

Historical contributions of research on reptiles to behavioral neuroendocrinology

David Crews*, Michael C. Moore

Section of Integrative Biology, University of Texas, Austin, TX 78712, USA
School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA

Received 8 February 2005; revised 2 April 2005; accepted 8 April 2005
Available online 24 May 2005

Abstract

Some of the first experiments in behavioral endocrinology in the 1930s were conducted with lizards, but events led to a hiatus that lasted for 30 years. In the 1960s, research resumed using techniques current at the time, but it was not until the mid-1970s that behavioral neuroendocrinology “discovered” reptiles as animal model systems. This historical review summarizes this period of work, illustrating an enormous increase in research that have led to conclusions such as (1) the phenomenon of dissociated reproductive strategies and hormone-independent behaviors, which have aided our understanding of how the “memory” of sex steroid actions is maintained. (2) Progesterone plays an important role in the organization and activation of sexual behavior in males. Progesterone also synergizes with T to control male courtship much as does estrogen and progesterone to control sexual receptivity in females. Thus, progesterone is as much a “male” hormone as it is a “female” hormone. (3) Use of cytochrome oxidase histochemistry to study the role of experience over the long term in modifying brain activity. (4) Hormone manipulations as a powerful tool to test hypotheses about the evolution of behavior in free-living animals.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Reptiles; Sexual behavior; Aggressive behavior; Cytochrome oxidase; Experience; Steroid hormones; Neuroendocrinology

“Natural experiments permit one to examine conditions that cannot...be created experimentally...and reveal the end results of ecological and evolutionary processes.” -J.M. Diamond, 1983

Introduction

The history of behavioral endocrinology research in reptiles parallels that in birds and mammals in many respects. In the 1930s, two visionaries, G. Kingsley Noble and Llewellyn T. Evans, working independently (for they did not hold one another in high regard), carried out studies on the effects of the recently discovered steroid and pituitary

hormones on the morphology and behavior of the then called American chameleon (*Anolis carolinensis*) (Evans, 1936; Greenberg and Noble, 1940, 1944).

Following the death of Noble (of pneumonia contracted during field work) and the inability of Evans to find a permanent position, there was a 30-year hiatus before, in the 1970s, there was a resurgence of interest in reptiles as model systems for the study of hormone–brain–behavior relationships from three different perspectives. First, following the discovery that sex steroids were concentrated in discrete brain areas, there was a flurry of studies with cats, rats, hamsters, and chickens in which small quantities of androgens were implanted directly into the preoptic area and anterior hypothalamus (POAH) to elicit mating behavior in castrated males. Robert D. Lisk pioneered intracranial work in lizards. While on sabbatical in 1965 in C.H. Sawyer’s laboratory at UCLA, he implanted a fiber optic strand into the POAH of the desert iguana (*Dipsosaurus*

* Corresponding author. Section of Integrative Biology, University of Texas, Austin, TX 78712, USA.

E-mail address: crews@mail.utexas.edu (D. Crews).

dorsalis), producing illumination using a “Grain of Wheat” light bulb (similar to Benoit’s classic studies in ducks). The ovaries of females that received a 14 hour per day illumination via the fiber optic (long-days) developed and laid eggs while the ovaries of females receiving short-day illumination failed to develop. Ironically, the undergraduate assisting him became an animal rights activist and refused to allow the data to be published. On return to Princeton University, Lisk continued the intracranial studies and published a study demonstrating that testosterone (T) into the POAH inhibited testicular recrudescence (Lisk, 1967). However, because he did not want to be stretched “too thin,” he concentrated on mammals thereafter. Ian Callard subsequently conducted a series of studies in the blue spiny lizard (*Sceloporus cyanogenys*), all of which focused on the role of negative feedback in regulating gonadal function. However, it was the careful neuroethological work of Neil Greenberg (1982, 2002, 2003) that paved the way for later studies in reptilian behavioral neuroendocrinology, a tradition carried on by his student Summers (2002).

Like Noble before him, Gary Ferguson was interested in behavioral correlates of ovarian state. In the mid-1960s, he described the formation of yellow spots and a sideway hopping movement in response to male courtship in the female collard lizard (*Crotaphytus collaris*) after ovulation. His student William Cooper then showed in the early 1970s that the injection of progesterone (P) not only induced the formation of these spots, but also the rejection behavior (Cooper and Greenberg, 1992).

With the development of radioimmunoassay (RIA) in the late 1960s came the ability to measure circulating concentrations of sex steroid hormones, followed by studies of steroid receptor kinetics, and Ian Callard pioneered such studies in reptiles (Callard et al., 1991). However, it was not until the 1970s that the changing levels in sex hormones were correlated with sexual behavior in males and female vertebrates.

The animal most often studied during this period was the green anole (the American chameleon of Noble and Evans). This animal was the subject of David Crews, working under the direction of Daniel Lehrman and Jay Rosenblatt. The focus of this work built on the conjectures of F. H. A. Marshall, who suggested in 1936 that “the sexual posturing of the male produces exteroceptive stimuli which act upon the anterior pituitary of the female through the hypothalamus, and so affects the necessary synchronization between the sexual processes of the male and female (Marshall, 1936, p. 445).” Of course the work of Daniel Lehrman and Robert Hinde served as models for the proximate studies. They had shown how the behavior of the male ringdove and canary, respectively, advanced the onset of nest-building behavior and reduced the latency to initiating incubation behavior. As a new graduate student at the now defunct Institute of Animal Behavior at Rutgers-Newark, Crews started with ringdoves under the direction of Lehrman, but found the colony rooms noisy, the birds uninteresting behaviorally, and felt that the

‘cream’ had already been skimmed by the earlier research (such is the naïveté and arrogance of a new researcher). What excited him was the work of the early researchers in behavioral endocrinology, which he referred to as “frontier behavioral endocrinology.” His discovery of the work of Noble and Evans combined with his childhood love of reptiles and fascination with the woods and swamps surrounding his home in Florida and South Carolina led him to propose to Lehrman that these would make suitable animals for a thesis project. It was only much later that he discovered that Lehrman had been a high school volunteer in the greenhouse atop the American Museum of Natural history where Noble conducted his lizard studies.

In his thesis research Crews validated Noble’s suggestion that regular patterns in ovarian state might underlie the cycles of receptivity in the female green anole (Crews, 1975, 1980). He also discovered that the act of copulation rapidly inhibited further receptivity in females which later work indicated to be a neuroendocrine reflex. Crews also demonstrated that the courtship behavior of the male green anole stimulated ovarian growth and, further, that this effect was due to the hormonal state of the male. He then determined that it was the display of the dewlap, rather than the body movement, that was the critical stimulus for this behavioral facilitation of ovarian state. Crews also discovered that females viewing aggressive behavior between males, inhibited ovarian growth, a response most likely due to stress.

After his doctoral work, Crews spent a postdoctoral with Paul Licht at University of California, Berkeley, where he learned RIA and conducted studies of turtle reproductive endocrinology. But it was at Harvard University that Crews returned to ‘frontier’ behavioral endocrinology of lizards. It was at this time that his work turned back to the green anole and the problem of brain mechanisms and, working with Joan Morrell, Donald Pfaff and Richard Tokarz, identified the sites of sex hormone concentrating sites in the green anole. Not surprisingly, these areas were located principally in limbic nuclei. At Harvard Crews worked primarily with undergraduates. With Abraham Morgentaler, he demonstrated that intracranial implants into the POAH of T and E induced mating behavior in castrated males. This latter finding was surprising, but consistent with other studies in the same time period that in mammals E could act centrally to stimulate sexual behavior. With James Wheeler, he demonstrated that lesions of the POAH abolished male courtship. During this same period, he worked on the hormonal basis of female sexual receptivity with Donald McNicol, and later June Wu, finding that while E at sufficient dosage might stimulate receptivity in ovariectomized females, P synergized with E, initially facilitating the onset of receptivity but some hour’s later suppressing receptivity. A similar biphasic action of P had previously been demonstrated in guinea pigs, hamsters, and rats. Other studies with Paul Valenstein examined the role of peripheral feedback from the ovaries on estrogen (E)-induced receptivity. It was at this time that Crews performed his

experiments on the role of peripheral feedback from the hemipenes and testes on male copulatory behavior.

It was a book review that galvanized Crews to focus on reptiles with unusual adaptations as a means of illuminating fundamental principles. In his review of the volume Herbert (1979) commented “Reader’s familiar with mammalian behaviour will turn with interest to Crews’ chapter on reptiles, expecting insights into new worlds. They will be disappointed, not by Crews’ writing, but by the astonishing similarity between the hormonal control of behaviour in reptiles and mammals. This gives a repetitive feeling to this chapter, for once undeserved. One is tempted to feel sorry for works on these species, condemned, it seems, to follow in the footsteps of those more preoccupied with rodents.” While it was true that the work with the green anole reflected more of the conservation of brain–behavior relationships, Crews knew that there was tremendous diversity in reproductive patterns in reptiles that challenged the dogma in behavioral neuroendocrinology and he began to document these ‘experiments of nature’. It has been this body of work that has likely had the greatest impact toward our present understanding of the plasticity of neuroendocrine mechanisms. A few years after Crews arrived at the University of Texas at Austin, Michael Moore joined the laboratory as a postdoctoral fellow. As a new faculty member at Arizona State University Moore began his own research program in reptilian behavioral endocrinology. Taken together, the body of work from these two laboratories have borne out the observation of Bartholomew (1982) that such experiments of nature “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.”

These discoveries may be grouped under the following categories: (1) Dissociated reproductive strategies and hormone-independence of sexual behavior, (2) Evolution of novel neuroendocrine mechanisms, (3) Phenotype engineering, and (4) Organization of within-sex phenotype differences. We cite mostly reviews and ask the reader to consult these for specific citations.

(1) Dissociated reproductive strategies and hormone independence of sexual behavior

Prior to 1980, all studies on the hormonal control of sexual behavior in vertebrates had been conducted on vertebrates, principally inbred laboratory rodents, in which mating behavior occurred when gonadal hormone secretion was high and the gametes mature. Crews (1984) referred to this as the Associated reproductive strategy (Fig. 1) and, indeed, the majority of vertebrates fall into this category. The idea that mating behavior is dependent upon the steroid hormones produced by the growing gonads became dogma. But, because we tend to study only what we know, we tend

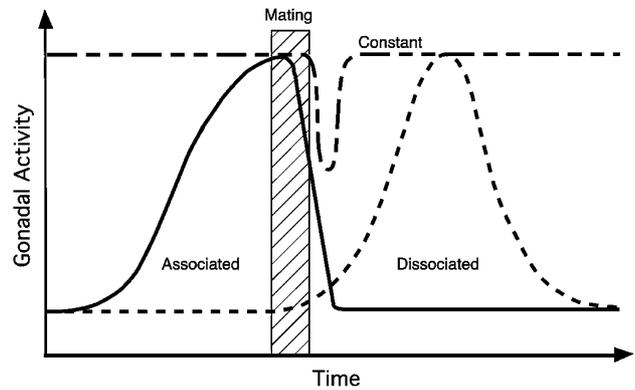


Fig. 1. Vertebrates display a variety of reproductive patterns. Here gonadal activity is defined as the development of eggs and sperm and/or increased sex steroid hormone secretion. Individuals exhibiting the associated reproductive pattern (solid line) live in temperate regions where seasonal cycles 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 (small dash 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 (long dash 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 Whittier and Crews, 1987).

to know only that which we study, which in this instance resulted in an overly narrow perspective of hormone–brain–behavior relationships. However, as indicated by the quote beginning this article, animals live in a variety of environments that have led to the evolution of fascinating neuroendocrine adaptations.

The first such example was the red-sided garter snake (*Thamnophis sirtalis parietalis*). This animal is found in Manitoba, Canada and has a very short breeding season, depending upon the weather ranging from 1 to 4 weeks (Fig. 2). This occurs when the animals emerge from winter hibernation in response to increasing temperatures. At this time, the testes are regressed. Studies of animals at the den revealed that circulating concentrations of T were low or basal during winter dormancy (there is no metabolism of steroids in hibernation when temperatures underground range from 0 to 5°C) and following emergence. [In those years where there is only a brief Fall, males may enter into hibernation with elevated plasma T concentrations and they emerge with elevated T levels but they decline rapidly on emergence.] Because of the requisite time to grow sperm, testicular recrudescence, spermatogenesis, and elevations in androgen concentra-

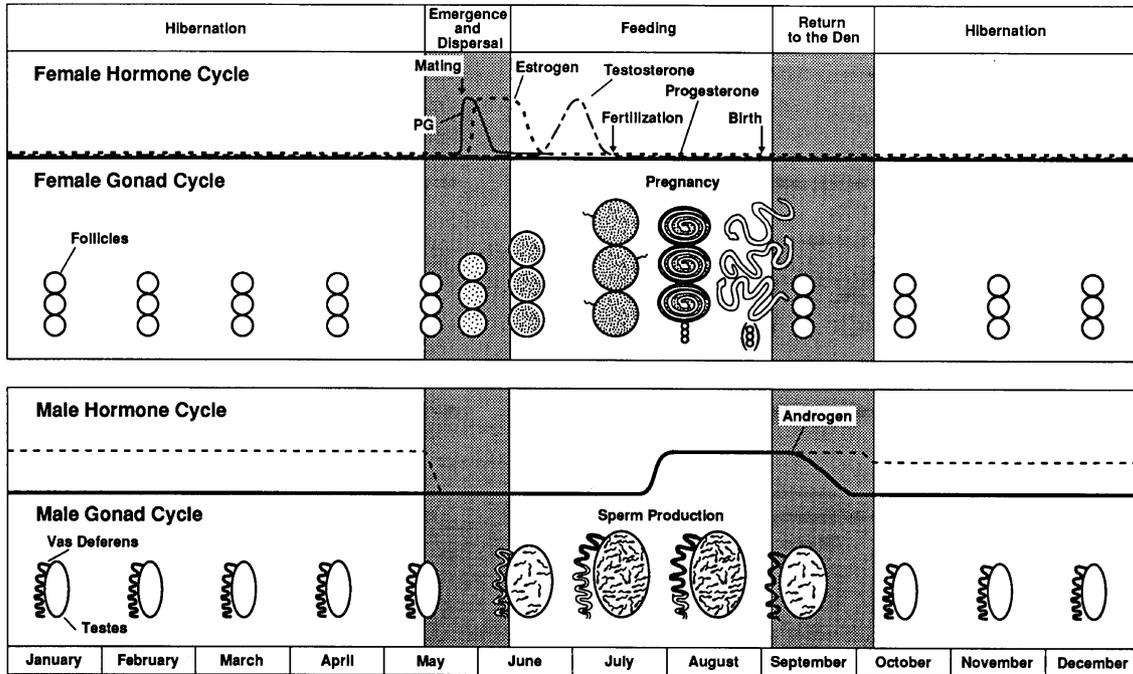


Fig. 2. The major physiological and behavioral events in the annual reproductive cycle of the red-sided garter snake in Canada. Animals spend most of the year underground. In the spring, they emerge and mate before dispersing to summer feeding grounds. In the female, mating initiates gonadal growth as 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 usually are 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. (Adapted from Crews, 1990).

tions occur in the summer, after the spring mating season has ended.

Thus, all garter snakes exhibit a pattern in which mating behavior is not associated with gonadal hormone secretion or spermatogenesis. This was termed a Dissociated reproductive strategy (Fig. 1). [A third category (Constant or Opportunistic reproductive strategy) is characteristic of animals living in extreme environments in which mating behavior is displayed within minutes of a particular change in the environment (e.g., rainfall in deserts) (Whittier and Crews, 1987). In such animals, the gonads are fully developed with gametes at or close to full maturation, and this state is maintained often times for years until the right conditions prevail. Only recently has the behavioral neuroendocrinology of opportunistic breeding species begun to be studied.]

The initial study on the hormonal control of sexual behavior in male red-sided garter snakes concluded that androgen activated male courtship (Crews, 1983, 1990). In that study, however, few animals were available and it was necessary to conduct a sequential or repeated measures design in which all animals were treated with androgen capsules during the period in which, we later learned, courtship behavior normally waxed and waned. Indeed, when sufficient animals became available to conduct a simultaneous or group treatment design, it was found that intact, castrated, or even hypophysectomized males would respond to the increased temperature following prolonged

hibernation with robust courtship behavior without androgen treatment. Other experiments demonstrated that circulating androgens are not involved in the stimulation of male courtship behavior in the spring, even if androgen is implanted directly into the POA! Instead, it is an environmental cue, increasing temperature in the spring following a prolonged period of cold dormancy that triggers courtship behavior.

Subsequent research found that androgens are important for courtship behavior in male red-sided garter snakes, but in an unusual way. Courtship behavior slowly declines over years in castrated males and, coincidentally, so to does the frequency and robustness of courtship behavior. This suggested that the elevated androgen levels later during the Summer may be important in re-organizing each season the temperature sensitivity of the neural substrates of mating behavior for the spring, a process called Trans-seasonal Organization (Crews, 1991). This was found to be the case; treatment of long-term castrates (3 years) with androgen for 8 weeks during the Summer (at the time when intact males usually experience a surge in androgens) leads to a significant increase in courtship behavior in the next spring following hibernation.

As in species with an associated pattern of reproduction, the POAH is integral to the display of courtship behaviors in species with a dissociated pattern. David Friedman found that lesions of the POAH continuum in the red-sided garter snake reduce courtship and copulatory behavior, and Ethan

Allen found increased metabolic activity in the POAH during courtship. We also recognized that the POA is also an evolutionarily conserved nucleus in the control of thermoregulation. Since temperature is the critical cue activating courtship behavior in this species Randy Krohmer lesioned the POAH to find that it not only abolished courtship behavior in males but also disrupted their thermoregulatory behavior.

Female, like male, red-sided garter snakes also mate when the follicles are previtellogenic and E levels are basal. Studies initiated by William Garstka, and continued by Joan Whittier and Mary Mendonça, established that mating initiates a neuroendocrine reflex that begins with an inhibition of receptivity and an immediate rise in prostaglandin $F_{2\alpha}$, followed by a rise in E that peaks 24 h later; follicular development then commences and ovulation occurs in about 6 week (Whittier and Tokarz, 1992). Andrew Halpert showed that mating was also important in sperm transport and storage.

Another line of research begun with Patrick Ross and Bill Garstaka, and continued by Robert Mason, Mary Mendonça, and Joan Whittier, focused on the role of pheromones as modulators of sex behavior in male and female red-sided garter snakes, but space does not allow justice to this interesting research (Mason, 1992; Whittier and Tokarz, 1992). Another perspective on the hormonal independence of behavior was contributed by the work on aggressive behavior in the mountain spiny lizard (*Sceloporus jarrovi*) by Moore et al. (see Moore and Marler, 1988 for review).

(2) Evolution of novel neuroendocrine mechanisms

Some lizard species consist entirely of females, reproducing by obligate parthenogenesis. These clonal lizard species arose from the hybridization of sexual species that still exist. In the summer of 1978, David Crews and Kevin Fitzgerald, working independently, observed in several parthenogenetic whiptail species (genus *Cnemidophorus*) what appeared to be “mating” behaviors, identical to the courtship and copulatory behavior of sexual whiptail species (Crews and Fitzgerald, 1980). This behavior was called pseudosexual behavior, since fertilization was not necessary for reproduction. Further, individuals alternate between displaying male-like pseudocopulatory behaviors and female-like receptive behaviors depending upon the stage of follicular development (Fig. 3). It struck Crews that this display of both male- and female-like pseudosexual behaviors *alternately by a single individual* demonstrated perfectly the fundamental bisexuality of the vertebrate brain. That is, because the parthenogens are a clone and consist only as female individuals experiencing the same endocrine environment, it becomes possible to examine simultaneously the neural circuits underlying typically dimorphic sexual behaviors.

Crews decided to concentrate on the parthenogenetic desert-grasslands whiptail (*C. uniparens*) because of its abundance and could be captured more easily than other some other members of the genus (if four investigators to catch each lizard in the desert under 120 °F can be called easy!). This species descended from a hybridization event

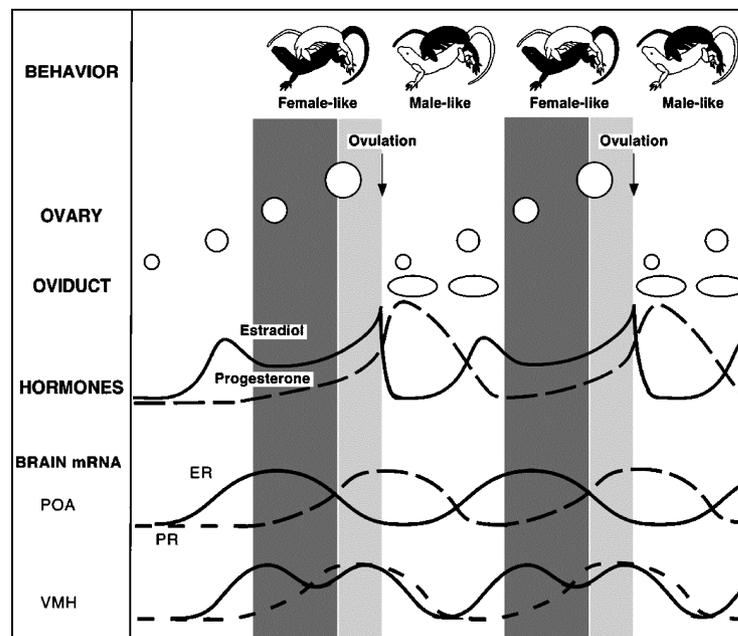


Fig. 3. Relation among male-like and female-like pseudosexual behavior, ovarian state, circulating levels of estradiol and progesterone during different stages of the reproductive cycle, and changes in the expression of genes coding for estrogen receptor (ER)-mRNA and progesterone receptor (PR)-mRNA in the preoptic area (POA) and the ventromedial hypothalamus (VMH), of the parthenogenetic whiptail lizard. The transition from receptive to mounting behavior occurs at the time of ovulation (arrow). (Redrawn from Crews, 1996).

between two sexually reproducing species, the rusty rumped whiptail (*C. burti*) and the little striped whiptail (*C. inornatus*). Thus, we had a snapshot of evolution in which we could compare the unisexual descendant with its direct sexual ancestors.

Early experiments indicated that engaging in pseudosexual behavior stimulated ovarian growth in the parthenogenetic whiptail just as male courtship behavior stimulates ovarian growth in its sexual ancestor (Crews et al., 1983). Early on, Michael Moore demonstrated that differences in the behavioral roles during pseudocopulation are paralleled by differences in the circulating levels of sex steroid hormones (Moore et al., 1985a,b). That is, individuals show primarily female-like behavior during the preovulatory stage when E concentrations are relatively high and P levels relatively low; in contrast, individuals display male-like behavior in the postovulatory phase when concentrations of E are low and P levels are high. The close parallel between the transition from female-to male-like pseudosexual behavior and from E dominance to P dominance in the plasma at ovulation (Fig. 3) led to experiments with Mark Grassman showing that the postovulatory surge in P may play a crucial role in controlling the expression of male-like pseudosexual behavior. Other studies indicated that there is both hormonal and regional specificity in function of the POAH and the VMH (Crews and Sakata, 2000).

This was followed by research with Jonathan Lindzey showing that in the ancestral sexual species there is individual variation in the capacity of P to induce male-typical behavior (=P-sensitivity). Lindzey then went on to demonstrate that P was acting as a native molecule and that the specificity and affinity of the androgen receptor was typical of mammalian androgen receptor. He also found that T and P synergize to stimulate sexual behavior in castrated males. Indeed, this individual variation in P-sensitivity in the sexual ancestor may have served as the substrate for the evolution of P-activation of male-like pseudosexual behavior in the descendant unisexual species. Work with green anole as well as rats and mice revealed a similar role of P and its receptor in modulating sexual behavior of male rodents (Phelps et al., 1998; Witt et al., 1994).

Coincident with Lindzey, Juli Wade revealed that there was a sexual dimorphism in the size of the POA and VMH (and in the soma size of neurons in these areas) of males of the ancestral species and, further, that these differences were under androgenic control. Surprisingly, the size of these brain areas did not differ when the parthenogens were displaying male-like and female-like pseudosexual behavior, even though Matt Rand had found previously significant differences in the metabolic activity of these areas in animals displaying the different pseudosexual behaviors.

Because the antibodies available at the time failed to cross-react with lizard tissue, Larry Young cloned and sequenced whiptail orthologs of the mammalian androgen (AR), estrogen (ER), and progesterone receptors (PR) and mapped the neuroanatomical distribution of these receptors.

He also conducted the first studies in reptiles detailing the patterns of gene expression in discrete brain areas during the follicular cycle and in response to exogenously administered sex steroid hormones. In addition, he found that the lower circulating levels of E in the parthenogen was correlated with higher abundance of ER gene expression in the POA, thereby accounting for the increased sensitivity to exogenous E in this species compared to females of the ancestral species. This led him to develop the 'sensitivity compensation' model, which holds that the increased level of ER gene expression in the POA results in greater sensitivity to the circulating concentrations of E, which in turn results in lower levels of circulating E through feedback effects. Comparisons of species with different hormone–brain–behavior relationships revealed three factors that explains these species differences in endocrine physiology and behavior: (i) sensitivity to exogenous sex steroid hormones, (ii) hormone-dependent regulation of sex steroid hormone receptor gene expression, and (iii) the neuroanatomical distribution of steroid receptor gene expression, especially in nonlimbic brain areas (Young and Crews, 1995).

John Godwin extended this work to the male, demonstrating a sex difference in PR expression in the VMH in response to exogenous E (Godwin and Crews, 1997). Godwin also conducted the experiment that showed exactly how the neuroendocrine mechanism controlling mounting behavior had changed in the evolution of the parthenogen. By injecting ovariectomized females of the sexual ancestor and the parthenogenetic descendant with E, he found that PR expression was upregulated in the POA in the descendant parthenogen, but not in the ancestral sexual species. Thus, as indicated in Fig. 3, with increasing levels of E during the follicular cycle in the parthenogen, the abundance of PR increases in the POA with the result that as the hormone ratio changes from E dominance to P dominance with ovulation, there is abundant PR in the brain area critical to pseudocopulatory behavior at the time P levels surge (Fig. 1, bottom panel). This contrasts with what occurs in the sexual ancestral species. Here, under a similar scenario in the female, as E increases in the circulation there is no change PR mRNA in the POA and no mounting behavior occurs following ovulation. In both species the rising level of E from the growing follicles upregulate PR mRNA in the VMH, the brain area involved in sexual receptivity. In males of the ancestral species, neither E nor P are normally secreted, yet a response similar to that of the female is observed, namely that E treatment fails to upregulate PR mRNA in the POA while P downregulates PR mRNA while upregulating AR mRNA in the POA of progesterone-sensitive males. Finally, in intact males, where androgen is normally the dominant hormone, T strongly upregulates PR mRNA in the POA (as well as in conspecific females and in the parthenogen).

Recent research has gone in two different directions, namely neurotransmitters and experience. Sarah Woolley

has examined the distribution of tyrosine hydroxylase in the brain and the evolutionary changes in dopaminergic modulation of courtship behavior in the two species of whiptails, while Jon Sakata has examined the role of experience in regulating behavior and metabolic capacity in the brain.

(3) Phenotype engineering

A central assumption of Darwin's Theory of Evolution is that natural selection results in present organisms that possess traits that better adapt them to their environment than do other possible traits. Most studies testing this fundamental assumption have had to use correlation approaches. A more direct test would be to create organisms that possessed alternative character traits and then test their fitness in the field against the wild type organisms. For a long time, this possibility seemed a fantasy. However, Andersson (1982) pioneered an approach now referred to as phenotype engineering (Ketterson et al., 1996). In this experiment, he modified the tail lengths of male long-tailed widowbirds (*Euplectes progne*) and showed that males with artificially shortened tails experienced reduced mating success, whereas those with artificially lengthened tails experience enhanced mating success.

As exciting as this experiment was, the number of character traits that can be mechanically manipulated in this fashion is limited to a few morphological traits. Marler and Moore (1988) expanded this approach to behavioral studies and pioneered behavioral phenotype engineering by using hormone implants to manipulate the behavior of free-living organisms and then to determine the fitness consequences of this manipulation in the field. In the initial experiment, male mountain spiny lizards (*Sceloporus jarrovi*) were given T implants to make them more aggressive and released back into the field. Mortality of these males was tracked over several months. The more aggressive males experienced increased annual mortality compared to males given sham implants.

This was the first manipulative experiment to use hormones to demonstrate directly that male aggressive behavior had a fitness cost. This experiment, although conceptually possible in many species, was greatly facilitated by the choice of a reptile model system primarily because these animals were easy to manipulate and follow in the field. Mountain spiny lizards are fairly large, diurnal animals that have small territories, recover quickly from surgery, have easily quantifiable behaviors, tolerate close approach by human observers and are easy to locate in the field. The large sample sizes required by this experiment to generate the statistical power necessary to compare survivorship curves would have been difficult to manage with many other organisms.

Subsequent experiments with this approach capitalized on the ease with which this system could be manipulated.

Marler and Moore (1989) showed that T implanted males were more active, spent less time in refugia and foraged less than sham-implanted males. This led to the hypotheses that the costs of aggression to these males were either due to (1) a negative energy balance because of time spent in activity and/or reduced foraging time or (2) increased exposure to predation. This led to a further experiment that capitalized on the ease with which these animals could be worked on in the field. This was a food supplementation experiment in which these two hypotheses were tested (Marler and Moore, 1991). Food supplementation is a common experiment in field biology, but it is often confounded by the fact that entire study plots have to be food supplemented leading to a loss of statistical power due to pseudoreplication. In many reptiles, it is possible to provide food to specific individuals and not others on one study plot which avoids the problems of pseudoreplication. In our experiment, individual males on the same study area but with noncontiguous territories were either hand fed mealworms or simply visited by the investigator as controls. Food supplementation allowed T-implanted males to survive as well as controls. This result (1) refuted the predation hypothesis since these males continued to be very active and (2) supported the negative energy balance hypothesis because survivorship improved with extra food. Finally, we were able to do an experiment directly measuring energy expenditure in T-implanted males. This experiment used the doubly labeled water technique and again capitalized on unique advantages of reptiles. Doubly labeled water can be used in birds and mammals to measure energy expenditure over time periods of a day or two, probably too short to detect many differences significant to fitness. Because of the lower metabolic rate of reptiles, doubly labeled water can be used in reptiles to measure energy expenditure over several weeks, probably a time interval more relevant to natural selection. Using this technique, Marler et al. (1995) were able to show that the energy expenditure of T-implanted males was more than 30% greater than non-implanted males. This difference was most likely due to activity differences rather than a direct effect of T because laboratory housed males given T did not show this increase. This study was one of the first to demonstrate an energetic cost to male aggression.

These experiments supported through direct experimental manipulations that more aggressive males paid a fitness cost and were able to take this further and specifically identify a negative energy balance, probably due to increased activity and decreased foraging, as the specific cost incurred by the more aggressive males. Although this approach of hormonal phenotype engineering has now been used in a wide variety of organisms and systems, especially birds (Ketterson and Nolan, 1999), there is no doubt that the initial efforts to establish this approach were greatly facilitated by the unique features of reptilian models.

(4) Organization of within-sex phenotype differences

One of the major foci for behavioral endocrinology has been trying to understand the mechanisms underlying the differences between the sexes. Work on this problem in the late 1950s in the laboratory of W.C. Young led to the formulation of one of the great pillars of the discipline, the organization–activation hypothesis (Phoenix et al., 1959). This hypothesis proposed that sex steroid hormones act during early development to establish or organize the patterns of sexual behavior and then again later in adult life they act again to turn on or activate the expression of those behavioral patterns. This hypothesis has structured much of the work in behavioral neuroendocrinology in the decades since it was proposed.

In addition to understanding the differences between the sexes, an equally important problem is understanding the origin of differences between individuals. This problem had intrigued many but much less progress had been made in understanding it. Both Crews (Crews and Bull, 1987; Rhen and Crews, 2002) and Moore (1991), working independently, became interested in using unique model systems to address this issue. Crews began working on the leopard gecko (*Eublepharis macularius*), which has temperature-dependent sex determination. As described below, this model allows gonadal sex and sex behavior to be studied separately and has much greater than normal phenotype variation as a result. Moore became interested in the use of animals with naturally occurring alternative male phenotypes as model systems for understanding the mechanisms underlying individual differences in behavior. The reasoning was that these organisms show exaggerated individual differences that would be easier to study. This led to the selection of the locally common tree lizard (*Urosaurus ornatus*) as a model system. The work on these two model systems is discussed below.

(a) Tree lizards

Animals with discrete alternative male phenotypes present interesting models for behavioral neuroendocrinology (Knapp, 2003; Moore, 1991; Rhen and Crews, 2002). In these species, males have several alternative phenotypes and often differ in morphology, behavior and reproductive physiology. This extreme form of individual variation is more easily studied than the subtle, continuous variation in phenotype present in more typical species.

Moore became interested in tree lizards when he captured animals in his backyard and noticed that they were highly polymorphic in the color of the throat fan (dewlap). At about the same time, an early study by Hover (1985) was published and pointed to the possibility that these were alternative male phenotypes that differed in their aggressive behavior. This was confirmed by Thompson and Moore (1991, 1992). Further studies, until very recently, focused on a single population of tree lizards that contained two

phenotypes, aggressive males with orange-blue dewlaps and nonaggressive males with orange dewlaps (Moore et al., 1998).

The first studies on the tree lizards tried to identify adult hormone differences between the phenotypes that could account for the differences in aggressive behavior (Moore et al., 1998). Frustratingly, these studies were negative and adult males of both types had identical seasonal patterns of T and corticosterone. Further reflection, inspired particularly by some early work of Elizabeth Adkins-Regan (Adkins, 1978), led to investigating the possibility that organizational differences might account for the differences between the males. After reviewing the few studies at the time of hormones in alternative male phenotypes, Moore (1991) formalized this idea as the Relative Plasticity Hypothesis. This hypothesis accounted for the then existing data that some alternative phenotypes had hormone differences, whereas others did not by proposing that species with developmental fixed phenotypes relied on organizational actions of hormones whereas species with developmentally plastic phenotypes relied on activational actions of hormones.

The work of Diana Hews and Rosemary Knapp tested this hypothesis in tree lizards (Hews et al., 1994). The phenotypes in tree lizards are developmentally fixed. The studies supported the predictions of the relative plasticity hypothesis that these fixed phenotypes would be organized by hormones by showing that castrated neonates were more likely to develop into the nonaggressive orange-dewlapped phenotype, whereas T-implanted neonates were more likely to develop into the aggressive orange-blue dewlapped phenotype. Although these initial studies implicated a role for T as the organizing agent, further studies have surprisingly suggested that P of adrenal origin may be involved (Jennings et al., 2004; Moore et al., 1998). Although confirming the role of P has proved challenging (Weiss and Moore, 2004), this subsequent work has been confounded by changes in the population structure of the study population induced by the severe drought in the southwestern United States in the last several years. This problem illustrates that while fieldwork has its benefits and attractions, it also presents unique challenges as well!

Subsequent studies by Rosemary Knapp revealed that there were plastic phenotypes within the tree lizard system as well (Knapp et al., 2003). Specifically, the nonaggressive orange males switch between sedentary behavior (satellite strategy) and nomadic behavior (nomad strategy) apparently in response to the conditions on the study area. Results of studies to test the predictions of the Relative Plasticity Hypothesis that this plastic change should be mediated by activational actions of hormones suggest that this switch is mediated by rises in corticosterone (B) when conditions are more stressful on the study area. This rise in B inhibits T, which apparently reduces site fidelity and causes the lizards to become nomadic. In the aggressive orange-blue males, no plasticity of behavior occurs. These males are always

territorial. Interestingly, the T levels of these males in much more resistant to being suppressed by B than are the T levels of orange males. The discovery of this difference in stress reactivity among the morphs led to the further discovery that the morphs differ in the levels of plasma proteins that bind the stress hormone B (Jennings et al., 2000). This work further suggests that the liver, which produces these binding proteins, may be a target for organizational actions of hormones.

The work on tree lizards was among the first to experimentally demonstrate an organizational role for steroid hormones in producing within sex differences in phenotype, a conclusion that was strongly supported by the parallel work in the leopard gecko discussed next. The work on the neuroendocrinology of tree lizard alternative male phenotypes has been followed by a number of ongoing similar studies of alternative male phenotypes in birds, amphibians, fish, and other reptiles (Knapp, 2003). Especially noteworthy is the work of Sinervo et al. (e.g., Sinervo et al., 2000) on the similar system in the side-blotched lizard (*Uta stansburiana*), which integrates neuroendocrine work into a rich context of the understanding of the natural history and selection on the different phenotypes.

(b) Leopard Geckos

The leopard gecko (*Eublepharis macularius*) lacks sex chromosomes, depending instead on the temperature experience during the mid-trimester of incubation to determine gonadal sex. [The Crews Lab has also conducted extensive experiments on the physiology and molecular endocrinology of temperature-dependent sex determination (Crews, 1996, 1999; Crews et al., 2001).] In the leopard gecko eggs incubated at 26°C result in only female hatchlings (=low-temperature females), whereas incubation at 30°C produces a female-biased sex ratio, and 32.5°C produces a male-biased sex ratio; incubation of 34–35°C again produces virtually all females (=high-temperature females).

In initial studies with William Gutzke, followed by the doctoral dissertations of Alan Tousignant, Patricia Coomber, Alice Fleming, Turk Rhen, and Jon Sakata, Crews has studied the functional outcomes of this mode of sex determination, finding that incubation temperature accounts for much of the phenotypic variation seen among adults both between and within the sexes (Crews, 1993; Crews et al., 1998; Sakata and Crews, 2004). For example, head size is 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, plasma E levels are significantly higher in males from a female-biased temperature compared to males from a male-biased temperature; among females, circulating E levels are significantly higher, and androgen levels significantly lower, in low-temperature females compared to females from a male-biased temperature.

Incubation temperature also has a major influence on the nature and frequency of the aggressive and sexual behavior

displayed by the adult leopard gecko (Crews, 2003; Crews et al., 1998; Sakata and Crews, 2004). 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 are 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 T to restore aggression. Following ovariectomy and T treatment, low-temperature females still do not exhibit increased levels of aggression toward males, whereas females from male-biased temperatures return to the high levels exhibited while gonadally intact. Other studies with Turk Rhen further indicate that incubation temperature influences how the individual responds to steroid hormones in adulthood.

Courtship 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 from low and male-biased temperatures are treated with T, they will begin to tail-vibrate toward female, but not male, stimulus animals; males appear to regard such females as male because they are attacked. Females from a male-biased temperature are less attractive than are females from lower incubation temperatures.

Working initially with Patricia Coomber and later with Jon Sakata, Crews discovered that the morphological, physiological, and behavioral phenotypes we have revealed reflected neural phenotypes. The first, and much to our surprise, discovery was that there are no statistically significant sexual dimorphism in the volumes of the POA and VMH between males and females at those incubation temperatures that produce both sexes. There are, however, consistent differences across incubation temperatures. For example, POA volume is larger in both males and females from the male-biased temperature compared to same sex animals from the female-biased temperature. We also measured metabolic capacity of limbic areas and found that males and females from the male-biased temperature have greater cytochrome oxidase (CO) activity in the POA compared to animals from the other incubation temperatures, whereas females from the female-biased temperature have greater CO activity in the VMH compared to females from the male-biased temperature (Crews, 2003; Crews et al., 1998; Sakata and Crews, 2004).

An important question is whether these differences in the volume and CO activity of brain nuclei are a consequence of a direct action of temperature, or an indirect result of

temperature's sex-determining function. This was tested using the classic gonadectomy and hormone replacement therapy approach, with the finding that incubation temperature of the embryo directly organizes the brain independent of gonadal sex.

Other studies focused on the role of adult experience on metabolic capacity of specific brain regions in males and females from different incubation temperatures. The results indicate that within-sex differences are dynamic in adulthood, changing as leopard geckos gain sociosexual experience, while the embryonic environment influences the nature and degree of these changes.

Conclusion

Crews and Moore (1986) reviewed some of the reproductive strategies found in vertebrates and the various hormonal, environmental, and social cues used to activate mating behavior. It was pointed out that the cue used by a particular species depends upon the environmental, physiological, and developmental constraints imposed by its particular reproductive strategy. This led in turn to widely applicable generalizations and providing a useful evolutionary perspective within which to conceptualize differences between species, populations, and individuals. In the last 20 years, and reflected in the pages of this journal, there has been an enormous increase in the study of naturally occurring species under ecologically relevant conditions that has revealed the plasticity of the proximate mechanisms underlying mating behaviors. Some the conclusions are:

- (1) The phenomenon of dissociated reproductive strategies and hormone-independent behaviors, which have aided our understanding of how the "memory" of sex steroid actions is maintained.
- (2) Progesterone plays an important role in the organization and activation of sexual behavior in males. Progesterone also synergizes with T to control male courtship much as does E and P to control sexual receptivity in females. Thus, P is as much a "male" hormone as it is a "female" hormone.
- (3) Use of cytochrome oxidase histochemistry to study role of experience over the long term in modifying brain activity.
- (4) Hormone manipulations as a powerful tool to test hypotheses about the evolution of behavior in free-living animals.

References

Andersson, M., 1982. Female choice selects for extreme tail length in a widowbird. *Nature* 299, 818–820.
 Adkins, E.K., 1978. Sex steroids and the differentiation of avian reproductive behavior. *Am. Zool.* 18, 501–509.

Bartholomew, G.A., 1982. Scientific innovation and creativity: a zoologist's point of view. *Am. Zool.* 22, 227–235.
 Callard, I.P., Etheridge, K., Giannoukos, G., Lamb, T., Perez, L., 1991. The role of steroids in reproduction in female elasmobranchs and reptiles. *J. Steroid Biochem. Mol. Biol.* 40, 571–575.
 Cooper, W.E., Greenberg, N., 1992. Reptilian coloration and behavior. In: Carl Gans, C., Crews, D. (Eds.), *Biology of the Reptilia, Physiology E. Hormones, Brain and Behavior*, vol. 18. University of Chicago Press, Chicago, Illinois, pp. 298–422.
 Crews, D., 1975. Psychobiology of reptilian reproduction. *Science* 189, 1059–1065.
 Crews, D., 1980. Interrelationships among ecological, behavioral and neuroendocrine processes in the reproductive cycle of *Anolis carolinensis* and other reptiles. In: Rosenblatt, J.S., Hinde, R.A., Beer, C.G., Busnel, M.C. (Eds.), *Advances in the Study of Behavior*, vol. 11. Academic Press, New York, pp. 1–74.
 Crews, D., 1983. Control of male sexual behavior in the Canadian red-sided garter snake. In: Balthazart, J., Pröve, E., Gilles, R. (Eds.), *Hormones and Behavior in Higher Vertebrates*. Plenum Press, London, pp. 398–406.
 Crews, D., 1984. Gamete production, sex hormone secretion, and mating behavior uncoupled. *Horm. Behav.* 18, 22–28.
 Crews, D., 1990. Neuroendocrine adaptations. In: Balthazart, J. (Ed.), *Hormones, Brain and Behaviour in Vertebrates*. S. Karger AG, Basel, pp. 1–14.
 Crews, D., 1991. Trans-seasonal action of androgen in the control of spring courtship behavior in male red-sided garter snakes. *Proc. Natl. Acad. Sci.* 88, 3545–3548.
 Crews, D., 1993. The organizational concept and vertebrates without sex chromosomes. *Brain Behav. Evol.* 42, 202–214.
 Crews, D., 1996. Temperature-dependent sex determination: the interplay of steroid hormones and temperature. *Zool. Sci.* 13, 1–13.
 Crews, D., 1999. Sexuality: the environmental organization of phenotypic plasticity. In: Wallen, K., Schneider, J. (Eds.), *Reproduction in Context*. M.I.T. Press, Cambridge, MA, pp. 473–499.
 Crews, D., 2003. The development of phenotypic plasticity: where biology and psychology meet. *Dev. Psychobiol.* 43, 1–10.
 Crews, D., Bull, J.J., 1987. Evolutionary insights from reptilian sexual differentiation. In: Haseltine, F.P., McClure, M.E., Goldberg, E.H. (Eds.), *Genetic Markers of Sexual Differentiation*. Plenum Press, New York, pp. 11–26.
 Crews, D., Fitzgerald, K.T., 1980. "Sexual" behavior in parthenogenetic lizards (*Cnemidophorus*). *Proc. Nat. Acad. Sci.* 77, 499–502.
 Crews, D., Moore, M.C., 1986. Evolution of mechanisms controlling mating behavior. *Science* 231, 121–125.
 Crews, D., Sakata, J.T., 2000. Evolution of brain mechanisms controlling sexual behavior. In: Matsumoto, A. (Ed.), *Sexual Differentiation of the Brain*. CRC Press, Boca Raton, FL, pp. 113–130.
 Crews, D., Gustafson, J.E., Tokarz, R.R., 1983. Psychobiology of parthenogenesis. In: Huey, R.B., Pianka, E.R., Schoener, T.W. (Eds.), *Lizard Ecology. Studies of a Model Organism*. Harvard University Press, Cambridge, pp. 205–231.
 Crews, D., Sakata, J.T., Rhen, T., 1998. Developmental effects on intersexual and intrasexual variation in growth and reproduction in a lizard with temperature-dependent sex determination. *Comp. Biochem. Physiol., Part C: Pharmacol., Toxicol. Endocrinol.* 119, 229–241.
 Crews, D., Fleming, A., Willingham, E., Baldwin, R., Skipper, J.K., 2001. Role of steroidogenic factor 1 and aromatase in temperature-dependent sex determination in the red-eared slider turtle. *J. Exp. Zool.* 290, 597–606.
 Diamond, J., 1983. Laboratory, field, and natural experiments. *Nature* 304, 586–587.
 Evans, L.T., 1936. Territorial behavior of normal and castrated lizards. *J. Genet. Psychol.* 48, 88–111.
 Greenberg, N., 1982. A forebrain atlas and stereotaxic technique for the lizard, *Anolis carolinensis*. *J. Morph.* 174, 217–236.

- Greenberg, N., 2002. Ethological aspects of stress in a model lizard, *Anolis carolinensis*. *Integr. Comp. Biol.* 42, 526–540.
- Greenberg, N., 2003. Sociality, stress, and the corpus striatum of the green Anolis lizard. *Physiol. Behav.* 79, 429–440.
- Greenberg, B., Noble, G.K., 1940. Testosterone propionate, a bisexual hormone in the American chameleon. *Proc. Soc. Exp. Biol. Med.* 44, 460–461.
- Greenberg, B., Noble, G.K., 1944. Social behavior in the American chameleon, *Anolis carolinensis*. *Physiol. Zool.* 17, 392–439.
- Godwin, J., Crews, D., 1997. Sex differences in the nervous system of reptiles. *Cell. Mol. Neurobiol.* 17, 649–669.
- Herbert, J., 1979. Old fashioned behaviour. In: Beyer, C. (Ed.), *Review of Endocrine Control of Sexual Behaviour*, Trends Neurosci, vol. 2. Raven Press, N.Y. (xviii–xix).
- Hews, D.K., Knapp, R., Moore, M.C., 1994. Early exposure to androgens affects adult expression of alternative male types in tree lizards. *Horm. Behav.* 28, 96–115.
- Hover, E.L., 1985. Differences in aggressive behavior between two throat color morphs in a lizard, *Urosaurus ornatus*. *Copeia* 1985, 933–940.
- Jennings, D.H., Moore, M.C., Knapp, R., Matthews, L., Orchinik, M., 2000. Plasma steroid-binding globulin mediation of differences in stress reactivity in alternative male phenotypes in tree lizards, *Urosaurus ornatus*. *Gen. Comp. Endocrinol.* 120, 289–299.
- Jennings, D.H., Painter, D., Moore, M.C., 2004. Role of the adrenal in early post hatching differentiation of alternative male phenotypes in the tree lizard (*Urosaurus ornatus*). *Gen. Comp. Endocrinol.* 135, 81–89.
- Ketterson, E.D., Nolan Jr., V., 1999. Exaptation, adaptation, and constraint: a hormonal perspective. *Am. Nat.* 153, S4–S25.
- Ketterson, E.D., Nolan Jr., V., Cawthorn, M.J., Parker, P.G., Ziegenfus, C., 1996. Phenotypic engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. *Ibis* 138, 1–17.
- Knapp, R., 2003. Endocrine mediation of vertebrate male alternative reproductive tactics: the next generation of studies. *Integr. Comp. Biol.* 43, 658–668.
- Knapp, R., Hews, D.K., Thompson, C.W., Ray, L., Moore, M.C., 2003. Environmental and endocrine correlates of tactic switching by non-territorial male tree lizards (*Urosaurus ornatus*). *Horm. Behav.* 43, 83–92.
- Lisk, R.D., 1967. Neural control of gonad size by hormone feedback in the desert iguana *Dipsosaurus dorsalis dorsalis*. *Gen. Comp. Endocrinol.* 8, 258–266.
- Marler, C.A., Moore, M.C., 1988. Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. *Behav. Ecol. Sociobiol.* 23, 21–26.
- Marler, C.A., Moore, M.C., 1989. Time and energy costs of aggression in testosterone-implanted free-living male mountain spiny lizards (*Sceloporus jarrovi*). *Physiol. Zool.* 62, 1334–1350.
- Marler, C.A., Moore, M.C., 1991. Energetic costs of aggression compensated for by food supplementation in testosterone-implanted male mountain spiny lizards. *Anim. Behav.* 42, 209–220.
- Marler, C.A., Walsberg, G.E., White, M.L., Moore, M.C., 1995. Increased energy expenditure due to increased territorial defense in male lizards after phenotype manipulation. *Behav. Ecol. Sociobiol.* 37, 225–232.
- Marshall, F.H.A., 1936. Sexual periodicity and the causes which determine it. *Philos. Trans. R. Soc. Lond. B* 226, 423–456.
- Mason, R.T., 1992. Reptilian pheromones. In: Gans, C., Crews, D. (Eds.), *Biology of the Reptilia, Physiology E. Hormones, brain and behavior*, vol. 18. University of Chicago Press, Chicago, Illinois, pp. 114–228.
- Moore, M.C., 1991. Application of organization–activation theory to alternative male reproductive strategies: a review. *Horm. Behav.* 25, 154–179.
- Moore, M.C., Marler, C.A., 1988. Hormones, behavior and the environment: an evolutionary perspective. In: Stetson, M.H. (Ed.), *Processing of Environmental Information in Vertebrates*. Springer-Verlag, New York, pp. 71–84.
- Moore, M.C., Whittier, J.M., Crews, D., 1985a. Sex steroid hormones during the ovarian cycle of an all-female, parthenogenetic lizard and their correlation with pseudosexual behavior. *Gen. Comp. Endocrinol.* 60, 144–153.
- Moore, M.C., Whittier, J.M., Billy, A.J., Crews, D., 1985b. Male-like behavior in an all-female lizard: relationship to ovarian cycle. *Anim. Behav.* 33, 284–289.
- Moore, M.C., Hews, D.K., Knapp, R., 1998. Evolution and hormonal control of alternative male phenotypes. *Am. Zool.*, 133–151.
- Phelps, S.M., Lydon, J., O'Malley, B.W., Crews, D., 1998. Regulation of male sexual behavior by progesterone receptor, sexual experience and androgen. *Horm. Behav.* 34, 294–302.
- Phoenix, C.H., Goy, R.W., Gerall, A.A., Young, W.C., 1959. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behaviour in the female guinea pig. *Endocrinology* 65, 369–382.
- Rhen, T., Crews, D., 2002. Variation in reproductive behavior within a sex; neural systems and endocrine activation. *J. Neuroendocrinol.* 14, 517–532.
- Sakata, J.T., Crews, D., 2004. Developmental sculpting of social phenotype and plasticity. *Neurosci. Biobehav. Rev.* 28, 95–112.
- Sinervo, B., Miles, D.B., Frankino, W.A., Klukowski, M., DeNardo, D.F., 2000. Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. *Horm. Behav.* 38, 222–233.
- Summers, C.H., 2002. Social interaction over time, implications for stress responsiveness. *Integr. Comp. Biol.* 42, 591–599.
- Thompson, C.W., Moore, M.C., 1991. Syntopic occurrence of multiple dewlap color morphs in male tree lizards, *Urosaurus ornatus*. *Copeia* 1991, 493–503.
- Thompson, C.W., Moore, M.C., 1992. Behavioral and hormonal correlates of alternative reproductive strategies in a polygynous lizard: tests of the relative plasticity and challenge hypotheses. *Horm. Behav.* 26, 568–585.
- Weiss, S.L., Moore, M.C., 2004. Activation of aggressive behavior by progesterone and testosterone in male tree lizards, *Urosaurus ornatus*. *Gen. Comp. Endocrinol.* 136, 282–288.
- Whittier, J.M., Crews, D., 1987. Seasonal reproduction: patterns and control. In: Norris, D.O., Jones, R.E. (Eds.), *Hormones and Reproduction in Fishes, Amphibians, and Reptiles*. Plenum Press, New York, pp. 385–409.
- Whittier, J.M., Tokarz, R.R., 1992. Physiological regulation of sexual behavior in female reptiles. In: Gans, C., Crews, D. (Eds.), *Biology of the Reptilia, Physiology E. Hormones, Brain and Behavior*, vol. 18. University of Chicago Press, Chicago, IL, pp. 24–69.
- Witt, D.M., Young, L.J., Crews, D., 1994. Progesterone and sexual behavior in males. *Psychoneuroendocrinology* 19, 553–562.
- Young, L.J., Crews, D., 1995. Comparative neuroendocrinology of steroid receptor gene expression and regulation: relationship to physiology and behavior. *Trends Endocrinol. Metab.* 6, 317–323.