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General and Comparative Endocrinology 148 (2006) 252–259
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

Social and photoperiod effects on reproduction in five species of *Peromyscus*

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Received 14 December 2005; revised 24 February 2006; accepted 11 March 2006
Available online 19 April 2006

Abstract

At temperate latitudes, mammals and birds use changes in day length to time their reproductive activities to coincide with seasonal fluctuations in the environment. Close to the equator, however, conditions permissive of breeding do not track changes in day length as well, so other cues may be more important than photoperiod. In a variety of vertebrates, social interactions regulate breeding condition. We hypothesized that individuals of different species of *Peromyscus* mice found closer to the equator would respond more strongly to housing with an opposite sex conspecific than they would to photoperiod. To test this hypothesis, we compared the effects of long and short day lengths versus 8 days of pair housing with a female on reproductive tissue weights and testosterone (T) concentrations in five species of *Peromyscus* (*P. aztecus*, *P. eremicus*, *P. maniculatus*, *P. melanophrys*, and *P. polionotus*). After 13 weeks of short days (8L:16D), *P. maniculatus*, *P. melanophrys*, and *P. polionotus* significantly reduced relative testes mass compared to long day (16L:8D) housed animals. Social housing, however, had no effect on tissue weights in any species. However, male *P. polionotus* paired with females for 8 days increased T concentrations compared to single-housed males, whereas paired *P. maniculatus* reduced T. These data suggest that mechanisms of photoperiodic and social regulation of reproductive function are mediated by different physiological mechanisms among closely-related species and that both phylogeny and environmental factors contribute to patterns of reproductive plasticity.

Keywords: Evolution; *Peromyscus*; Photoperiod; Social experience; Testosterone; Testes

1. Introduction

Animals living in temperate or boreal environments display extensive seasonal variability in many physiological and behavioral traits. Presumably, this variability exists to help animals persist and reproduce in dynamic, but generally predictable environments. In temperate climates, many vertebrates show extensive changes in reproductive activity over the year. One of the most common environmental stimuli used to regulate reproduction is photoperiod (day length). In nontropical habitats photoperiod reliably predicts changes in temperature and precipitation. In general, species that inhabit tropical climates have expanded breeding seasons compared to closely related species that live in temperate climates. Several studies have observed that short days do not inhibit reproduction in tropical rodents (Demas and Nelson, 1998; Heideman and Bronson, 1990; Nunes et al., 2002), although this outcome does not necessarily reflect an inability to detect slight changes in photoperiod in these species (Hau et al., 1998). In the tropics, photoperiod may be a less informative predictor of impending climate, so other cues may be more useful for timing reproductive activity. In many species of vertebrates, social experience modulates activation of the reproductive axis.

Responsiveness to social stimuli may substitute or modify responsiveness to photoperiod. For example, male *Peromyscus aztecus* failed to reduce testes mass when housed in short days (8L:16D), but males that were housed with a female in long day lengths had larger testes and epididymides than singly housed males (Demas and Nelson, 1998). Male Siberian hamsters (*Phodopus sungorus*) housed with a

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0016-6480/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2006.03.006

female did not undergo testicular regression in short days (Hegstrom and Breedlove, 1999). At a neuroendocrine level, photostimulation of male hamsters with long days triggers an FSH surge within 3–5 days (Wolfe et al., 1995), but an LH surge is delayed unless hamsters are simultaneously housed with a female (Anand et al., 2002). In white-footed mice (*Peromyscus leucopus*), photoperiod has similar context-dependent effects; males paired with ovariectomized females for 13 weeks increased testes mass in long day conditions compared to individually-housed males, but pair-housing did not affect testes mass in short days (Pyter et al., 2005b).

Work in other vertebrate species supports the hypothesis that social cues modify reproductive state outside of the context of photoperiod. In voles (*Microtus pennsylvanicus*) ovulation is induced by exposure to male urine (Clulow and Mallory, 1970). Subordinate males exposed to aggressive behavior (social subjugation) may respond with suppression of reproduction at either a behavioral or physiological level (Yamaguchi et al., 2005; but see Koyama and Kamimura, 2003). In rhesus macaque (*Macaca mulatta*) subordinate males have smaller testes than dominant males (Bercovitch and Nurnberg, 1996), although it is unclear whether this is a cause or consequence of social status. Additionally, the presence or absence of parental care can modify the effects of social dominance. In non-parental cichlid fish (*Astatotilapia burtoni*) dominant males have larger testes than subordinates (Francis et al., 1993). In contrast male gobies (*Zosterisessor ophiocephalus*) provide paternal care upon assuming dominant social status, and this transition is associated with a decrease in testes mass (Scaggiante et al., 2004). Thus, a variety of social experiences can modulate reproductive state.

In the present study, we assessed the effects of photoperiod and social housing on reproductive tissue masses and testosterone concentrations in five species of *Peromyscus*. In the first experiment, males of each species were housed in either long or short day conditions for 13 weeks, and then reproductive organs and free testosterone were compared among and within species. Although the effects of photoperiod on testosterone have been measured previously in *Peromyscus maniculatus*, we are not aware of any study that has observed the effects of photoperiod on biologically active testosterone. In the second experiment, males were randomly assigned to single or pair housing for 8 days. Investigation of the effects of photoperiod and social experience in five different species in these contexts allowed us to examine the effects of latitude, photoperiod, and social stimulation on reproductive function in a phylogenetic context (Brooks and McLennan, 1991).

2. Methods

2.1. Study species

Peromyscus species are found across much of North America, and they inhabit a wide range of habitats and have extensive variation in ecology and life history characters (King, 1968). Deer mice, *P. maniculatus*, are distributed broadly and can be found as far north as the Northwest Territories of Canada and as far south as northern Mexico. Populations of this species exhibit differential sensitivity of reproductive activity to photoperiod, and breeding season duration varies depending on the population (Bronson, 1985). The *P. maniculatus* (*bairdii*) used in this study originated from a population that was trapped in central Michigan (latitude: 42°N). The other species in the present study have more restricted distributions. Old-field mice (*P. polionotus subgriseus*) are found in the southeast US and inhabit recently disturbed habitats such as old fields or sand dunes (29°N). *P. polionotus* reproduction has been observed through-out the year (Blair, 1951), although reproductive activity peaks in late fall (Caldwell and Gentry, 1965). Cactus mice (*P. eremicus*) are found primarily in southern California (32°N) and northern Mexico (Riddle, 2000) in a xeric environment. One study suggests that *P. eremicus* can breed year-round, but that hot and dry conditions tend to inhibit reproduction (Abbott, 1969). Aztec mice (*P. aztecus*) inhabit mesic cloud forests (19°N) in central Mexico (Vazquez et al., 2001). Reproduction has been observed throughout the year in this species, with a peak occurring at the end of the dry seasons (May) and during the wet season (August) (Vazquez et al., 2000). Plateau mice (*P. melanophrys*) inhabit desert highlands (22°N) in central Mexico (Hooper, 1955), and relatively little is known about their breeding season. Phylogenetic analyses indicate that *P. polionotus* and *P. maniculatus* are more closely related to each other than they are to *P. aztecus*, *P. eremicus*, and *P. melanophrys* (Carleton, 1980; Stangl and Baker, 1984). Additional details on these populations are available at the *Peromyscus* Stock Center website (http://stkctr.biol.sc.edu/).

2.2. Experiment 1: Species differences in the effects of photoperiod on reproductive system

Virgin male *P. aztecus*, *P. eremicus*, *P. maniculatus*, *P. melanophrys*, and *P. polionotus* of similar age (50 days < age <90 days) were obtained from the *Peromyscus* Genetic Stock Center (Columbia, SC). Upon arrival to our facility, all males were individually housed and randomly assigned to long (16L:8D) or short day length (8L:16D) conditions for 13 weeks. We chose the 13 week time period because prior work in *P. leucopus* suggested that gonadal regression was most stable at this time point (Pyter et al., 2005a). Over this period, all animals were provided with filtered tap water and Harlan Teklad 8640 ad libitum. After 13 weeks, males were anesthetized with isoflurane and rapidly decapitated. Testes, epididymides, and epididymal fat pads were dissected, cleaned of connective tissue, and weighed to the nearest 0.1 mg. Trunk blood was centrifuged, and plasma was removed for radioimmunoassay (RIA). Free testosterone was measured with a ^{125}I ImmuChem double antibody RIA kit (MP Biomedicals, Costa Mesa, CA), and all samples were run in a single assay. The intra-assay CV was 11.0%, and detection limit was 0.1 ng ml^{-1}. Samples that were below the standard curve were set to the detection limit as conservative estimates. Animals were maintained in accordance with the recommendations of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*, and all procedures were approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee.

2.3. Experiment II: Effects of social experience on reproductive tissue and testosterone

As above, a different set virgin male mice of similar age were obtained from the *Peromyscus* Genetic Stock Center, but in this experiment, all animals were housed for 13 weeks in long day conditions (16L:8D). After this period, an age-matched female (obtained from the same location and housed singly for the same period of time in the same photoperiod) was introduced to the males mice in the experiment; the other half of the mice remained singly housed. Males were randomly assigned to either pair-housed or single-housed groups. Eight days after this treatment, all male mice were rapidly decapitated under isoflurane, trunk blood was collected, and reproductive tissue masses cleaned and measured. Trunk blood was

centrifuged, and plasma was removed for RIA. Total testosterone was measured with a ^{125}I testosterone RIA kit (DSL-4100; Diagnostic Systems Laboratories, Webster, TX). These samples were run in two assays, the inter-assay CV was 9.6%, the average intra-assay CV was 3.9%, and the detection limit was 0.1 ng ml^{-1}.

2.4. Statistical analyses

Before testing for effects of treatments, all reproductive tissue masses were divided by body mass to obtain relative values. For analyses of reproductive tissues we used two-way ANOVA to test for effects of species, photoperiod, and the interaction. To test the hypothesis that photoperiod affected reproductive tissue, we used planned comparisons to compare long day and short day animals in each species. Because there were species × photoperiod interactions for some variables, we tested for species differences using only long day animals with one-way ANOVA followed by post hoc Duncan with $\alpha = 0.05$. In the social experience experiment we used two-way ANOVA to test for effects of species, social experience, and the interaction. We used planned comparisons to test for effects of social experience in each species. All analyses of testosterone concentrations were conducted on log transformed data.

3. Results

3.1. Experiment I: Species differences in the effects of photoperiod on reproductive system

In a two-way ANOVA of body mass-adjusted testes, there was a significant species × photoperiod interaction ($F_{4,68} = 3.90$, $p < 0.01$, Table 1). Planned comparisons revealed that *P. maniculatus*, *P. polionotus*, and *P. melanophrys* had larger body mass-adjusted testes mass in long day compared to short days, but *P. aztecus* and *P. eremicus* did not differ between photoperiod treatments (Fig. 1A). There were also species differences in mass-adjusted testes size of long day animals ($F_{4,42} = 174$, $p < 0.001$). Post hoc Duncan tests indicated that *P. melanophrys* had the largest mass-corrected testes mass and that *P. eremicus* and *P. aztecus* had the smallest (Fig. 1A).

In a two-way ANOVA on paired epididymides there was a significant effect of species ($F_{4,68} = 85.56$, $p < 0.01$), but no effect of photoperiod ($F_{1,68} = 3.38$, $p < 0.07$), or interaction ($F_{4,68} = 1.85$, $p = 0.13$). Only *P. polionotus* had larger paired epididymides in long day compared to short days (Fig. 2B). One-way ANOVA of long day animals showed significant species differences in mass-corrected epididymides ($F_{4,42} = 34.3$, $p < 0.001$). Post hoc Duncan tests indicated that *P. melanophrys* had the largest mass-corrected epididymides and that *P. eremicus* had the smallest (Fig. 2B).

In two-way ANOVA of body-mass corrected epididymidal fat pad size there was a significant interaction between species and photoperiod ($F_{4,68} = 2.84$, $p < 0.03$). Fat pads were larger in long days for *P. eremicus* and *P. polionotus* but not affected by photoperiod in *P. maniculatus*, *P. melanophrys* or *P. aztecus* (Fig. 1C). One-way ANOVA of long day animals showed significant species differences in mass-corrected epididymidal fat pads ($F_{4,42} = 34.3$, $p < 0.001$). Post hoc Duncan tests indicated that *P. melanophrys* and *P. eremicus* had larger fat pads than *P. polionotus*, *P. maniculatus*, or *P. aztecus* (Fig. 1C).

In two-way ANOVA on free T concentrations there was a significant effect of species ($F_{1,68} = 12.18$, $p < 0.01$) and non-significant effect of photoperiod ($F_{4,68} = 7.28$, $p < 0.07$) with no interaction ($F_{4,68} = 0.89$, $p = 0.48$). Although both *P. maniculatus* and *P. melanophrys* had lower free T concentrations in short days, planned comparisons indicated that only *P. polionotus* had significantly lower free T in short days. Post hoc Duncan tests indicated that *P. aztecus* and *P. eremicus* had the lowest free T concentrations and that *P. polionotus*, *P. maniculatus*, or *P. melanophrys* had the highest free T concentrations (Fig. 2).

3.2. Experiment II: Effects of social experience on reproductive tissue and testosterone

As in experiment 1, two-way ANOVA's detected significant species differences in body mass-adjusted testes ($F_{4,60} = 149$, $p < 0.01$), epididymides ($F_{4,60} = 81.1$, $p < 0.01$), and fat pad mass ($F_{4,60} = 13.7$, $p < 0.01$), and testosterone concentration ($F_{4,60} = 28.5$, $p < 0.01$). However, no significant effects of social housing or interactions were detected on any of these measures. Planned comparisons indicated that paired *P. polionotus* had significantly higher T concentrations compared to single housed animals, whereas in paired *P. maniculatus*, T concentrations were higher in single-housed mice ($p < 0.05$ in both cases, Fig. 3). T values of paired *P. aztecus* did not differ from unpaired males ($p = 0.12$). Pair housing had no effect on T in *P. melanophrys* or *P. eremicus* ($p > 0.05$ in each case).

4. Discussion

Latitude of origin had no consistent effects on responsiveness to photoperiod or social housing in five species of *Peromyscus* in this study. We expected that if latitude influenced the degree of gonadal regression in response to short

Table 1
Means (± standard error) for reproductive tissue masses in Experiment II

	Testes		Epididymides		Fat pad		Body size	
	Single	Pair	Single	Pair	Single	Pair	Single	Pair
P. aztecus	389 ± 21	381 ± 39	179 ± 21	176 ± 22	317 ± 82	201 ± 34	46 ± 2.6	42 ± 2
P. maniculatus	277 ± 8	254 ± 8	103 ± 6	89 ± 8	97 ± 11	83 ± 6	19 ± 0.4	19 ± 0.3
P. melanophrys	2348 ± 130	2116 ± 124	588 ± 36	549 ± 41	615 ± 134	578 ± 68	53 ± 3	51 ± 3
P. eremicus	271 ± 60	225 ± 39	68 ± 11	73 ± 10	175 ± 66	141 ± 28	24 ± 1	22 ± 0.8
P. polionotus	173 ± 8	189 ± 10	41 ± 3	39 ± 5	57 ± 4	53 ± 5	15 ± 0.5	14 ± 0.5

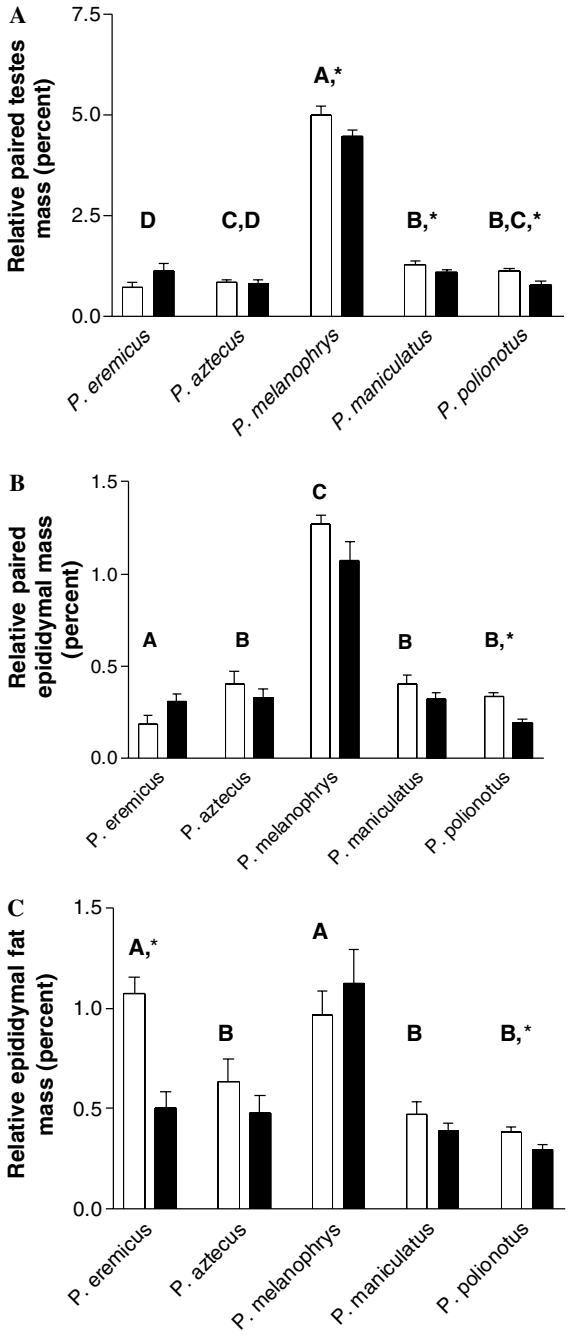

Fig. 1. Effects of photoperiod and species differences on reproductive tissues. (A) Relative paired testes mass is divided by body mass. (B) Relative epididymal mass is divided by body mass. (C) Relative epididymal fat pad mass is divided by body mass. Open bars: long day, black bars, short day. In each panel, letters reflect outcomes of post hoc comparisons of long day animals. $N = 6$–8 per group. Bars with the same letter are not significantly different. $^*p < 0.05$ within species planned comparison test of photoperiod.

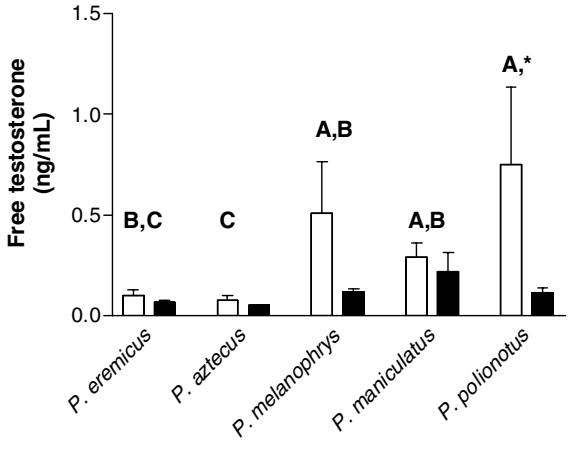

Fig. 2. Effects of photoperiod and species differences on free testosterone. Open bars: long day, black bars, short day. Letters reflect outcomes of post hoc comparisons of long day animals. $N = 6$–8 per group. Bars with the same letter are not significantly different. $^*p < 0.05$ within species planned comparison test of photoperiod.

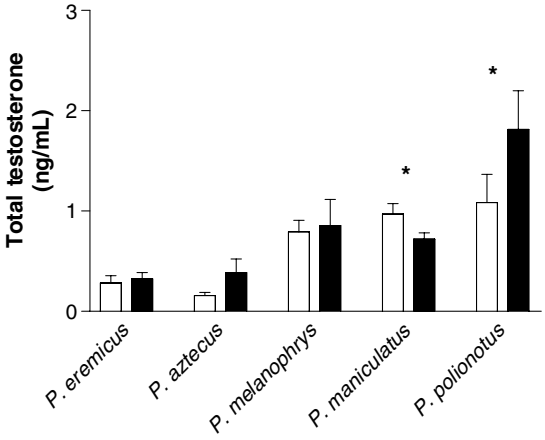

Fig. 3. Effects of social housing on total testosterone. $N = 6$–8 per group. Open bars: single housed, black bars, pair housed. $^*p < 0.05$ within species planned comparison test of photoperiod.

days, then *P. polionotus*, *P. aztecus*, and *P. melanophrys* would respond to short days with relatively modest gonadal regression, and *P. maniculatus* would respond with stronger gonadal regression. Instead, *P. maniculatus*, *P. melanophrys*, and *P. polionotus* exhibited gonadal regression in short days whereas the other two species did not. Work in several species has suggested that social stimuli can regulate reproductive function in the absence of photoresponsiveness, so species that did not respond to short days were expected to exhibit reproductive responses to pair housing with a female. Instead, we observed that only male *P. maniculatus* and *P. polionotus*, which also responded to photoperiod, exhibited changes in T when housed with a female. Our data indicate that the reproductive systems of different species of *Peromyscus* vary in how they respond to social and environmental signals. Species differences were also observed. The very large size of testes and epididymides in *P. melanophrys* was notable because they were five times larger than next largest species (*P. maniculatus*). Below we consider how variability in phylogeny, ecology, and behavior may impact plasticity in reproductive function.

Reproductive suppression in response to short days was observed in two lower latitude species (*P. polionotus*

and *P. melanophrys*) and one higher latitude species (*P. maniculatus*). Consistent with previous observations (Demas and Nelson, 1998), *P. aztecus* did not show reproductive suppression in short days. *P. eremicus* also did not exhibit reproductive suppression in short days, similar to closely related *Peromyscus californicus* (Nelson et al., 1995). Based on these data and observations of reproductive suppression in short days in other species from the same family (Cricetidae), it seems likely that photoperiod-responsiveness is the ancestral state for *Peromyscus* (Frost and Zucker, 1983). If this is the case, then the most parsimonious explanation for the evolution of photoperiod-responsiveness in *Peromyscus* is that photoperiod-responsiveness was lost twice within the genus, once in the subgenus *Haplomylomys* (including *P. californicus* and *P. eremicus*) and once in *P. aztecus* (Fig. 4). This interpretation suggests that there is a shared genetic basis for non-responsiveness in the *Haplomylomys* group, which could differ from *P. aztecus*. Our use of captive populations of *Peromyscus* raises the possibility that inbreeding or genetic drift could contribute to our results, as previous work has demonstrated that rapid change in reproductive parameters can occur in captive populations (Mauricio et al., 2005). However, *P. maniculatus* and *P. polionotus* have been bred in captivity since 1948 and 1952, respectively, yet these species exhibited the strongest responses to photoperiod. In contrast, *P. aztecus* and *P. eremicus* have been bred in captivity since 1986 and 1993, respectively, and did not respond to short days. In our view these dates do not support the hypothesis that inbreeding resulted in the observed species differences in photoresponsiveness.

Previous analyses of latitudinal differences in seasonal reproduction in mammals focused on variation at the population level. For example Bronson (1985) summarized studies in *P. maniculatus*, lagomorphs (*Lepus* sp. and *Sylvilagus* sp.), and deer (*Odocoileus sp.*), and reported that although populations of rabbits and deer maintained seasonal rhythms in breeding at different latitudes, *P. maniculatus* had elongated breeding seasons at low latitudes. Extended breeding seasons at low latitudes has also been observed in *P. leucopus* (Heideman et al., 1999). This population variation in breeding season length could reflect genetic variation in photoperiod-responsiveness to short days. Indeed, genetic variation in photoperiod-responsiveness has been identified within several rodent species (reviewed in Prendergast et al., 2001). Non-responders, or individuals that do not respond to short days with gonadal regression have been identified in *P. maniculatus* and *P. leucopus*, and selection experiments have determined that there is a genetic basis for this phenotype (Desjardins et al., 1986; Heideman et al., 1999). Polymorphisms for specific candidate genes or regulatory genes have not yet been identified. Our data suggest that species comparisons of candidate genes or regulatory regions may be a useful strategy for identifying the genetic loci that contribute to photoperiodic regulation of reproduction. Regardless of the mechanisms involved it is clear that some, but not all, *Peromyscus* respond to photoperiod.

The genus *Peromyscus* is one of the few mammalian groups in which systematic studies of behavior have been conducted. In a series of studies, Dewsbury and colleagues studied the mating behavior of several species of *Peromyscus* including several outgroups (reviewed in Langtimm and Dewsbury, 1991). All *Peromyscus* species examined were distinct from other neotomine species because males and females did not form a copulatory lock, whereas the species in the *Haplomylomys* subgenus (*P. eremicus* and *P. californicus*) could be distinguished from the *Peromyscus* subgenus by the presence of intra-vaginal thrusting during mating (Dewsbury, 1975). *P. melanophrys* differed from

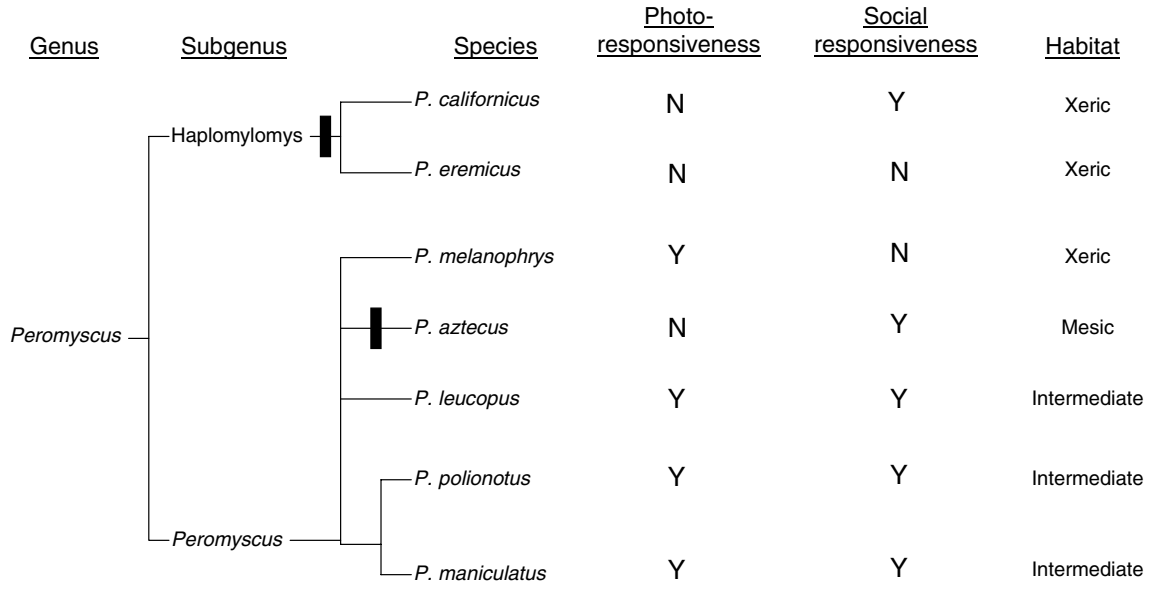

Fig. 4. Influences of phylogeny, photoperiod, and social interaction on reproductive activity in *Peromyscus*. Black bars indicate loss of reproductive photoresponsiveness.

other *Peromyscus* species included in the present study in that its copulatory behavior lacked several behavioral characteristics including a stereotyped dismount after mating (Dewsbury, 1979). We observed that *P. melanophrys* had massive testes and epididymal masses relative to body size (Fig. 1A, five times greater than the next species, *P. maniculatus*). It is not known if testis size is similarly large in *Peromyscus mexicanus*, a closely related species that shares this less complex mating behavior (Dewsbury, 1979). The large testes size in *P. melanophrys* was not associated with increased T (Fig. 3). *P. melanophrys* also had relatively large epididymal fat pads, a trait shared by *P. eremicus*. These species are in different subgenera, but live in similar environments (desert), which may reflect a connection between fat pads and arid climates.

Previous studies on the effect of social experience on the male reproductive system examined the interaction with short days photoperiod. Housing male *P. maniculatus* with a female in short days prevented gonadal regression (Whitsett and Lawton, 1982); a similar phenomenon was detected in Siberian hamsters (Hegstrom and Breedlove, 1999). The present study examined the effect of social housing on reproductive tissues and circulating T in long days. Although no significant changes in organ sizes were detected over the 8 day study, it is possible that changes may occur over a longer time scale. We did observe changes in T in response to pair housing with a female in both *P. maniculatus* and *P. polionotus*. The direction of these responses differed; male *P. polionotus* exhibited increased T whereas male *P. maniculatus* exhibited decreased T. Although most studies report that exposure to females increases T (at least in the short term), our finding in *P. maniculatus* is consistent with one prior study. Male *P. maniculatus* exposed to aggressive females during development had reduced testes growth as adults (Whitsett and Miller, 1985). This effect is not likely to be due to chemical stimulation, as prior studies observed no effect of female urine exposure on male testes growth in *P. maniculatus* (Lawton and Whitsett, 1980). One hypothesis for the species difference in T in response to paired housing is that T responses to pair housing reflect variability in social systems. A paternity study on free-living *P. polionotus* suggested that the dominant mating strategy in this species is the formation of stable mating pairs (Foltz, 1981). Paternity studies on *P. maniculatus* indicate that this species is promiscuous (Ribble, 1996). In socially or genetically monogamous species mating behavior may be delayed due to a period of pair bonding. Thus increases in T due to engaging in sexual behavior may occur later in *P. polionotus* compared to *P. maniculatus*, which would not be expected to form pair ponds. Indeed, reports of male *P. polionotus* intromission latencies in tests with hormone primed females are six times longer than those observed for *P. maniculatus* (Dewsbury, 1971). A study on *P. leucopus* reported that males paired with ovariectomized females in long days had higher T than single housed males (Pyter et al., 2005b). Presumably these males did not mate with their female cage-mates. This raises a related hypothesis that species differences in T responses reflect motivation to mate (Macrides et al., 1975), and that after mating occurs, T subsides. In biparental *P. californicus* T increases 2 weeks after initial pairings and decreases 2 weeks after the birth of pups and post-partum estrus (Trainor et al., 2003). A similar pattern occurs in biparental *Phodopus campbelli*, T gradually increases prior to and including post-partum estrus and subsequently declines (Reburn and Wynne-Edward, 1999). This hypothesis predicts that male *P. maniculatus* would mate within the first few days of pairing and that this would be associated with a decrease in sexual motivation (and thus T).

Two species (*P. aztecus* and *P. eremicus*) did not exhibit reproductive responses to photoperiod or social housing. A previous study reported that components of male *P. aztecus* reproduction did not respond to photoperiod, but that males increased testes mass and T when housed with an intact female for 8 days (Demas and Nelson, 1998). In our study, male *P. aztecus* did not respond to photoperiod and did not show significant responses to social housing. Our T measurements for paired and unpaired male *P. aztecus* match previously reported means for these groups almost exactly, but higher variation in our paired group made this difference statistically non-significant. An eight day period for pairing may be more sensitive for detecting changes in circulating hormones than changes in tissue weights, as we observed no significant effect of social housing on tissue weights in any species. Both *P. aztecus* and *P. eremicus* had relatively smaller testes masses relative to body size compared to the other species in this study. Although some laboratory studies have led to speculation that *P. eremicus* may form pair bonds (Dewsbury, 1974; Eisenberg, 1963), we are not aware of any field data describing social organization for either species.

These data show that there is a considerable diversity in the environmental regulation of the male reproductive system in the genus *Peromyscus*. It appears that photoperiod non-responsiveness has evolved at least twice within the genus, which provides an interesting opportunity for comparative work to determine if the same mechanisms preventing photoperiod-responsiveness have evolved in the *Haplomylomys* subgenus and *P. aztecus*. It is unclear whether *P. eremicus* is an aseasonal breeder or simply unresponsive to the cues used in this study. Another member of the subgenus *Haplomylomys*, *P. californicus*, does not respond to short day, but responds to water restriction by reducing testes size (Nelson, 1993). It would be interesting to determine if other desert dwelling species (e.g., *P. eremicus* and *P. melanophrys*) regulate reproductive output in response to water availability. Even *P. aztecus*, which live in relatively wet cloud forests, may be affected by water, as more females in breeding condition (as assessed by condition of nipples and presence of palpable embryos) were trapped during the rainy season (July–October) than during the rest of the year (Vazquez et al., 2000).

Phylogeny, photoperiod, and social interactions are important influences of reproductive activity in *Peromyscus* (Fig. 4). Further studies are necessary to understand the effects of environmental signals on reproductive condition among *Peromyscus*. Available evidence points towards species differences in the time course of mating as an important variable regulating T, but this hypothesis has not yet been tested consistently in multiple species. Also, it would be informative to investigate more fully the physiology, behavior, and general ecology of *P. melanophrys* given its remarkable testes size relative to the other species studied here. Within *Peromyscus*, both social and environmental factors appear to have influenced the evolution of plasticity in the male reproductive system.

Acknowledgments

The authors thank A.G. Trainor and S.L. Kidder for technical help and Zachary Weil for comments on the manuscript. This research was supported by NIH MH076313 to B.C.T. and MH57535 to R.J.N.

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