor. Attractive as it is, Thornton (2001) has provided an elegant and convincing challenge to this hypothesis.

The gene sequences of the steroid hormone receptors have been determined for several dozen species. Using bioinformatics and statistical techniques, Thornton reconstructed the gene sequence of the ancestral hormone receptor at the root of the steroid receptor evolutionary tree. Analysis of that sequence indicates that the ancestral steroid hormone was an ER (figure 7.19A). Thus, estrogen, the last hormone in the steroid biosynthetic pathway, appears to have been the first hormone to function through a steroid receptor. Thus, ER appears to have been the first steroid hormone receptor and coopted estrogen as a signaling molecule. Since estrogen synthesis and maturation of the egg is linked (for reasons as yet unknown), this union served as a reliable indicator to the brain to coordinate the reproductive process. In so doing, all of the intermediate steroid molecules in the steroidogenic pathway leading to estrogen became available as possible chemical signals for receptors that emerged by gene duplication (figure 7.19B). Thornton (2001) calls this co-evolutionary dance ligand exploitation, noting its similarity to the already discussed sensory exploitation model for the evolution of sexual signals. It is also possible that after the initial estrogen–estrogen receptor signaling system had evolved, subsequent receptors created by gene duplication were shaped by the intermediates themselves. That is, the fixed molecular structure of the steroid intermediates shaped through selection the gene sequence to render receptors having the greatest affinity/specificity for these intermediates. PR was the next steroid receptor to evolve, and its ancestral function may have been in the control of ovulation because it is associated with the ovulation. The role of androgen in development of a sexually dimorphic phenotype in males was a relatively recent evolutionary novelty, requiring the later emergence of the androgen receptor by gene duplication. The more ancient nature of estrogen receptors would explain the seeming paradox of why estrogen is the active molecule that "masculinizes" the brain in male mammals and birds.

Reconstructing evolutionary scenarios is useful because they help place systems in a larger context. Such scenarios can also lead to predictions or point the investigator toward potential areas of research. For example, a complete understanding of the role of the pituitary in vertebrate reproduction is aided by studies of primitive chordates in which the pituitary is exposed directly to the environment through a hole in the roof of the mouth. Reconstructed scenarios also help to place in perspective such apparent anomalies as dissociated reproductive patterns or environmental sex determination. Are these phenomena specialized adaptations or are they representative of more primitive conditions?

Similarly, the union of phylogenetic systematics and molecular genetics not only suggests solutions to questions of when and how molecules evolved but also broadens our understanding of existing problems. For example, the

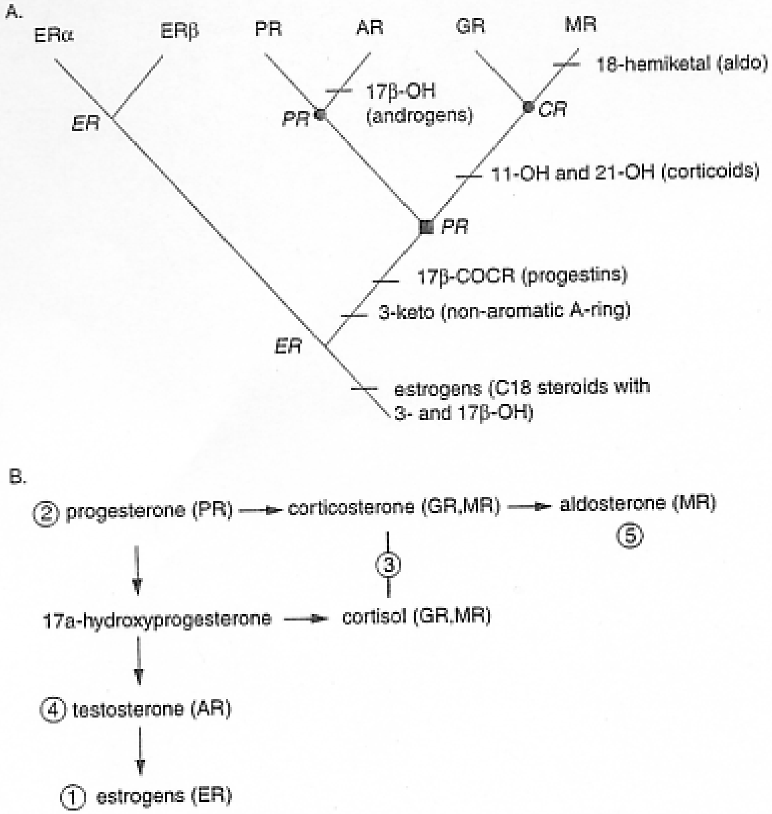


Figure 7.19 Evolution of hormone-specific steroid receptors from an ancestral estrogen receptor by ligand exploitation. (*A*) Phylogenetic tree of vertebrate steroid receptors, inferred from amino acid sequences of extant receptors. Branching points represent gene duplications; dark circles indicate a genome expansion after the divergence of lamprey from jawed vertebrates, and the dark square indicates a genome expansion before this divergence. Ancestral receptors at each node are shown based on reconstructions of ancestral sequences and characteristics. Bars on branches show the emergence of each receptor's capacity to bind ligands with the specific chemical structures. (*B*) The terminal hormone in the synthesis pathway for steroid hormones was the first for which a receptor evolved. A simplified form of the biochemical pathway for synthesis of mammalian steroid hormones is shown. The receptor bound by each hormone is shown, and the circled numbers shows the order in which those receptors emerged during evolution. Natural selection for estrogen-ER signaling also selected for the synthesis of progesterone and testosterone as intermediates, before there were receptors for these steroids. Subsequent gene duplications created novel receptors, which evolved high affinity for these steroids, transforming them into bona fide hormones. ER, estrogen receptor; PR, progesterone receptor; CR, corticoid receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; AR, androgen receptor; aldo, aldosterone.

conclusion that estrogen is the most ancient steroid hormone would account for its apparent endocrine role in a wide variety of taxa, from sea stars and amphioxus to vertebrates. This in turn would account for why so many species are profoundly affected when exposed to estrogenic pesticides and industrial organochlorines early in development.

Conclusions

With the great variety of animals, particularly their behaviors and their physiologies, are there any generalities? With the establishment of evolutionary theory, evidence that there is "unity in diversity" has come with discoveries of common anatomical features, the cell cycle, conservation of intermediary metabolism, and the genetic code, to name but a few. To this can be added that in vertebrates there is a conservation of the neural circuits underlying sexual behavior. Based on the examples in this chapter alone, it does not appear that the same can be said of the hormonal mechanisms underlying behavior.

This particular chapter has documented how some widely held assumptions are generalities only in a very restricted sense. I have shown how much of our conceptual understanding of the behavioral endocrinology stems from extensive studies on relatively few species. According to Beach (1979), there are

> *two cardinal rules that should govern not only the construction of animal models for human behavior, but for all interspecific comparisons regardless of the behavior and the species involved. The first rule is that meaningful comparisons are based not upon the formal characteristics of behavior, but upon its causal mechanisms and functional outcomes.... The second rule is that the validity of interspecific generalization cannot exceed the reliability of intraspecific analysis. Significant comparison of a particular type of behavior in two different species is impossible unless and until the behavior has been adequately analyzed in each species by itself. Only after independent, intraspecific analysis is achieved can we properly interpret the nature and degree of interspecific similarities and differences."* (pp. 113–114)

To these two rules, a third and a fourth might be added. Third, make the consideration of the organism's natural history the first and last step in any biological study of behavior. Fourth, open the way to new insights by questioning traditional paradigms.

Applying an evolutionary perspective to behavioral endocrinology challenges the breadth and validity of assumptions about the mechanisms that control species-typical behaviors. This is not the same thing as saying that no unitary explanations apply to all mammals (versus nonmammals), endotherms (versus ectotherms), or even vertebrates (versus invertebrates). As this volume testifies, we have gathered considerable information about the neuroendocrine bases of behavior in a few species. To uncover truly broad generalizations, however, we must begin looking with equal intensity and rigor at other organisms.

Thus, the ecological and evolutionary perspective points the way to a variety of natural experiments or "experiments of nature." The pattern of evolution is best illustrated in the diversity of organisms. By studying closely related species that live in different habitats, we see how each has become adapted. By studying distantly related species that live in the same habitat, we can see if the solutions to similar problems are different or analogous.

The unique qualities of each species also give us a deeper understanding of the constraints and the potential for change in fundamental processes. Where basic conflicts exist, control mechanisms adapt or the species goes extinct. To echo the beginning admonition of Bullock, to ignore comparative research would greatly limit our understanding of the evolution of hormone-brain-behavior relations.

mary

1. The timing of reproduction is subject to various constraints, including environmental, developmental, physiological, social and behavioral, and evolutionary factors.
2. These constraints have resulted in some animals in a dissociation between gonadal activity and sexual behavior in one or both of the sexes. In other animals they have led to the development of alternative mating strategies in males.
3. The behavioral interactions between the mating partners are important in facilitating and synchronizing the respective physiologys and advancing through the reproductive cycle.
4. There are a variety of sex-determining mechanisms in vertebrates, including genotypic, temperature, and behavioral sex determination. This means that the factors controlling the development of sexual dimorphisms may vary in fundamental ways in animals exhibiting different mechanisms of sex determination.
5. Sexuality resides in the brain, not in the gonad.
6. The adult phenotype results from a combination of hormonal and nonhormonal stimuli both early in development and later in adulthood. These might include the hormones or temperature experienced by an embryo or the behavior of the mother toward the young. Later in life these stimuli may include the behavioral experiences of the individual.
7. The neural circuits in the limbic forebrain the regulate social and sexual behavior are ancient and conserved among vertebrates.
8. Species differences in the distribution of sex steroid hormone–concentrating neurons are rare, but species differences in the abundance and regulation of sex steroid hormone receptors are common.
9. Progesterone and its receptor are important in the regulation of sexual behavior in male vertebrates.

10. Androgen and progesterone synergize in the control of the copulatory behavior of males much as estrogen and progesterone synergize in the control of the sexual receptivity of females.

Study Questions

1. Behavior and the neuroendocrine mechanisms that underlie it are subject to selection pressures. What environmental constraints shape the timing of reproductive behavior?
2. What is sexual selection and how might it be a factor in shaping the brain mechanisms that govern species-typical courtship behaviors?
3. What is phenotypic plasticity and what are some of the factors that influence it?
4. Reproduction requires behavioral and physiological synchronization of the participating animals. Explain what this means and give examples.
5. The organizational concept was developed from research on organisms that possess sex chromosomes. Does it apply to organisms that lack sex chromosomes? Discuss.

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Notes

1. Energy metabolism in the brain can be mapped using several techniques two of which are: 2-deoxyglucose uptake determines acute changes in tissue metabolic activity due to energy use over a period of minutes, and cytochrome histochemistry which assesses the chronic alteration in tissue metabolic capacity resulting from sustained metabolic demands over a period of weeks or months.
2. The fundamental difference between sexual and unisexual organisms makes it important to have subtle but necessary semantic rules. (i) Strictly speaking, it is inaccurate to refer to "male sexual behavior" (i.e., mounting and intromission behavior) and "female sexual behavior" (i.e., receptive behavior); each individual usually displays the behaviors characteristic of its gonadal sex (or homotypical behaviors), but has the capacity to exhibit behaviors characteristic of the opposite gonadal sex (or heterotypical behaviors). Thus, it is more accurate to refer to male-typical and female-typical sexual behaviors. (ii) Although parthenogenetic whiptails have only ovaries and lack male genitalia, it is not appropriate to refer to them as females. The term "female" only has meaning in the context of "male." (iii) Parthenogenetic whiptails exhibit behaviors seen commonly in sexually active male whiptails during mating. (These I term "pseudosexual" behaviors.) Since male-typical and female-typical sexual behaviors can only refer to those behavioral displays associated with males and females in gonochoristic species (e.g., intromission and receptive behaviors, respectively), it is not appropriate to use this terminology when describing the pseudosexual behaviors of parthenogenetic species. Therefore, because two sexes usually do not occur in unisexual organisms, the terms "male-like" and "female-like" rather than "male-typical" and "female-typical" are used to refer to the appropriate pseudosexual behaviors.

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