Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 9547–9550, December 1986
Evolution

Behavioral facilitation of reproduction in sexual and unisexual whiptail lizards

(reptile/behavior/parthenogenesis/evolution/endocrinology)

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Communicated by Hampton L. Carson, September 8, 1986

ABSTRACT **All-female, parthenogenetic species afford a unique test of hypotheses regarding the nature and evolution of sexuality. Mating behavior accomplishes the transfer of gametes and stimulates the coordination of reproductive activity of the male and female. *Cnemidophorus uniparens*, a parthenogenetic species, is believed to have resulted from the hybridization of two extant gonochoristic species, *Cnemidophorus inornatus* and *Cnemidophorus gularis*. *C. uniparens* regularly and reliably perform behaviors identical in form to those performed during mating by male *C. inornatus*. We have determined experimentally that individuals of the parthenogenetic species demonstrating male-like pseudosexual behavior also share a similarity in function with males of the sexually reproducing species. The number of female *C. inornatus* ovulating increases, and the latency to ovulation decreases, if a sexually active conspecific male is present. A similar facilitatory effect on ovarian recrudescence occurs in the all-female *C. uniparens* in the presence of a male-like individual. These results show that behavioral facilitation of ovarian recrudescence is important in sexual and unisexual species. This may represent a potent selection pressure favoring the maintenance of male-typical behaviors, thus accounting for the display of behavioral traits usually associated with males in unisexual species of hybrid origin.**

In sexual species, a female's reproduction is triggered by various environmental factors, including social stimuli arising from the courtship and copulatory behaviors of conspecific males. In this regard it is important to distinguish conceptually the essential from the facilitatory functions of males. In gonochoristic species, sperm are necessary for successful reproduction, whereas the mating behaviors typical of the male can act as neuroendocrine primers to facilitate female reproductive activity; in many instances, the female will not undergo normal ovarian growth in the absence of sexually active males (1–4). Similar phenomena are known for female affects on males (5–10).

This dependence on males for stimulation of reproduction, as well as for sperm, presents an obstacle to the evolution of all-female parthenogenesis. Parthenogenesis has often been studied to understand how eggs initiate development in the absence of sperm and thus overcome this particular dependence on males. Parthenogenesis has rarely been studied with respect to the female's dependence on males for stimulation of reproductive events prior to the onset of development.

Dependence on male stimulation in a parthenogenetic species is a trait that would seem disadvantageous. However, the nature of the origin of the species and the abrupt reduction in genetic variance accompanying parthenogenesis may greatly retard the loss of such characters that suddenly become disadvantageous. For example, gynogenetic species retain a dependence on sperm to trigger development, even though the male genome is not incorporated (11). It is equally plausible that a parthenogenetic species might retain a capacity to respond to male behavior in accelerating their reproduction. The present study investigates this phenomenon in sexual and parthenogenetic whiptail lizards of the genus *Cnemidophorus*. Specifically, we compare the effects of the presence and hormonal condition of conspecifics on the induction of follicular growth and the latency to first ovulation in representatives of a gonochoristic species, *Cnemidophorus inornatus*, and a parthenogenetic species, *Cnemidophorus uniparens*, two species that share a very recent common ancestry.

One-third of the whiptail lizard species (*Cnemidophorus*) consist only of females that reproduce by true parthenogenesis (12, 13). The parthenogenetic species are known to have evolved from gonochoristic species; cytogenetic and electrophoretic studies indicate that two diploid gonochoristic species, whose immediate descendants are *C. inornatus* and *Cnemidophorus gularis*, hybridized to form the triploid parthenogen, *C. uniparens* (14–16); restriction endonuclease analyses of mitochondrial DNA indicate that *C. inornatus* is the maternal ancestor of the first generation diploid hybrid. *C. inornatus* then backcrossed with the hybrid female, thus contributing two-thirds of the triploid genome of *C. uniparens* (L. D. Densmore, C. Moritz, J. W. Wright, and W. M. Brown, personal communication). Because representatives of all three species exist today, it is possible to study the evolution of reproductive controlling mechanisms by comparing the parthenogenetic species with descendants of its direct evolutionary ancestors.

Reproductively active *C. uniparens* regularly and reliably exhibit pseudocopulatory behaviors if housed together (17, 18) that are identical in form to the mating behavior of *C. inornatus* (19–21). Crews and Fitzgerald (17) raised the question of why unisexual *C. uniparens* exhibit behaviors identical to the mating behaviors of their sexual ancestral species. Are these pseudosexual behaviors a useless vestige of their sexual ancestry or do they serve a biological function? The present results suggest that a continued need for behavioral facilitation of reproduction may have led to the maintenance of behavioral traits associated with reproduction in this parthenogenetic species.

In nature, *C. uniparens* usually lay 2–3 clutches each breeding season (22, 23). In captivity, we have observed that individuals lay an average of 2.3 ± 0.1 (mean ± SEM) clutches each breeding season (n = 52 animals, 119 clutches). Fecundity, however, varies markedly, but with the same magnitude of variance, according to social conditions (19, 24). *C. uniparens* housed in isolation or with an ovariectomized cagemate lay fewer clutches of eggs (averaging 0.7 and 1.0 clutches, respectively) than do individuals housed with an intact cagemate (1.5 clutches); individuals housed with an

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Abbreviation: df, degrees of freedom.

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ovariectomized individual treated with androgen, which will also induce male-like pseudosexual behavior (19, 24), lay on the average 2.6 clutches (19). Significantly, the evolution of the parthenogenetic mode of reproduction has not been accompanied by an alteration in the morphological progression and hormonal profile of the reproductive cycle (19, 25, 26). The latency to first ovulation following emergence from hibernation is similar in the two species (37.7 ± 1.9 days for *C. inornatus* and 37.4 ± 1.6 days for *C. uniparens*; mean ± SEM).

Reproductively mature *C. inornatus* and *C. uniparens* were collected during the reproductive season in Arizona. The animals were toe-clipped for permanent identification, and transported to the laboratory. The two species were housed in separate microprocessor-controlled environmental chambers that were programed to simulate the changes in photoperiod, temperature, and humidity characteristic of southeastern Arizona during the lizard's reproductive season (27). Animals were housed in 75 × 30 × 30 cm glass aquaria with 5 cm of sand as substrate. Isolated *C. inornatus* females were housed in aquaria divided into thirds, whereas the groups occupied the entire aquarium; pairs of *C. uniparens* were housed in aquaria divided in half with poster board. Radiant heat was provided by a 50-W heat lamp suspended 24 cm above the sand. In addition to room lights, each case received illumination from one ultraviolet light (GE-F20T12-BL) and a Chroma 50 fluorescent light suspended 61 cm above the sand. Lizards were fed either crickets or mealworms dusted with calcium and phosphates three times weekly. Water was provided in bowls *ad libitum*.

In the experiment with *C. inornatus*, females were housed either in isolation or in groups of three containing only intact females, two females and a castrated male *C. inornatus*, or two females and a castrated male *C. inornatus* that had received Silastic capsules containing exogenous dihydrotestosterone or testosterone. Stimulus male *C. inornatus* were castrated at least 5 months prior to the beginning of the experiment. Seven males received two Silastic capsules, one containing testosterone [5 mm × 0.64 mm (inner diameter) × 1.19 mm (outer diameter)] and the other dihydrotestosterone [8 mm × 1.47 mm (inner diameter) × 1.96 mm (outer diameter)]. These androgen treatments reliably induce sexual behaviors in castrated male *C. inornatus* (20). For purposes of analysis, one female was randomly excluded *a priori* from the all-female group.

In the experiment with *C. uniparens*, individuals were housed either in pairs consisting of two intact individuals, an intact individual and an ovariectomized individual implanted with a Silastic capsule containing progesterone, or an intact individual and an ovariectomized individual implanted with a blank capsule. Stimulus *C. uniparens* either had intact ovaries or were ovariectomized 2 weeks prior to the beginning of the experiment. Hormone-treated stimulus animals were implanted with a Silastic capsule containing progesterone [10 mm × 1.47 mm (inner diameter) × 1.96 mm (outer diameter)]. Ovariectomized *C. uniparens* do not exhibit pseudosexual behavior (19, 24), but administration of exogenous progesterone will reliably induce male-like pseudosexual behavior in ovariectomized individuals (28). An empty capsule was used as a blank control. These implant sizes have been shown to result in plasma hormone concentrations comparable to those normally seen in intact lizards captured in the field (25, 26). One individual from each pair in the group consisting of two intact individuals was randomly excluded *a priori*.

Ovarian activity was monitored by twice-weekly palpations of the abdomen (methods in ref. 18). The latency to ovulation was determined from two consecutive positive identifications of the postovulatory stage. The log likelihood test (29) was used to evaluate differences in the proportion of postovulatory lizards among treatment groups. Many individuals failed to ovulate during the study (*C. inornatus*: 23/43; *C. uniparens*: 17/32). Therefore, within each group, individuals that ovulated were ranked according to the latency to ovulation, whereas individuals that failed to ovulate were assigned an equal rank. Differences between groups were then compared using a one-way analysis of variance applied to ranks that is equivalent to the Kruskal–Wallis test (30).

None of the *C. inornatus* housed in isolation ovulated (Table 1). Some of the females housed in all-female groups (33%) or with a castrated male *C. inornatus* (25%) ovulated [log likelihood test (G) = 3.3; degrees of freedom (df) = 2; $P > 0.05$]. Although the numbers of females ovulating in these two groups were not significantly different from the isolation group, this was probably due to the small sample size for isolated females. A larger proportion of the females (93%) housed with castrated males treated with exogenous androgens ovulated during the course of the study ($G = 23.4$; df = 3; $P < 0.01$). The latency to ovulation varied with housing conditions (Fig. 1). Females housed alone with other females, or with a castrated male, exhibited longer latencies to ovulation than females housed with an androgen-treated, castrated male [analysis of variance (F) = 8.6; df = 3; 39; $P < 0.01$].

Individual *C. uniparens* also exhibited a differential probability of ovulating depending upon social housing (Table 1). Fewer (18%) of the individuals housed with an untreated, ovariectomized cagemate ovulated compared to 58% of the individuals housed with another intact individual and 66% of the individuals housed with an ovariectomized cagemate that had received a Silastic capsule containing progesterone ($G = 6.2$; df = 2; $P \leq 0.05$). Again, the latency to ovulation varied with housing conditions (Fig. 1). Housing with an ovariectomized cagemate or with an intact cagemate resulted in a significantly longer latency to ovulation compared to housing with an ovariectomized cagemate treated with progesterone ($F = 5.0$; df = 2, 29; $P < 0.05$). In those cages where both individuals were intact and reproductively active, analysis of the palpation records reveals that a complementarity was established in physiology and behavior such that one individual was postovulatory and exhibited male-like behavior while the other was preovulatory and exhibited female-like behavior.

We have demonstrated that the presence and hormonal condition of conspecifics can facilitate the induction of ovarian growth in the gonochoristic whiptail lizard *C.*

Table 1. Facilitation of ovulation in captive gonochoristic and parthenogenetic *Cnemidophorus* lizards

Treatment group	Ovulating, no.	Not ovulating, no.
C. inornatus		
Isolate	0	5
All-female	4	8
Castrate	3	9
Castrate + androgen	13	1
C. uniparens		
Ovariectomized + blank	2	9
Intact	7	5
Ovariectomized + progesterone	6	3

Presented is the number of females *C. inornatus* ovulating when housed in isolation (Isolate), with other females (All-female), with a castrated, untreated male *C. inornatus* (Castrate), or with a castrated, androgen-treated male *C. inornatus* (Castrate + androgen). In the study with *C. uniparens*, intact individuals were either housed together with an ovariectomized cagemate (Ovariectomized + blank), in pairs (Intact), or with an ovariectomized, progesterone-treated cagemate (Ovariectomized + progesterone).

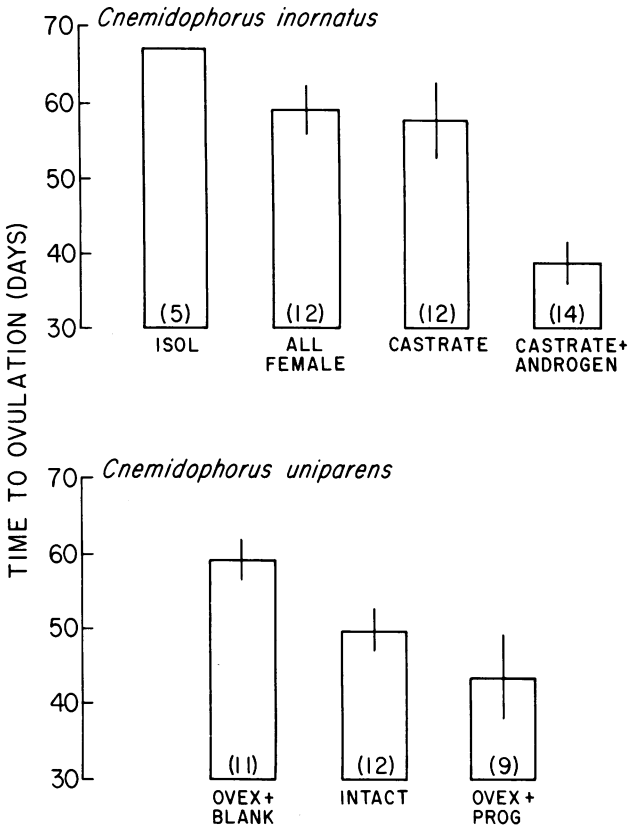

FIG. 1. Behavioral facilitation of ovulation in captive gonochoristic *C. inornatus* and parthenogenetic *C. uniparens*. Depicted is the mean latency (with standard error) to ovulation in females housed under different social conditions. Except in the isolation group (ISOL), intact female *C. inornatus* were housed either with other females (ALL FEMALE), with a castrated, untreated male *C. inornatus* (CASTRATE), or with a castrated, androgen-treated male *C. inornatus* (CASTRATE + ANDROGEN). In the study with *C. uniparens*, intact individuals were either housed together in pairs (INTACT) or with an ovariectomized cagemate who had received either a blank Silastic implant (OVEX + BLANK) or an implant containing progesterone (OVEX + PROG). Sample sizes are in parentheses. Individuals that failed to ovulate were assigned the maximum latency plus 15 days to distinguish them from individuals that ovulated on the last day of the study.

inornatus. Similarly, in a parthenogenetic descendant, *C. uniparens*, the presence and hormonal condition of cagemates has a profound effect on the onset of reproduction. This is a clear example in which a strong selection pressure (behavioral facilitation of ovarian activity) has maintained a trait (pseudosexual behaviors) in a species that lacks males.

Female-like pseudosexual behavior is limited to the preovulatory stage of the follicular cycle, whereas the expression of male-like pseudosexual behavior occurs most frequently during the postovulatory stages of the cycle (17, 18). Further, intact individuals housed together will quickly establish and maintain a complementarity in their reproductive conditions and alternate in their roles in pseudocopulatory encounters as they progress through their respective reproductive cycles (17, 18). This alternation in behavioral roles is paralleled by transitions in the circulating concentrations of sex steroid hormones produced by the ovary (25). Preovulatory animals expressing female-like pseudosexual behavior are characterized by an elevated concentration of estradiol and a moderate concentration of progesterone, whereas postovulatory animals exhibiting male-like pseudosexual behavior have a lower concentration of estradiol (by a factor of 2) and a greater concentration of progesterone (by a factor of 3) (25). The circulating concentrations of dihydrotestosterone and testosterone are not detectable at any stage of the reproductive cycle. We have demonstrated that although sexual behavior in male *C. inornatus* is dependent upon testicular androgens (20), it is the surge in progesterone following ovulation that triggers male-like pseudosexual behavior in *C. uniparens* (28). The same neural circuitry is probably involved in the control of male-like pseudosexual behavior in *C. uniparens* (35) and courtship and copulatory behaviors in male *C. inornatus* (J. C. Rozendaal and D.C., unpublished data). Recent studies suggest the neuroendocrine mechanisms underlying this shared behavioral display are different in *C. uniparens* and *C. inornatus* (J.L., K. Matt, and D.C., unpublished data).

This persistence of pseudosexual behaviors and behavioral facilitation of reproduction in *C. uniparens* is perhaps due to the fact that the species arose from the hybrid union of two gonochoristic species. This conclusion is based on similar experiments with gonochoristic and parthenogenetic strains of *Drosophila mercatorum* demonstrating that, in contrast to the unisexual whiptail, both traits are absent in unisexual *D. mercatorum* (31, 32). However, unlike parthenogenetic *Cnemidophorus*, the parthenogenetic strain of *Drosophila* arose from virgin females of a single species (33). This suggests that the functional association (34) between gonadal sex and behavioral sex is not intrinsic but dependent on the origin of the species. In *Cnemidophorus* these traits, which may be controlled by separate genetic systems, are linked because of the historical behavioral interaction between a male and a female (hybridization), whereas in *D. mercatorum* they are not.

We thank J. J. Bull, E. R. Pianka, and M. Kirkpatrick for reading the manuscript. This research was supported by National Science Foundation Grant BNS 85-12362, a grant from the Whitehall Foundation, National Institute of Mental Health Research Scientist Development Award MH00135 to D.C., and by National Institutes of Health National Research Service Award HD06618 to M.G.

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