

Courtship in Unisexual Lizards: A Model for Brain Evolution

An all-female species of whiptail lizards presents a unique opportunity to test hypotheses regarding the nature and the evolution of sexual behavior

by David Crews

Courtship of females by males is a necessary part of life for most animal species; if successful, it ends in copulation, thereby ensuring fertilization and the perpetuation of the species. In the majority of animals studied so far, however, courtship serves a function unrelated to fertilization: it stimulates ovarian development and reproductive activity on the part of the female and increases the overall efficiency of reproduction [see "The Reproductive Behavior of Ring Doves," by Daniel S. Lehrman; *SCIENTIFIC AMERICAN*, November, 1964]. Thus it may not seem odd that in species that lack males, male-like behavior continues to be exhibited.

This is true for an unusual species of whiptail lizard, *Cnemidophorus uniparens*, which inhabits the southwestern U.S. The species is derived from a "bisexual" ancestor (one that consists of males and females) but is itself "unisexual" (there are no males). The females reproduce by parthenogenesis, that is, reproduction without fertilization, and therefore copulation between its members is not directly related to the production of offspring. Nevertheless, the females in this species actively engage in lengthy courtship behaviors that are virtually identical with those observed between male and female whiptail lizards.

What mechanisms would allow such a behavior to persist in the absence of males? The brain, which controls mating behavior in males and females, not only has adapted to a new set of stimuli in this species but also has mediated a switch to females of behavioral patterns that are normally associated with males. This reinforces the observation that the

brain is equipped with neural circuits for both male and female behavioral repertoires, regardless of biological sex. By investigating the manner in which that has come about, using unisexual lizards as my model, I have gained insight into the ability of the brain to adjust to changing conditions during the course of evolution.

I became interested in the subject almost 10 years ago while studying the influence of hormones on the courtship of anole lizards [see "The Hormonal Control of Behavior in a Lizard," by David Crews; *SCIENTIFIC AMERICAN*, August, 1979]. I had been sent six adults of *C. uniparens* and wanted to compare their hormone cycles with those of the male and female anoles I had been studying for several years.

While working in my laboratory I happened to glance at the cage containing the whiptail lizards and noticed two individuals engaged in courtship with each other. The observation, made purely by accident, stunned and intrigued me. Over several weeks the behavior was repeated many times, following the same pattern on each occasion. This clearly was not random interaction between individuals but a highly ritualized form of behavior. How might it be explained? It seemed unlikely that parthenogenetic females would expend so much time and energy on an activity that had no apparent purpose. Was the behavior a useless vestige of the species' sexual history or did it have some real biological significance?

In order to answer that question I decided to compare the pseudosexual behaviors of these unisexual lizards with the sexual behavior of their

closest bisexual relatives. I needed to do this for two reasons: first, I wanted to determine what behavioral differences, if any, existed between these species and how they might have been altered in the switch from bisexuality to unisexuality; second, I wanted to ascertain whether or not courtship between male and female whiptails serves a function other than insemination. If it does, this would suggest that a similar function might be served by the courtship seen between parthenogenetic females.

One way to test this hypothesis would be to compare the number of offspring produced by females that engage in courtship behavior with the number produced by females that do not. If courtship among unisexual females could be shown to result in greater reproductive output, I would have reason to believe it is not merely a useless vestige of the past but a strategy critical to the continuing success of the species.

To understand the mechanisms by which pseudosexual activity among unisexual females evolved requires a look at the neuronal and hormonal factors that mediate it. It is well known that courtship is controlled by the central nervous system: sex hormones synthesized by males and females act directly on certain areas of the brain to trigger sex-typical behaviors. How is it that male-typical courtship behavior (which is mediated by male hormones) shifted from males to females during the course of evolution? Two possible explanations seem reasonable. Either the mechanisms that control male behavior in the ancestral sexual species were retained but modified in some way in the descendant species, or an entirely new and unknown mecha-

nism had evolved to control male-like behavior in unisexual females.

The whiptail lizards (genus *Cnemidophorus*) have several unique characteristics that make them ideal subjects for a study of this kind. There are 45 species in the genus; of these 15 have been identified as all-female species derived from existing bisexual ones. The bisexual species consist of males and females in a normal one-to-one ratio and reproduce sexually (as the vast majority of vertebrates do). The unisexual species are formed from the hybridization of closely related species; they consist solely of females that reproduce parthenogenetically. The offspring in such species are clones: they are genetically identical with their mothers and with one another [see "Unisexual Lizards," by Charles J. Cole; SCIENTIFIC AMERICAN, January, 1984].

Because these unisexual lizards are the result of a hybrid union, formed by the fusion of gametes from two existing species, both the ancestral (that is, the parental) and the descendant (the unisexual) species can be identified using molecular tech-

niques. This is a subtle but critical point. The existence of an ancestral species provides a control group against which the reproductive strategies of its descendant species can be compared. By comparing the DNA sequences of various whiptail lizards, Llewellyn D. Densmore III, Craig C. Moritz and Wesley M. Brown of the University of Michigan were able to determine that the maternal ancestor of *C. uniparens* is the bisexual species *C. inornatus*. Although they were unable to identify the paternal ancestor, the identification of one parental ancestor was sufficient for the purposes of this study.

I began my investigation by comparing mating in both species. In *C. inornatus*, as in *C. uniparens*, courtship follows a well-defined series of steps: the male typically approaches a female and probes her body with his tongue; if she is sexually receptive, he grips her with his jaws by grabbing the skin on either her neck or her foreleg. That act appears to pacify the female and provides the male with an opportunity to climb on her back, straddling her with his legs.

Once mounted, the male scratches the sides of the female with his fore and hind legs, pressing her body into the substrate as he does so. After several minutes the male maneuvers his tail under that of his female partner, thereby bringing his cloaca into contact with hers.

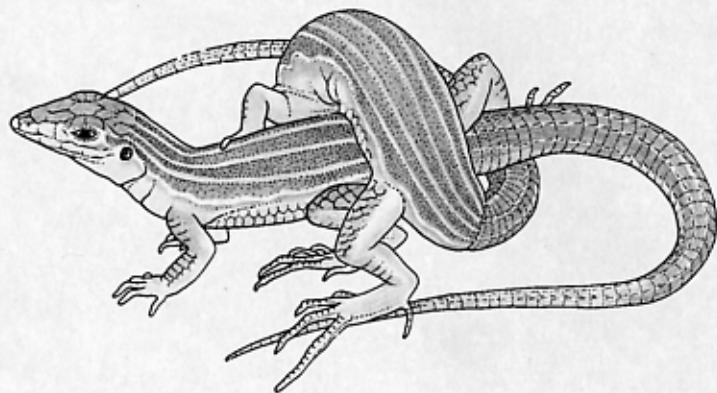
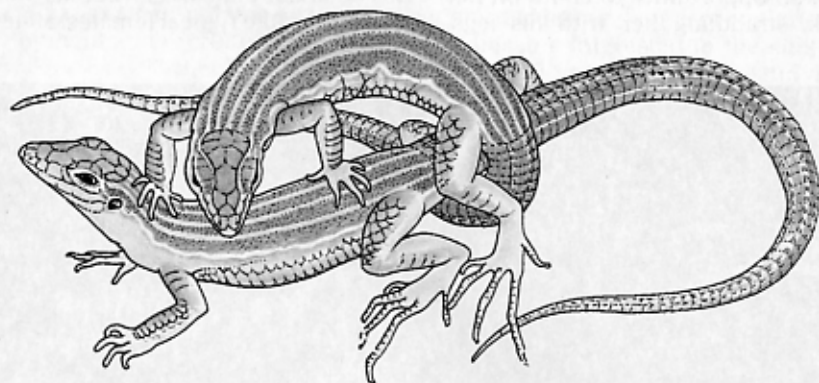
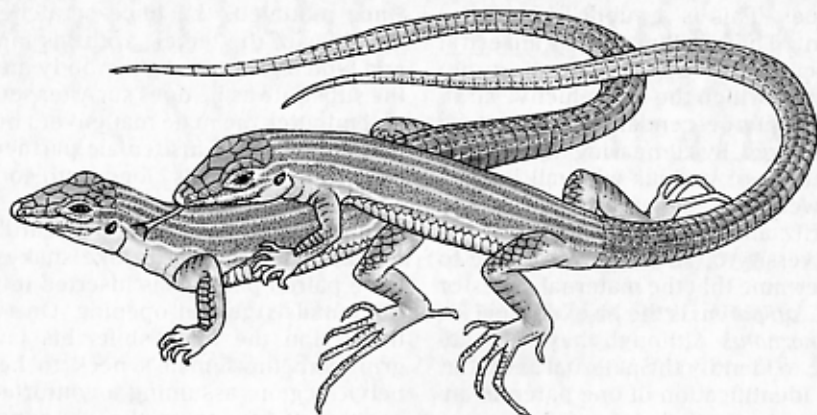
During mating one of the lizard's two hemipenes (lizards, like snakes, have paired penises) is inserted into the female's genital opening. On intromission the male shifts his jaw grip from the female's neck to her pelvic region, assuming a contorted position I refer to as the doughnut posture. He maintains this position for between five and 10 minutes, during which time ejaculation occurs. The male then rapidly dismounts and leaves the female.

Members of the descendant unisexual species *C. uniparens* exhibit a courtship ritual almost identical with that of their bisexual ancestors, except that some females assume the role of the male (I call this behavior male-like) whereas others behave like typical females. Male-



WHIPTAIL LIZARD, *Cnemidophorus uniparens*, from the southwestern U.S. is one of 15 species in the genus that are known to

consist only of females. In six of the species females have been observed participating in courtship behavior with one another.



COURTSHIP RITUAL between females in the unisexual species *C. uniparens* is virtually identical with that of its bisexual ancestor, *C. inornatus*. The "male" (the female that displays male-like behavior) will approach a female (one displaying typical female-like behavior) (*top*) and investigate her with its tongue. If the female is receptive, the "male" will mount her (*top center*), grabbing her by the skin of her neck as it does so. After a few minutes the "male" will swing its tail under that of the female (*bottom center*), a move that places their cloacal openings in apposition. The "male" then shifts its jaw grip to the pelvic region of the female (*bottom*), forming the characteristic doughnut posture. This step, also known as pseudocopulation, lasts between five and 10 minutes.

like females approach and mount sexually receptive females, gripping them with their jaws as they do so; after a few minutes they swing their tails under those of their partners and shift their jaw grips to form the characteristic doughnut posture (I call this behavior pseudocopulation). The only difference between pseudocopulation and true copulation is that the unisexual lizards are morphologically female (they lack hemipenes) and so intromission cannot occur between them.

Having determined that the courtship displays of these two species of whiptail lizards are virtually identical, I needed to determine what impact, if any, courtship (without insemination) has on reproduction. Among many species, including human beings, courtship has a primer effect: it serves to synchronize the reproductive physiologies of the male and female and to regulate normal ovarian development in females. If I could find evidence that courtship by males stimulates ovarian development in *C. inornatus*, I would have reason to suspect that a similar phenomenon might be taking place in *C. uniparens*, only with females substituting for males.

With Jonathan K. Lindzey, a graduate student in my laboratory, I began examining rates of ovulation in *C. inornatus* females reared under three different conditions: alone, with other females, or with males. The results were consistent with those found for other vertebrates: these females will not ovulate in isolation; if caged with other females, only a few will ovulate and they produce fewer eggs. In the presence of males, however, their ovaries grow much more rapidly, so that there is more frequent ovulation and greater total egg production. This represented a major finding: the results clearly indicate that courtship behavior in the ancestral species is a potent physiological force and is critical to the reproductive success of the species.

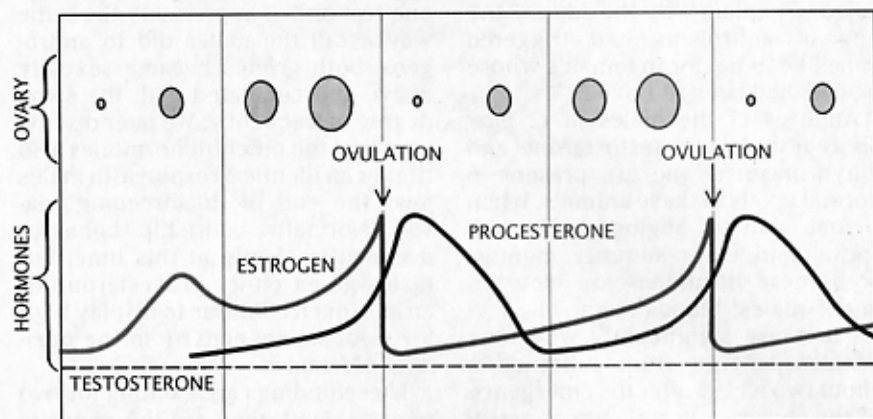
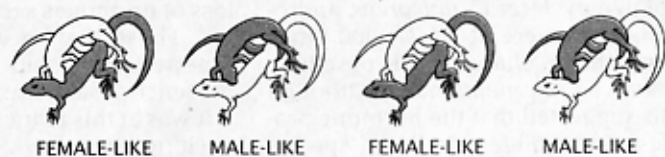
I wanted to see if a similar physiological effect might be operating in *C. uniparens*. In its natural habitat a female of this species will lay two or three egg clutches, each containing an average of two or three eggs, during the course of the breeding season (which lasts from spring until mid-summer). I was able to demonstrate, with the help of Jill E. Gustafson, an undergraduate in my laboratory, that females reared in isolation laid an average of only .8 clutch over

the course of the breeding season, whereas those reared in the presence of other sexually active females (with whom they engaged in pseudocopulation) produced more than three times that number, or an average of 2.5 clutches over the same period.

Thus we successfully proved that courtship behavior enhances reproduction in both bisexual and unisexual lizards. One major question remained unanswered, however: What underlying physiological mechanisms control sexual activity in these two species? If I could answer that question, I would know whether or not the same hormones responsible for male behavior in *C. inornatus* trigger male-like behavior in *C. uniparens*. This in turn would enable me to understand the role of the brain in sexual behavior and how it may have evolved in response to unisexuality.

I sought first to test the response of *C. uniparens* females to elevated levels of the male sex hormones testosterone and dihydrotestosterone (collectively called androgens). I operated on these lizards to remove their ovaries (the primary source of sex hormones in females) and then administered androgens to them. By doing so I found I could consistently induce male-like behavior in ovariectomized females. Although this seemed unusual (androgens are normally synthesized by the testes), the implications were intriguing. If male hormones were in fact responsible for male-like behavior, then the hormone profiles of these females must have been radically altered over the course of evolution. Had the ovaries, which normally produce female sex hormones, assumed the role of the testes? Were they producing male hormones to compensate for the absence of males? To prove such a hypothesis would require a thorough investigation into the physiological basis for sexual activity in both the unisexual and the bisexual species.

I began that undertaking by mapping the ovarian cycle, the focal point of reproduction in females and the process by which eggs are produced. We had already established, on the basis of egg production, that *Cnemidophorus* females undergo two or three discrete ovarian cycles (each approximately from three to four weeks in duration) during the three or four months of the mating season. Each of these cycles in turn can be divided into two distinct phases: the follicular, or preovulatory, phase, and the luteal, or postovulatory,



SEXUAL ACTIVITY in *C. uniparens* fluctuates in accordance with the ovarian cycle. Females that are preovulatory display female-like courtship behavior; those that are postovulatory exhibit behavior that is male-like. The change from female to male sex roles is mediated by changes in the circulating levels of sex hormones: high levels of estrogen are associated with female-like behavior; a sharp drop in estrogen (following ovulation) and a rapid increase in progesterone are associated with the switch to male-like behavior. The cycle is repeated two or three times during the breeding season.

phase. During the follicular phase yolk critical to the development of the embryo is deposited in the follicles, which reach a maximum diameter of 10 millimeters in six to eight days. The luteal phase follows ovulation when corpora lutea (the remnants of the follicles) form in the ovary and the eggs pass into the oviducts. Once in the oviduct the eggs acquire a hard outer shell and are usually laid between seven and 14 days after ovulation.

Having mapped the ovarian cycle, we now needed to compare it with fluctuations in sexual activity. Lindsey found that females of the ancestral species, *C. inornatus*, are receptive to males only during the follicular phase, when they are preovulatory. During the luteal phase, when they are postovulatory, they will aggressively reject the advances of males. We found a similar pattern of activity in *C. uniparens*. Female-like behavior is only expressed by females in the preovulatory phase, whereas male-like behavior appears consistently in females that have recently ovulated and are in the postovulatory phase.

As with sexual activity, patterns of hormone secretion vary according to the ovarian cycle. The female hormones, estrogen and progesterone (which are produced by the follicles

and the corpora lutea respectively), fluctuate in these lizards much as they do in most vertebrate females. Michael C. Moore, a postdoctoral fellow in my laboratory, found that in both *C. uniparens* and *C. inornatus* circulating concentrations of estrogen increase during the follicular phase, and reach peak levels at the time of ovulation. Progesterone levels, which are low during most of the follicular phase, are at their highest during the luteal phase and then decline rapidly when the corpora lutea are resorbed in from seven to 10 days. A new cycle begins in both species about a week later. The similarities between the estrogen and progesterone cycles in the two species indicate that their hormone profiles have remained stable over the course of evolution.

Contrary to what I had expected based on the results of my early experiment with ovariectomized females, we found that circulating levels of the male hormones testosterone and dihydrotestosterone in male-like females were uniformly low and in most instances could not be detected in our radioimmunoassay tests. There was no indication, even during or after pseudocopulation, of transient surges in their concentration. During the entire repro-

ductive cycle of *C. uniparens*, androgen levels were at least 1,000 times lower than is characteristic of sexually active *C. inornatus* males. Although this suggested that the hormone profiles of this all-female lizard species had not been profoundly altered, it failed to explain why the administration of androgens had triggered male-like behavior in females whose ovaries had been removed.

Analysis of the males of *C. inornatus* reveals that testosterone and dihydrotestosterone are present in normal levels in these animals. When sexual activity begins during the spring and early summer months, both these hormones are elevated in the males' bloodstream. The levels increase significantly when the females emerge from hibernation about two weeks after the emergence of the males, a correlation suggesting that the presence of females increases hormonal levels in male whiptails. Similarly, concentrations of the female hormones estrogen and progesterone are low, a pattern that conforms to the one found in most males of bisexual species.

I still needed to prove that testicular hormones control courtship and copulation in the males of *C. inornatus*. To do this, Lindzey and I surgically removed the testes from a group of sexually active males and then placed the males in cages with sexually receptive females. The results were unambiguous: castrated males court females significantly less often than intact males do. Moreover, by administering sex hormones to the castrated males, we were able to obtain direct evidence that the decline in sexual activity was linked to the

loss of hormones secreted by the testes. Those that received hormones renewed courtship activity in the presence of sexually active females.

It was at this point that we made an exciting discovery. Some of the castrated males treated with progesterone responded in precisely the same way as all the males did to androgens: both groups became sexually active and copulated with the same degree of frequency. We later discovered that the different hormones also trigger an identical response in males near the end of the breeding season. Normally courtship behavior declines markedly at this time, but males given either progesterone or an androgen continue to display high levels of sexual activity in the presence of females.

These findings are exciting for two reasons. First, they are the opposite of what has been found in mammals and birds (in those animals progesterone is a potent suppressor of sexual activity in males; here progesterone stimulates sexual activity), and second, they suggest that this sensitivity to the female hormone progesterone may have set the stage for the evolution of pseudocopulation in unisexual whiptails.

Notwithstanding, pseudocopulation remained a mystery. We knew that the loss of males in *C. uniparens* had been accompanied by a significant loss of testosterone and dihydrotestosterone, but we did not know what had taken their place as regulators of male-like behavior. The fact that male-like courtship was retained in this species indicated that the neural circuits underlying male

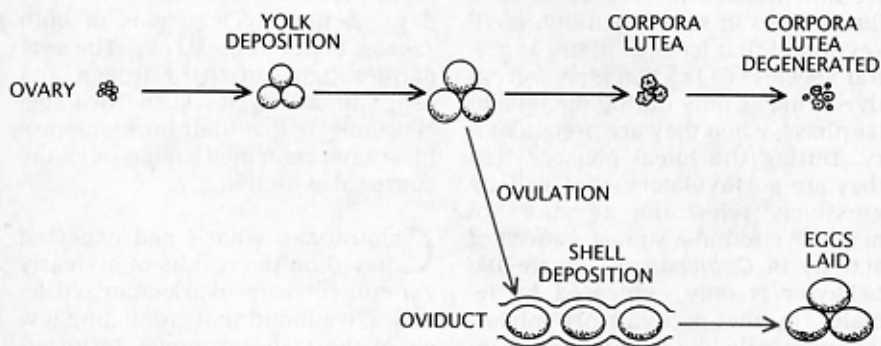
behavior had been retained, but the hormones had changed.

We knew that androgens trigger courtship behavior in *C. inornatus* males, but we could find no evidence for either testosterone or dihydrotestosterone during any phase of the ovarian cycle in *C. uniparens*. Thus we suspected that male-like behavior in that species is triggered by another cue, most likely a hormone produced by the ovary. We had several reasons for believing this was the case. Pseudocopulation had never been observed in females that were either reproductively inactive or had had their ovaries removed. Moreover, female- and male-like behaviors are clearly defined by the ovarian cycle: female-like behavior is limited to the follicular phase, male-like behavior to the luteal phase.

In many animals, transitions in sexual behavior correspond to hormonal changes. In these lizards, during the transition from female- to male-like behavior, circulating levels of estrogen drop by a factor of three, whereas levels of progesterone increase by a factor of nine. Could this abrupt shift in hormone concentration play a crucial role in the expression of pseudosexual behavior? Mark Grassman, a postdoctoral fellow in my laboratory, and I hypothesized this was so. We guessed that progesterone might be the endogenous stimulus responsible for male-like behavior and estrogen the stimulus for female-like behavior.

Furthermore, we surmised that if pseudocopulation were to operate as an effective reproductive strategy, complementarity would have to exist, that is, at any one time during the breeding season some females would be in the follicular phase and others would be in the luteal phase. We could test this by removing the ovaries from a number of females and then implanting capsules in them that contained either estrogen, progesterone or nothing. By pairing them in different combinations and monitoring their ensuing sexual activity, we could correlate behavior with specific hormones.

The results were unequivocal. When females that received progesterone were paired with females that received estrogen, pseudocopulation ensued, with the former individuals consistently performing the role of males and the latter the role of females. Pairs in which one individual received an empty capsule and the other a capsule containing



SUCCESSIVE CHANGES in the ovaries of whiptail lizards take place during the course of the ovarian cycle. At the beginning the ovaries are small, but they become progressively larger as the eggs inside them develop and acquire greater amounts of yolk. When the eggs are mature, they are released into the oviducts (this step is called ovulation), where they acquire hard shells, a process that lasts for from seven to 14 days. During this period the corpora lutea in the ovaries degenerate, a step that readies the ovaries for another round of egg production. The entire cycle, which lasts for from three to four weeks, is repeated several days after the first batch of eggs is laid.

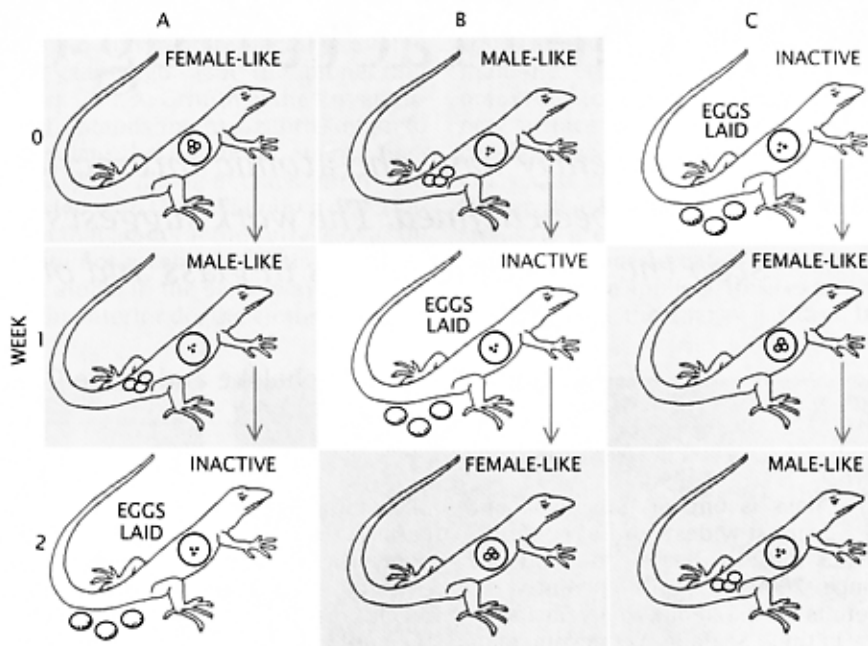
either progesterone or estrogen did not engage in sexual activity. I had previously determined that individuals housed together quickly establish and maintain this type of complementarity in their reproductive states. By alternating sex roles they maximize fecundity and increase the efficiency of reproduction.

The data from these experiments fit nicely into existing notions about the ability of the brain to adjust to different hormonal influences. It has often been said that the brain is initially bisexual and it is only later in development that it becomes sexually dimorphic. We wanted to explore this notion further, believing that in doing so we might finally solve the mystery of unisexual courtship.

We knew from studies on bisexual vertebrates that two distinct neural circuits mediate sexual behavior between males and females: one includes the anterior hypothalamus-preoptic area (AH-POA) of the brain and controls mounting and copulation in males; the other includes the ventromedial hypothalamus (VMH) and controls sexual receptivity in females. Moreover, both these areas of the brain have receptors for the corresponding male or female sex hormones.

Would the same distinctions hold true for unisexual lizards? With help from Mark Mayo, an undergraduate, and Juli Wade, a graduate student in my laboratory, I sought to determine which areas of the brain control male- and female-like behavior in *C. uniparens*. We implanted minute quantities of sex hormones directly into the brain of individuals that were sexually inactive (we had surgically removed their ovaries). The results were clear-cut. Androgens and progesterone implanted into the AH-POA activated male-typical pseudo-sexual behavior (estrogen did not); conversely, estrogen implanted into the VMH stimulated female-typical behavior (androgen and progesterone did not).

Thus we knew the neuronal circuits that mediate sexual behavior in *C. uniparens* were the same ones that control sexual behavior in bisexual vertebrates. We hypothesized that progesterone was able to stimulate male behavior in the AH-POA by binding to androgen receptors present in the neural circuits. Because these animals lack androgen almost entirely, there would be little competition for the binding sites and progesterone would bind readily to them.



COMPLEMENTARITY in the ovarian cycles of *C. uniparens* females makes courtship in this species possible. During the breeding season females will synchronize their activities so that when some individuals are preovulatory, others are postovulatory. This is represented here by three females whose behavior was observed over the course of several weeks. As a female changes from a state of preovulation (indicated by enlarged ovaries) to one of postovulation (indicated by the presence of eggs in the oviduct) her behavior changes from female-like to male-like. Because these lizards are reproductively out of phase (when one is female-like, her partner is male-like), courtship activity is continuous during the mating period. Sexually inactive females (those that have just laid their eggs) resume female-like behavior at the start of their next ovarian cycle. Individuals engaged in courtship with one another during a given week are shown in color.

Experiments Lindzey and I have carried out with Kathleen Matt of Arizona State University on bisexual lizards suggest that is the case. By radioactively labeling androgens and progesterone we were able to determine that progesterone is effective in displacing androgen from hormone receptors in the AH-POA. These experiments are only suggestive, however, and do not conclusively prove that progesterone binds to androgen receptors in the brain of *C. uniparens* females.

Some other possible mechanisms by which male-like behavior may be expressed by females must be considered. One is that progesterone is converted in the brain into androgen. This is unlikely, however, because we have recently found that R5020, a synthetic progesterone analogue, which cannot be converted into androgen in the brain, will stimulate sexual behavior in castrated *C. inornatus* males. Another possibility is that progesterone binds to progesterone receptors that are functionally linked to the AH-POA. But this is also unlikely, because it suggests there is a fundamental difference between

the progesterone receptor of whiptail lizards and all other vertebrates studied to date.

By dissecting the elements of pseudo-sexual behavior in a unisexual species of whiptail lizards we have shown how behavior critical to ovarian development and reproductive success can be retained when the conditions under which it originally evolved change. In the case of *C. uniparens* the loss of males meant the loss of male hormones that normally control male-typical mating behavior. Nevertheless, the persistence of male-like behavior in a unisexual lizard was possible because particular features of the brain of its ancestor, *C. inornatus* (namely the presence of dual neural circuits mediating male- and female-typical behaviors, and the sensitivity of its androgen receptors to progesterone), were co-opted to serve new functions in the absence of males. In this way courtship behavior between females has provided new insights into behavioral evolution and the means by which the neuroendocrine mechanisms that control behavior adapt to changing conditions.