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Testosterone response to courtship predicts future paternal behavior in the California mouse, *Peromyscus californicus*

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ABSTRACT

In the monogamous and biparental California mouse (*Peromyscus californicus*), paternal care is critical for maximal offspring survival. Animals form pair bonds and do not engage in extrapair matings, and thus female evaluation of paternal quality during courtship is likely to be advantageous. We hypothesized that male endocrine or behavioral response to courtship interactions would be predictive of future paternal behavior. To test this hypothesis, we formed 20 pairs of California mice, and evaluated their behavior during the first hour of courtship interactions and again following the birth of young. We also collected blood from males at baseline, 1 hr after pairing, 3 weeks paired, and when young were 4 days old to measure testosterone (T). We found that male T-response to courtship interactions predicted future paternal behavior, specifically the amount of time he huddled over young when challenged by the temporary removal of his mate. Males that mounted T increases at courtship also approached pups more quickly during this challenge than males who had a significant decrease in T at courtship. Proximity of the male and female during courtship predicted paternal huddling during a 1-hr observation, and a multiple regression analysis revealed that courtship behavior was also predictive of birth latency. We speculate that male T-response to a female in *P. californicus* is an honest indicator of paternal quality, and if detectable by females could provide a basis for evaluation during mate choice.

Courtship behavior has been described in a wide variety of taxa including birds, reptiles, insects, amphibians, and fish (Adkins-Regan, 1981; Lindzey and Crews, 1986; Sturtevant, 1915; Wagner, 1989; Wootton, 1984). Courtship behaviors themselves are diverse, but it is generally accepted that courtship serves as a tool to assess a potential mate and determine whether to proceed with the act of mating (Adkins-Regan, 2005). Although courtship can provide basic information regarding sex and species of the signaler, it can also be used to evaluate a potential partner for desired qualities. An animal may select a mate on the basis of direct benefits such as nuptial gifts or access to resources, or indirect benefits including good genes or offspring defense (Andersson, 1994). Classically, females have been considered the "choosy sex"; due to the contribution of costly ova and in many cases larger parental investment, females are predicted to benefit from cautious evaluation of males and their ability to provide direct or indirect benefits that will ultimately produce surviving young (Trivers, 1985). For example, female barn swallows benefit indirectly from mating with males who possess the largest ornaments, given that ornament size is positively correlated with enhanced offspring viability (Moller, 1994). In a monogamous, biparental species, we might predict that paternal care is an important benefit and that a male would be able to signal his competence as a future parent. Ostlund and Ahnesjo (1998) have shown that 15-spined stickleback females prefer males that shake their bodies more frequently during courtship, and the frequency of body shakes is in turn correlated with more frequent egg fanning bouts and increased egg hatching success.

Biparental care as a component of a breeding system can heighten the consequences of choosing an unsuitable mate. If a pair cannot coordinate its behavior to provide adequate resources to young, the associated reproductive consequences may be severe. Moreover, the opportunity to remate following the death of a partner may not exist (Thomas and Wolff, 2004). Although rare, monogamy and biparental care occur in approximately 3–5% of mammalian species and may enhance offspring survival (Kleiman, 1977; Wright, 2006). The California mouse, *Peromyscus californicus*, is a prime example of a species whose behavioral ecology implicates the need for careful evaluation of potential mates during courtship. First, California mice are strictly monogamous. DNA fingerprinting studies have shown no evidence of extra-pair fertilizations in the field (Ribble, 1991), and the majority of animals do not mate with a novel individual when presented with the opportunity in a laboratory setting (Gubernick and Nordby, 1993). The presence of the father also appears to enhance offspring survival. In the field, only 26% of young born to father-absent

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families reach weaning age, whereas 81% of father-present pups do (Gubernick and Teferi, 2000). Similarly, laboratory studies have found that male care enhances offspring survival in cold environmental conditions and when parents are required to wheel-run for food (Cantoni and Brown, 1997; Gubernick et al., 1993). Overall, these data present a strong case for careful evaluation of prospective partners in the California mouse, and suggest that quality of paternal behavior may be evaluated in the mate choice process.

Testosterone (T) promotes paternal care in *P. californicus* and is one possible endocrine mechanism that could link courtship interactions with future paternal behavior (Trainor and Marler, 2001; Trainor and Marler, 2002). Testosterone supports courtship components of male sexual behavior in a variety of species, including ultrasonic courtship calling in rodents (Floody et al., 1979; Nyby et al., 1977; Pomerantz et al., 1983), and courtship singing in birds; in all avian species studied to date, T metabolites play a role in the expression of courtship behavior (Harding et al., 1983). As such, T may provide an endocrine link between behaviors and resources that are of interest to females. As an example, female grey partridges select males on the basis of calling and vigilance behavior, the latter of which is androgen-dependent (Fusani et al., 1997). Thus, males that demonstrate high levels of vigilance are behaviorally signaling that they are in good physical condition and will be able to protect their mate and offspring from predators.

In the current study, we tested the hypothesis that endocrine and behavioral measures during courtship predict future paternal behavior in the California mouse. Given the strong selection pressure associated with paternal care in this species, courtship may be an important time period in which indicators of paternal behavior are assessed by potential mates. We hypothesized that these predictors could be either behavioral, biological (specifically via T measures) or a combination of both behavioral and endocrine courtship variables. Based on recent work with the dark-eyed junco showing that natural, individual variation in T responsiveness is associated with paternal care (McGlothlin et al., 2007), we were particularly interested in whether T responsiveness might also be related to paternal behavior in the California mouse. We paired sexually naive male and female California mice and observed their behavior during the first hour of pairing, once weekly until the birth of young, and during expression of paternal care in both challenge (female temporarily removed) and strictly observational paradigms. The use of a challenge paradigm is particularly important because paternal care may be more essential when the female is absent from the nest. Blood samples were drawn from males at baseline, following 1 hr of courtship, at 3 weeks paired and 4 days following the birth of young. Finally, we looked for associations between courtship variables and paternal behaviors.

Methods and materials

Subjects

We used 20 male and 20 female reproductively inexperienced *P. californicus* reared in a laboratory colony at the University of Wisconsin, Madison. Animals were maintained in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*, and the University of Wisconsin-Madison IACUC approved all procedures. Mice were weaned at 30 days old and housed in same-sex groups of two to four, in 48.3-cm-long × 26.7-cm-wide × 15.6-cm-high cages. Animals had free access to Purina 5015 mouse chow and water. The testing room was maintained at 25 °C under a 14:10 light/dark cycle with lights on at 2200 h, and all behavioral observations were conducted at 1300 h under dim red light. Testing began at 6–8 months of age, when we randomly assigned males and females to pairs. No male siblings were used, and no male/female pair shared common ancestry for a minimum of two prior generations.

Pairing procedure

One day prior to introduction, fur was shaved on both flanks or on the lower back of each male and female for identification on videotape. The male was then placed in a 91-cm-long × 46-cm-wide × 43-cm-high clear polycarbonate home arena equipped with a water bottle, mouse chow, a wooden nest box (12.7 cm long × 11.4 cm wide × 8.3 cm high), a cotton nestlet, aspen bedding, and a red transparent tube 15 cm long × 4.8 cm in diameter. During pilot studies, we observed that using a smaller testing arena resulted in primarily aggressive interactions (E.D.G., personal observation), and thus we chose testing arenas approximately three times larger than a standard housing cage. We placed males in testing arenas for 24 hrs before the introduction of a female, allowing the males to establish territories (Bester-Meredith et al., 1999; Trainor and Marler, 2001); by introducing a pair within the male's territory, we sought to mimic the female-biased dispersal pattern of the California mouse (Ribble, 1991). At pairing, the female was placed in the male's home cage, and 1 hr of videotape was recorded. Behavioral observations were recorded for 1 hr weekly until the pair delivered pups. An observer blind to pair identity scored the 1-hr courtship period for all behaviors that occurred; those that were consistently observed were selected for statistical analysis (Table 1). Although we intended to separate any pair displaying life-threatening aggression at pairing, no interventions were required. Courtship behaviors were subsequently scored by a reviewer blind to pair identity.

Paternal behavior assessment

We chose to assess paternal behavior using two separate observational paradigms, including a paternal "challenge," as well as an unmanipulated observation. On post-natal day 3 (PND3), we conducted a pup displacement challenge (PDC) to measure paternal behavior in the absence of the female. An experimenter removed the mother and pups from the testing arena 90 s before the start of the trial, returning pups to the location in the testing arena furthest from the nest (generally the opposite corner of the cage). At the conclusion of the 10-min trial, the mother was returned to the family. Latency to approach young, duration of huddling over young, duration of licking and grooming young, overall time spent in contact with young, and the number of retrievals were recorded. Huddling was defined as sitting crouched with an arched back over young, with minimal movement. Retrievals, although rare at this early stage in development (Bester-Meredith et al., 1999), were defined as grasping the pup by the skin of its neck or back and carrying it to another location in the testing arena.

On PND4, we conducted a 1-hr observation of paternal behavior without manipulation to assess paternal behavior in the presence of

Table 1
Description of behaviors consistently observed across pairs during the courtship period.

Behavior	Description
Proximity	The amount of time that animals are within the same quadrant of the testing arena
Following	The female walks slowly around testing arena and the male follows and investigates her anogenital region. The female may pause briefly, but continues to allow investigation by the male
Nose sniff	The male and female simultaneously approach, touch noses and engage in mutual nose-to nose investigation
Wrestle	Aggression developing into a tumble, in which animals attempt to pin one another
Chase	One animal pursues the other while running, attempting to make contact
Defensive jab	A fast, outward motion with the forepaw to deter an approaching animal, the result of which is typically a retreat by the offender. Only observed in females