

David Crews

Institute of Reproductive Biology,
Departments of Zoology and Psychology,
University of Texas, Austin, Tex., USA

The Organizational Concept and Vertebrates without Sex Chromosomes

Key Words

- Steroid hormones
- Organizational concept
- Vertebrates
- Gonochorism
- Hermaphroditism
- Parthenogenesis
- Sex determination
- Sexual differentiation

Abstract

The diversity in vertebrate reproductive patterns provides natural experiments that yield new insights into behavioral endocrinology. Discussed here is the generality of the concept of an organizing sex during sexual differentiation. In its present form the Organizational Concept emphasizes hormonally induced organization of the male phenotype, with the female phenotype being the neutral or default condition. Does this concept extend to vertebrates lacking genotypic sex determining mechanisms? The answer appears to be No. In species with temperature-dependent sex determination, each embryo has an equal probability of developing into either a male or a female; there is no heritable genetic predisposition for sex determination. In species with behavior-dependent sex determination, sex-change occurs during adulthood as a result of perceived alterations in the social environment. In parthenogenetic species, only female individuals exist, yet they display both male-like and female-like 'sexual' behaviors. In contrast to the contemporary view of the Organizational Concept, let us assume that the male pattern is derived and imposed upon the ancestral female pattern. If this perspective is taken, several avenues of study are identified: (i) the importance of sexual similarities; (ii) extending the principle of complementarity of sexual behaviors to the brain; (iii) temperature modulation of sexual differentiation, and (iv) the role of the brain in sex determination.

In mammals, birds, and many other gonochoristic vertebrates (separate sexes in separate individuals), gonadal sex is determined by specific chromosomes inherited from the parents at fertilization (fig. 1, top panel). This is referred to as genotypic sex determination (GSD). There have been remarkable advances in molecular genetics over the last two years, resulting in the identification of the testis determining gene (SRY) on the Y chromosome in mice and man. SRY-like genes have been found in a variety of vertebrates, but their involvement in testis determination is not apparent. Indeed, comparative studies indicate that SRY is a large family of transcription factors and their presence is

not correlated with testis development in a variety of non-mammalian vertebrates [Tiersch et al., 1991, 1992].

Steroid hormones produced by the differentiated gonad are known to sculpt the differences between male and female, but they are not believed to be involved in sex determination [Wilson et al., 1981]. This modifying action of hormones during early development is referred to as the organizing action of sex steroids. Evidence of this fact comes from studies with a variety of eutherian mammals (but not metatherian mammals [Renfree, 1993]), demonstrating that perinatal castration of genetic males results in adults having female-typical morphology, physiology, and

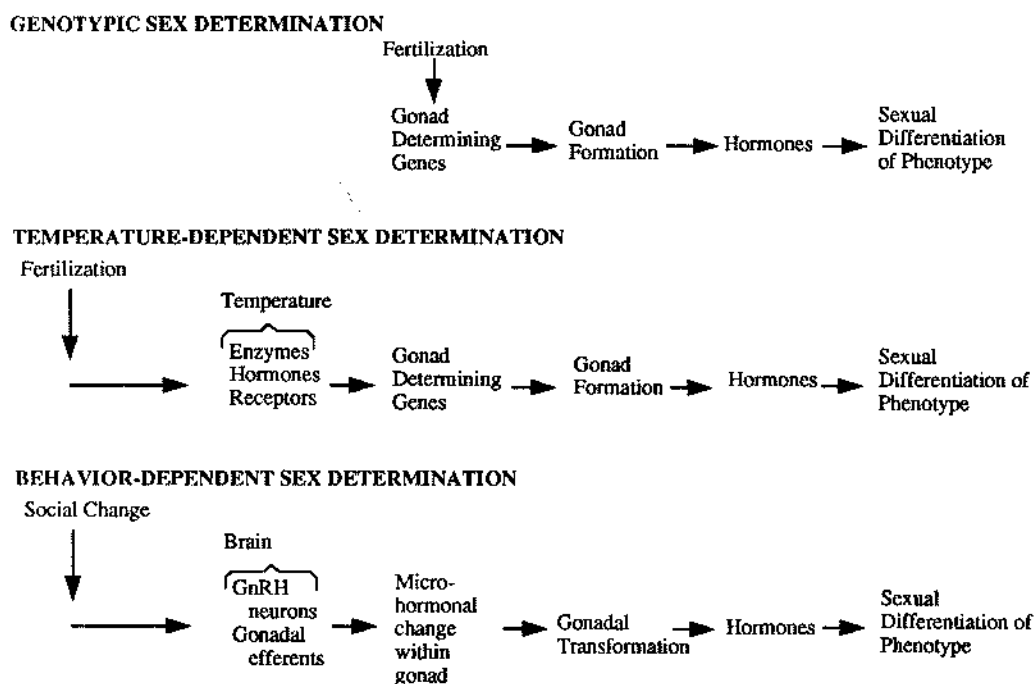


Fig. 1. In the current model of vertebrate sex determination and sexual differentiation gonadal sex is fixed at fertilization by the union of specific chromosomes (top panel). Only after the gonad is formed do hormones begin to exert an influence, sculpting specific structures that eventually will differ between the sexes. Research on reptiles with temperature-dependent sex determination (TSD) indicates that sex determination in TSD species is fundamentally different in at least one way (middle panel). 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 enzymes and hormone receptors such that sex-specific hormone milieus created in the urogenital system of the developing embryo determine gonad type. Research on fish with behavior-dependent sex determination (BSD) suggests that social stimuli encountered by the adult leads to sex change via the brain, acting first on hypothalamic GnRH-producing cells which, in turn, act on neurons that project to the gonads (bottom panel). The activity of these neurons modifies the endocrine environment within the gonad, bringing about gonadal transformation.

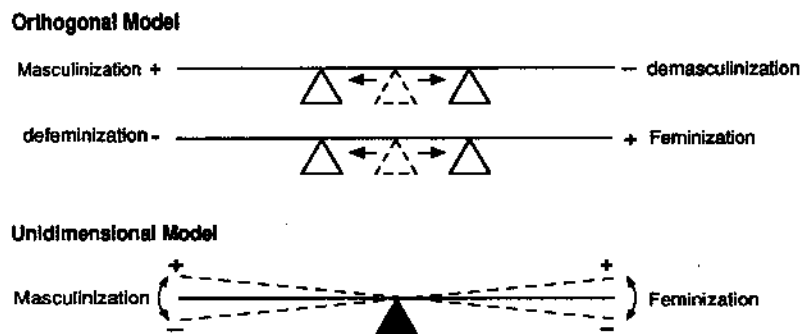
behavior, whereas perinatal androgen treatment of genetic females results in adults with a masculinized phenotype. This often is referred to the hormonal theory of sexual differentiation (hereafter termed the Organizational Concept), which holds that the male is the organized sex, while the female is the neutral or default sex; in birds the opposite is believed to pertain.

In 1974 Richard Whalen gathered together the then already large literature on the role of steroid hormones in the sexual differentiation of behavior [Whalen, 1974]. His article formalized speculation that reached as far back as the early 1900's that each individual was initially bisexual. This concept, termed the 'orthogonal model of sexual differentiation', holds that masculinization and feminization – the components of sexuality – are not ends of a unitary

dimension but rather separate and independent processes (fig. 2, top panel). Taking advantage of genetic anomalies and experimental studies with exogenous hormone treatments, it was shown that each individual possesses both dimensions and it is the relative development of each dimension that distinguishes males from females in their sexual behavior.

Thus, the essential features of the Organizational Concept then are (i) sex is determined at fertilization by chromosomes inherited from the parents, (ii) maleness is a consequence of testicular hormones acting throughout the body; (iii) the female is the neutral or default sex, whereas the male is the organized sex, and (iv) the differentiation of maleness and femaleness is reflected in two dimensions (masculinization ↔ demasculinization and

Fig. 2. Two models of sexual differentiation. The top panel depicts the orthogonal model in which the dimensions of maleness and femaleness are separate and independent. This model applies to GSD species. The bottom panel depicts the unidimensional model in which maleness and femaleness are at opposite ends of the same continuum. This model may apply to TSD species.



feminization ↔ defeminization), both residing within each individual.

Only comparative studies will inform us of the generality of the Organizational Concept. In this regard atypical organisms can be especially useful, for their unusual adaptations illustrate alternative solutions to particular problems. Also, they 'often force one to abandon standard methods and standard points of view' with the result that, 'in trying to comprehend their special and often unusual adaptation, one often serendipitously stumbles on new insights' [Bartholomew, 1982]. For example, in the present context, we now know the hormonal theory of sexual differentiation is not universal to vertebrates or even to all mammals. In marsupial mammals some somatic features (e.g., the pouch and scrotum) develop independently of hormones, under direct genetic control [Renfree, 1993]. This raises the question of whether the Organizational Concept can be applied to vertebrates that exhibit other modes of sexual inheritance?

Some gonochoristic vertebrates can be grouped collectively as having environmental sex determination. These vertebrates lack heritable chromosomes that determine gonadal sex. Instead, sex is determined after fertilization either by the environment in which the embryo develops or by the environment in which the adult lives. The first instance is common in oviparous reptiles where the incubation temperature of the egg determines gonadal sex. In temperature-dependent sex determination (TSD), incubation temperature modulates the steroid hormone microenvironment of the developing gonads to determine gonadal sex (fig. 1, middle panel). The second instance, behavior-dependent sex determination (BSD), is common in many coral reef fishes where the social environment determines gonadal sex. In BSD the individual's perception of the social environment is transduced into neural stimuli that change gonadal sex (fig. 1, bottom panel). In both forms of environmental sex determination, the individual (embryo

or adult) is equipotent and can develop either into a male or into a female.

Another mode of reproduction found in vertebrates is parthenogenesis. Parthenogenetic species consist only of individuals possessing a female phenotype; males are absent or exceedingly rare. There are two forms of parthenogenesis known in vertebrates: facultative or male-dependent parthenogenesis and obligate or true parthenogenesis. In facultative parthenogenesis the sperm initiate embryonic development and the parthenogen must mate with the male of a closely related sexual species: in hybridogenesis the male genome combines with the maternal genome in the zygote, whereas in gynogenesis, the male genome does not combine and the embryo develops under the influence of the maternal genome entirely. In obligate parthenogenesis only female individuals exist, and there is no need for the parthenogen to mate with males to initiate embryonic development.

I will first sketch the state of our knowledge of each of these forms before addressing the question of whether the Organizational Concept be applied to such species.

Temperature-Dependent Sex Determination

Egg temperature during the middle of embryogenesis determines sex in all crocodylians, many turtles, and some lizards, a process known as temperature-dependent sex determination (TSD) [Bull, 1980; Ewert and Nelson, 1991; Wibbels et al., 1992]. This effect of temperature is not due to differential mortality and is permanent, extending through adulthood [Bull, 1987; Gutzke and Crews, 1988]. Further, sex chromosomes have not been observed in any of the many species known to have TSD, although they are found in other reptiles [Bull, 1980]. Lastly, the effect of temperature is easily demonstrated in the laboratory and

also has been shown many times in the field [Erguson and Joanen, 1982; Bull, 1985].

Temperature operates in an all-or-none fashion; at intermediate temperatures the sex ratio varies rather than intersexes being formed [Wibbels et al., 1991a]. Temperature acts by modifying the temporal and spatial distribution of enzymes and hormone receptors. Hydroxysteroid dehydrogenase and aromatase enzyme levels are greatest during the period of sex determination. Interestingly, these enzymes are not present in the gonad, but are concentrated in the adrenal and mesonephros, tissues directly proximate to the developing gonad [Merchant-Larios et al., 1989; Thomas et al., 1992]. Temperature also modifies the expression and quantity of estrogen receptor (ER) in the adrenal and mesonephros [J. Bergeron, T. Wibbels and D. Crews, observ.]. Thus, the resulting temperature-specific steroid microenvironment [Dorizzi et al., 1991; Thomas et al., 1992; White and Thomas, 1992] is the proximate cue that determines gonadal sex.

It is widely assumed, although there is suggestive evidence to the contrary [George and Wilson, 1988], that in GSD species steroid hormones play no role in sex determination and application of exogenous steroid hormones do not alter the primary sex ratio. If estrogen (E) is administered to the eggs of reptiles with TSD, while they are being incubated at a temperature that normally produces only males, all of the hatchlings will have ovaries [Bull et al., 1988, Crews et al., 1989, 1991]. Not only does E share the same window of sensitivity as temperature, but incubation temperature and hormones synergize; less E is required to produce females as the all-female temperature is approached. This suggests that steroid hormones operate as the physiological equivalent of incubation temperature [Wibbels et al., 1991b]. The sexual competence of estrogen-determined females is comparable to normal temperature-determined females in all respects [D. Crews, observ.].

Since sex is determined by temperature, could the temperature that produces one sex be more potent than the temperature that produces the opposite sex, operating essentially as an organizing/default system? Both male-producing and female-producing temperatures appear to have a similar potency [Bull et al., 1990]. Instead, the effect of temperature is cumulative. Switching eggs from a temperature that produces one sex to a temperature that produces the opposite sex late in the temperature-sensitive period will have no effect unless the second temperature is more extreme than is normally necessary [Bull et al., 1990; Wibbels et al., 1991b].

Steroid hormones are not similar in potency however. Although E can override the effects of an all-male producing temperature, androgens or their agonists do not over-

come the effects of an all-female producing temperature. Administration of testosterone (T), dihydrotestosterone (DHT) or synthetic androgen receptor agonists to eggs incubated at a temperature that normally produces only females have consistently failed to produce males [Wibbels and Crews, 1992]. But is this finding equivalent functionally to an organizing vs. neutral temperature? Turtle eggs incubated so as to produce a 50:50 sex ratio are sensitive to both estrogens and androgens; exogenous E will result in females, whereas exogenous DHT induces development of male individuals [Wibbels et al., 1992]. Simultaneous administration of E and DHT to eggs incubating at this intermediate temperature results in gonadal hermaphrodites [T. Wibbels and D. Crews, unpubl. observ.].

Behavior-Dependent Sex Determination

Behavior-dependent sex determination (BSD) refers to socially-induced changes in gonadal sex. In simultaneous hermaphrodites the social situation directly determines the behavioral and gametic sex of the individual. Simultaneous hermaphrodites possess ovotestes and are bisexual in physiology and behavior and they commonly alternate in the display of behavioral and gametic sex, often while pairing with a single partner through several spawnings or the entire breeding season; it is rare for an individual to self-fertilize with the single exception to this rule being *Rivulus*, a small brackish water fish [Demski, 1987]. In sequential hermaphrodites the sex change is usually long-lasting and in the final stage, permanent. In sequential hermaphrodites, individuals are either first-male-and-then-female (protandry) or first-female-and-then-male (protogyny). In some species there may also be some individuals that never change sex.

Exogenous hormone treatment will cause a functional sex reversal in many fish species (in both directions), but this transformation takes weeks to complete. Thus, it is unlikely that sex-change in hermaphroditic fish is induced by steroid hormones. In a simultaneous hermaphrodite the time scale is simply too rapid to involve classical nuclear forms of steroid hormone receptors (see below). Even in sequentially hermaphroditic fish, the change in sexual behavior (whether from female-to-male or male-to-female) often is seen within minutes or hours after mating or removal of the dominant male (or female), yet detectable shifts in hormonal profiles do not occur until many days or weeks later; gonadal change takes weeks or months [Robertson, 1972; Demski, 1987; Reinboth, 1988; Warner and Swearer, 1991]. Indeed, years of intensive study have failed to show any role of steroid hormones in the *initiation* of sex-change [Reinboth, 1988].

There are lizards that reproduce by obligate parthenogenesis rather than by sexual means. These species, which descended from gonochoristic ancestors having GSD, consist now of only female individuals. In obligate parthenogenesis there is no need to mate with males of closely related sexual species to initiate embryonic development as occurs in gynogenesis and hybridogenesis (male-dependent parthenogenesis). In obligate parthenogenesis, the presence of males or a dependence on sperm (as in male-dependent parthenogenesis) are not necessary for reproduction. The sexual ancestral species of unisexual whiptail lizards (genus *Cnemidophorus*) is believed to have an XX:XY sex determining system [Bull, 1978], and incubation temperature has no detectable effect on sex determination in *Cnemidophorus* lizards [Crews, 1989a].

Parthenogenetic whiptails exhibit behaviors that appear identical to the mating behavior of related sexual species [Crews and Fitzgerald, 1980; Crews, 1989b]. This 'sexual' behavior is under hormonal control [Crews, 1989b] and serves some of the same functions as does sexual behavior in sexual species, most notably the facilitation of environmentally induced ovarian growth [Crews et al., 1986]. Further, the same brain regions that control mounting and receptive behavior in sexual whiptail lizards [Rozendaal and Crews, 1989; Wade and Crews, 1991a] regulate male-like and female-like 'sexual' behavior in unisexual whiptail lizards [Mayo and Crews, 1987; Crews, 1989b; Wade and Crews, 1991a]. The volume of nuclei in these brain areas [Crews et al., 1990; Wade and Crews, 1991b], as well as the soma size of individual cells therein [Wade and Crews, 1992] are sexually dimorphic in the sexual ancestral species. Interestingly, the brains of parthenoforms are uniformly female-like yet continue to manifest both male-like and female-like behavioral traits. There is no evidence for cyclic changes in brain nuclei or in neuron somata size correlated with changes in ovarian state; preovulatory (= female-like in behavior) parthenogens are equivalent to postovulatory (= male-like in behavior) parthenogens.

Does the Organizational Concept Extend to Vertebrates without Sex Chromosomes?

In its present formulation the Organizational Concept appears to be restricted to those gonochoristic vertebrates with GSD. In reptiles with TSD, the physical environment, rather than the genetic constitution an individual inherits from its parents, determines gonadal sex. In hermaphro-

ditic 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 'sexual' behaviors.

In its present form the Organizational Concept does not appear to apply to vertebrates lacking sex chromosomes. But a slightly broader view would include *all* vertebrates. [I wish to emphasize that this alternative view in no way challenges interpretations already assigned to empirical data from studies on vertebrates with GSD.]

There are two aspects of this alternative view (fig. 3). The first aspect has to do with evolution. There can be little doubt that males evolved only after the evolution of self-replicating (= female) organisms. Males have repeatedly been gained and lost, but females have remained. When speaking of mechanisms, therefore, and not outcomes such as the presence of male and female individuals, consider the female as the ancestral sex (rather than the neutral sex) and the male as the derived sex (rather than the organized sex). Unlike the present Organizational Concept developed from research largely on mammals, this formulation assumes that female development is an active process rather than a passive state. The second aspect has to do with emphasis. The present Organizational Concept emphasizes sex differences, or how males and females differ. However, if we are to understand fully sex differences, we must first appreciate the *similarities* in the neuroendocrine mechanisms subserving female-typical and male-typical sexual behavior.

When we consider this evolutionary sequence of female first, male second and, secondly, this shift in emphasis from the differences to the similarities between the sexes, we see from another angle this repeated observation that maleness is imposed upon a female phenotype, not vice versa. In the atypical vertebrates with ESD discussed here, the trigger initiating the sex determination cascade is not a genetic signal like SRY but nongenetic signals derived from other sources. (Even in protandrous fish Douglas Shapiro [1992] has concluded that males first pass through a modified ovarian stage before developing testes.) When the problem of sexual differentiation is viewed in this manner, the intriguing possibility emerges that males may be more like females than females are like males. This brings to mind the common finding that in mammals it is relatively easy to masculinize individuals compared to the difficulty of defeminizing individuals [Baum, 1987].

This alternative perspective identifies several problems worthy of study: (i) the importance of sexual similarities; (ii) extending the principle of complementarity of sexual behaviors to the brain; (iii) temperature modulation of

sexual differentiation, and (iv) the role of the brain in sex determination.

The Importance of Sexual Similarities

Presently the usual method in investigations of sex differences is to study the behaviors typical of each sex, or the neural circuits or the hormones underlying these behaviors. For example, the focus in most behavioral endocrinology investigations is the study of mounting in gonadal males or receptivity in gonadal females, or homotypical behaviors. Thus, while each circuit has been examined in detail, this almost always has been done in isolation of its complement. More study is needed of heterotypical behaviors or the display of male-typical behaviors in gonadal females and the display of female-typical traits in male individuals.

Is there any evidence that study of similarities between the sexes would yield new insights? One well documented example concerns the role of E in the control of sex behavior. In most female vertebrates E secreted by the growing ovarian follicles activates sexual receptivity. However, in males T secreted by the testes is aromatized to E in the brain of sexually active males and, in some species, E is the active molecule in males as well as in females [Sachs and Meisel, 1988]; recent evidence with songbirds suggests that the brain is the primary source of estrogen [Schlinger and Arnold, 1992].

Another example of sexual similarities in psychoneuroendocrine mechanisms may prove to be the role of progesterone (P) in facilitating hormone-induced sexual behaviors in both sexes. In many vertebrates P synergizes with E to facilitate the onset and intensity of receptivity in females; in species as diverse as rats, hamsters, guinea pigs, and the green anole lizard, P first facilitates and then inhibits E-induced receptivity [Pfaff and Schwartz-Giblin, 1988]. Estrogen promotes the synthesis of oxytocin receptors in the ventromedial hypothalamus, an area critical to the display of receptivity, while P facilitates the spread of oxytocin receptors to behavioral activating areas [Schumacher et al., 1990]. Oxytocin, along with E and P, plays an important role in the control of receptivity [Pfaff and Schwartz-Giblin, 1988].

Could P have a similar effect in males? It is widely assumed that progestagens inhibit androgen action in males. For example in a variety of birds and mammals, as well as in the green anole lizard [Young et al., 1991], the administration of exogenous P will inhibit sexual activity in intact males. Indeed, progestagens are used as a form of

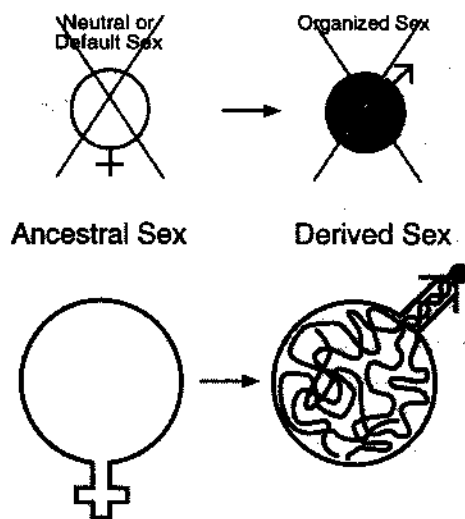


Fig. 3. Alternative views of sexual differentiation. The top portion depicts the present Organizational Concept as it is used to describe the sexual differentiation process. The female is regarded as the neutral or the default sex, whereas the male is regarded as the organized sex; in sexual differentiation research today, the emphasis is on the differences between the sexes. For reasons detailed in the text, this paradigm does not apply to vertebrates without sex chromosomes. The bottom portion depicts an alternative paradigm which combines the sequence in the evolution of sex and a change in emphasis. That is, the female is regarded as the ancestral sex, whereas the male is regarded as the derived sex. In species with genotypic sex determination, the trigger for male development is represented on the small Y chromosome, whereas in species lacking sex chromosomes, the trigger initiating the genetic cascade resulting in male development is non-genetic. This raises the possibility that males may be more like females than females are like males. Thus, an important avenue of research is to explore the similarities between the sexes as well as the differences.

'chemical castration' in felony rape conviction to decrease male libido.

However, all of the studies supporting this generalization that P is an antiandrogen and inhibits sexual behavior in males have relied on pharmacological dosages of P. What of the physiology of P in males? Male rats show a pronounced diurnal rhythm in P secretion, with the peak in P levels coinciding with the onset of dark, the period of greatest copulatory activity [Kalra and Kalra, 1977]. Further, low dosages of P have been found to be androgenic in vitro studies using some secondary sex structures [Bardin et al., 1984]. In gonochoristic whiptail lizards exogenous P or synthetic progestin will stimulate sexual behavior in some castrated males [Lindzey and Crews, 1986, 1988]; in both the whiptail and the green anole lizard, low dosages of P will synergize with T to facilitate androgen-induced mounting behavior in castrated males [Young et al., 1991; Lind-

zey and Crews, 1993]. In situ hybridization studies using homologous oligonucleotide probes for progesterone receptor (PR) show large amounts of PR-mRNA in the anterior hypothalamus-preoptic area (AH-POA) of intact, sexually active male *C. inornatus* [L. Young and D. Crews, unpubl. observ.].

If this finding of an involvement of P in male sexual behavior is ever to make it out of the comparative endocrine literature, I must show that P facilitation of mounting behavior occurs in male rats. Recent studies indicate that physiological dosages of P can reinstate mounting behavior in some castrated male rats, although it is not as effective as physiological levels of T (table 1). However, administration of the anti-progestin RU486 to intact males leads to a deficit in sexual behavior; this is not due to an action on androgen-mediated events as castrated males treated with both T and RU486 show levels of mounting and intromission similar to those of castrated males treated with T alone [D. Witt, T. Insell, L. Young, and D. Crews, unpubl. observ.]. Thus, P is both a 'female' hormone and a 'male' hormone. If this parallel is valid, the well-known oxytocin facilitation of mounting behavior in male rats [Arletti et al., 1985] may also be modulated by P.

Extending the Principle of Complementarity of Sexual Behaviors to the Brain

Normally, mating is a behavior made possible only by mutual consent, and if fertilization is to occur, two individuals must interact in a complementary fashion. 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. Indeed, the coordination of complementary behaviors between the sexes is necessary to normal physiological processes [Crews, 1992; Crews et al., 1992]. Thus, complementary behaviors are of fundamental importance in vertebrate reproduction, even in hermaphroditic and parthenogenetic (all-female) species.

Beach [1979] referred to this as the Principle of Stimulus-Response Complementarity. According to this principle, successful mating will occur if and only if both partners are in the appropriate physiological and behavioral condition *and* if the behaviors of each partner elicit the appropriate behavioral response. This can be restated as two simple phrases. 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. This sounds simple, but the principle has broad implications. It

Table 1. Role of progesterone in the control of mounting and intromission behavior of castrated male rats

Group	No.	% Mntg	% Intro
Intact	7	100	100
Int + RU486	8	63	80
*BL+BL	6	0	0
*BL+P	11	64	86
*BL+T	8	75	83
*P+T	7	100	71
*T+RU486	7	71	40

Expressed is percentage of animals mounting in standardized behavioral tests; % Intro indicates percentage of males that mounted that also achieved intromission. Hormones administered by a subcutaneous silastic capsule that delivered physiological (in the case of progesterone) or threshold (in the case of testosterone) levels of the respective hormones as determined by radioimmunoassay. The experiment was conducted in two replicates; the replicates did not differ and the results are combined here. * = Castrated males; BL = blank implant; P = progesterone implant; T = testosterone implant [D. Witt, L. Young, and D. Crews, unpublished observations.]

is independent of the genetic or gonadal sex of the participants, positing instead that homotypical stimuli are transduced into heterotypical responses and vice versa. This means that female (or male) associated responses tend to evoke masculine (or feminine) responses in both males and females. The display of 'sexual' behavior in all-female parthenogenetic species as well as in gonochoristic bisexual and hermaphroditic species means this behavioral complementarity, not just sperm and eggs, is fundamental to successful reproduction.

Can this principle of behavioral complementarity be extended to the brain? A number of researchers have found that the AH-POA, which we know to be involved in the control of mounting behavior in males, is sexually dimorphic in size and in the number and type of connections of individual neurons in these areas. It has been more difficult to establish a similar relationship for the VMH and receptivity. The gonochoristic whiptail lizards provide a particularly good example of such complementary sexually dimorphic hypothalamic areas [Crews et al., 1990].

It is common to hear, but not easy to find in the primary literature, that sex differences in behavior are *caused* by sex differences in brain structure. This intuitive leap, attractive as it is, has only been established conclusively for telencephalic areas such as the 'song' nuclei in passerine birds [Kelley and Brenowitz, 1992], and the motor neurons in the spinal cord controlling the penis in mammals [Breedlove, 1992]. A comparable causal *structure-activity* relationship

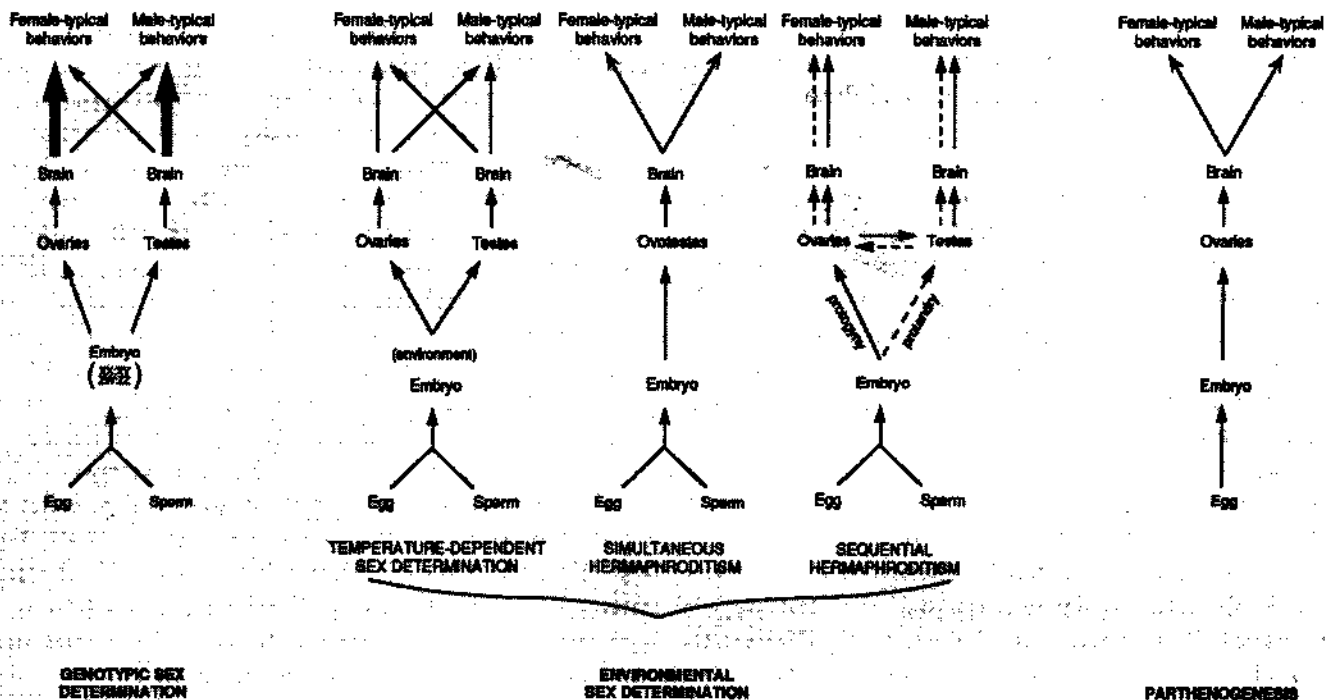


Fig. 4. All vertebrates display complementary sexual behavior patterns. However, they accomplish this by different means. Illustrated are the paths to the display of sexually dimorphic behaviors in the various forms of reproduction exhibited by vertebrates. Even in unisexual species, pseudosexual matings are seen. In both genotypic sex determination and environmental sex determination, it is important to separate effects due to the presence (or absence) of specific chromosomes or environmental factors from the effects of sex hormones. In genotypic sex determination the width of the arrows connotes early organizational effects of sex hormones on the probability that female-typical behaviors and male-typical behaviors will be shown by the breeding adult. Since comparable information is not available for the other forms of reproduction, all lines in other modes of reproduction are of equal width.

in brain and behavior has yet to be demonstrated convincingly for any limbic structure.

In this regard, the parthenogenetic whiptail lizards are particularly instructive. These animals exhibit complementary 'sexual' behaviors *without* showing corresponding changes in the hypothalamic areas known to be involved in the control of these behaviors [Wade and Crews, 1991b]. Indeed, administration of exogenous androgens either early in life or later in adulthood, which causes a parthenogen to exhibit a male-like phenotype and display frequently male-like mounting behaviors, has no obvious effect on the volumes of these brain areas or on the soma size of neurons contained therein [Wade et al., 1993]. Importantly, the same results are obtained when comparing these parameters between courting and noncourting males of the sexual ancestral species of similar physiological states [Wade et al., 1993]. Results such as these raise the possibility that

behavioral dimorphisms need not be determined by structural dimorphisms in the hypothalamus.

However, as shown in figure 4, while the outcome may be the same, the mechanisms achieving these outcomes must differ in species that exhibit environmental sex determination or in parthenogenetic species. In reptiles with TSD, each individual egg has an equal probability of becoming male or female. In simultaneous hermaphrodites, an individual possesses an ovotestis and releases sperm (and displays male-typical behavior) or eggs (and displays female-typical behavior) alternatively with its mate. In sequential hermaphrodites the individual develops first as one sex, complete with the appropriate behavior, and is then transformed to the other sex and displays the appropriate behavior. In the parthenogens, only female individuals occur, yet they exhibit both male-like and female-like 'sexual' behaviors.

In TSD the genetic constitution inherited from the parents does not determine gonadal sex. This raises the question of whether the process of sexual differentiation of behavior is according to the orthogonal model or whether sexuality is represented by a single continuum of male ↔ female (fig. 2, bottom panel). That is, increases in male-typical traits will be linked to decreases in female-typical traits. This hypothesis can be tested by studies of species in which (i) one sex is produced (represented) over a wide range of temperatures and (ii) incubation temperature is dissociated experimentally from gonadal sex. When this is done we see that temperature can modify sexually dimorphic traits without modifying gonadal sex. Maintaining eggs of the leopard gecko at relatively low (26 °C) or at very high (34–35 °C) and ordinarily lethal incubation temperatures produces only females [Viets et al., 1993]. At intermediate temperatures both males and females are produced, but the sex ratio varies: 30 °C yields a female-biased sex ratio (80:20) and 32.5 °C yields a male-biased sex ratio (20:80).

The temperature experienced during incubation has a profound effect on development of the adult phenotype. For example, adult leopard geckos have marked sexual dimorphisms in morphology, physiology, and behavior. One secondary sex character is the specialized secretory pores located anterior to the cloaca. In males, as well as in females from male-biased incubation temperatures, these pores are open, while in females from low, or female-biased incubation temperature, they are closed. Head size also is sexually dimorphic, with males having wider heads than females. However, within each sex, the higher the incubation temperature, the wider the head of the adult [Crews, 1988].

As in many mammalian species, male leopard geckos are the larger sex. At 65 weeks of age (adulthood), a male from a 32.5 °C incubation temperature will weigh about 50 g, whereas a female from a 26 °C incubation temperature will weigh about 35 g. The temperature experienced during incubation has a profound effect on both growth rate and adult body weight (fig. 5) [Crews et al., 1993]. Females from a male-biased incubation temperature (32.5 °C) grow larger than do females from a female-biased incubation temperature (30 °C), indeed as large and as rapidly as 30 °C males (fig. 5, top). In mammals, castration early in life attenuates growth, whereas ovariectomy has no effect [Wade, 1976]. However, if the ovaries are removed from female leopard geckos from 26 °C incubation conditions on the day of hatching, they will grow as would a male from a 32.5 °C incubation temperature. The fundamental importance of temperature is seen best in individuals that receive hormone treatment during embry-

onic development. As in other TSD reptiles, administration of estrogen to the egg insures female development [Bull et al., 1988]. Such estrogen-determined females grow as rapidly as do males from the same incubation temperature (fig. 5, bottom).

Incubation temperature also modifies the endocrine physiology of adult leopard geckos. As would be expected, there is a difference in the nature and pattern of concentrations of sex steroids between the sexes. Among females, androgen levels are significantly higher, and estrogen levels significantly lower, in individuals incubated at male-biased incubation temperatures compared to individuals from a female-biased incubation temperature [Gutzke and Crews, 1988]. Males from a female-biased incubation temperature have significantly higher levels of estrogen than males from a male-biased incubation temperature [Tousignant and Crews, 1992].

In sexually differentiated species, does maleness develop independently of femaleness (and vice versa)? The Organizational Concept regards the female as the neutral or default sex and the male as the organizing sex (that is, if you are not male, you are a female), and maleness and femaleness as separate and independent dimensions residing within each individual. However, even in GSD species genetic males naturally tend to be masculinized and defeminized, which contradicts this premise of the independence of sex-typical mechanisms. On the basis of evolutionary theory, Jim Bull predicted that a mutation that is beneficial to one sex may be harmful to the opposite sex. William Rice recently has demonstrated in empirical studies that this indeed occurs in fruitflies [Rice, 1992]. This raises the question of whether, in the brain, specifically in the neuroendocrine events that underlie the expression of sex-typical displays, one mechanism (e.g., that controlling male-typical behaviors or physiology) interferes with the adaptive evolution of another mechanism (e.g., that controlling female-typical behaviors or physiology)?

The sexual behaviors of individual geckos from different incubation temperatures also vary. In this species, attractiveness, the ability of a stimulus animal to elicit high-intensity courtship from a male, is a female-typical trait. Aggression toward another individual is a male-typical trait. Females from a low all-female producing incubation temperature (26 °C) are highly attractive to males, readily exhibit receptivity when courted, and show no evidence of aggression toward either male or female stimulus animals. Aggression begins to be exhibited by females from higher incubation temperatures, with females from male-biased temperatures (32.5 °C) being the most aggressive [Flores et al., 1992]. Further, females from higher incubation temperatures (30.0 and 32.5 °C) are significantly less attractive than 26 °C females, with those from 32.5 °C being the least attractive.

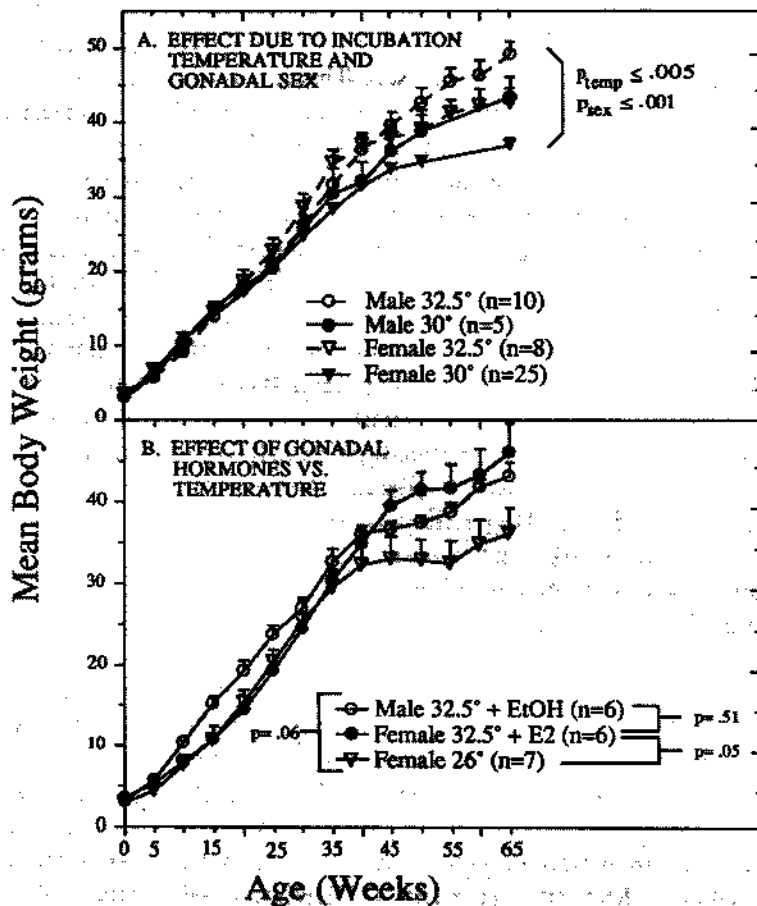


Fig. 5. The relative influence of incubation temperature and gonadal sex in the leopard gecko, a species with temperature-dependent sex determination. Illustrated is the average body weight (\pm standard error) from different incubation temperatures or hormonal manipulations; 26.0 °C produces all females, 30.0 °C produces a female-biased sex ratio, and 32.5 °C produces a male-biased sex ratio. Each individual was weighed every five weeks from hatching until adulthood. All animals are raised in isolation at a 14:10 h/30:18 °C daily photothermal regimen and fed a standard diet. Top: Males and females incubated at either 30.0 or 32.5 °C. Bottom: Animals manipulated as eggs.

Taken together these results suggest that both temperature and internal secretions interact in the development of sexual dimorphisms. The development of sexuality in the leopard gecko thus may fit a unidimensional model of the differentiation of sexual behavior rather than the orthogonal model that has proven so useful with GSD species. These results also show how both prenatal environmental and endocrine factors can serve to organize the adult phenotype. In the leopard gecko a hierarchy of controls exist in the sexual differentiation process, with incubation temperature being of primary importance and gonadal steroids of secondary importance.

Bull [1980] proposed that sex determining mechanisms such as TSD are an evolutionary precursor to GSD mechanisms. If true, there should be remnants of TSD-like mechanisms in GSD species, even though homeotherms are buffered from changes in temperature. That is, even in mammals, temperature may be capable of modulating the organizing action of steroid hormones. We tend to think

that young mammals and birds develop under constant conditions. However, it is important to realize that even in mammals, temperatures vary both within individuals (e.g., diurnal variation; pregnant vs. non-pregnant states) and between individuals (e.g., individual differences). Several laboratories, notably those of Satinoff [1991] and Williams [1991], find that by altering the temperature slightly the behavioral capabilities of rat pups are dramatically affected. This finding that temperature can play such an important role is significant, for it appears that temperature may be an important but overlooked variable in sexual differentiation in GSD species.

The Role of the Brain in Sex Determination

In behavior-dependent sex determination (BSD), an individual's perception of the social environment ultimately changes its gonadal sex (fig.1, bottom panel). What

mechanism could underlie this transformation? The almost immediate change in behavior following either spawning (in a simultaneous hermaphrodite) or the removal of the suppressing fish (in a sequential hermaphrodite) means that the brain must be centrally involved in the process of sex-change. Davis and Fernald [1990] demonstrated that in a gonochoristic African cichlid fish neurons containing gonadatropin-releasing hormone (GnRH) in the preoptic area of males change in size according to the individual's status (territorial vs. nonterritorial) [see Francis, 1992, for review]. These same populations of GnRH cells occur in hermaphroditic fish [Grober and Bass, 1991; Grober et al., 1991]. These neurons in turn might synapse on other hypothalamic neurons projecting to the gonad.

In all vertebrates, including hermaphroditic fish, there are afferent and efferent neural connections between the gonad and hypothalamus. Denervation of the gonad leads to gonadal atrophy [Burden and Lawrence, 1977; Gerendai et al., 1978; Lipner, 1988], and changes in the gonad are paralleled by changes in neurochemistry in discrete hypothalamic areas [Subhedar and Alandikar, 1982; Subhedar et al., 1987; Jones et al., 1990; Lara et al., 1990; Subhedar and Rama Krishna, 1990]. The efferent nerves terminate on steroidogenic cells in the gonad [Baumgarten and Holstein, 1968; Gresik, 1973; Unsicker, 1973; Jones et al., 1990; Lara et al., 1990] and electrical stimulation of appropriate hypothalamic areas can either increase or decrease gonadal steroid secretion [Kawakami et al., 1981; Damber, 1990]. Further, electrical stimulation in different and specific hypothalamic areas results in sperm or egg release in a simultaneous hermaphroditic sea bass [Demski, 1987]. Finally, gonadal afferents have been found to dictate sexual behavior in both males and females [Crews, 1978, 1980].

Thus, five facts are now well-established: (i) Sex steroid hormones will functionally sex-reverse fish, but only over a prolonged time course. (ii) GnRH-containing cells in the brain are sensitive to changes in social status. There is (iii) direct brain-gonad innervation, and some of these nerves (iv) terminate on steroidogenic cells in the gonad, which are (v) proximate to the gonial cells. Taken together this suggests that an individual's perception of the environment may act ultimately via these brain-gonad pathways to mediate the behavioral role adopted (in a simultaneous hermaphrodite) or the process of sex-change (in a sequential hermaphrodite).

Neural innervation of primary and secondary sex characters may ultimately account for a variety of other well-known phenomena. For example, removal of the left ovary in chickens often results in the vestigial right gonad becoming a functional testis [van Tienhoven, 1961]. The removal

of hormonal suppression is the traditional interpretation of these results. However, in these studies manipulated animals were raised together. It is equally plausible that the social environment of such individuals may determine what the vestigial gonad will become. That is, a testis may develop if the individual is housed with gonadally intact females, or an ovary if the individual is housed with gonadally intact males. Similarly, in male toads there is a rudimentary ovary, attached to the testes, called the Bidder's organ. Removal of the testes in male toads causes the Bidder's organ to become a functional ovary [Noble, 1954]. It is assumed that testicular androgens suppress Bidder's organ and prevent it from becoming a functional ovary. However, the necessary castration-hormone replacement studies have not been done, so one cannot discount the possible role of neural innervation of Bidder's organ to account for its transformation following castration. Lastly, neural mediation may be the mechanism by which significantly female-biased sex ratios are produced. Experimental tests of these hypotheses would be straight-forward.

Conclusion

Viewing diverse vertebrates as natural experiments extends and expands our understanding of the sexual differentiation process. Alternative model systems can reveal overlooked phenomena, such as the possibility of progesterone being as much a 'male' hormone as it is a 'female' hormone, the possibility that sexually dimorphic limbic areas need not be related to sexually dimorphic behaviors, that temperature may be an important factor in sexual differentiation, that the brain may be involved in sex determination, and how a comparative perspective can open new areas of investigation.

Atypical animals can also raise new questions about old answers. In TSD incubation temperature determines sex in an all-or-none fashion and each embryo can develop into either a male or female. While sexual differentiation appears similar to that of GSD species, the trigger initiating the process differs. Thus, prenatal environmental factors together with steroid hormones influence the development of sexually dimorphic traits. Is it reasonable to speculate that the same processes occur in mammals and birds but, because of homeothermy, are masked? The possibility of a central role for brain-gonad connections in sex-changing fish points to the potential for other similar relationships in behavioral neuroscience. All-female animals exhibiting bisexual behavior speaks to the retention of male-specific elements in evolution.

Acknowledgments

I thank J. Alcock, M. Baum, J. Bull, L. Eisenberg, J. Herbert, E.B. Keverne, M. Kirkpatrick, M. Konishi, J. Lindzey, F. Nottebohm, M. Ryan, J. Rosenblatt, A. Tousignant, J. Vandenberg, J.

Wade, W. Wilczynski, and L. Young for discussion of these ideas and reading previous versions of this paper. Thanks also to Dr. Thomas Karger for his continuing support of these Workshops. Supported by a NIMH Research Scientist Award.

References

- Arletti, R., C. Bazzani, M. Castelli, and A. Bertolini (1985) Oxytocin improves male copulatory performance in rats. *Horm. Behav.*, 19: 14-20.
- Bardin, C.W., T. Brown, V.V. Isomaa, and O.A. Jame (1984) Progestins can mimic, inhibit and potentiate the actions of androgens. *Pharm. Ther.*, 23: 443-459.
- Bartholomew, G.A. (1982) Scientific innovation and creativity: A zoologist's point of view. *Amer. Zool.*, 22: 227-235.
- Baum, M.J. (1987) Hormonal control of sex differences in the brain and behavior of mammals. In *Psychobiology of Reproductive Behavior: An Evolutionary Perspective* (ed. by D. Crews), Prentice-Hall, Englewood Cliffs, pp. 231-257.
- Baumgarten, H.G., and A.F. Holstein (1968) Adrenerge Innervation im Hoden und Nebenhoden vom Schwan (*Cygnus olor*). *Z. Zellforsch.*, 91: 402-410.
- Beach, F.A. (1979) Animal models for human sexuality. In *Sex, Hormones and Behavior* (ed. by R. Potter, and J. Whelan), Excerpta Medica, Amsterdam, pp. 113-143.
- Breedlove, S.M. (1992) Sexual differentiation of the brain and behavior. In *Behavioral Endocrinology* (ed. by J.B. Becker, S.M. Breedlove, and D. Crews), The MIT Press, Cambridge, Massachusetts, pp. 39-68.
- Bull, J.J. (1978) Sex chromosome differentiation in reptiles. *Can. J. Genet. Cytol.*, 20: 205-209.
- Bull, J.J. (1980) Sex determination in reptiles. *Q. Rev. Biol.*, 55: 3-21.
- Bull, J.J. (1985) Sex ratio and nest temperature in turtles: comparing field and laboratory data. *Ecol.*, 66: 1115-1122.
- Bull, J.J. (1987) Temperature-dependent sex determination in reptiles: validity of sex diagnosis in hatchlings. *Can. J. Zool.*, 65: 1421-1424.
- Bull, J.J., W.H.N. Gutzke, and D. Crews (1988) Sex reversal by estradiol in three reptilian orders. *Gen. Comp. Endocrinol.*, 70: 425-428.
- Bull, J.J., T. Wibbels, and D. Crews (1990) Sex determining potencies vary among female incubation temperatures in a turtle. *J. Exp. Zool.*, 256: 339-341.
- Burden, H.W., and I.E. Lawrence Jr. (1977) The effect of denervation on compensatory ovarian hypertrophy. *Neuroendocrinology*, 23: 368-378.
- Crews, D. (1978) Hemipenile preference: Stimulus control of male mounting behavior in the lizard, *Anolis carolinensis*. *Science*, 199: 195-196.
- Crews, D. (1980) Interrelationships among ecological, behavioral and neuroendocrine processes in the reproductive cycle of *Anolis carolinensis* and other reptiles. In *Advances in the Study of Behavior* (ed. by J.S. Rosenblatt, R.A. Hinde, C.G. Beer, and M.C. Busnel), Academic Press, New York, pp. 1-74.
- Crews, D. (1988) The problem with gender. *Psychobiology*, 16: 321-334.
- Crews, D. (1989a) Absence of temperature-dependent sex determination in congeneric sexual and parthenogenetic *Cnemidophorus* lizards. *J. Exp. Zool.*, 252: 318-320.
- Crews, D. (1989b) Unisexual organisms as model systems for research in the behavioral neurosciences. In *Evolution and Ecology of Unisexual Vertebrates* (ed. by R.M. Dawley, and J.P. Bogart), New York State Museum, Albany, NY, pp. 132-143.
- Crews, D. (1992) Behavioral endocrinology and reproduction: An evolutionary perspective. In *Oxford Reviews in Reproductive Biology* (ed. by S. Milligan), Oxford University Press, pp. 303-370.
- Crews, D., K.T. Fitzgerald (1980) 'Sexual' behavior in parthenogenetic lizards (*Cnemidophorus*). *Proc. Natl. Acad. Sci. USA*, 77: 499-502.
- Crews, D., J.J. Bull, and T. Wibbels (1991) Estrogen and sex reversal in turtles: dosages producing both sexes produces few intersexes. *Gen. Comp. Endocrinol.*, 81: 357-364.
- Crews, D., M. Grassman, and J. Lindzey (1986) Behavioral facilitation of reproduction in sexual and unisexual whiptail lizards. *Proc. Nat. Acad. Sci. USA*, 83: 9547-9550.
- Crews, D., A. Tousignant, and T. Wibbels (1993) Some considerations for inducing reproduction in captive reptiles. In *Captive Management and Conservation of Amphibians and Reptiles* (ed. by J.B. Murphy, and J. T. Collins), Society for the Study of Amphibians and Reptiles, in press.
- Crews, D., J. Wade, and W. Wilczynski (1990) Sexually dimorphic areas in the brain of whiptail lizards. *Brain, Behav. Evol.*, 36: 262-270.
- Crews, D., T. Wibbels, and W.H.N. Gutzke (1989) Action of sex steroid hormones on temperature-induced sex determination in the snapping turtle (*Chelydra serpentina*). *Gen. Comp. Endocrinol.*, 75: 159-166.
- Damber, J.E. (1990) The effect of guanethidine treatment of testicular blood flow and testosterone production in rats. *Experientia*, 46: 486-487.
- Davis, M.R., and R.D. Fernald (1990) Social control of neuronal cell size. *J. Neurobiol.*, 21: 1180-1188.
- Demski, L.S. (1987) Diversity in reproductive patterns and behavior in teleost fishes. In *Psychobiology of Reproductive Behavior: An Evolutionary Perspective* (ed. by D. Crews), Prentice Hall, Inc., Englewood Cliffs, pp. 2-27.
- Dorizzi, M., T.-M. Mignot, A. Guichard, G. Desavages, and C. Pieau (1991) Involvement of oestrogens in sexual differentiation of gonads as a function of temperature in turtles. *Differentiation*, 47: 9-17.
- Ewert, M.A., and C.E. Nelson (1991) Sex determination in turtles: Diverse patterns and some possible adaptive values. *Copeia*, 1991: 50-69.
- Ferguson, M.W.J., and T. Joanen (1982) Temperature of egg incubation determines sex in *Alligator mississippiensis*. *Nature*, 296: 850-853.
- Flores, D.L., A.J. Tousignant, and D. Crews (1992) Influence of incubation temperature on aggressive behavior and attractiveness in the female leopard gecko, *Eublepharis macularis*. *Am. Zool.*, 32: 9A.
- Francis, R.C. (1992) Sexual lability in teleosts: Developmental factors. *Q. Rev. Biol.*, 67: 1-18.
- George, F.W., and J. Wilson (1988) Sex determination and differentiation. In *The Physiology of Reproduction* (ed. by E. Knobil, and J.D. Neill), Raven, Press, New York, pp. 3-26.
- Gerendai, I.B., S. Maugeri, M.A. Roxas, and U. Scapagnini (1978) Prevention of compensatory ovarian hypertrophy by local treatment of the ovary with 6-OHDA. *Neuroendocr.*, 27: 272-278.
- Gresik, E.W. (1973) Fine structural evidence for the presence of nerve terminals in the testis of the teleost, *Oryzias latipes*. *Gen. Comp. Endocrinol.*, 21: 210-213.
- Grober, M.S., and A.H. Bass (1991) Neuronal correlates of sex/role change in labrid fishes: LHRH-like immunoreactivity. *Brain Behav. Evol.*, 38: 302-312.
- Grober, M.S., I.M.D. Jackson, and A.H. Bass (1991) Gonadal steroids affect LHRH preoptic cell number in a sex/role changing fish. *J. Neurobiol.*, 22: 734-741.
- Gutzke, W.H.N., and D. Crews (1988) Embryonic temperature determines adult sexuality in a reptile. *Nature*, 332: 832-834.
- Jones, R.E., P.H. Desan, K.H. Lopez, and H.B. Austin (1990) Asymmetry in diencephalic monoamine metabolism is related to side of ovulation in reptile. *Brain Res.*, 50: 187-191.
- Kalra, U.S., and S.P. Kalra (1977) Circadian periodicities of serum androgens, progesterone, gonadotropins and luteinizing hormone-releasing hormone in male rats: the effects of hypothalamic deafferentation, castration and adrenalectomy. *Endocrinology*, 101: 1821-1827.

- Kawakami, M., K. Kubo, T. Uemura, M. Nagase, and R. Hayashi (1981) Involvement of ovarian innervation in steroid secretion. *Endocrinology*, 109: 136-145.
- Kelley, D.B., and E. Brenowitz (1992) Hormonal influences on courtship behaviors. In *Behavioral Endocrinology* (ed. by J.B. Becker, S.M. Breedlove, and D. Crews), The MIT Press, Cambridge, Massachusetts, pp. 187-216.
- Lara, H.E., D.F. Hill, K.H. Katz, and S.R. Ojeda (1990) The gene encoding nerve growth factor is expressed in the immature rat ovary: effect of denervation and hormonal treatment. *Endocrinology*, 126: 357-363.
- Lindzey, J., and D. Crews (1986) Hormonal control of courtship and copulatory behavior in male *Cnemidophorus inornatus*, a direct ancestor of a unisexual, parthenogenetic lizard. *Gen. Comp. Endocrinol.*, 64: 411-418.
- Lindzey, J., and D. Crews (1988) Effects of progestins on sexual behavior in castrated lizards (*Cnemidophorus inornatus*). *J. Endocrinol.*, 119: 265-273.
- Lindzey, J., and D. Crews (1993) Effects of progesterone and dihydrotestosterone on stimulation of androgen-dependent sex behavior, accessory sex structures, and *in vitro* binding characteristics of cytosolic androgen receptors in male whiptail lizards (*Cnemidophorus inornatus*). *Horm. Behav.*, 27: 269-281.
- Lipner, H. (1988) Mechanisms of mammalian ovulation. In *The Physiology of Reproduction* (ed. by E. Knobil, and J.D. Neill), Raven Press, New York, pp. 455-459.
- Mayo, M.L., and D. Crews (1987) Neural control of male-like pseudocopulatory behavior in the all-female lizard, *Cnemidophorus uniparens*: Effects of intracranial implantation of dihydrotestosterone. *Horm. Behav.*, 21: 181-192.
- Merchant-Larios, H., I.V. Fierro, and B.C. Urruiza (1989) Gonadal morphogenesis under controlled temperature in the sea turtle *Lepidochelys olivacea*. *Herpetol. Monog.* 3: 43-61.
- Noble, G.K. (1954) *The Biology of the Amphibia*. McGraw-Hill Book Co., Inc., New York, 577 pp.
- Pfaff, D.W., and S. Schwartz-Giblin (1988) Cellular mechanisms of female reproductive behaviors. In *The Physiology of Reproduction* (ed. by E. Knobil, and J.D. Neill), Raven Press, New York, pp. 1487-1568.
- Reinboth, R. (1988) Physiological problems of teleost ambisexuality. *Env. Biol. Fishes.* 2: 455-488.
- Renfree, M. (1993) Gonadal and genital differentiation: the sexual takeover of the excretory system. In *The Differences Between the Sexes* (ed. by R. Short, and E. Balaban), in press.
- Rice, W.R. (1992) Sexually antagonistic genes: experimental evidence. *Science*, 256: 1436-1439.
- Robertson, D.R. (1972) Social control of sex reversal in a coral reef fish. *Science*, 177: 1007-1009.
- Rozendaal, J.C., and D. Crews (1989) Effects of intracranial implantation of dihydrotestosterone on sexual behavior in male *Cnemidophorus inornatus*, a direct sexual ancestor of a parthenogenetic lizard. *Horm. Behav.*, 23: 194-202.
- Sachs, R.D., and R.L. Meisel (1988) The physiology of male sexual behavior. In *The Physiology of Reproduction* (ed. by E. Knobil, and J.D. Neill), Raven Press, New York, pp. 1393-1485.
- Satinoff, E. (1991) Developmental aspect of behavioral and reflexive thermoregulation. In *Developmental Psychobiology: New Methods and Changing Concepts* (ed. by H.N. Shair, G.A. Barr, and M.A. Hofer), Oxford University Press, New York, pp. 169-188.
- Schlinger, B.A., and A.P. Arnold (1992) Circulating estrogens in a male songbird originate in the brain. *Proc. Natl. Acad. Sci. USA*, 89: 7650-7653.
- Schumacher, M., H. Coirini, D.W. Pfaff, and B.S. McEwen (1990) Behavioral effects of progesterone associated with rapid modulation of oxytocin receptors. *Science*, 250: 691-694.
- Shapiro, D.Y. (1992) Plasticity of gonadal development and proterandry in fishes. *J. Exp. Zool.*, 261: 194-203.
- Subhedar, N., and V.V. Alandikar (1982) Effect of electrical stimulation of the testis on the nucleus praepopticus magnocellularis of the frog, *Rana tigrina*. *Cell Tissue Res.*, 224: 45-53.
- Subhedar, N., and N.S. Rama Krishna (1990) The response of nucleus praepopticus neurosecretory cells to ovarian pressure in the frog, *Rana tigrina*. *Gen. Comp. Endocrinol.*, 80: 438-450.
- Subhedar, N., N.S. Rama Krishna, and M.K. Deshmukh (1987) The response of nucleus praepopticus neurosecretory cells to ovarian pressure in the teleost, *Clarias batrachus* (Linn.). *Gen. Comp. Endocrinol.*, 68: 357-368.
- Thomas, E.O., P. Licht, T. Wibbels, and D. Crews (1992) Development of hydroxysteroid dehydrogenase activity in sexually undifferentiated and differentiated embryos of the turtle *Trachemys (Pseudemys) scripta*. *Biol. Reprod.*, 46: 140-145.
- Tiersch, T.R., M.J. Mitchell, and S.S. Wachtel (1991) Studies on the phylogenetic conservation of the SRY gene. *Hum. Genet.*, 87: 571-573.
- Tiersch, T.R., B.A. Simco, K.B. Davis, and S.S. Wachtel (1992) Molecular genetics of sex determination in channel catfish: studies on SRY, ZFY, Bkm and human telomeric repeats. *Biol. Reprod.*, 47: 185-192.
- Tousignant, A., and D. Crews (1992) Incubation temperature and gonadal sex affect growth and reproductive physiology in the leopard gecko, *Eublepharis macularius*. *Am. Zool.*, 32: 21A.
- Unsicker, K. (1973) Innervation of the testicular interstitial tissue in reptiles. *Z. Zellforsch.*, 146: 123-138.
- van Tienhoven, A. (1961) Endocrinology of reproduction in birds. In *Sex and Internal Secretions* (ed. by W.C. Young), The Williams and Wilkins Co., Baltimore, pp. 1088-1169.
- Viets, B.E., A. Tousignant, M.A. Ewert, C.E. Nelson, and D. Crews (1993) Temperature-dependent sex determination in the leopard gecko, *Eublepharis macularius*. *J. Exp. Zool.*, 265: 679-683.
- Wade, G.N. (1976) Sex hormones, regulatory behaviors, and body weight. In *Advances in the Study of Behavior* (ed. by J.S. Rosenblatt, R.A. Hinde, E. Shaw, and C. Beer), Academic Press, New York, pp. 201-267.
- Wade, J., and D. Crews (1991a) The effects of intracranial implantation of estrogen on receptivity in sexually and asexually reproducing female whiptail lizards, *Cnemidophorus uniparens* and *C. inornatus*. *Horm. Behav.*, 25: 342-353.
- Wade, J., and D. Crews (1991b) The relationship between reproductive state and 'sexually' dimorphic brain areas in sexually reproducing and parthenogenetic whiptail lizards. *J. Comp. Neurol.*, 309: 507-514.
- Wade, J., and D. Crews (1992) Sexual dimorphisms in the soma size of neurons in the brain of whiptail lizards (*Cnemidophorus* species). *Brain Res.*, 594: 311-314.
- Wade, J., J.-M. Huang, and D. Crews (1993) Hormonal control of sex differences in the brain, behavior, and accessory sex structures of whiptail lizards (*Cnemidophorus* species). *J. Neuroendocrinol.*, 5: 81-93.
- Warner, R.R., and S.E. Swearer (1991) Social control of sex change in the bluehead wrasse, *Thalassoma bifasciatum* (Pisces: Labridae). *Biol. Bull.*, 181: 199-204.
- Whalen, R.E. (1974) Sexual differentiation: models, methods, and mechanisms. In *Sex Differences in Behavior* (ed. by R.C. Friedman, R.H. Richart, and R.L.V. Wiele), John Wiley & Sons, New York, pp. 467-484.
- White, R.B., and P. Thomas (1992) Adrenal-kidney and gonadal steroidogenesis during sexual differentiation of a reptile with temperature-dependent sex determination. *Gen. Comp. Endocrinol.*, 88: 10-19.
- Wibbels, T., and D. Crews (1992) Specificity of steroid hormone-induced sex determination in a turtle. *J. Endocrinol.*, 133: 121-129.
- Wibbels, T., J.J. Bull, and D. Crews (1991a) Chronology and morphology of temperature-dependent sex determination. *J. Exp. Zool.*, 260: 371-381.
- Wibbels, T., J.J. Bull, and D. Crews (1991b) Synergism between temperature and estradiol: A common pathway in turtle sex determination? *J. Exp. Zool.*, 260: 130-134.
- Wibbels, T., J.J. Bull, and D. Crews (1992) Hormone-induced sex determination in an amniotic vertebrate. *J. Exp. Zool.*, 262: 454-457.
- Williams, C.L. (1991) Development of a sexually dimorphic behavior: hormonal and neural controls. In *Developmental Psychobiology: New Methods and Changing Concepts* (ed. by H.N. Shair, G.A. Barr, and M.A. Hofer), Oxford University Press, New York, pp. 206-222.
- Wilson, J.D., F.W. George, and J.E. Griffen (1981) The hormonal control of sexual development. *Science*, 211: 1278-1284.
- Young, L.J., N. Greenberg, and D. Crews (1991) Antiandrogenic and synandrogenic effects of progesterone on sexual behavior in male green anole lizards (*Anolis carolinensis*). *Horm. Behav.*, 25: 477-488.