

A plastic interval timer synchronizes pubertal development of summer- and fall-born hamsters

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Gorman, Michael R. A plastic interval timer synchronizes pubertal development of summer- and fall-born hamsters. *Am J Physiol Regulatory Integrative Comp Physiol* 281: R1613–R1623, 2001.— Summer and fall decreases in day length induce reproductive regression in adult hamsters and delay reproductive maturation of their young. The following year pubertal development is triggered by an interval timer (IT) that renders animals refractory to inhibitory short day lengths after ~25 wk. Timing of gonadal and somatic development was examined among offspring born to Siberian hamsters in early-August vs. late-September day lengths. Pubertal maturation was delayed in both groups until late winter. Gonadal growth occurred at significantly later ages among August- vs. September-born males as did late-winter spurts in ponderal growth of both sexes. Timing of reproductive and somatic development depended on postnatal rather than prenatal photoperiod exposure and was unrelated to the circadian entrainment status of dams. When developmental patterns were assessed in relation to time of year, group differences were largely eliminated. Because the IT triggers these developmental events, its duration must be plastic. This plasticity facilitates a relative synchronization or entrainment of developmental milestones in hamsters born into different late-summer/early-fall photoperiods.

photorefractoriness; seasonality; photoperiodism; melatonin; nonresponsiveness

SIBERIAN HAMSTERS (*Phodopus sungorus*, sometimes also called Djungarian hamsters) and a host of other rodent species have evolved mechanisms to concentrate breeding effort in the fraction of the year with the most abundant energy supplies (3). Changes in thermoregulatory functions, feeding, and sociosexual behaviors parallel these variations in reproductive capacity. The entire suite of annual rhythms is readily induced in the laboratory by simulating the annual variation in day length (DL) (13). A summer phenotype, including robust laboratory breeding, is provoked by long photoperiods characteristic of late spring and early summer, whereas reproductive suppression occurs in short DLs of late fall or early winter. Under natural or simulated natural conditions, the transition to the winter phenotype is driven by reductions in DL below a so-called critical DL, estimated in Siberian hamsters to be ~13 h of light/day (13L) (19). Offspring born near the end of

the summer breeding season defer pubertal development until the following year.

For the vast majority of mammals examined, measurement of DL depends on the circadian system, which differentially entrains to long and short DLs. In short days and long nights, the suprachiasmatic nuclei program a relatively long duration of elevated nighttime locomotor activity (α) and interval of elevated pineal melatonin secretion (6). By contrast, in short nights of summer, the duration of nighttime locomotor activity and of pineal melatonin secretion is compressed. The duration of elevated melatonin determines the seasonal phenotype, with long and short melatonin durations orchestrating winter and summer adaptations, respectively (1, 10).

Not all individuals of a rodent species respond similarly to decreasing or short photoperiods (12, 21, 28). Whereas the majority may undergo reproductive suppression, a sizable fraction of the population may continue breeding into late summer or early fall, particularly if environmental conditions are mild. In most species studied, this photononresponsiveness reflects insensitivity to short-day patterns of melatonin secretion. The Siberian hamster may be exceptional in this regard insofar as photononresponsiveness reflects a failure of the circadian system to adopt a short-day entrainment pattern (14, 29). Because such hamsters generate a short-duration melatonin signal, they remain reproductively competent (30). Attempted fall breeding may confer a selective advantage particularly among older hamsters unlikely to survive the winter to breed the following spring. However, the young of such animals, having high expected winter survival, would likely benefit from delaying reproductive activity until spring.

For both adults and prepubertal young of the previous breeding season, the transition from reproductive suppression in fall and winter to reproductive competence in spring and summer is initiated well in advance of long spring DLs (31, 35). The timing of this transition depends on an interval timer (IT) mechanism that renders animals insensitive, or refractory, to short inhibitory DLs. The presumed function of the IT is to anticipate optimal breeding conditions in spring so

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that reproduction can occur as soon as conditions are favorable (31, 35). There have been few tests of this proposition, and little is known about the formal or physiological bases of this component of photoperiodic systems. Moreover, the fact that photononresponsive hamsters may persist in bearing litters into short autumnal photoperiods constrains the potential utility of an IT mechanism for timing springtime puberty in their offspring. For example, assume that the IT triggers gonadal development in males after a fixed interval of 25 wk of short-DL exposure. If a pup is born into inhibitory DLs in early August, gonadal development would begin in early February. Mature spermatozoa would be available in March, and the first litters sired by this male could appear in early April. In contrast, the same 25-wk IT would trigger reproductive development of a hamster born in early October only in early April. Sperm would mature in mid-May, and birth of this male's offspring would be delayed until early June. Clearly, in at least one of these two example cases, the IT is not optimally timing parturition to coincide with earliest favorable spring conditions.

To be of maximal utility from an ecological perspective, the IT duration should be flexible to compensate for the time of year into which offspring of the previous season are born. An earlier study employing static DLs demonstrated that the duration measured by the IT is indeed plastic under certain conditions (16). Male hamsters gestated and raised in 12L initiated gonadal development and pubertal increases in body weight at a significantly later age than did hamsters gestated and raised in 10L. I hypothesized that hamsters born in 12L represented hamsters born at the autumnal equinox, whereas those born in 10L represented a cohort born 4 wk later in late October. The difference in age at gonadal development, 3.6 wk, roughly coincided with the time required for DLs to decrease at the latitude of origin ($\sim 55^\circ$ N) from 12L to 10L. Because hamsters in that study were exposed to unchanging DLs of unknown ecological significance, however, this interpretation awaited testing under more naturalistic DL conditions as in the present study.

The present experiment confirms an earlier report that female Siberian hamsters will readily breed into decreasing summer/fall DLs in the laboratory (37). I therefore tested the hypothesis that photoperiod alters the length of the IT such that pubertal development in spring is synchronized among offspring born into various short photoperiods of the previous year. Because mothers may communicate DL information to their offspring prenatally, whereas offspring may respond directly to environmental cues postnatally, the present experiment assessed the relevance of pre- vs. postnatal cues on duration of the IT. Finally, I characterized the degree to which the photoresponsiveness of mothers influenced developmental patterns of their pups raised under simulated natural photoperiods.

METHODS

Siberian hamsters, *Phodopus sungorus*, were housed at $22 \pm 2^\circ\text{C}$ and provided with food (mouse chow 5015, Purina

Mills, St. Louis, MO) and water ad libitum. At weaning at 18–21 days of age, individuals were maintained in same-sex groups (2–3 hamsters) in polypropylene cages ($27 \times 16 \times 13$ cm). Male and female adult hamsters previously maintained on a 15-h photoperiod (15L; lights out at 1800, 100- to 400-lx light intensity) were paired and transferred to 13L (lights out at 1700). At 26–28 days of age, 48 female offspring (F1) of these pairs were transferred to a 13-h 25-min DL (13:25L) representing a simulated natural photoperiod (SNP) of April 15 for 50° N latitude (SNP50). Henceforth, all given dates correspond to photoperiod simulations, and all are out of phase with the actual year. Thereafter, DL changed daily to mimic the seasons of 50° latitude. Females were housed singly at the summer solstice and on July 15 (DL = 15:30L) were paired for 16 days with male hamsters previously maintained in 15L.

On the day of birth of F2 offspring in an August photoperiod, 12 litters and their dams were transferred to a SNP50 phase-advanced by 6 wk (Aug \rightarrow Sept group; Fig. 1; Table 1). The remainder of dams and pups was maintained on the original yearly photocycle (Aug group; Fig. 1). All adult females that remained in the original SNP were paired again with males for 16 days beginning on late September 1, and a second cohort of offspring was born into September photoperiods (Sept group; Fig. 1). Table 1 lists average DL conditions experienced at birth, after transfer, and at postnatal *day 15* when photoperiodic mechanisms may begin to respond to DL directly. When DLs had decreased to 8L at the winter solstice, male and female offspring were maintained thereafter on that photoperiod (Fig. 1). In addition, a subsample of male offspring from Aug and Sept treatment groups experienced naturally increasing DLs after the winter solstice to assess the role of the IT vs. increasing DLs on pubertal development.

Pups were weighed at weaning and weekly from 5 wk of age, and testis size was estimated every second week beginning at 16 wk. Hamsters were first lightly anesthetized with methoxyflurane (Metofane; Pitman Moore, Mundelein, IL) or isoflurane vapor (Aerrane; Fort Dodge Animal Health, Fort Dodge, IA). Inguinal fur was shaved, and the gonads were manipulated into the scrotal sac. Length (*L*) and width (*W*) of the left testis were measured externally with calipers. Esti-

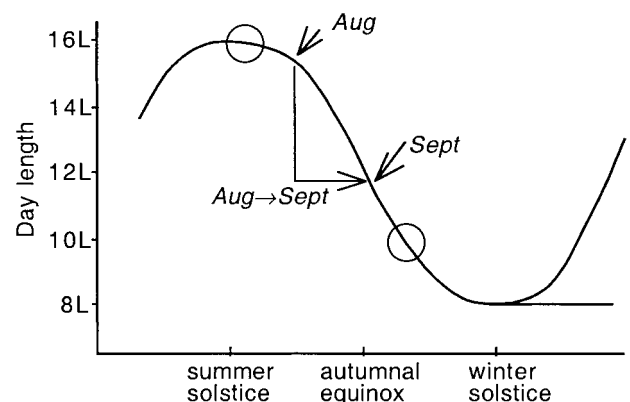


Fig. 1. Schematic representation of photoperiodic stimuli employed in the present experiment. Females raised in a simulated natural photoperiod of 50° N latitude bore litters in early August (Aug treatment group) or late September (Sept treatment group) or had litters born in August transferred at birth to September day lengths (DLs) (Aug \rightarrow Sept treatment group). At the winter solstice, offspring remained in the natural photoperiod or were maintained thereafter in 8 h of light/day (8L). Circles indicate intervals of monitoring of locomotor activity of dams.

Table 1. *Photoperiodic conditions of male and female pups at birth and postnatal day 15*

Group	Born On	DL at Birth	DL at Postnatal Day 15	Transferred at Birth to	DL at Transfer	DL at Postnatal Day 15
<i>Males</i>						
Aug	Aug 10 ± 0.6	14:19L	13:29L			
Aug→Sept	Aug 3 ± 0.1	14:44L		Sept 14	12:20L	11:22L
Sept	Sept 21 ± 0.4	11:49L	10:55L			
<i>Females</i>						
Aug	Aug 3 ± 0.5	14:44L	13:54L			
Aug→Sept	Aug 3 ± 0.2	14:44L		Sept 14	12:20L	11:22L
Sept	Sept 23 ± 0.7	11:41L	10:47L			

DL, day length; L, hours of light/day.

mated testis volume (ETV) was calculated as $L \times W \times W$, yielding an index that correlates highly with paired testis weight with correlation coefficients r routinely exceeding 0.90 (16). Measurement has no marked effect on testis size as the weight at autopsy of the repeatedly measured left testis varies by <5% from the weight of the previously unmeasured right testis. Below ~100 mg, paired testis weight is not reliably assessed externally, but growth above this value is readily distinguishable from the completely regressed state where $ETV < 150$ U (unpublished observations). Thus the first time point at which $ETV > 150$ U was designated as the onset of testis maturation.

To assist with identification of photononresponsive hamsters, pelage coloration was rated on a four-point scale similar to that used by Duncan and Goldman (4) that distinguished dark summer pelage from three degrees of winter molt.

Locomotor activity. Before the initial pairing with males, and again after litters were weaned and DL had decreased below 11L, home cage locomotor activity of F1 females was monitored with passive infrared motion detectors (Coral Plus; Visonic, Tel-Aviv, Israel) mounted on a filter top placed on top of the home cage. Movement across 3 or more of 27 detection zones activated a closed contact relay that was logged in 10-min bins by VitalView hardware and software (Minimitter, Sun River, OR). Actiview software was used to prepare 24-h histograms of activity levels over 10–14 days. Mean number of counts per 10 min over this interval (including both active and inactive periods) was calculated for each animal. Activity onset was defined as the first point in the dark phase at which activity levels were 150% of the 10-min average and sustained at that level for at least three of the following six 10-min intervals. Activity offset was defined as the last point during the dark period at which activity levels were 150% of the 10-min average and sustained at that level for at least four of the preceding six 10-min intervals. Activity duration (α) was obtained by subtracting time of activity onset from time of activity offset.

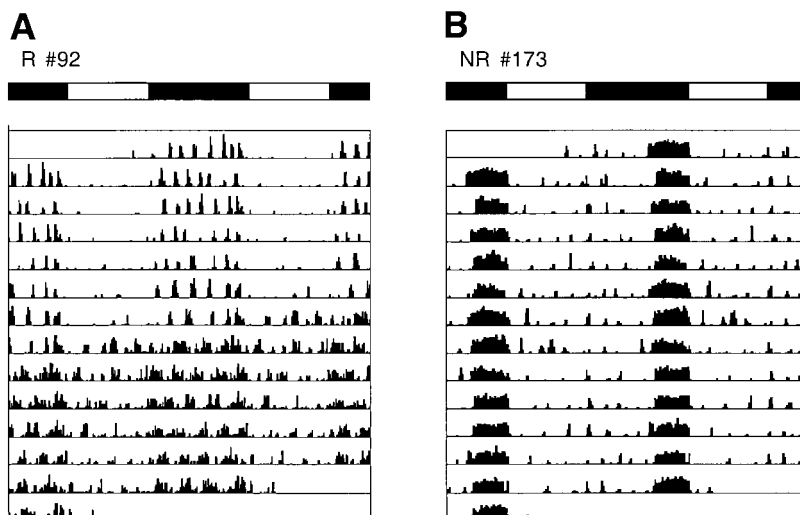
Photoreponsiveness. Nonresponsiveness to short DLs of the adult female breeding stock was assessed on the basis of locomotor activity profiles. Dams were designated nonresponsive if α failed to expand by at least 1.5 h in short compared with long DLs (a threshold was chosen based on the between-subject variability observed under long-DL conditions). Individual assessment of pup activity rhythms was not logistically feasible, and thus nonresponsiveness was inferred through a joint consideration of pelage coloration and gonad size in males. Some male offspring exhibited substantial gonadal maturation and summer coloration when first measured at *week 16* and thereafter. Because these animals might be either photononresponsive hamsters

or hamsters becoming refractory within the first 16 wk of life, earlier body weight data were examined in an attempt to distinguish between these possibilities. Hamsters gonadally unresponsive to short DLs from birth typically show accelerated body weight gains (unpublished observations), whereas those becoming refractory would be expected to have average weights just before becoming refractory. Thus hamsters with summer phenotypes at *week 16* and exceeding mean body weight of their treatment group by more than 1.5 SDs before *week 12* were designated nonresponders. Hamsters not meeting this body weight criterion were considered to be photoreponsive and to have developed early photorefractoriness. For these males, onset of gonadal development was coded as *week 16* because this was the first point assessed. For females, nonresponders met this body weight criterion and exhibited summer pelage at *week 16* and thereafter.

Analyses. Body weight differences between groups were assessed using two types of comparisons. First, groups were compared for differences in body weight at equivalent ages. However, because hamsters were born at different times of the year, they did not experience the same photoperiods at the same age. Thus body weights were also compared between groups matched for the phase of year or ambient photoperiod. To minimize group differences introduced by the rapid spurt of initial growth, analysis was confined to body weights measured on or after November 30 photoperiods. By then, the youngest hamsters were 10 wk old and had reached mature, although winter, size. For each type of analysis, repeated-measures ANOVAs (Statview 5.0; SAS Institute; Cary, NC) were performed, and main effects of cohort (Aug, Sept, and Aug→Sept groups) are reported. As would be expected, in all analyses body weights varied significantly over time (main effect of time), and these statistics are not reported. Cohort \times time interactions, which indicated group differences in the pattern of somatic growth over time, are reported whenever present. Where omnibus repeated-measures ANOVAs yielded significant main effects or interactions of treatment groups, these were followed up with pairwise repeated-measures ANOVAs between cohorts. Additionally, group differences at individual time points were assessed using between-subject ANOVAs and Student's t -tests. Because the onset of gonadal development was not normally distributed in all cases, nonparametric Kruskal-Wallis H -tests were employed to assess group differences.

Measures among all three groups were compared to assess whether the duration of the IT was influenced by photoperiodic conditions. To assess the specific relevance of DLs before and after birth, Aug→Sept hamsters were contrasted with groups under similar conditions prenatally (Aug group) or postnatally (Sept group). Finally, the importance of maternal nonresponsiveness of IT function was evaluated by compar-

Fig. 2. Representative double-plotted actograms of photoresponsive (R; A) and nonresponsive (NR; B) dams as measured by passive infrared motion detectors in short DLs. Dark bars above actograms represent time of lights off. Note in the nonresponsive hamster the relatively short period of nighttime activity, which is restricted to the second half of the daily dark period.



ing development of young born of photoresponsive vs. nonresponsive dams.

RESULTS

Breeding. Thirty-seven of 48 adult females (77%) bore litters within a 16-day interval beginning on a simulated August 2 photoperiod. Mean birth dates and corresponding photoperiods of Aug and Aug→Sept litters are shown in Table 1. Values for male and female offspring differed because only a selected fraction of females was retained for these experiments. On pairing a second time with males, 32 of 36 adult females

(89%) bore litters within a 13-day interval (Sept group; Table 1). These birth dates approximated, but did not exactly match, the 6-wk advanced postnatal conditions of Aug→Sept hamsters. Of 43 dams yielding clear circadian activity records, 26 (46%) were designated nonresponsive to short DLs on the basis of their locomotor activity records (Fig. 2). Neither sex ratio of litters ($F = 0.7$, $df = 2$, $P > 0.45$) nor the number of pups per litter ($F = 1.1$, $df = 2$, $P > 0.30$) differed between the three cohorts of hamsters.

Male offspring. In contrast to the population of adult dams, the vast majority of male offspring (150 of 160,

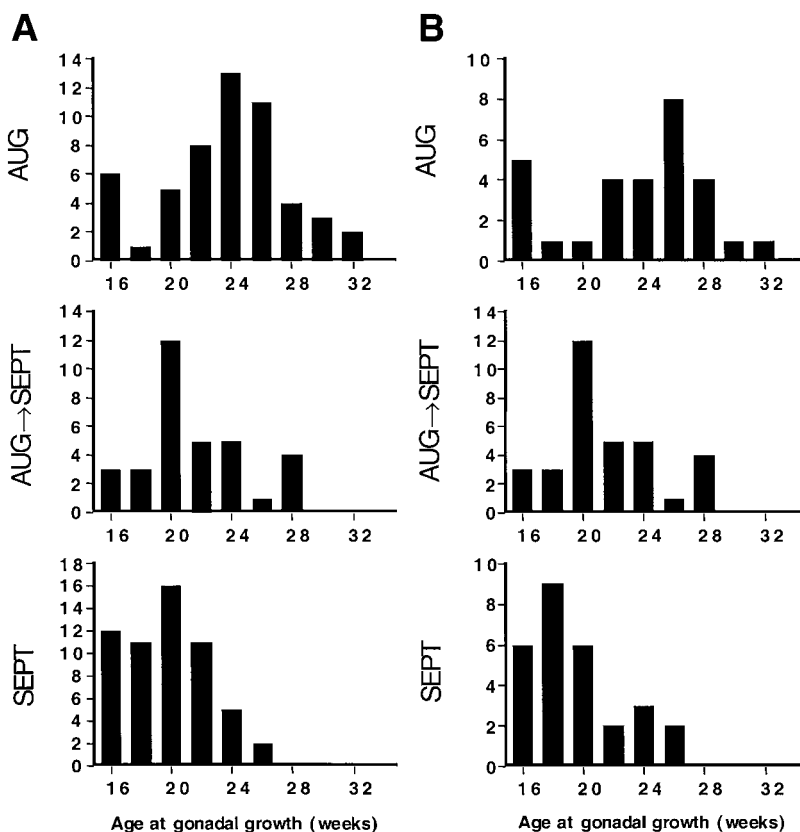


Fig. 3. Frequency distributions of age (wk) at onset of gonadal development in photoresponsive male hamsters exposed to the 3 experimental conditions described in METHODS. A: all responder hamsters, regardless of whether DLs increased or remained short after the winter solstice. B: only those responder hamsters maintained on 8L after the winter solstice. Not included are 7 males that died before initiation of gonadal development.

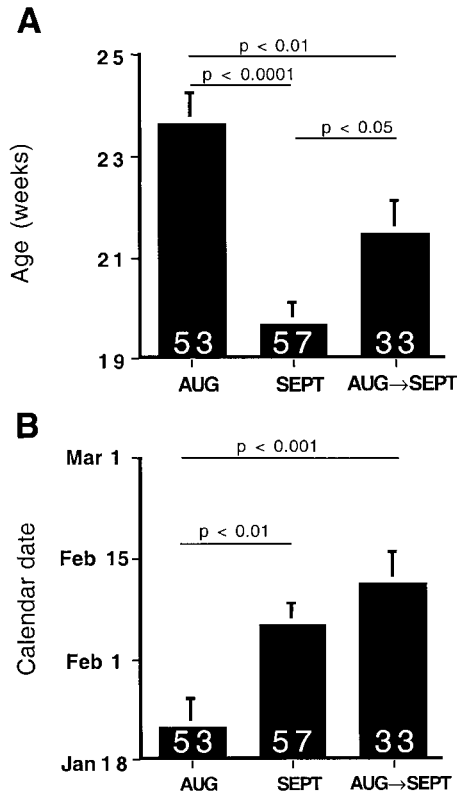


Fig. 4. A: mean + SE age (wk) at onset of testicular development in photoresponsive male hamsters born and raised in the 3 photoperiod regimes described in METHODS. Hamsters exposed to increasing DLs after the winter solstice and those clamped on 8L are considered jointly. B: mean + SE calendar date of the year at onset of testicular development in the same cohort of hamsters. Sample sizes are noted inside bars.

94%) exhibited a photoperiod-induced winter phenotype. On the basis of pelage and testis size at *week 16* and earlier body weights, 10 males were designated as photononresponsive. These nonresponders were observed disproportionately in Aug treatment group (8 of 10, $\chi^2 = 7.3$, $df = 2$, $P < 0.05$) but were no more likely to be born of nonresponder than of responder dams ($\chi^2 = 0.2$, $df = 1$, $P > 0.6$). Nonresponsive offspring were omitted from all subsequent analyses.

For neither Aug nor Sept groups did the post-winter solstice DL treatment (gradually increasing vs. clamped at 8L) affect the age at onset of testicular growth among photoresponsive hamsters ($P > 0.80$, $P > 0.60$, respectively). Thus this factor was eliminated in further analyses except as noted below. Among photoresponsive males, onset of gonadal growth of Aug hamsters was significantly more variable than in Sept hamsters ($F = 2.2$, $df = 56,52$, $P < 0.01$; equality of variance *F*-test; Fig. 3A) because of a bimodal distribution of this measure in Aug males. The three groups, moreover, initiated gonadal development at different ages ($H = 28.1$, $df = 2$, $P < 0.001$; Kruskal-Wallis *H*-test; Fig. 4A), with Aug hamsters delaying maturation by 4 wk beyond that observed in Sept males ($P < 0.0001$). Aug->Sept animals were intermediate between Aug and Sept cohorts ($P < 0.05$). Consideration

of only males kept in 8L after the winter solstice yielded the same pattern (Fig. 3B), and group differences in average gonadal onset persisted ($H = 14.0$, $df = 2$, $P < 0.001$, not shown).

When assessed at comparable times in the yearly photocycle, omnibus group differences in gonadal development were diminished but still evident ($H = 11.5$, $df = 2$, $P < 0.01$; Fig. 4B). August-born males (Aug group) initiated gonadal onset 2.0 wk earlier than did Sept hamsters ($P < 0.001$) and nearly 3.0 wk earlier than Aug->Sept ($P < 0.001$) males. The same general result was obtained when analysis was restricted to hamsters clamped on 8L ($H = 5.1$, $df = 2$, $P < 0.08$, data not shown).

Among photoresponsive male hamsters, birth cohort also affected somatic growth. Early body weights (*weeks 3–9*) differed significantly between groups (main effect of cohort, $F = 3.1$, $df = 2$, $P < 0.05$; see Fig. 6A, inset), with Aug->Sept males weighing significantly less than Aug animals ($F = 4.9$, $df = 1$, $P < 0.05$). The pattern of growth (cohort \times time interaction), however, did not differ among groups during this early interval ($F = 1.1$, $df = 10$, $P > 0.35$). A plateau in body weights was reached at ~ 7 wk of age regardless of condition.

Increasing DLs after the winter solstice stimulated somatic growth in both Aug ($F = 5.5$, $df = 20$, $P < 0.001$; Fig. 5A) and Sept hamsters ($F = 3.0$, $df = 22$,

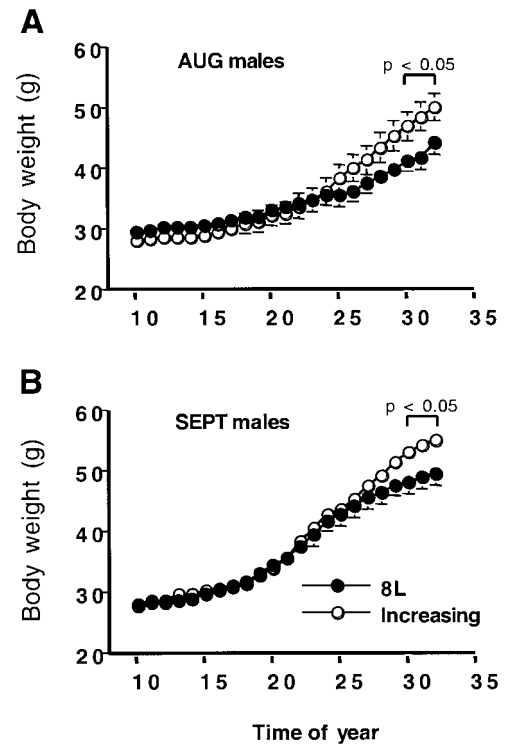


Fig. 5. Mean \pm SE body weight of male responder hamsters born into Aug (A) or Sept (B) photoperiods. After the winter solstice, hamsters were exposed either to increasing DLs (\circ , $n = 22–26$, $n = 26–29$ for Aug and Sept groups, respectively) or 8L (\bullet , $n = 20–34$, $n = 28$ for Aug and Sept groups, respectively). In some cases, SEs are contained within the symbol indicating mean values. Brackets indicate weeks where groups differ significantly from one another ($P < 0.05$).

$P < 0.001$; Fig. 5B) relative to 8L males as reflected in post-winter solstice DL \times time interactions during weeks 10–32. For both cohorts, increasing DLs generated significantly higher body weights from 30 wk of age ($P < 0.05$). The two postsolstice treatment conditions were therefore treated separately in further analyses of body weight data. For the three groups of hamsters clamped at 8L, overall body weights between 10 and 32 wk of age did not differ between cohorts, but the cohort \times time interaction was highly significant ($F = 8.0$, $df = 44$, $P < 0.001$; Fig. 6A). Although initially somewhat heavier than other groups, hamsters born into August DLs (Aug group) increased body weight at later ages than did hamsters born into September DLs (Sept group) (pairwise repeated-measures ANOVA, $F = 11.0$, $df = 20$, $P < 0.001$) or transferred to September DLs at birth (Aug \rightarrow Sept group) ($F = 14.2$, $df = 22$, $P < 0.001$). The latter two groups showed similar developmental trajectories. Aug and Sept hamsters exposed to naturally increasing DLs after the winter solstice also showed significantly different patterns of growth ($F = 5.7$, $df = 22$, $P < 0.001$; Fig. 6B). When assessed on a week-by-week basis, Aug males weighed significantly less than other groups beginning at 23 wk of age ($P < 0.05$) regardless of whether DLs increased after the winter solstice (Fig. 6, A and B).

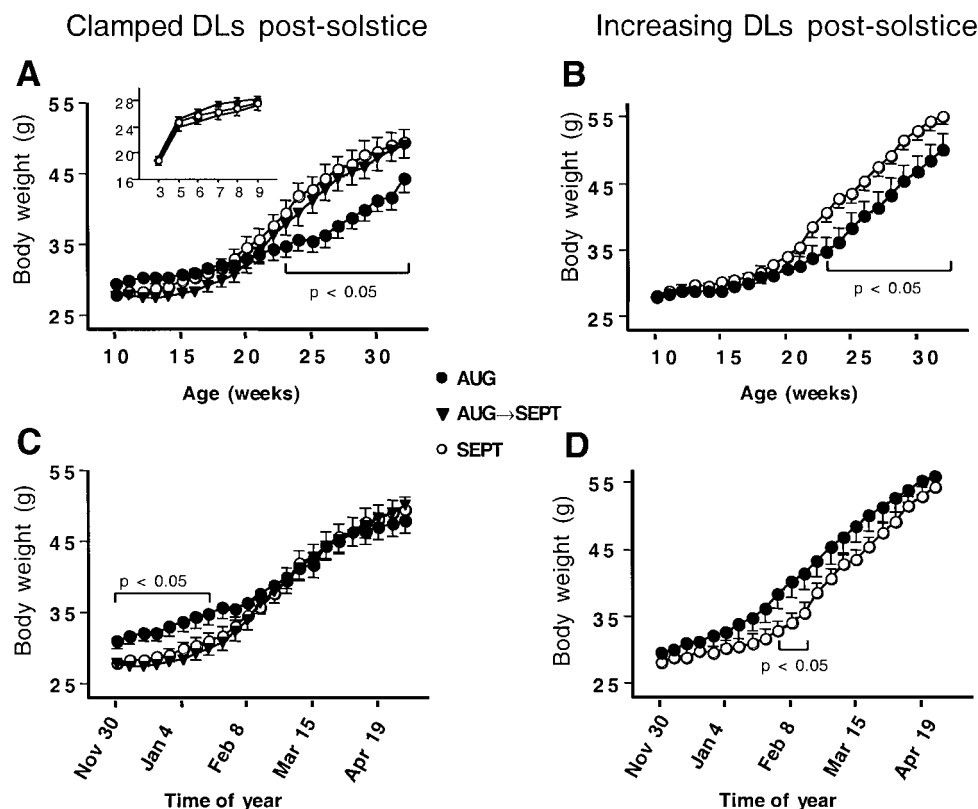
When matched for phase of the year, statistical differences in body weight trajectories persisted, but group differences diminished in spring. Among hamsters clamped on the solstice DL, the cohort \times time interaction during weeks 10–32 was highly significant ($F = 3.1$, $df = 44$, $P < 0.001$; Fig. 6C). This omnibus

result reflected the different body weight trajectory of Aug males compared both with Sept ($F = 3.4$, $df = 22$, $P < 0.001$) and Aug \rightarrow Sept males ($F = 6.7$, $df = 22$, $P < 0.001$). Similarly, among hamsters experiencing natural increases in DL after the solstice, the pattern of body weights differed between Aug and Sept males ($F = 1.7$, $df = 22$, $P < 0.001$; Fig. 6D). At individual time points, however, the groups differed significantly only for 3 wk in early February ($P < 0.05$). Among the subsample clamped at 8L, Aug hamsters were heavier than other groups from late November through mid-January (Fig. 6C) but not after that point.

Maternal responsiveness had influence on neither onset of testicular development nor male somatic development in weeks 3–9. In later somatic development, moreover, maternal nonresponsiveness had no effect among the Sept or Aug \rightarrow Sept cohorts. Among Aug males clamped at 8L after the solstice, however, body weights increased earlier among offspring of photononresponsive dams compared with those of photoresponsive dams ($F = 3.1$, $df = 22$, $P < 0.001$, data not shown). The same effect was not seen in Aug males experiencing gradually increasing DLs ($F = 0.7$, $df = 22$, $P > 0.75$).

Female offspring. Like their male siblings, the vast majority of female offspring (56 of 62, 90.3%) exhibited the winter phenotype. Only six females were designated nonresponders on the basis of summer pelage in short DLs and accelerated growth rates. These individuals were not differentially distributed among treatment groups ($\chi^2 = 0.9$, $df = 2$, $P > 0.60$).

Fig. 6. Mean \pm SE body weights of photoresponsive male hamsters from Aug, Sept, or Aug \rightarrow Sept treatment cohorts. Subgroups of hamsters remained on the 8L after the solstice photoperiod (A and C; $n = 20$ –34, $n = 28$, and $n = 29$ –33 for Aug, Sept, and Aug \rightarrow Sept males, respectively). Others were exposed to natural increases in DL after the solstice (B and D; $n = 22$ –26 and $n = 26$ –29 for Aug and Sept males, respectively). Mean weights are plotted as a function of age in wk (A and B) or in terms of the calendar date of the ongoing or prior simulated natural photoperiod (C and D). Brackets denote weeks where body weights of Aug hamsters differ from those of other groups. A, inset: body weights from 3 to 9 wk of age.



Among photoresponsive females, no differences in average body weight or pattern of growth over time were found between cohorts from 3 to 9 wk of age (Fig. 7A, inset). All groups approached their maximum body weights by 5 wk of age. Markedly different body weight trajectories were seen after *week 10*, however (Fig. 7A). A main effect of cohort ($F = 4.5$, $df = 2$, $P < 0.05$) reflected lower average body weights of Aug vs. Sept females ($F = 16.4$, $df = 1$, $P < 0.001$, pairwise comparison). A main effect of cohort was not found with other pairwise comparisons. Additionally, a significant cohort \times time interaction ($F = 4.2$, $df = 48$, $P < 0.001$; Fig. 7A) indicated different patterns of growth over time for the three groups. Qualitatively, Sept females increased body weight from plateau levels beginning at ~ 16 wk of age, whereas Aug hamsters remained at plateau levels until ~ 24 wk of age and increased thereafter. Hamsters phase-advanced at birth (Aug \rightarrow Sept group) showed an intermediate pattern of weight gain, with gradual rather than abrupt increases from plateau body weights. The pattern of growth did not differ significantly between Aug \rightarrow Sept and Sept females. Between Aug hamsters and the other two groups, however, the body weight

trajectory did differ significantly ($F = 9.8$, $df = 1$, $P < 0.001$ vs. Sept; $F = 3.7$, $df = 1$, $P < 0.001$ vs. Aug \rightarrow Sept hamsters). Finally, Sept females weighed significantly more than Aug females at each time point from 17 to 34 wk of age and were heavier than Aug \rightarrow Sept females from 25 to 29 wk ($P < 0.05$).

When differences between groups were assessed by matching for time of year (Fig. 7B), the main effect of cohort failed to reach statistical significance ($F = 1.7$, $df = 2$, $P < 0.20$) as did the cohort \times time interaction ($F = 1.0$, $df = 48$, $P < 0.50$). This interaction, moreover, would not reach significance in any pairwise comparisons between treatment groups ($P > 0.30$ in all cases). Body weights of Sept females exceeded those of Aug females ($P < 0.05$) for 11 wk from mid-January to late March (as well as during single weeks in December and May). No other groups differed at any single time point.

Maternal responsiveness had no significant effect on early (*weeks 3–9*) or later (*weeks 10–34*) development in female offspring.

Post hoc analysis. Because the unexpected bimodality in the onset of testis growth among Aug hamsters (Fig. 3A) suggested alternative developmental strategies in that group, post hoc analyses were conducted to determine whether exclusion of the secondary mode would affect the overall conclusions drawn from the male data. Six male hamsters deemed responsive on the basis of the criteria described above but with developed testes by *week 16* (see Fig. 3A) were excluded, and the data were reanalyzed. Excluding these hamsters, Aug males initiated gonadal growth at 24.6 ± 0.5 wk of age or 4.9 wk later than did Sept hamsters ($P < 0.001$) and 3.1 wk later than did Aug \rightarrow Sept males ($P < 0.001$). In terms of the season of the year, Aug hamsters initiated gonadal growth on Jan 29 ± 3 days compared with Feb 5 ± 3 days for Sept hamsters (difference not significant) and Feb 11 ± 4 days for Aug \rightarrow Sept hamsters ($P < 0.01$). For males exposed to increasing DLs after the solstice, body weights between Aug and Sept cohorts differed significantly only at a single week in mid-February ($P < 0.05$). Among hamsters clamped at 8L after the solstice, Aug males had higher body weights than Aug \rightarrow Sept males from early December to mid-January. No other comparisons yielded significant differences.

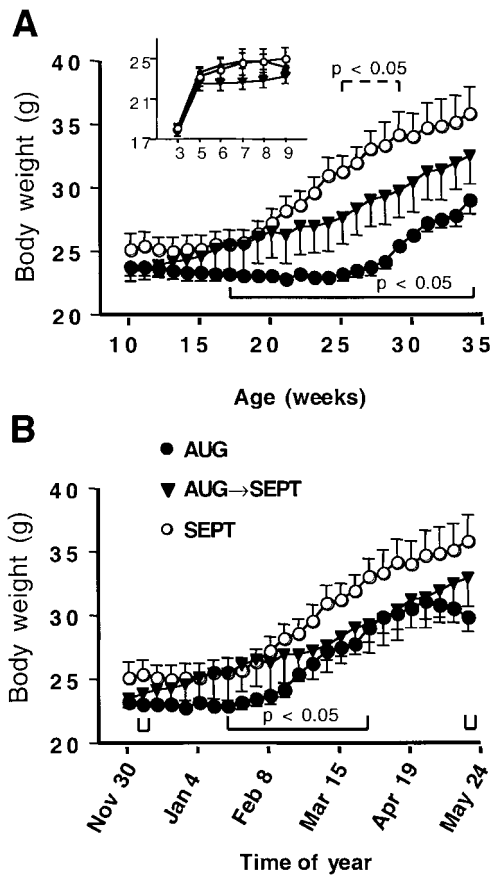


Fig. 7. Mean \pm SE body weights of photoresponsive female hamsters from Aug ($n = 19$), Sept ($n = 17$ – 19), and Aug \rightarrow Sept ($n = 18$) treatment cohorts and exposed to 8L after the winter solstice. Mean weights are plotted as a function of age in wk (A) or in terms of calendar date of the prior simulated natural photoperiod (B). Brackets indicate times of significant differences between Aug and Sept hamsters (solid brackets) and between Sept and Aug \rightarrow Sept females (dashed bracket). A, inset: body weights from 3 to 9 wk of age.

DISCUSSION

Adult female Siberian hamsters readily mated throughout decreasing summer DLs, bearing litters into different developmentally inhibitory photoperiods. Rates of maturation differed for offspring born into simulated early-August (Aug group) and late-September (Sept group) conditions and were little influenced by prenatal conditions. Specifically, winter increases in body weight occurred at different ages in Aug and Sept cohorts but were nearly synchronous when considered with respect to the time of year. Similarly, the onset of gonadal development in males was delayed to a later age in Aug compared with Sept hamsters. As these

effects were obtained whether or not DLs increased after the winter solstice, they reflect changes in the operation of an IT mechanism rather than a principal synchronizing effect of increasing winter DLs per se. A plastic IT mechanism may enable animals born into different types of inhibitory summer or fall DLs to synchronize reproductive effort in the next breeding season. This IT plasticity may be a prerequisite for cohorts of animals born into a reproductive "shoulder season" in fall if they are to time their reproductive effort to coincide with the earliest favorable conditions in spring.

Short or decreasing DLs inhibit reproductive capacity of male and female Siberian hamsters (19, 25), but breeding in the laboratory and the field continues at least into September (37). Correspondingly, nearly all dams born into an April SNP remained fertile into late summer when DLs had decreased to ~13L. In shorter DLs, many of these females also failed to entrain locomotor activity patterns in the canonical short-day fashion in which activity duration, α , is expanded. The entrainment pattern that generates a long α is associated with a long-duration peak of nocturnal melatonin secretion necessary for reproductive suppression and other short-day traits (6). While photononresponsiveness has a genetic basis as revealed by artificial selection experiments (9, 22), expression of this trait depends on prior exposure to long DLs, which fixes the circadian system in a state resembling that in long DLs (11, 14). The incidence of nonresponsiveness among dams was comparable to that reported for males from a similarly maintained colony unselected for or against this trait but routinely exposed to long DLs (14).

A genetic influence on nonresponsiveness notwithstanding, nonresponsive dams were able to produce pups that were reproductively inhibited by short DLs as in prior reports (32). Because pups were almost uniformly responsive when exposed to late-September photoperiods at birth, the higher incidence of nonresponsiveness among the August-born males suggests an environmental induction by DL of photoresponsiveness in pups as well as dams. In prior studies with adult males, exposure to 18L resulted in high rates of nonresponsiveness, whereas 14L produced virtually no nonresponders despite equivalent stimulation of reproductive and somatic growth by the two long DLs (14). Although rates of nonresponsiveness were still fairly low among Aug males, DLs shorter than 14:19L (the maximum postnatal DL experienced by Aug animals) appear to be sufficient to induce nonresponsiveness in at least some pups. No higher incidence, however, was seen in female offspring in the same photoperiod. A photoperiod history-dependent mechanism, unlike an exclusively genetically determined one, would avoid the potentially disadvantageous outcome whereby a nonresponsive dam might produce pups insensitive to short DLs. Because short-lived rodents born early in the year are unlikely to survive to breed the following spring, there may be selection pressures for older adults to continue breeding indefinitely into the fall. Their pups, which are likely to overwinter successfully

on the other hand, may be under strong selection pressure to delay reproductive effort until favorable conditions arrive in spring.

As rodent dams communicate photoperiodic information to their offspring in utero (33, 36), maternal nonresponsiveness, having a circadian basis and influencing melatonin secretion patterns, might alter the maturational trajectories of the pups. Indeed, in other studies of hamsters raised in 16L, body weight and gonad size differed for young born of nonresponsive vs. responsive dams (32). In the present study, the offspring of nonresponsive dams differed from those of responsive dams only among males born into August DLs and left in 8L after the solstice. Because males experiencing gradual increases in DL after the solstice did not show the same effect, this phenomenon would likely have little significance in the field where DLs are not clamped at 8L. Prenatal photoperiod more generally, as assessed by comparison of Aug→Sept and Sept cohorts, likewise exerted only minor effects on later development: Aug→Sept males were 2 wk older than Sept males at onset of gonadal development, and Aug→Sept females weighed less than Sept females from 25 to 29 wk of age. Although postnatal photoperiodic conditions were intended to be identical, the two groups were raised in SNPs differing in phase by 1 wk (Table 1). Both of the aforementioned effects disappeared when groups were matched for calendar date of the simulated yearly photocycle (analyses not shown), suggesting that postnatal, rather than prenatal, factors account for these differences. The analytic utility of this comparison, however, may be limited because many Sept young were born to nonresponsive mothers expressing short α , and their prenatal melatonin exposure may therefore mimic that of Aug→Sept hamsters. Maternal responsiveness, however, had no effect on Sept hamsters. Thus large effects of prenatal photoperiod previously reported after abrupt postnatal changes in DL or in the absence of postnatal melatonin exposure (20, 33, 34) may be muted in a more naturalistic context or may be quite specific to the photoperiods used in earlier studies.

DLs after the winter solstice also exerted only minor effects on development. Naturally increasing DLs led to higher body weights relative to males maintained on 8L, but the groups did not diverge significantly until March 8 and April 19 for the Aug and Sept cohorts, respectively. The timing of testis development, which is initiated many weeks in advance of these dates, moreover, was not influenced by the postsolstice photoperiod. Similarly, increases from plateau body weights were well under way before increasing DLs significantly stimulated growth. Together, these results suggest that even in SNPs the transition to the spring/summer reproductive phenotype is programmed by the IT rather than by increasing DLs per se. Winter and spring increases in DL likely modulate the pattern of body weight gain, and according to other studies, reverse the refractoriness to short DLs (16, 35).

In contrast, the different varieties of inhibitory DLs presented between birth and the winter solstice appear

to have exerted marked effects on development. Initially, Aug and Sept cohorts were equally suppressed in terms of somatic development. This inhibition is confirmed by a post hoc comparison of both groups with hamsters photostimulated by postnatal 15L (cf Figs. 6A and 7A with *week 5* body weight in 15L: 31.0 ± 0.6 g and 27.9 ± 0.6 g for males and females, respectively; $P < 0.001$). Despite the initial parallel developmental track in Aug and Sept cohorts, transition to the reproductive phenotype was initiated at significantly earlier ages in the later-born hamsters. Because August-born animals transferred to September conditions (Aug→Sept group) also initiated gonadal development at an earlier age than Aug hamsters, postnatal DLs are particularly implicated and discount a primary determining role of maternal parity on the length of the IT. Thus the type of short-day pattern an animal experiences developmentally affects it when it becomes photorefractory to short DLs and undergoes pubertal maturation. These results thus extend to a more naturalistic context the modulation of the IT mechanism earlier shown with static DLs (16). In several species, life histories vary for young born early or late in the breeding season (24, 26, 27) in large part because these cohorts are exposed to stimulatory and inhibitory photoperiods, respectively. The present results are novel insofar as they demonstrate a deferred effect on developmental milestones by different regimens of DLs equally inhibitory from birth. The role of additional factors distinguishing seasonal cohorts in the field (e.g., maternal age and parity, ambient temperature, food availability, etc.) remains to be determined and was not addressed here.

Photoperiod-sensitive plasticity in the duration of the IT would appear to achieve a relative synchronization of reproductive activity in spring, although fertility per se was not assessed in this experiment. Employing a conservative definition of photoresponsiveness, Aug and Sept males initiated gonadal development only 2 wk apart in terms of calendar dates and were completely synchronized when early-developing outliers were eliminated from the Aug group. Although body weight does not closely index reproductive status, it is controlled by overlapping photoperiodic mechanisms, and this measure too was better related to calendar date than to animal age in both males and females. Groups matched for calendar date differed significantly at many fewer time points than when matched for age, especially as the experiment progressed. If both body weight and onset of testis growth are synchronized between cohorts, it is likely that fertility too is relatively synchronized. It is not possible to say, however, whether natural selection acted on IT mechanisms specifically to synchronize fertility. Nevertheless, the results are consistent with such a functional interpretation.

On the other hand, a comparable synchronization was not achieved for Aug and Aug→Sept hamsters who initiated gonadal development 2–3 wk apart in January/February depending on whether or not any Aug hamsters were excluded from the analysis. Early

body weights of Aug→Sept males also differed from those of Aug males. Together, these results suggest that the abrupt and unecological phase advance of the photocycle in the former group may have compromised the ability of these animals to appropriately process ambient DLs. This would imply that prenatal photoperiods prepare animals for proper interpretation of postnatal conditions. As animals would never experience these conditions in nature, failure to synchronize with Aug hamsters does not argue against the functional explanation proposed.

Synchronization of seasonal cohorts by photoperiod was not observed in laboratory-bred white-footed mice, *Peromyscus leucopus* (8). In that study, mice born into simulated September and October photoperiods and maintained in the natural yearly photocycle thereafter exhibited gonadal development at comparable ages with no evidence of springtime reproductive synchrony. In contrast to the present study, dams of those offspring were uniformly maintained in long DLs (14L) until midpregnancy, at which point they were transferred to simulated seasonal cycles. As entrainment to shorter DLs can require weeks or months, any maternally communicated photoperiodic information would likely have been a long-DL signal throughout gestation and into early postnatal life. When pups matured to process DL information independently of their dams, those first photoperiodic signals would reflect the short ambient DLs. Although established for other species in the genus (2), the importance of prenatal photoperiod is unknown for *P. leucopus*. In hamsters, moreover, exposure to abrupt, unecological jumps in photoperiod can mask the influence of ecologically meaningful signals associated with the naturalistic pattern of gradual photoperiodic change (15). Thus it is possible that the unecological photoperiodic transfers of *P. leucopus* obscured the capacity of seasonal cohorts to synchronize their reproductive development. Whether *P. leucopus* both fully gestated and raised in natural photoperiods would synchronize reproductive development remains an open question. Alternatively, the *P. leucopus* population studied derived from a lower latitude (42° N) than did the *Phodopus* colony (55°), and seasonal environmental variation may be correspondingly less extreme. As a result, selection pressures on the timing of spring breeding may be weaker (see Ref. 7), and deer-mice may not have evolved the capacity for developmental synchrony or may have achieved it by means other than sensitivity to photoperiod.

In contrast to other groups, males of the Aug cohort had available to them multiple developmental strategies. An identifiable fraction was photononresponsive and showed rapid somatic growth and full gonadal maturation by late November or earlier. If autumn conditions were mild, these males may have been able to breed even in the season of their birth. Other males were not clearly identified as nonresponsive because they did not show particularly rapid somatic growth but nonetheless were gonadally mature by 16 wk of age. Present data unfortunately do not permit resolution of the issue of whether they were photononrespon-

sive or merely refractory to short DLs within 16 wk of exposure. Nonetheless, they clearly reflect a strategy discontinuous with the majority of the Aug cohort as indicated by the bimodal distribution in onset of testis growth. The general conclusions are not substantially altered by the assigned status of these males, but a somewhat better synchronization with the Sept cohort is achieved when they are not considered. In some other species for which extensive field data are available (e.g., deermice, *P. maniculatus*), complete synchrony of spring breeding is not observed. A small fraction anticipates the main breeding season by several weeks and generally suffers higher mortality than do female mice breeding later (7). This greater cost, however, is balanced by a greater inclusive fitness among those breeding successfully, suggesting that there need not be a single optimal breeding time for the species. Analogously, nonresponsive August-born males (Aug group) or those becoming photorefractory at an early age may represent an alternative reproductive strategy similarly maintained as a balanced polymorphism. Despite this polymorphism within the Aug group, the successive litters of late-summer/early-fall young do not mature in distinct waves to comprise separate spring breeding cohorts.

What formal mechanisms might underlie the developmental synchronization of the different seasonal cohorts of offspring? The IT has been conceptualized as an hourglass triggered by an environmental event. If the IT were triggered at different ages in the seasonal cohorts, it would not need to time different durations to synchronize developmental milestones. For example, shortening DLs in early August might photoinhibit hamsters from birth, but the IT itself might only be triggered after DL decreased to some shorter critical DL such as 12L (5, 17). Relative synchrony would occur because September-born animals would reach this critical photoperiod at an earlier age. This mechanism, however, insufficiently accounts for the different intervals timed in unchanging 10L and 12L (16). Alternatively, the duration measured by the IT, once triggered, might depend on ambient photoperiod. This latter conception shares formal similarities with the entrainment of endogenous circannual rhythmic processes that may be advanced or delayed by light and by melatonin (18, 38, 39). Future studies will address whether modulation of the IT by photoperiod shares physiological mechanisms of entrainment elucidated in circannual species.

Perspectives

A strict critical DL concept whereby organisms distinguish only between stimulatory and inhibitory DLs above and below a threshold value, respectively, has been increasingly supplanted by the recognition that the natural progression of gradually changing DLs provides animals with rich signals to extract information about the time of year (23). For example, photoperiod-driven changes in reproductive condition may occur over a broad range of DLs depending on the

temporal patterning of prior DL signals. This study extends the critique of the critical DL concept to the second major component of photoperiodic systems, the interval timing mechanism mediating the photoperiod-independent transition to reproductive condition. In a naturalistic context, this system discriminates among different versions of developmentally inhibitory DL patterns to synchronize springtime development of Siberian hamster pups.

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