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Genetic and Environmental Influences on Short-Day Responsiveness in Siberian Hamsters (*Phodopus sungorus*)

Sharry L. Goldman, Krishnan Dhandapani,² and Bruce D. Goldman¹

Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269, USA

Abstract Siberian hamsters are photoperiodic rodents that typically exhibit several physiological changes when exposed to a short-day photoperiod. However, development of the winter phenotype in short days is largely conditional on prior photoperiod history: Hamsters that have been reared in an exceptionally long day length (18 L) do not usually exhibit the winter phenotype after transfer to short days, whereas animals reared under “moderately” long days (16 L) are more variable in responsiveness to subsequent short-day exposure, with 20% to 30% generally failing to exhibit winter-type responses. Hamsters reared exclusively in an “intermediate” day length (14 L) are almost uniformly responsive to short photoperiod. In the present study, the authors examine the influence of photoperiod history on short-day responsiveness in a breeding line of hamsters that has been subjected to artificial selection for resistance to the effects of short days. The results demonstrate that photoperiod history is an important determinant of short-day responsiveness in both random-bred (UNS) hamsters and animals artificially selected and bred for nonresponsiveness to short photoperiod (PNR). The PNR hamsters have a reduced requirement for long-day exposure to evoke a state of unresponsiveness to short days. The results are discussed in relation to possible significance for the origin of population and species differences in photoperiod responsiveness.

Key words photoperiodism, hamster, genetic, polymorphic

There are numerous species differences among mammals in responsiveness to photoperiod cues. For example, even closely related species may exhibit different critical day length requirements for maintaining reproductive activity (Elliott, 1976; Hong et al., 1986). In some species, photoperiod influences several traits; in other species, the effects of photoperiod are more restricted (Goldman and Nelson, 1993). Differences in photoperiodic response also occur between different breeding populations within a species.

White-footed mice (*Peromyscus leucopus*) collected in Connecticut exhibited reproductive inhibition when exposed to short days in the laboratory; mice of the same species collected in Georgia did not show this short-day response. This difference in photoperiod response persisted when mice from the two populations were born and reared under identical conditions in the laboratory (Lynch et al., 1981; Carlson et al., 1989). Similarly, differences in photoperiod responsiveness were found between deer mice (*Peromyscus*

1. To whom all correspondence should be addressed.

2. Present address: Department of Physiology and Endocrinology, Medical College of Georgia, Augusta, GA 30912, USA

maniculatus) captured at different latitudes (Dark et al., 1988). Differences in photoperiod responsiveness between species and between different breeding populations within a species undoubtedly represent evolved adaptations to different environments.

Variations in photoperiod responsiveness have also been observed between individual members of a single breeding population. Laboratory-bred deer mice, derived from a single wild-caught breeding population, showed variations in response to short days. Individual mice that failed to undergo gonadal regression in short days also failed to regress when treated with a dose of melatonin that induced regression in short-day responsive deer mice (Blank and Freeman, 1991). It has been suggested that variations in photoresponsiveness within a breeding population may represent a balanced polymorphism that permits opportunistic winter breeding by a fraction of the population. Winter reproduction by a portion of the breeding population has been described for a number of small rodents (Christian, 1980).

Laboratory bred Siberian hamsters (*Phodopus sungorus*) exhibit considerable individual variations in responsiveness to photoperiod. Most individuals undergo gonadal regression, molt to a winter pelage, and decrease body and lipid mass when exposed to short days (Goldman and Nelson, 1993). However, 20% to 30% of the animals may fail to exhibit any of these responses. The basis for the variation in photoresponsiveness among Siberian hamsters is different from that described for deer mice and white-footed mice, where nonresponsive individuals produce typical short-day melatonin patterns but do not exhibit winter-type changes in response to these patterns. Short-day nonresponsive Siberian hamsters exhibit longer taus as compared to their photoresponsive counterparts. This leads to different phase angles of entrainment to short-day photoperiods and a subsequent failure to show the expanded durations of melatonin secretion that are required for initiating the various winter-type responses in this species (Freeman and Goldman, 1996a; Puchalski and Lynch, 1986).

Through artificial selection, it has been possible to develop breeding lines of Siberian hamsters with much larger percentages of animals that are nonresponsive to short days (Freeman and Goldman, 1996 a,b; Kliman and Lynch, 1992), demonstrating a heritable component to this trait. Our laboratory has developed a breeding line of photoperiod non-responsive hamsters (PNR) in which more than 80% of

the adult males fail to exhibit testis regression when exposed to short days. However, responsiveness to short photoperiod appeared to be at least partly age related, since when PNR hamsters were exposed to short days beginning at 19 days of age, substantial numbers of animals did show short-day responses. Hamsters derived directly from our general breeding colony (UNS, unselected breeding line) also exhibited a larger proportion of short-day nonresponders when tested as adults than when tested as juveniles. These observations suggested that the older animals may be the members of the population that would be most likely to engage in winter breeding in the field (Freeman and Goldman, 1996b).

In Siberian hamsters, photoperiod history may be more important than age per se for determining whether an individual will exhibit winter responses when exposed to short days. When male UNS Siberian hamsters were exposed to very long days (18 L) for 10 weeks and then shifted to short days, few animals showed reproductive inhibition; in contrast, hamsters that were raised in an intermediate day length (14 L) consistently showed the species-typical inhibition of reproductive activity when transferred to short days (Gorman and Zucker, 1997). These results, taken in conjunction with other studies employing simulated natural photoperiods (Gorman and Zucker, 1995), suggested the following scenario: Hamsters born early in the breeding season would be exposed to the longest days of summer, which exceed 18 L in the natural habitat of this species. This photoperiod experience would render most of the animals nonresponsive to short days, so that these individuals would be most likely to engage in winter breeding. Hamsters born later in the breeding season and not exposed to mid-summer day lengths would be highly responsive to short days and would therefore be reproductively inhibited through the winter (Gorman and Zucker, 1997). The end result would be that the oldest animals in the winter population would be those most likely to attempt winter breeding, whereas the younger hamsters would gamble on survival through the winter and, if successful, breed the following spring.

The present study utilized our breeding line (PNR) of hamsters that was selected for nonresponsiveness to short days to further explore the role of photoperiod history in short-day responsiveness. The experiments were designed to determine (a) whether PNR hamsters require some amount of long-day exposure to become nonresponsive to short days and (b) whether the effects of long-day exposure to induce nonrespon-

siveness to short days are quantitatively different in PNR and UNS hamsters, respectively.

MATERIALS AND METHODS

Animals used in these experiments were obtained from two breeding colonies maintained in our laboratory. The main colony of random-bred hamsters (UNS, or unselected) was derived from stock provided by Klaus Hoffmann. The second breeding line (PNR, or photoperiod nonresponsive) was derived from the UNS hamsters by artificial selection for the trait of nonresponsiveness to a short-day photoperiod (10 L). Our breeding colonies are routinely maintained under a 16:8 LD photoperiod at a room temperature of 20 to 22 °C. Artificial selection that led to the development of our PNR colony was carried out by raising hamsters in 16 L, testing them for responsiveness to a short-day photoperiod, and establishing breeding pairs from among the males and females that failed to respond. Further details of the method of artificial selection and comparisons of the PNR and UNS hamsters with respect to photoperiodic response and circadian parameters have been reported (Freeman and Goldman, 1996 a,b).

Food (Agway Prolab 3500 RMH) and water are provided ad libitum for all hamsters. All animals used as breeders in these experiments were raised from birth in 16 L. At the time that adults were paired for use as experimental breeders, they were either left in 16 L or transferred to 18 L or 14 L (Experiments 1, 3, and 4). Adult males and females were paired and left together for breeding and during pregnancy and lactation. Hamster pups are routinely weaned at 18 to 20 days of age in our colonies. In some of the present experiments, pups were weaned at 14 days postpartum (day of birth = day 1, or D₁). In these cases, the breeding pairs and their pups were given supplemental feeding with sunflower seeds for a few days prior to weaning, and freshly weaned hamsters continued to receive sunflower seeds in addition to the standard diet for a few more days. This contributed to more rapid growth of the young.

Experiment 1: Effect of Photoperiod of Rearing on Short-Day Responsiveness of UNS and PNR Hamsters in Adulthood

This experiment was designed to determine the effects of different photoperiods during rearing on

adult responsiveness to short days in hamsters from both breeding lines. Breeding pairs of both UNS and PNR hamsters were housed in 14 L (lights on 0300-1700 h), 16 L (lights on 0200-1800 h), or 18 L (lights on 0100-1900 h). Pups were weaned and group housed by sex at 18 days postpartum. All hamsters remained in the same photoperiod that had been in effect during gestation. Only male offspring were used in this experiment.

At 60 days of age, hamsters either remained in the photoperiod of gestation/rearing or were moved to 10 L (lights on 0500-1500 h; short-day photoperiod). Eight weeks later, all animals were autopsied. Pelage condition was scored (Duncan and Goldman, 1984), and animals were considered to have displayed a winter molt if they exhibited any indication of the molt. Since Siberian hamsters usually require 10 to 12 weeks of short days to complete a winter molt, these animals generally showed only beginning stages of molt. Nevertheless, this is a reliable indicator of response to short days because hamsters housed exclusively in long days show no indications of winter pelage development. To assess reproductive status, the testes were removed and weighed. Male hamsters raised in long days rarely exhibit paired testes weights < 500 mg by 3 to 4 months of age (Freeman and Goldman, 1996b). Therefore, paired testes weights < 500 mg were considered to indicate reproductive inhibition.

Experiment 2: Effects of Different Amounts of Long-Day Exposure in Early Life on Subsequent Response to Short Days in PNR Hamsters

In a preliminary study, male PNR hamsters ($n = 10$) that were gestated and reared postnatally in 10 L all showed retarded testis development at 1 month of age. Also, PNR males ($n = 7$) gestated in 16 L and transferred with their parents to 10 L on the day of birth all had small testes at 1 month (Eric Anderson, unpublished data). These observations suggest that PNR hamsters may require postnatal exposure to long days to become unresponsive to short days; alternatively, it may be that resistance to the reproductive inhibitory effects of short days simply does not develop until later in life. Experiments 2 and 3 were designed to determine whether PNR hamsters require some period of postnatal exposure to long days to become unresponsive to the reproductive inhibitory effects of short days.

Only PNR hamsters were used in this experiment. Our preliminary results suggested that PNR males raised from birth in short days were uniformly responsive, and other studies have suggested that photoperiods experienced during the first 2 weeks after birth do not influence subsequent photoperiod responsiveness in Siberian hamsters (Stetson et al., 1989). Therefore, we decided to test whether long-day exposure after the second week of life is required to induce a state of nonresponsiveness to short days. Adults were paired and bred in 16 L, and breeding pairs remained with their litters in the same room until the pups were weaned at D_{14} of age (day of birth = D_1). In the first part of this experiment, half the animals in each litter were transferred to 10 L on the day of weaning; the remaining pups were left in 16 L for 6 more days and were moved to 10 L at D_{20} . In the second part of the experiment, additional litters were produced by breeding pairs in 16 L, and littermates were either moved to 10 L at weaning (D_{14}) or at D_{25} . After 8 weeks in 10 L, all hamsters were weighed, scored for molt to winter pelage, and autopsied to determine testis or uterine weights.

Experiment 2 was repeated in its entirety several months following completion of the first run. The repeat was undertaken because in a subsequent experiment (Experiment 3) we observed somewhat different frequencies of short-day responders under conditions virtually identical to those employed in Experiment 2.

Experiment 3: Effects of Photoperiod of Gestation and during D_{14-24} after Birth on Responsiveness to Short Days

The results of Experiment 2 revealed that PNR hamsters transferred from 16 L to 10 L at D_{14} were almost uniformly responsive to short days. This observation was compatible with the hypothesis that long-day exposure subsequent to D_{14} is required for the development of nonresponsiveness to short days in the PNR hamsters. However, it remained possible that age per se had a role in the results—that is, perhaps hamsters are less likely to respond to short days at D_{25} than at D_{14} , regardless of photoperiod history. It is also possible that the photoperiod of gestation might influence short-day responsiveness, since gestation photoperiod can have a potent effect on postnatal photoperiodic responses in this species

(Reppert et al., 1985; Stetson et al., 1986). To further explore these possibilities, we made use of previous observations indicating that for Siberian hamsters 14 L is an “intermediate” photoperiod—in that transfer to this day length may evoke either gonadal regression or gonadal stimulation, depending on whether the previous photoperiod was longer or shorter (Hoffmann et al., 1986).

One set of breeding pairs of PNR hamsters was placed in 16 L. Litters produced by these pairs were reared to postnatal D_{14} in 16 L. At this time, litters were weaned and the weanlings were divided into three treatment groups. One group was left in 16 L until D_{24} ; another group was transferred to 14 L until D_{24} . Both these groups were transferred to 10 L on D_{24} and were autopsied 8 weeks later. A control group was transferred to 14 L at D_{14} and remained in 14 L until they were autopsied at the same age as the two previous treatment groups.

A second set of breeding pairs of PNR hamsters was placed in 14 L. Offspring of these animals were raised to postnatal D_{14} in 14 L and were then apportioned among three treatment groups. One group remained in 14 L until D_{24} ; another group was transferred to 16 L until D_{24} . Both these groups were transferred to 10 L on D_{24} and were autopsied 8 weeks later. A control group remained in 14 L until they were autopsied at the same age as the two previous groups.

Experiment 4: Transmission of Photoperiod Information from Mother to Fetus in PNR Hamsters

Pregnant UNS hamsters transmit photoperiod information to their fetuses. Experiment 3 included groups of PNR hamsters gestated in 16 L and 14 L, respectively, and these treatments were included to assess the possibility that the photoperiod of gestation might influence short-day responsiveness in the offspring of PNR hamsters. However, it is not clear whether PNR dams would be able to provide their fetuses with differential photoperiod information relevant to gestation photoperiods of 16 L and 14 L, since most adult PNR hamsters do not exhibit short-day responses and may themselves be incapable of distinguishing between long and short day lengths. Therefore, we designed an experiment to resolve this issue. Breeding pairs of PNR hamsters were housed in 16 L or 14 L. On the day of birth, 16 L pairs and their litters were transferred to 14 L; the pairs that had been in 14 L

during gestation remained in 14 L. Pups were weaned at D₁₄ and remained in 14 L until autopsy at D₃₈.

Statistics

Body weights and organ weights in each experiment were first analyzed by two-way analysis of variance (ANOVA). In cases where significant differences were indicated by ANOVA, appropriate pairs of group means were compared using the two-tailed Student's *t* test in Experiments 1 and 4. For Experiments 2 and 3, ANOVA was followed by Tukey's HSD test to evaluate the significance of differences between pairs of treatment groups.

Data for percentage of animals failing to exhibit regressed testes and data for percentage of animals failing to begin winter molt were analyzed using Statistica's two-sided difference between two proportions test (based on chi-square distribution). Comparisons were made between appropriate pairs of treatment groups.

RESULTS

Experiment 1: Effect of Photoperiod of Rearing on Short-Day Responsiveness of UNS and PNR Hamsters in Adulthood

When gestated and reared in our "standard" 16 L photoperiod and then transferred to 10 L in adulthood, 31% of the UNS males failed to exhibit testicular regression and 55% had not begun to molt to winter pelage after 8 weeks (Table 1). In contrast, more than 80% of the PNR males gestated and reared in 16 L failed to show both types of short-day responses after 8 weeks in 10 L. These differences between UNS and PNR hamsters reared in 16 L were statistically significant ($p < 0.01$). When gestated and reared in 14 L rather than 16 L, the percentages of both UNS and PNR males failing to exhibit responsiveness to short days were reduced ($p < 0.01$ for comparisons within each breeding line for animals reared in 16 L vs. 14 L). For UNS males reared in 14 L, only 5% failed to show testicular regression in 10 L and only 9% failed to begin a winter molt. When reared in 18 L, a greater proportion of the UNS hamsters were unresponsive to short days as compared to UNS males reared in 16 L (67% of the 18 L reared males failed to regress vs. 31% of the 16 L reared males, $p < 0.01$). The proportion of PNR males that

were unresponsive to short days was not different between animals reared in 18 L versus 16 L. Both UNS and PNR males that were reared throughout the experiment in either 18 L or 16 L had uniformly large testes at autopsy. Most males kept in 14 L until the end of the study also had large testes, but some of the animals in 14 L showed testicular regression (30% UNS, 12% PNR).

Experiment 2: Effects of Different Amounts of Long-Day Exposure in Early Life on Subsequent Response to Short Days in PNR Hamsters

In the first run of Experiment 2, PNR hamsters that were gestated in 16 L and reared to 25 days of age in the same photoperiod were mostly unresponsive to short days when transferred to 10 L at Day 25 (Figs. 1 and 2). The percentages of short-day nonresponsive males in this group (94% failed to show testis regression) were similar to what was observed for PNR males raised to adulthood in 16 L before exposure to 10 L (Experiment 1; 81% failed to regress). This contrasted sharply with the results for PNR hamsters that were transferred to 10 L at D₁₄. For this group, only 3% of the males failed to exhibit testis regression and only 6% failed to begin a molt to winter pelage; PNR females transferred to short days at D₁₄ had reduced uterine weights (compared to those transferred to 10 L at D₂₅, $p < 0.01$), and only 23% of the females failed to begin a winter molt (compared to 83% of the PNR females transferred to 10 L at D₂₅, $p < 0.01$).

In the second run of Experiment 2, the treatment effects were similar to those obtained in the first run (Figs. 1 and 2). However, there were quantitative differences between the two runs; for groups of males transferred to short days at D₂₀ or D₂₅, respectively, there were higher frequencies of nonresponders in the first run as compared to the second.

Experiment 3: Effects of Photoperiod of Gestation and during D₁₄₋₂₄ after Birth on Responsiveness to Short Days

For male PNR hamsters, there was an approximately 50% reduction in the percentage of short-day nonresponders when animals were exposed to 14 L at D₁₄₋₂₄ rather than 16 L during the same period of development (Table 2). This was true regardless whether the photoperiod during gestation and for the first two

Table 1. Effect of photoperiod of rearing on short-day responsiveness of adult Siberian hamsters derived directly from our general breeding colony (UNS, unselected breeding line) and those of the photoperiod nonresponsive (PNR) breeding line.

Breeding Line (n)	Photoperiod Treatment	Paired Testes Weights (mg)	Percentage Failing to Regress	Percentage Failing to Molt
UNS (10)	18 L (→) 18 L*	1058 ± 69 [†]	100	100
PNR (16)	18 L (→) 18 L	834 ± 30	100	100
UNS (33)	18 L (→) 10 L	616 ± 65 ^a	67 ^a	100 ^{+,a}
PNR (26)	18 L (→) 10 L	627 ± 58 ^b	77 ^b	85 ^{+,b}
UNS (10)	16 L (→) 16 L	946 ± 60	100	100
PNR (10)	16 L (→) 16 L	798 ± 54	100	100
UNS (68)	16 L (→) 10 L	315 ± 48 ^a	31 ^{a,d}	55 ^{a,d}
PNR (47)	16 L (→) 10 L	657 ± 44 ^c	81 ^{c,d}	89 ^{c,d}
UNS (10)	14 L (→) 14 L	657 ± 84	70	100
PNR (17)	14 L (→) 14 L	687 ± 50	88	94
UNS (58)	14 L (→) 10 L	119 ± 30 ^a	5 ^{a,e}	9 ^{a,e}
PNR (63)	14 L (→) 10 L	401 ± 47 ^{b,c}	44 ^{b,c,e}	50 ^{b,c,e}

NOTE: Group means with the same letters are significantly different, $p < 0.01$. * Group means are significantly different, $p < 0.05$. UNS and PNR groups subjected to 18 L (→) 18 L were not significantly different from each other. These groups were not compared to the corresponding 18 L (p) 10 L groups. The same was true for the UNS and PNR 16 L (→) 16 L and 14 L (→) 14 L groups.

*18 L (→) 18 L: animals remaining in 18 L from day of birth until autopsy at 16.5 weeks of age; 18 L (→) 10 L: animals transferred from 18 L to 10 L at 60 days of age and autopsied at 16.5 weeks of age.

[†]Mean ± SEM.

postnatal weeks was 14 L or 16 L ($p < 0.05$ in both cases). For each of the three types of treatments administered subsequent to D₁₄, there were no significant differences in reproductive or pelage parameters between comparison groups gestated and raised until D₁₄ under 14 L or 16 L, respectively. It is noteworthy that 35% of the PNR males that were exposed exclusively to 14 L during gestation and rearing were unresponsive to short days when transferred to 10 L at D₂₄. This is very similar to the results in Experiment 1, where PNR males raised from birth to adulthood in 14 L exhibited a 44% incidence of short-day nonresponders. Short-day responsiveness of hamsters gestated and reared to D₂₄ in 16 L (Tables 2 and 3) was similar to that for animals gestated and reared to D₂₅ in 16 L in Experiment 2 (Figs. 1 and 2). Results for female PNR hamsters (Table 3) followed the same patterns as observed for males.

Experiment 4: Transmission of Photoperiod Information from Mother to Fetus in PNR Hamsters

PNR males that were gestated in 16 L and transferred to 14 L at birth exhibited retarded testis development (on D₃₈) as compared to males gestated in 14 L and left in the same photoperiod during postnatal development (Table 4; $p < 0.01$).

DISCUSSION

Most small rodents are subject to high rates of death from predation and disease. It is unlikely that many of these animals survive for more than 1 year in the field. Overwinter mortality in deer mice is reported to be 50% higher for adults as compared to young mice (Millar and Teferi, 1993). Age-related winter breeding of small rodents could evolve as a strategy that involves a gamble: Older members of the population that are unlikely to survive until the following spring may gamble by attempting reproduction even under unfavorable conditions, whereas younger animals may experience a greater chance of success by conserving energy over the winter and reproducing under more favorable conditions the following spring. The odds for successful winter breeding would be expected to shift from year to year, depending largely on the severity of winter conditions. Thus, year-to-year variations in winter conditions might be expected to exert varying selection on genetic components of short-day responsiveness. This could lead to the apparent genetic polymorphisms for short-day responsiveness that have been reported for some rodents (Freeman and Goldman 1996b). It has been suggested that balanced polymorphisms with respect to photoperiodic traits could be at least one basis for the striking variations in population density that

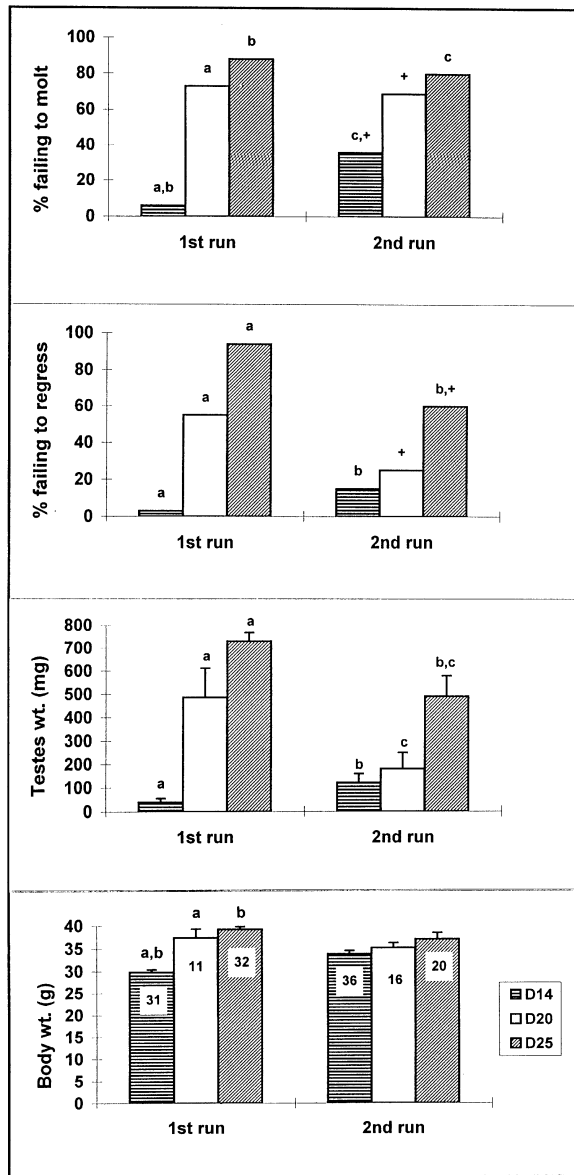


Figure 1. Effects of different amounts of long-day exposure in early life on responsiveness to short days in male photoperiod nonresponsive (PNR) hamsters. Hamsters were exposed to 16 L until D₁₄, D₂₀, or D₂₅, and were then transferred to 10 L for 8 weeks. The four panels show, from bottom to top, data for body weight, testes weight, percentage of males failing to show testis regression, and percentage of animals failing to molt. The three bars on the left of each panel display data from the first run of this experiment; the three bars on the right show data from the second run. The *n* for each group is displayed inside the bars in the body weight (bottom) panel. SEM is indicated above the bars for body and testes weights. Letters above bars indicate statistically significant differences; groups with the same letter were different from each other at $p < 0.01$, whereas + or ++ denotes groups that were different at $p < 0.05$.

occur from year to year in some microtine rodents (Nelson, 1987). Genetically based differences in

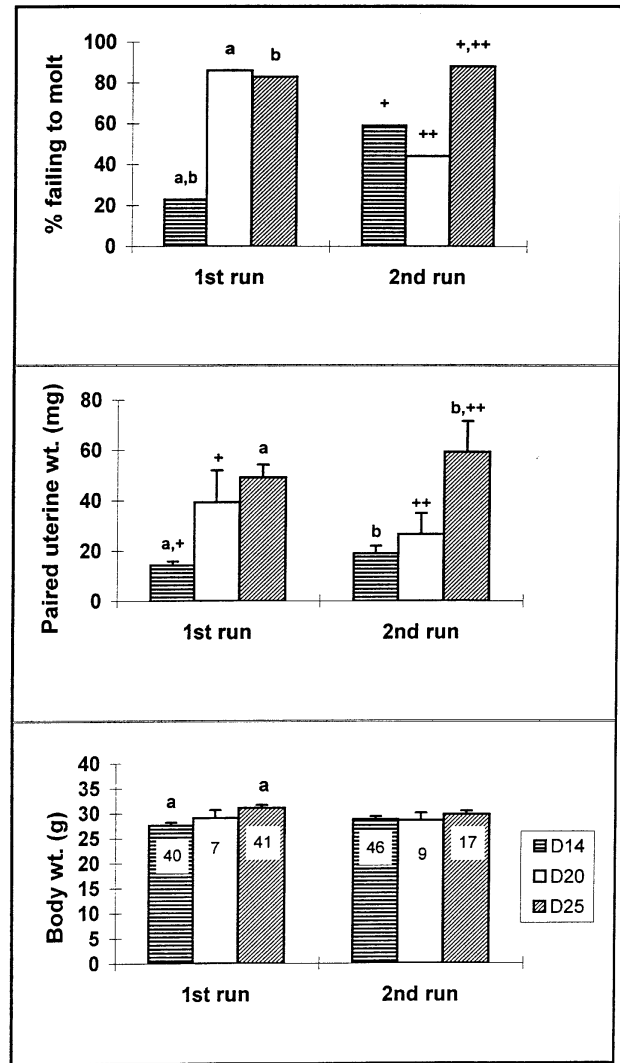


Figure 2. Effects of different amounts of long-day exposure in early life on responsiveness to short days in female photoperiod nonresponsive (PNR) hamsters. Treatments were the same as for Figure 1. The three panels show, from bottom to top, data for body weight, uterine weight, and percentage of females failing to molt. The *n* for each group and statistical differences are indicated as in Figure 1.

photoresponsiveness within single breeding populations are likely to serve as the basis for the evolution of major differences in photoresponsiveness between breeding populations and between species.

Siberian hamsters, like a number of other small, long-day breeding rodents, exhibit interindividual variability in short-day responsiveness. When exposed to short days, most Siberian hamsters exhibit reproductive inhibition, lose body weight and body fat, and molt to a winter-type pelage. These responses are observed in virtually all animals when they are exposed to short days beginning around the time of

Table 2. Effect of photoperiod during postnatal D₁₄₋₂₄ on short-day responsiveness in male photoperiod nonresponsive (PNR) hamsters gestated and raised to weaning in 16 L.

G	D ₁₄₋₂₄	D ₂₄₋₈₀	N	Body Weight (g)	Testis Weight (mg)	Percentage Failing to Regress	Percentage Failing to Molt
16 L	16 L	10 L	34	38.7 ± 1.1	515 ± 61 ⁺	59 ⁺	85 ^a
16 L	14 L	10 L	28	36.7 ± 1.1	274 ± 62 ^{+,a}	32 ^{+,a}	43 ^{a,b}
16 L	14 L	14 L	21	40.7 ± 0.8	686 ± 44 ^a	91 ^a	100 ^b
14 L	16 L	10 L	33	38.1 ± 1.1	531 ± 64	64 ⁺⁺	85 ⁺
14 L	14 L	10 L	20	35.8 ± 1.1 ^a	308 ± 80 ^b	35 ^{++,b}	55 ^{+,c}
14 L	14 L	14 L	16	41.7 ± 0.7 ^a	779 ± 24 ^b	100 ^b	94 ^c

NOTE: Group means with the same letters are significantly different, $p < 0.01$. ⁺ Group means are significantly different, $p < 0.05$.

Table 3. Effect of photoperiod during postnatal D₁₄₋₂₄ on short-day responsiveness in female photoperiod nonresponsive (PNR) hamsters gestated and raised to weaning in 16 L.

G	D ₁₄₋₂₄	D ₂₄₋₈₀	N	Body Weight (g)	Uterine Weight (mg)	Percentage Failing to Molt
16 L	16 L	10 L	20	32.2 ± 1.0	51.6 ± 9.1	75
16 L	14 L	10 L	27	31.1 ± 0.8 ⁺	39.2 ± 6.9 ^a	60 ^a
16 L	14 L	14 L	20	34.9 ± 0.8 ⁺	88.4 ± 12.9 ^a	100 ^a
14 L	16 L	10 L	27	32.8 ± 0.9 ⁺⁺	62.2 ± 13.7 ^b	93 ^b
14 L	14 L	10 L	26	28.9 ± 1.0 ^{++,b}	18.2 ± 3.7 ^{b,c}	42 ^{b,c}
14 L	14 L	14 L	19	35.3 ± 0.8 ^b	70.5 ± 11.0 ^c	100 ^c

NOTE: Group means with the same letters are significantly different, $p < 0.01$. ⁺ or ⁺⁺ Group means are significantly different, $p < 0.05$.

Table 4. Effect of gestation photoperiod on reproductive maturation in male photoperiod nonresponsive (PNR) hamsters raised from birth in 14 L.

G	D ₁₋₃₈	N	Body Weight (g)	Testis Weight (mg)	Percentage Individuals with Testis Weight > 350 mg
16 L	14 L	11	26.5 ± 0.8 ⁺	125 ± 27 ^a	0 ^a
14 L	14 L	14	29.9 ± 1.0 ⁺	526 ± 63 ^a	79 ^a

NOTE: Group means with the same letter are significantly different, $p < 0.01$. ⁺ Group means significantly different, $p < 0.05$.

weaning; however, if short-day exposure is initiated after hamsters have reached adulthood, a significant number of animals fail to show the typical constellation of short-day responses (Freeman and Goldman, 1996 a,b; Kliman and Lynch, 1992; Puchalski and Lynch, 1986). These observations pertain to hamsters reared under a 16 L photoperiod, which has been the day length most commonly used as a standard long day in laboratories examining photoperiod responses in this species. In contrast to the results obtained when animals were reared in 16 L, 27 out of 29 Siberian hamsters were unresponsive to short days following 10 weeks of exposure to very long days (18 L), whereas only 2 out of 31 animals raised in an intermediate day length (14 L) failed to exhibit typical winter-type responses following subsequent short-day exposure.

These results reveal that responsiveness to short photoperiod is strongly influenced by photoperiod history in this species; exposure to very long days during maturation reduces the probability that a hamster will exhibit the species-typical winter responses during subsequent exposure to a short photoperiod (Gorman and Zucker, 1997).

The ability of a long day (18 L) photoperiod history to influence subsequent short-day responsiveness in Siberian hamsters is a pineal-independent effect (Prendergast and Freeman, 1999). This is an exception to the more typical situation in mammals, whereby most photoperiodic effects are mediated by specific patterns of pineal melatonin secretion (Goldman and Elliott, 1988; Goldman and Nelson, 1993). The photoperiod history effect on short-day responsive-

ness may involve more direct effects of day length on properties of the SCN; specifically, short-day nonresponders generally have longer free-running period lengths (τ) as compared to responders (Freeman and Goldman, 1996a; Margraf et al., 1991; Prendergast and Freeman, 1999; Puchalski and Lynch, 1986). The longer τ of the nonresponders leads to delayed phase angles of entrainment in short-day photoperiods and a subsequent failure to show the expanded duration of pineal melatonin secretion that is required to evoke winter-type responses in this species (Goldman and Nelson, 1993; Puchalski and Lynch, 1986).

The results of the present experiments confirm and extend earlier findings in revealing that photoperiod history is influential in determining short-day responsiveness in both random-bred (UNS) hamsters and hamsters artificially selected for nonresponsiveness to short days (PNR). Our UNS hamsters probably were similar to the animals used by Gorman and Zucker (1997) and those used by Prendergast and Freeman (1999), because the breeding colony that provided subjects for those studies was founded with animals supplied from our laboratory in 1985, and circadian characteristics of the short-day nonresponders reported in these publications are similar to those for our nonresponsive animals (Freeman and Goldman, 1996a).

The following conclusions may be drawn from the present results:

1. Photoperiod history contributes very significantly to determining whether individual Siberian hamsters will exhibit the short-day nonresponsive phenotype. This is true for both UNS and PNR hamsters.
2. For UNS hamsters, exposure to 18 L is required to induce a predominance (67%) of the short-day nonresponsive phenotype in adulthood; only 31% of the UNS animals reared in 16 L, and 5% of those reared in 14 L, were short-day nonresponsive. For PNR hamsters, exposure to 16 L was adequate to induce the short-day nonresponsive phenotype in 81% of the animals. Therefore, the process of artificial selection used to generate the PNR line has resulted in a relaxation of the requirement for "very long days" for induction of the state of short-day nonresponsiveness.
3. A substantial proportion (56%) of the PNR hamsters that were reared in 14 L remained unresponsive to short days. This could mean that (a) even 14 L is a sufficiently long day for inducing a state of short-day nonresponsiveness in PNR hamsters or (b) that a large number of the PNR hamsters are constitutively nonresponsive to short days in adulthood, irrespective of photoperiod history.

4. The photoperiod experienced between postnatal D₁₄ and D₂₄ (14 L vs. 16 L) influences the frequency of expression of the short-day nonresponsive phenotype in PNR hamsters. However, the photoperiod experienced during gestation and prior to D₁₄ does not alter the frequency of short-day nonresponsiveness in these animals.
5. In experiments where both male and female hamsters were used, the results for the two sexes were generally similar; both sexes exhibited effects of photoperiod history on development of the short-day nonresponsive phenotype.

Many mammals exhibit effects of photoperiod on reproduction, but others do not appear to be photoperiodic for reproductive responses (Goldman and Nelson, 1993). This variability among species must have a genetic basis, but it is not clear what forms this may take. The present study reveals a relationship between the genetic basis for individual differences in photoperiod responsiveness and photoperiod history effects on photoperiod response. Animals (PNR) selected for resistance to the effects of short days proved to exhibit an increase in the frequency of the short-day nonresponsive phenotype after rearing in 16 L. In addition, it is clear that some of the PNR hamsters develop the short-day nonresponsive phenotype even when raised exclusively in an intermediate photoperiod (14 L), raising the possibility that short-day nonresponsiveness may be a constitutive condition (irrespective, at least, of photoperiod history) in these individuals.

The observation that PNR hamsters that have been reared to adulthood in 16 L generally fail to exhibit the species-typical winter responses during exposure to 10 L, might be taken to indicate that these animals have a poor ability to "discriminate" between different day lengths. However, male offspring born to PNR dams and raised from birth in 14 L exhibited different rates of testis maturation depending on whether gestation had occurred in 16 L or 14 L. This result strongly suggests that PNR dams are capable of discriminating between 16 L and 14 L during gestation and that they can transmit day length information to their fetuses. Indeed, the results obtained in this paradigm with PNR hamsters are virtually identical to those obtained in an earlier study using UNS hamsters gestated in 16 L or 10 L and reared in 14 L (Shaw and Goldman, 1995). In Siberian hamsters, the transmission of day length information from mother to fetus appears to depend on the mother's pattern of pineal melatonin secretion (Elliott and Goldman, 1989; Weaver and Reppert,

1986; Weaver et al., 1987). The mother's melatonin rhythm may be directly imposed on the fetus via the maternal/fetal circulation (Reppert et al., 1979; Yellon and Longo, 1987), and melatonin may stimulate target sites in fetal tissues (Carlson et al., 1991). We did not assess melatonin patterns in the present study; it is possible that melatonin patterns of the PNR dams were sufficiently different in 16 L versus 14 L to allow for maternal signaling of photoperiod information to the pups, as is known to be the case for UNS hamsters.

It should be noted that the inhibition of testis development for hamsters (UNS) gestated in 16 L and raised in 14 L from birth is transient. Although these animals exhibit retarded testis development at 27 to 42 days of age, the testes are fully developed by 62 days. The retarded testis development in these animals is quite different from the typical short-day response observed for hamsters exposed to shorter day lengths; for example, males kept under 10 L from birth still had completely regressed testes at 62 days (Shaw and Goldman, 1995), and testicular maturation in short photoperiod does not occur until approximately 150 days (Hoffmann, 1978). Therefore, it cannot be assumed that the retarded testis development in our PNR animals that were gestated in 16 L and reared from birth in 14 L is an indication that these animals are short-day responsive.

Artificial selection may generate genotypes (and corresponding phenotypes) that would not be produced under conditions of natural selection. Nevertheless, outcomes of artificial selection regimes may reveal genetic variation that could result in new phenotypes given suitable natural selection pressures. It is important to note a difference in results between our artificial selection for short-day nonresponders in Siberian hamsters and similar selection studies that were performed in other laboratories in Siberian hamsters (Kliman and Lynch, 1991, 1992) and white-footed mice (Heideman and Bronson, 1991). In the other instances, artificial selection simply altered the frequencies of two alternative phenotypes (short-day responsive vs. short-day nonresponsive in adulthood) that were already present in significant numbers in the starting population. In contrast, our artificial selection regime not only altered the frequencies of preexisting phenotypes but also resulted in the appearance of a new phenotype—hamsters that fail to respond to short days when tested at 20 to 25 days of age. This new phenotype now characterizes about 80% of the PNR population (Freeman and Goldman, 1996b; Fig. 1). Animals of our source population (UNS) exhibit

virtually 100% responsiveness to short days at weaning, so that we had to begin the selection process by screening adults for response to short days. The fact that we have obtained a new phenotype—most probably by recombination of genes that already were present in the UNS population—is of special significance because it demonstrates that novel “photoperiod response” phenotypes could likely be obtained within a few generations in nature (without relying on the occurrence of mutations) if selection pressures were sufficiently robust. Also, it is important to emphasize that the mode of artificial selection used here may bear similarities to what probably occurs in the field; that is, short-day nonresponsive hamsters must breed with individuals of the same phenotype during the winter, as these would be the only animals available for breeding during that season.

Our results expand on the information regarding photoperiod history effects in determining responsiveness to short photoperiod in Siberian hamsters. Nevertheless, the results do not exclude the possibility that age may also be a factor in determining the probability of response to short-day exposure, as has been suggested by others (Bernard et al., 1997). Substantial numbers of PNR hamsters failed to exhibit short-day responses, regardless of photoperiod history, except when transfer to short days was accomplished at D_{14} after birth. In the latter case, only 3% to 15% of the males were nonresponsive. The increase in percentage of short-day nonresponsive hamsters between D_{14} and D_{24} , even for animals raised in an intermediate day length (14 L), could reflect an age effect on short-day responsiveness. Alternatively, it may be that for the PNR hamsters even 14 L acts like a long photoperiod with respect to inducing a state of short-day nonresponsiveness. Other studies have suggested that Siberian hamsters may not be responsive to photoperiod information that is present during the first 2 weeks after birth (Stetson et al., 1989). The small number of hamsters that are short-day nonresponsive even at this early age may reflect a constitutive (that is, independent of both photoperiod history and age) nonresponsiveness in a small proportion of the PNR population. As mentioned earlier, a preliminary experiment in our laboratory indicated that PNR males were uniformly responsive to short days when transferred to 10 L on the day of birth. In an ongoing study that expands on this preliminary observation, 24 out of 24 PNR males that were transferred to 10 L at birth exhibited complete testicular regression at 60 to 62 days of age (mean testis wt. = 20.1 ± 0.4 mg; S. L.

Goldman, unpublished observation). This observation suggests that all PNR hamsters are capable of exhibiting typical reproductive responses to short days, but does not definitively indicate whether age at first exposure may be a factor in addition to the well-documented photoperiod history effect.

The photoperiod history-dependent mechanism that influences short-day responsiveness in Siberian hamsters would appear to favor winter breeding by animals that were born the preceding spring. These individuals would have been exposed to the longest day lengths of summer and would thus be least likely to exhibit reproductive inhibition in response to short days of late summer/fall. Hamsters born later in the breeding season and having less exposure to long summer days would be more responsive to decreased day length and would probably not be reproductively active during winter. Thus, this photoperiod history-based system probably favors winter breeding by the older members of the population, whereas younger animals are more likely to forgo reproduction during winter and conserve resources in a gamble on survival until the return of more favorable environmental conditions.

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REFERENCES

- Bernard DJ, Loose-Olson S, and Turek FW (1997) Age-related changes in the photoperiodic response of Siberian hamsters. *Biol Reprod* 57:172-177.
- Blank JL and Freeman DA (1991) Differential reproductive response to short photoperiod in deer mice: Role of melatonin. *J Comp Physiol [A]* 169:501-506.
- Carlson LL, Weaver DR, and Reppert SM (1991) Melatonin receptors and signal transduction during development in Siberian hamsters (*Phodopus sungorus*). *Dev Brain Res* 59:83-88.
- Carlson LL, Zimmerman A, and Lynch GR (1989) Geographic differences for delay of sexual maturation in *Peromyscus leucopus*: Effects of photoperiod, pinealectomy, and melatonin. *Biol Reprod* 41:1004-1013.
- Christian JJ (1980) Regulation of annual rhythms of reproduction in temperate small rodents. In *Testicular Development, Structure, and Function*, A Steinberger and E Steinberger, eds, pp 367-380, Raven, New York.
- Dark J, Johnston PG, Healy M, and Zucker I (1988) Latitude of origin influences photoperiodic control of reproduction of deer mice (*Peromyscus maniculatus*). *Biol Reprod* 28:213-220.
- Duncan MJ and Goldman BD (1984) Hormonal regulation of the annual pelage color cycle in the Djungarian hamster, *Phodopus sungorus*: I. Role of the gonads and the pituitary. *J Exp Zool* 230:89-95.
- Elliott JA (1976) Circadian rhythms and photoperiodic time measurement in mammals. *Fed Proc* 35:2339-2346.
- Elliott JA and Goldman BD (1989) Reception of photoperiodic information by fetal Siberian hamsters: Role of the mother's pineal gland. *J Exp Zool* 252:237-244.
- Freeman DA and Goldman BD (1996a) Evidence that the circadian system mediates photoperiodic nonresponsiveness in Siberian hamsters. *J Biol Rhythms* 12:100-109.
- Freeman DA and Goldman BD (1996b) Photoperiod nonresponsive Siberian hamsters: Effect of age on the probability of nonresponsiveness. *J Biol Rhythms* 12:110-121.
- Goldman BD and Elliott JA (1988) Photoperiodism and seasonality in hamsters: Role of the pineal gland. In *Processing of Environmental Information in Vertebrates*, MH Stetson, ed, pp 203-218, Springer-Verlag, New York.
- Goldman BD and Nelson RJ (1993) Melatonin and seasonality in mammals. In *Melatonin: Biosynthesis, Physiological Effects, and Clinical Applications*, HS Yu and RJ Reiter, eds, pp 225-252, CRC Press, Boca Raton, FL.
- Gorman MR and Zucker I (1995) Seasonal adaptations of Siberian hamsters: II. Pattern of change in day length controls annual testicular and body weight rhythms. *Biol Reprod* 53:116-125.
- Gorman MR and Zucker I (1997) Environmental induction of photoperiod nonresponsiveness in the Siberian hamster, *Phodopus sungorus*. *Am J Physiol* 272:R887-R895.
- Heideman PD and Bronson FH (1991) Characteristics of a genetic polymorphism for reproductive photoperiod responsiveness in the white-footed mouse (*Peromyscus leucopus*). *Biol Reprod* 44:1189-1196.
- Hoffmann K (1978) Effect of short photoperiods on puberty, growth and moult in the Djungarian hamster (*Phodopus sungorus*). *J Reprod Fertil* 54:29-36.
- Hoffmann K, Illnerova H, and Vanecek J (1986) Change in duration of the nighttime melatonin peak may be a signal driving photoperiodic responses in the Djungarian hamster (*Phodopus sungorus*). *Neurosci Lett* 67:68-72.
- Hong SM, Rollag MD, and Stetson MH (1986) Maintenance of testicular function in Turkish hamsters: Interaction of photoperiod and the pineal gland. *Biol Reprod* 34:527-531.
- Kliman RM and Lynch GR (1991) Evidence for independence of circadian characters and extent of photoperiod responsiveness in the Djungarian hamster, *Phodopus sungorus*. *J Biol Rhythms* 6:159-166.
- Kliman RM and Lynch GR (1992) Evidence for genetic variation in the occurrence of the photoperiod response of the Djungarian hamster, *Phodopus sungorus*. *J Biol Rhythms* 7:161-173.
- Lynch GR, Heath HW, and Johnston CM (1981) Effect of geographical origin on the photoperiodic control of repro-

- duction in the white-footed mouse, *Peromyscus leucopus*. Biol Reprod 25:475-480.
- Margraf RR, Zlomanczuk P, Liskin LA, and Lynch GR (1991) Circadian differences in neuronal activity of the suprachiasmatic nucleus in brain slices prepared from photo-responsive and photo-nonresponsive Djungarian hamsters. Brain Res 544:42-48.
- Millar JS and Teferi T (1993) Winter survival in northern *Peromyscus maniculatus*. Can J Zool 71:125-129.
- Nelson RJ (1987) Photoperiod-nonresponsive morphs: A possible variable in Microtine population-density fluctuations. Am Naturalist 130:350-369.
- Prendergast BJ and Freeman DA (1999) Pineal-independent regulation of photo-nonresponsiveness in the Siberian hamster (*Phodopus sungorus*). J Biol Rhythms 14:62-71.
- Puchalski W and Lynch GR (1986) Evidence for differences in the circadian organization of hamsters exposed to short day photoperiod. J Comp Physiol [B] 159:7-11.
- Reppert SM, Chez RA, Anderson A, and Klein DC (1979) Maternal-fetal transfer of melatonin in the non-human primate. Pediatr Res 13:788.
- Reppert SM, Duncan MJ, and Goldman BD (1985) Photic influences on the developing mammal. In *Melatonin and the Pineal Gland*, Ciba Foundation Symposium, D Evered and S Clark, eds, pp 116-128, Pitman, London.
- Shaw D and Goldman BD (1995) Gender differences in influence of prenatal photoperiods on postnatal pineal melatonin rhythms and serum prolactin and follicle-stimulating hormone in the Siberian hamster (*Phodopus sungorus*). Endocrinology 136:4237-4246.
- Stetson MH, Elliott JA, and Goldman BD (1986) Maternal transfer of photoperiodic information influences the photoperiodic response of prepubertal Djungarian hamsters (*Phodopus sungorus sungorus*). Biol Reprod 34:664-669.
- Stetson MH, Ray SL, Creyaurmiller N, and Horton TH (1989) Maternal transfer of photoperiodic information in Siberian hamsters: II. The nature of the maternal signal, time of signal transfer, and the effect of the maternal signal on peripubertal reproductive development in the absence of photoperiodic input. Biol Reprod 40:458-465.
- Weaver DR, Keohan JT, and Reppert SM (1987) Definition of a prenatal sensitive period for maternal-fetal communication of day length. Am J Physiol 253:E701-E704.
- Weaver DR and Reppert SM (1986) Maternal melatonin communicates daylength to the fetus in Djungarian hamsters. Endocrinology 119:2861-2863.
- Yellon SM and Longo LD (1987) Melatonin rhythms in fetal and maternal circulation during pregnancy in sheep. Am J Physiol 252:E799-E802.