Interaction of photoperiod and testes development is associated with paternal care in *Microtus pennsylvanicus* (meadow voles)

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Abstract

During the summer breeding season, free-living meadow voles do not engage in paternal care. However, in fall when female territoriality declines, social nesting and breeding activity may overlap and adult males nest with females and young. In the laboratory, meadow voles housed under short day (SD) lengths exhibit more and better quality paternal care than those housed under long day (LD) lengths. This observation is commensurate with the hypothesis that SD paternal care may increase fitness by decreasing pup mortality during colder months. However, SD males also demonstrate variability in paternal care. We hypothesize that this variability may be due to male fertility status; SD infertile males, incapable of siring offspring, should be less likely to care for pups than fertile males, for whom paternal care may confer fitness benefits. The goal of this experiment was to determine whether paternal behavior differed between fertile LD males, fertile SD males (i.e. males that were gonadally photoperiod-unresponsive to SD lengths), and infertile SD males (i.e. males that were gonadally photoperiod-responsive to SD lengths), as indexed by paired testes weights and behavioral evaluation. Fertile SD males exhibited proportionally more paternal behavior than infertile SD males or fertile LD males, which did not differ from each other. Fertile SD males also exhibited paternal behavior faster, spent more time in contact with pups, and engaged in longer and more frequent bouts of pup-directed grooming and huddling than either infertile SD males or fertile LD males. Collectively, these data suggest that photoperiod and fertility status may interact to exert both inhibitory and permissive control over the expression of paternal behavior in adult meadow voles. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

During the summer primary breeding season, female *Microtus pennsylvanicus* (meadow voles) are territorial, and reproductive males are rarely found in field nests with females or seen caring for young [1]. However, during the colder months of the year, meadow voles, like other small rodents [2], live communally to conserve heat. In some autumn populations, social nesting and breeding activity overlap in time, and scrotal males may be found nesting with a lactating female and her young [3,4]. During mild winters, reproductive activity may continue [5,6], but under low temperatures, reproduction ceases and nesting constellations consist of ascrotal males, nonlactating females, and juveniles [7].

Parental investment theory [8] predicts that during summer months the expression of paternal behavior in meadow voles should be rare, as under most conditions (when maternal care suffices), males of this predominantly polygynous species can best maximize reproductive success by seeking copulations with multiple mates, rather than selectively investing in one female and her offspring. However, under some ecological conditions, selective investment in a mate and paternal provisioning might confer increased fitness for reproductive male meadow voles [9]. For example, under low-density populations, selective affiliation with a known breeding female may secure postpartum matings

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when mates are scarce, and during the colder months of the year, the presence of the male in the nest may increase offspring survivorship by ensuring adequate thermoregulation of the litter.

Although no field research has tested the prediction that free-living reproductive meadow voles may initiate facultative paternal behavior in fall, other typically nonpaternal rodents engage in facultative care under similar circumstances (*Peromyscus maniculatus* [10]; *Marmota caligata* [11]; *P. leucopus* [12]; *Phodopus sungorus* [13]). Research on captive meadow voles also supports the assertion that under long, summer day lengths (LD) and short, fall day lengths (SD), photoperiod is a powerful regulator of the amount and quality of paternal behavior in meadow voles [14]. Specifically, more adult SD males exhibit paternal behavior than adult LD males, and SD males engage in retrieval, contacting, grooming and huddling of pups significantly more often and for longer durations than their LD male counterparts.

Although photoperiod has a permissive effect on paternal behavior expression, SD males show greater variability than LD males in whether or not they engage in paternal care [14]. One possible explanation for this phenomenon is that fertile SD males may be 'primed' to engage in paternal behavior in fall and winter nesting constellations, whereas infertile SD males, incapable of siring offspring, may be less likely to paternally respond to pups. From an adaptive level of analysis, such behavioral differences would be consistent with whether the male may have fathered the pups in the nest. Thus, the goal of this experiment was to determine whether fertile LD males, fertile SD males (i.e. males that were gonadally photoperiod-unresponsive to SD lengths), and infertile SD males (i.e. males that were gonadally photoperiod-responsive to SD lengths) differed in paternal behavior expression (as indexed by paired testes weights and behavioral evaluation).

2. Methods

2.1. Subjects

Subjects, derived from wild-caught voles indigenous to Northwestern Pennsylvania and Southwestern New York, were born to breeding pairs housed under LD lengths (14 h light/day) in an established colony at the University of Michigan. Weanling meadow vole pups were removed from the dam and sire at 19 days of age and housed alone in either LD or SD (10 h light/day) conditions. Subjects (total $N = 67$; $n = 31$ LD males, $n = 36$ SD males) were housed in polypropylene cages ($26.67 \times 21.59 \times 13.97$ cm) on pine shaving bedding with food (Purina mouse chow #5015) and water available ad libitum. Animal rooms were maintained at 21 ± 2 °C with low ambient noise conditions. Subjects remained so housed until the beginning of the experimental procedure (11–13 weeks of age).

2.2. Paternal behavior testing

As described previously [14,15], each sexually and parentally inexperienced male was placed in a novel polypropylene cage ($48.26 \times 26.67 \times 20.32$ cm) with fresh bedding. Males were allowed to become familiar with the new environment for 5 min, and then a 2–5-day-old alien pup was introduced to the opposite end of the cage from the male. Each test was conducted during the lighted phase of the light cycle and was videotaped for 10 min with a Panasonic camera and wide-angle lens on a time lapse VCR. An experimenter was always present during the test. If any male was overtly aggressive to a pup (causing pup vocalization or injury), the pup was immediately removed from the cage and the test terminated. Attacked pups were rarely injured, and consequently, they were returned to the home cage and reared normally by the parents (personal observation).

2.3. Testes removal and classification

Immediately following paternal behavior testing, study animals were sacrificed. Both testes were removed, placed in 2 ml of saline, and weighed on plastic weigh boats. SD and LD testes weights were coded as categorical variables. Previous research in our laboratory and others [16,17] has shown that testes weight is a reliable indicator of the presence or absence of spermatogenesis, and hence, fertility, in meadow voles. Grady and Lee (unpublished data) also found significantly higher testosterone concentrations in males with testes weights > 600 mg (1.90 ± 0.21 ng/ml) than males with testes weights < 600 mg (0.66 ± 0.29 ng/ml). In accord with these findings, males with paired testes weights below 600 mg were coded as infertile, whereas males with testes weights above 600 mg were coded as fertile. All LD males were coded as fertile, whereas 58.3% of SD males were coded as fertile, and 41.7% were coded as infertile.

2.4. Data analysis

As described previously [14,15], male behavior was scored categorically as pup-aggressive (rough handling/charging pup, resulting in pup vocalization and/or injury), pup-unresponsive (brief investigatory sniffing or no contacting/interacting with pup), or paternal (retrieval and/or grooming/huddling). Chi-square (χ^2) analysis was used to determine whether testes weight (e.g. infertile SD males, fertile SD males, fertile LD males) predicted the probability of male behavioral interactions with pups. Specific types of interactions (e.g. both the number and duration of sniffing, contacting, grooming, and huddling over the pup, in addition to time spent near the pup, time spent alone far from the pup, and the number of approaches and retrievals) were compared between infertile SD males, fertile SD males, and fertile LD males using one-way ANOVA, with Tukey post hoc pairwise comparisons to test for significant group differences. Finally, the latency to engage in any aggressive
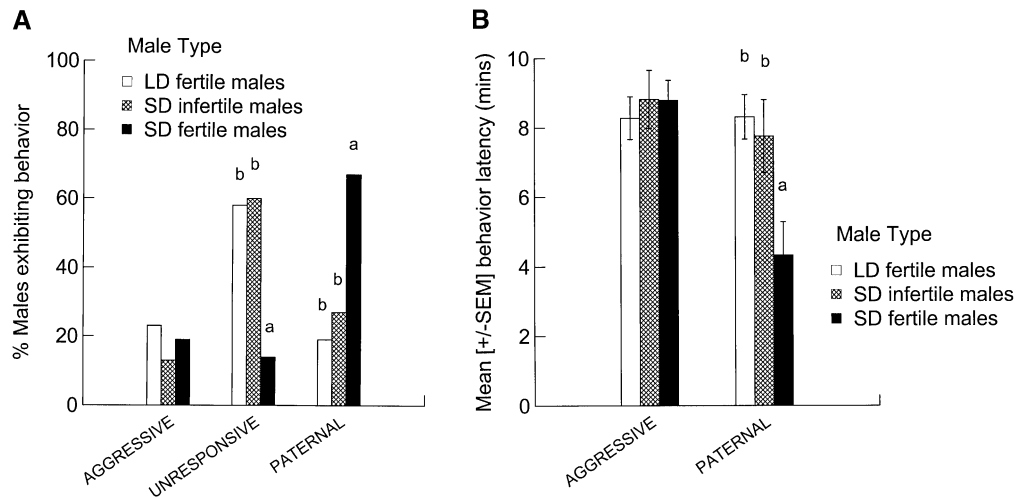
Fig. 1. Proportion of LD fertile males, SD infertile males, and SD fertile males that exhibited pup-aggressive, pup-unresponsive or paternal behavior toward 2–5-day-old neonates during 10-min paternal behavior tests (A). Mean (± S.E.M.) aggressive and paternal latencies during 10-min behavior tests (B). 'a' and 'b' indicate a significant difference ($P < .05$) between groups (within behavior type), where groups with no shared letters differ significantly and groups with shared letters do not.

behavior and the latency to behave in any paternal behavior (see above) were compared between groups using survival analysis, a statistical test that accounts for the probability of a behavioral event occurring, during a fixed elapsed time period (Systat 9.0).

3. Results

Meadow vole groups significantly differed on pup-directed behaviors (Pearson $\chi_2^2 = 15.082$; $P=.005$) (Fig. 1A). Specifically, fertile SD males exhibited categorically more paternal behavior than either infertile SD males ($P=.018$) or fertile LD males ($P=.001$), whereas the latter two groups did not differ ($P=.573$). Fertile SD males also exhibited less pup-unresponsive behavior than either infertile SD males ($P=.004$) or fertile LD males ($P=.002$), whereas the latter two groups did not differ ($P=.901$). Survival analysis demonstrated that males from these groups differed on how

quickly they initiated paternal behavior ($\chi_2^2 = 14.259$; $P=.001$), such that fertile SD males exhibited paternal behavior faster than either infertile SD males ($P=.033$) or fertile LD males ($P=.001$), whereas the latter two groups did not differ ($P=.580$) (Fig. 1B). The percentage of males that exhibited aggressive behavior did not differ by group nor did males from these groups differ on latency to become aggressive. Finally, LD fertile males, SD infertile males, and SD fertile males significantly differed on many specific measures of paternal behavior (Table 1).

4. Discussion

Data from this experiment indicate that males housed under SD lengths that have large testes (likely fertile) exhibit proportionally more paternal behavior than SD males that have small testes (likely infertile), or males housed under LD lengths that always have large testes (purportedly

Table 1
Mean (± S.E.M.) frequency and duration of male behavioral interactions with pups

Behavior	Measure	LD fertile males ($n=31$)	SD infertile males ($n=15$)	SD fertile males ($n=21$)	F	P
Sniffing	Frequency *	1.58 ± 0.50^a	2.40 ± 1.42^{ab}	5.10 ± 1.24^b	3.90	.025
	Duration†	0.11 ± 0.04	0.08 ± 0.05	0.17 ± 0.03	0.98	.383
Retrieving	Frequency	0.02 ± 0.02	0.30 ± 0.24	0.62 ± 0.31	2.83	.066
Contacting	Frequency	1.52 ± 0.62^a	5.40 ± 2.83^{ab}	11.67 ± 2.17^b	10.27	.001
	Duration	0.41 ± 0.22^a	0.66 ± 0.44^a	2.34 ± 0.52^b	8.03	.001
Grooming and huddling	Frequency	0.71 ± 0.27^a	1.53 ± 0.82^a	6.57 ± 1.35^b	15.12	.001
	Duration	0.20 ± 0.12^a	0.74 ± 0.40^a	1.95 ± 0.44	9.88	.001
Approaching	Frequency	1.07 ± 0.34^a	2.40 ± 1.24^a	5.81 ± 1.01^b	10.65	.001
	Duration	0.58 ± 0.29	0.18 ± 0.09	0.44 ± 0.09	0.63	.537
Alone	Duration	8.70 ± 0.48^a	8.33 ± 0.78^a	5.11 ± 0.74^b	9.82	.001

Superscripts a, b, ab = groups with no shared letters differ significantly ($P < .05$; Tukey post hoc tests), whereas groups with shared letters do not.

　* Frequency = mean (± S.E.M.) frequency of occurrence per 10-min test.

　† Duration = mean (± S.E.M.) duration of occurrence per 10-min test.

fertile). 'Fertile' SD males also exhibit paternal behavior faster, spend more time in contact with pups, and engage in longer and more frequent bouts of pup-directed grooming and huddling than either 'infertile' SD males or 'fertile' LD males (which do not differ from each other). Collectively, these data suggest that photoperiod and fertility status may interact to exert both inhibitory and permissive control over the expression of paternal behavior in adult meadow voles under specific environmental circumstances.

In mammals, paternal behavior is rare, as mating effort typically provides a larger genetic return for males [9,18]. Furthermore, paternal care may actually create mating opportunity costs, as allocating care to offspring typically precludes searching for additional mates. However, the failure to care for related young under marginal environmental conditions may carry negative fitness consequences for males. For meadow voles, these seasonably variable ecological circumstances may be particularly relevant, and may help explain the intraspecific variation in male parenting behavior reported herein.

Under 'modal' summer field conditions, female meadow voles typically have plentiful resources to rear young alone and population densities are sufficiently high for males to enhance reproductive success by seeking multiple mates. According to parental investment theory [8], summer meadow vole males would be unlikely to engage in paternal care. To our knowledge, there are no reports of free-living meadow voles exhibiting paternal care or even nesting with a female under summer field conditions [1]. Moreover, this lack of field evidence for paternal care is supported by laboratory data. In this study, over 80% of captive LD males without parental experience failed to exhibit paternal behavior, and these data are consistent with other laboratory reports [19–23].

In contrast to the breeding circumstances enjoyed by LD males, the decreased population densities and lower temperatures which characterize SD free-living conditions present more challenging circumstances under which to reproduce. Frequently, meadow vole breeding activity ceases in fall [7], presumably because the associated breeding costs are prohibitive. However, in some fall and winter populations, up to 50% of free-living meadow vole females are found with litters in the field [6,7]. Although adult SD males often undergo testicular regression in response to SD lengths [7], and adolescent males may delay puberty until the subsequent spring [1], the aforementioned presence of parous females in some breeding populations suggests that some proportion of adult males may remain fertile, and this assertion is supported by field observations of reproductive males nesting and sleeping with females and young [4]. Fertile (and infertile) SD males most likely join these extended maternal families for the thermoregulatory benefits afforded by group living [2]. However, additional fitness benefits may be associated with communal nesting for fertile, but not infertile, males. For example, under low-density populations, selective affiliation with a known breeding female may secure postpartum matings when mates are scarce, and during the colder months of the year, biparental huddling may increase offspring survivorship by ensuring adequate thermoregulation of the litter.

In light of these field data, one possible explanation for our laboratory findings (i.e. likely fertile SD males exhibit appreciable paternal care, whereas likely infertile SD males do not) is that fertile SD males may be 'primed' to care for their own pups during fall and winter nesting, whereas infertile SD males, incapable of siring offspring, are less likely to be paternally responsive to pups sired by other males. In a variety of rodent species [24–26], including meadow voles [14,23,27], social cues that are associated with reproductive activity (e.g. copulation, mate guarding, cohabitation with a pregnant mate, exposure to pups) inhibit pup-directed aggression and promote the onset of paternal behavior. Because SD males that remain reproductive may be more likely to initiate such social interactions with females and young, they may, in turn, exhibit more paternal responsiveness than infertile males (which would be less likely—by definition—to pursue such social interactions).

In conclusion, this experiment demonstrated that photoperiod and testes development are associated with the presence and quality of paternal behavior in captive meadow voles. Although the evolutionary rationale for the observed behavioral differences in these three groups of meadow voles has been well elucidated [8,28], exactly how these proposed seasonal parenting strategies are differentially regulated by central neuroanatomical and neurotransmitter systems (in particular, testosterone), is unknown. However, in Syrian hamsters, replacement testosterone does not instate the same sociosexual behaviors in LD and SD males, even when testosterone concentrations are identical [29], and SD, but not LD, males exhibit altered aromatase activity in some brain areas independent of circulating testosterone [30]. Taken together, these data suggest an interesting interpretation of our behavioral findings. In meadow voles, day length may influence how fertile males 'interpret' testosterone signals in particular brain areas. Thus, under SD lengths, when paternal behavior is most likely to accord fitness benefits, central testosterone, in combination with the aforementioned social cues, may exert a permissive effect on paternal behavior expression in fertile males. However, under LD lengths, when paternal behavior may be less adaptive and males are less likely to be exposed to the social cues that induce paternal care, central testosterone may exert an inhibitory effect on paternal behavior expression.

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