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Evidence for Genetic Variation in the Occurrence of the Photoresponse of the Djungarian Hamster, *Phodopus sungorus*

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Abstract The Djungarian hamster generally responds to a short-day photoperiod with a complex syndrome of physiological and behavioral changes; however, not all hamsters are photoresponsive. The phenotypic difference is, in part, genetically determined. Parent–offspring regression on a number of continuous and discontinuous measures indicated significant heritability for photoresponsiveness. Four generations of replicated bidirectional selection on a photoresponse index (PI) resulted in significant shifts in the percentage of responsive hamsters, although the average PI of responsive individuals was not significantly changed. Eight estimates of heritability ranged from 0.20 to 0.52. We hypothesize that the circadian system is responsible for the occurrence of the photoresponse, but that the extent of photoresponse is controlled by a separate functional system.

Key words *Phodopus sungorus*, photoperiodism, heritability, quantitative genetics, selection

In temperate and arctic regions, there are predictable seasonal fluctuations in the habitats of many terrestrial organisms. Survival often depends on the ability to adjust physiologically and/or behaviorally to the changing environmental conditions. In some species, such as the montane vole, *Microtus montanus*, chemical signals in the diet regulate seasonal reproduction (Negus and Berger, 1977; Berger et al., 1981). Other species, such as the golden-mantled ground squirrel, *Spermophilus lateralis*, possess an endogenous annual clock (Mrosovsky, 1980a,b). However, many species are also photoperiodic (Nelson, 1987), such that exposure to certain daylengths elicits species-specific seasonal adjustments. Photoperiod is a very dependable seasonal cue, since the sinusoidal annual pattern of daylength at a given latitude does not vary.

In preparation for winter, the Djungarian hamster, *Phodopus sungorus*, displays an adaptive complex of physiological changes. These changes include a decrease in body mass, molt to lighter fur color and greater fur thickness, spontaneous daily torpor, gonadal regression, and increased nonshivering thermogenesis (Hoffmann, 1973; Heldmaier and Steinlechner, 1981; Heldmaier et al., 1982; Elliott et al., 1987). Exposure of Djungarian hamsters to a short-day photoperiod elicits these physiological adjustments (Hoffmann, 1973; Heldmaier et al., 1981; Steinlechner et al., 1983), as well as modifications of thermoregulatory behavior (Puchalski et al., 1988a). Substantial evidence indicates that the daily rhythm of synthesis of the pineal hormone melatonin is under the control of the circadian system, and that a

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change in this rhythm triggers the winter adaptations (for a review, see Bartness and Goldman, 1989).

Like certain individuals in a number of other rodent species (Nelson, 1987), not all Djungarian hamsters respond to short-day conditions. Some characteristic differences between responsive and nonresponsive hamsters are known. Whereas responsive hamsters extend the duration of the nighttime melatonin peak upon transfer from a long-day to a short-day photoperiod (Goldman et al., 1982), the duration of the melatonin peak in nonresponders remains short (Puchalski and Lynch, 1986). Nonresponsive short-day-exposed hamsters can be made to respond with molt and decreased body mass by injection of melatonin 3 hr after lights-off, suggesting that the effector system mediating responses to melatonin is intact (Puchalski et al., 1988b). Furthermore, circadian organization differs between these phenotypes. Nonresponders have a longer free-running period, a shorter duration of activity, and a unique phase response curve relative to responders (Puchalski and Lynch, 1986, 1988, 1991a; Kliman and Lynch, 1991). The daily firing profile in the suprachiasmatic nucleus (SCN), as measured in *in vitro* brain slices, also differs between responders and nonresponders (Margraf et al., 1991a), although daily melatonin injections can influence the firing profile of a nonresponder to resemble that of a responder (Margraf et al., 1991b).

Is there a genetic basis for differences in photoresponsiveness? Quantitative genetic analyses could be helpful in addressing this question. One hallmark of quantitative genetics is that phenotypic variance (V_P) can be partitioned into genetic and environmental variances and the interaction of the two. Genetic variance can be further partitioned into additive, dominance, and epistatic components. Of these, additive genetic variance (V_A) is particularly useful, since it is this component that makes closely related individuals tend to resemble one another (e.g., parent and offspring). Two points need to be highlighted. First, this extent of resemblance between relatives can be quantified as "narrow-sense" heritability (h^2), which is defined as V_A/V_P . Given that h^2 is a ratio, it ranges from 0.0 to 1.0. Second, if a physiological trait is heritable, then the mean of that trait in a population can be altered by either natural or artificial selection. The strength of the response to selection reflects not only the magnitude of the h^2 for that trait, but also the intensity of selection (Falconer, 1981). Although knowledge of heritability can be useful for both evolutionary and mechanistic deductions, interpretations based solely on this genetic parameter must be made with caution. Estimates of genetic parameters such as heritability are population- and environment-specific, so, strictly speaking, they apply only to the population and environment in which the measurements were taken. Furthermore, a low or zero heritability need not imply the absence of genetic influence on a trait. If most of the genetic variance can be attributed to dominance, this will not contribute to heritability. In the extreme case, highly inbred populations, such as isogenic strains of laboratory mice commonly employed by physiologists, exhibit no heritability within strain (all individuals being genetically identical). However, the pronounced differences evident between strains demonstrate the presence of significant amounts of genetic influence.

Previous studies have suggested that degree of photoresponsiveness (i.e., the extent of molt and decrease in mass) is heritable in Djungarian hamsters (Lynch and Lynch, 1986; Lynch et al., 1989). This detection of additive genetic variation for photoresponsiveness, a trait that is supposedly critical for winter survival, raises an important evolutionary issue. Fisher (1930) argued that directional selection should decrease the additive genetic variance affecting a trait; yet our preliminary data for photoresponsiveness indicate that considerable

additive genetic variance remains. Similarly, Garland et al. (1990) estimated unexpectedly high heritabilities for antipredatory locomotor traits in a natural population of garter snakes (*Thamnophis sirtalis fitchi*).

Although previous studies demonstrate that the degree of photoresponsiveness is heritable, they do not adequately address how nonresponsiveness might be inherited. There are two possibilities. One view is that there is a continuous distribution of degrees of photoresponsiveness, which crosses a threshold beyond which no response is observed (i.e., nonresponders). Here, the combinations of genes and environmental influences that determine whether an individual responds also determine to what degree it responds. A second possibility is that the photoresponse may be controlled by genetic and environmental influences that determine whether an individual responds, but not how well it responds. In the latter case, the degree of response is under independent genetic control. Quantitative genetic methods can help us to distinguish these different models.

In the presents study, we examined, in greater detail, additive genetic influences on the short-day photoresponse of Djungarian hamsters. We used two different methods to estimate h^2 : (1) quantifying the similarity between offspring and parents by means of regression analysis, and (2) studying the response to artificial selection for or against photoresponsiveness. This study has established that (1) the likelihood of responding to short-day photoperiod is a heritable, threshold character; and (2) the response to short days and the extent of that response are under independent genetic control.

MATERIALS AND METHODS

HOUSING AND FEEDING

All hamsters in this study were laboratory-reared in a long-day photocycle (LD 16:8) at 21–22°C. They were weaned at 19–21 days of age and group-housed by sex in polypropylene cages (length, 30 cm; width, 18 cm; depth, 13 cm; one to six hamsters per cage). Occasionally, same-sex individuals from different families were combined in a single cage. Food (Wayne Rodent Blox) and water were available *ad libitum*. Mated pairs were kept in polypropylene cages (30 × 18 × 13 cm). Food (Wayne Breeder Blox) and water were available *ad libitum*. The diet was also supplemented weekly with either a seed mix or apple slices and cottage cheese. Lighting in the animal room was provided by Sylvania cool white fluorescent lights (250–750 lux at the level of the cage).

HAMSTER STOCKS

The “American” stock of Djungarian hamsters was derived from eight breeding pairs obtained from Dr. Bruce Goldman (Division of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06268). The “German” stock was derived from nine breeding pairs obtained from Dr. Gerhard Heldmaier (Zoology Department, Phillips University, 3550 Marburg, Germany). Both stocks were originally derived from that of Dr. Klaus Hoffmann (see Figala et al., 1973). Heldmaier subsequently bred additional wild-caught individuals into his original stock; the “German” stock, therefore, should have greater genetic variability than the “American” stock. These stocks differ in their response to chronic short-day exposure (Lynch et al., 1989).

PHOTOPERIOD TREATMENT AND PRODUCTION OF BASE POPULATION

Hamsters from both stocks, born in LD 16:8 (lights-on 0800–2400 hr), were used to construct our parental (P_1) population. Adults were single-housed and transferred to a short-day photoperiod of LD 9:15 (lights-on 0800–1700 hr). Body mass and molt index (described later) were measured biweekly for 14 weeks, after which hamsters were returned to LD 16:8 for breeding. Eight successful German–American crosses produced an F_1 generation, which was then treated in the same manner as the parental hamsters, and randomly bred to produce 98 females and 71 males in the F_2 (= G_0) generation. At 80–100 days of age, G_0 hamsters were transferred to short-day conditions and measured in the same manner as previous generations. A “photoresponse index” (PI) score (described below) was determined for each individual. The G_0 generation was used as the base population for replicated bidirectional selection on PI.

MOLT AND PHOTORESPONSE INDEXES

In this study, we modified the molt index of Figala et al. (1973) so that the 6-point index ranged from 0 (grey-brown summer pelage) to 5 (white winter pelage), rather than from 1 to 6, as originally defined. We measured molt after 12 weeks of short-day exposure, referred to hereafter as “M12.” We assessed body mass upon transfer to short-day conditions and biweekly thereafter. The decrease in body mass over the first 12 weeks in short-day photoperiod was termed “DBM.” Because an increase in body mass is not a response to short-day photoperiod, an individual that increased in body mass during this 12-week period was assigned a DBM score of 0. The PI was defined as follows:

$$PI = DBM + [2 \times M12]$$

We chose this index for several reasons. Both molt and body mass are easily measured on large numbers of animals. We doubled molt index in an effort to weight it approximately equally with decrease in mass. We have observed a number of hamsters that molt in short-day conditions without decreasing mass, and vice versa. Calculating PI as described above would mean that these animals would still be considered responsive to short-day conditions. We have not observed hamsters displaying other short-day responses (daily torpor, gonadal regression) without either decreasing body mass or molting. Most animals respond by 12 weeks in short-day conditions.

ARTIFICIAL SELECTION ON PI

Six lines were established from the G_0 population. First, individuals were randomly chosen from each family for matings used to produce two control lines. From the remaining hamsters, the female and male with the highest and lowest PI in each family were chosen for construction of replicated high and low lines. The chosen hamsters were crossed randomly within line, with the exception that no individuals with common grandparents were mated. The lines were closed after the first generation of selection.

Hamsters from the subsequent generations (G_1 , G_2 , G_3 , and G_4) were born in LD 16:8 (lights-on 0430–2030 hr). Because of the shift in the LD cycle in the breeding room (for reasons independent of this selection experiment), all of these individuals were transferred

to LD 16:8 (lights-on 0800–2400 hr) at 70–90 days of age. After 10 days, they were single-housed and transferred to short-day photoperiod; body mass and molt index were measured in the same manner as in previous generations. In all, we measured photoresponse in 479, 705, 530, and 573 individuals from the six lines over the four successive generations of selection. In an effort to maintain genetic variability and to minimize the chance of a genetic “bottleneck” in any of the lines, it was sometimes necessary to take more than one individual of a particular sex from some families. This was necessary, since not all matings produced offspring and some successful matings did not produce both female and male offspring.

HERITABILITY ESTIMATES AND ADDITIVE GENETIC CORRELATIONS

Since sex differences have been observed for the characters studied, we estimated all heritabilities separately for females and males. We estimated h^2 for DBM, M12, and PI by parent–offspring regression, using the replicate control lines (see Falconer, 1981) and weighting each parent–offspring point in the regression by the number of same-sex offspring per parent. We also estimated the heritabilities of occurrence of molt by week 12 in short-day photoperiod (i.e., $M12 > 0$) and occurrence of photoresponse (i.e., $PI > 0$) (Falconer, 1965; Cavalli-Sforza and Bodmer, 1971). Over four generations, 39 families producing 397 male offspring were analyzed as Control Line 1; similarly, 61 families producing 691 offspring were analyzed as Control Line 2. Regression was carried out by summing parent–offspring sums of products across generations to produce the numerator of the regression expression, and by summing parent sums of squares across generations to produce the denominator. This was required to account for possible generational differences in nonadditive genetic or environmental effects. Regressions were calculated for each control line, although the data from all control families in the first generation were used in both h^2 estimates, since there was no genetic basis for considering separate control lines until generation 2. Significance of regression values (i.e., $b > 0$) was determined by a t test, where $t_s = b/s_b$ (for $n - 2$ degrees of freedom, where n is the number of families, b is the regression value, and s_b is the standard error of the regression value).

We also estimated the heritability of PI from the direct response to selection in the replicate high and low lines (after Falconer, 1981) relative to the mean of the control line values at each generation, correcting for changes in control line variance. Although heritability estimates may be different in high or low lines after many generations of selection (Falconer, 1981), estimates based on the first four generations should be comparable. Therefore, we calculated mean female and male heritabilities and standard errors using the estimates from all four selected lines. Significance of the heritability estimates was determined by t test (where $t_s = \text{mean/standard error}$, with three degrees of freedom).

DATA ANALYSIS

We performed multivariate analysis of variance (MANOVA; SPSS-X, 1988) on the fourth generation of selection to test for the effects of selection (high vs. low lines), replicate line (nested within selection), family (nested within replicate line), and sex on PI and M12. For the PI MANOVA, we included only those hamsters with PI scores greater than 0; similarly, for M12, we included only those that molted by week 12 in short-day photoperiod. We used the rules for the pooling of error terms described by Sokal and Rohlf (1969). We examined

the effects of selection (high vs. low lines), replicate line (nested within selection), family (nested within replicate line), and sex on the percentage of hamsters with $PI > 0$ and the percentage of those with $M12 > 0$ by loglinear analysis (CATMOD Procedure; SAS Institute, 1985).

RESULTS

SUCCESS OF SELECTION

Bidirectional selection on PI created lines divergent for that character, relative to randomly bred control lines (Fig. 1A); similar patterns of divergence were seen for DBM and M12 (Figs. 1B, 1C). Selection also increased the percentage of responders (those hamsters with $PI > 0$) in the high lines, and decreased this percentage in the low lines, relative to controls (Fig. 1D); the percentage of hamsters molting by week 12 in short-day conditions changed in a similar manner (Fig. 1E). The response to selection was apparent after one generation of selection. Although randomly bred, the control lines did not maintain a steady mean PI or percentage of responsiveness, indicating possible environmental effects across generations. Still, the divergence between the high and low lines appeared to increase through the fourth generation. There was no consistent effect of selection on mass at weeks 0 or 12 in short-day conditions (Figs. 1F, 1G), indicating that the observed changes in DBM in the selected lines did not result from inadvertent selection on either of these measures.

HERITABILITY ESTIMATES

Parent-offspring regression revealed significant sire-son heritabilities for DBM, M12, PI, occurrence of molt by week 12 in short-day conditions, and occurrence of photoresponse (as measured by PI) in both control lines (Table 1). Significant dam-daughter regressions were estimated for occurrence of molt in both control lines, and for M12 and occurrence of photoresponse in Control Line 1. In all cases, estimates of heritability from Control Line 1 were greater than the corresponding estimates from Control Line 2 (Table 1).

The realized heritability of PI in females and males was also measured by direct response to artificial selection in each of the selected lines (Table 2). Heritability estimates ranged from 0.204 in High Line 2 males to 0.522 in High Line 1 females. Mean h^2 calculated from the four selected lines was 0.378 ± 0.056 in females and 0.358 ± 0.063 in males, with both values significantly greater than 0 (Table 2).

MULTIVARIATE ANALYSIS OF VARIANCE

When hamsters with $PI = 0$ were excluded from the analysis, there was no significant effect of selection on PI, although there were significant sex and family effects (Table 3). When only hamsters that molted by week 12 in short-day conditions were analyzed, we found no significant effect of selection on M12, although there was a significant sex \times selection interaction effect and marginally significant sex and family effects. The latter MANOVA showed, in particular, that the selection effect accounted for only 0.1% of the total variance in M12, indicating that selection on PI had no effect on the degree of molt in responding hamsters (Table 3).

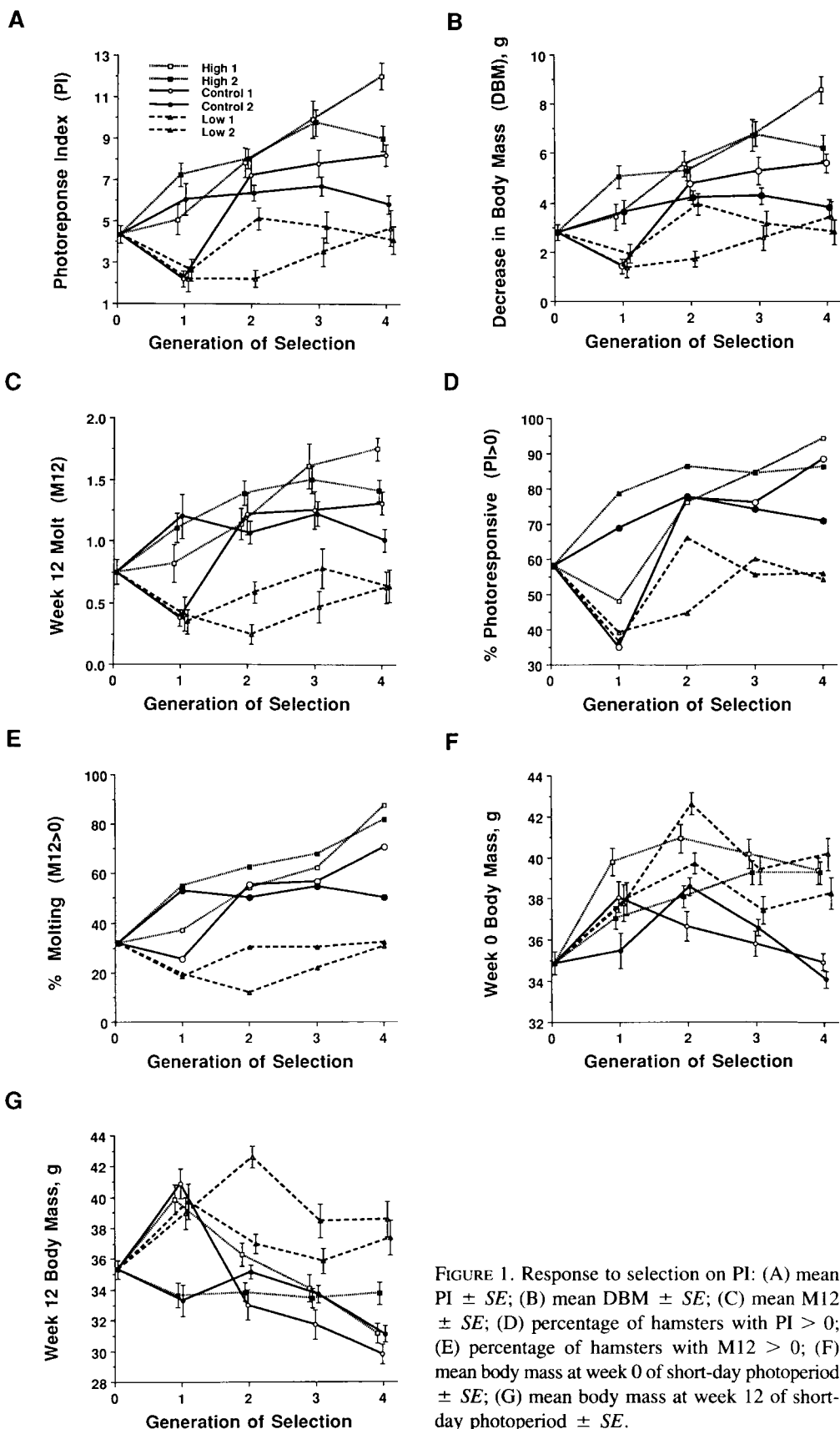


FIGURE 1. Response to selection on PI: (A) mean PI \pm SE; (B) mean DBM \pm SE; (C) mean M12 \pm SE; (D) percentage of hamsters with PI > 0; (E) percentage of hamsters with M12 > 0; (F) mean body mass at week 0 of short-day photoperiod \pm SE; (G) mean body mass at week 12 of short-day photoperiod \pm SE.

TABLE 1. Heritability Estimates ($\pm SE$) from Parent–Offspring Regression

| | Control Line 1 | Control Line 2 |
|--------------------------------------|--------------------|-------------------|
| PI | | |
| Female | 0.21 \pm 0.14 | 0.13 \pm 0.10 |
| Male | 0.62 \pm 0.18** | 0.26 \pm 0.12* |
| DBM | | |
| Female | 0.22 \pm 0.15 | 0.14 \pm 0.10 |
| Male | 0.52 \pm 0.20** | 0.28 \pm 0.13* |
| M12 | | |
| Female | 0.24 \pm 0.14* | 0.13 \pm 0.10 |
| Male | 0.72 \pm 0.15*** | 0.36 \pm 0.12** |
| Occurrence of photoresponse (PI > 0) | | |
| Female | 0.45 \pm 0.22* | 0.06 \pm 0.15 |
| Male | 0.43 \pm 0.19* | 0.31 \pm 0.14* |
| Occurrence of molt (M12 > 0) | | |
| Female | 0.43 \pm 0.20* | 0.36 \pm 0.12** |
| Male | 0.53 \pm 0.20** | 0.44 \pm 0.14** |

Note. Control Line 1 estimates based on 209 female offspring from 39 dams, and 188 male offspring from 39 sires. Control Line 2 estimates based on 339 female offspring from 61 dams, and 352 male offspring from 60 sires.

* $p \leq 0.05$. ** $p \leq 0.01$. *** $p \leq 0.001$.

LOGLINEAR ANALYSIS

The effect of four generations of selection on both the percentage of hamsters with PI > 0 and the percentage molting by week 12 in short-day photoperiod was highly significant ($p \leq 0.001$), indicating that selection had a major effect on the likelihood of responding to short-day conditions (Table 4). In high and low lines, respectively, 89.6% and 55.1% of the hamsters had PI > 0. Similarly, 78.8% and 31.4% of the high- and low-line hamsters, respectively, molted by week 12 in short-day conditions. No significant effect of sex, replicate line, or family was found, and there were no significant interaction effects (Table 4).

DISCUSSION

Photoresponsiveness was found to be a heritable character in this population of Djungarian hamsters. We carried out replicated, bidirectional selection on PI for four generations, and in all four selected lines, mean PI (including that of nonresponders) changed in the predicted direction relative to that of controls (Fig. 1A). This was expected, in light of a preliminary

TABLE 2. Realized Heritability Estimates from Selection Study

| | Females | Males |
|---------------|---------------------|--------------------|
| High Line 1 | 0.522 | 0.505 |
| High Line 2 | 0.249 | 0.204 |
| Low Line 1 | 0.386 | 0.397 |
| Low Line 2 | 0.354 | 0.326 |
| Mean $\pm SE$ | 0.378 \pm 0.056** | 0.358 \pm 0.063* |

* $p \leq 0.05$. ** $p \leq 0.01$.

GENETIC VARIATION IN HAMSTER PHOTORESPONSE

TABLE 3. Results of MANOVA on PI and M12

| Effect | df | % | MS | F |
|-----------------|-----|------|--------|----------------------------------------|
| PI | | | | |
| Sex | 1 | 2.9 | 245.74 | 9.49 _(1, 177) ** |
| Sel | 1 | 7.3 | 612.70 | 4.74 _(1, 2) |
| Rep (Sel) | 2 | 3.1 | 129.17 | 1.93 _(2, 29) |
| Fam (Rep) | 29 | 23.1 | 67.02 | 2.59 _(29, 177) *** |
| Sex × Sel | 1 | 0.0 | 2.04 | 0.02 _(1, 2) |
| Sex × Rep (Sel) | 2 | 0.3 | 13.10 | 0.19 _(2, 29) |
| Sex × Fam (Rep) | 24 | 8.7 | 30.54 | 1.18 _(24, 177) |
| Within | 177 | 54.5 | 25.91 | — |
| M12 | | | | |
| Sex | 1 | 1.6 | 0.95 | 3.35 _(1, 132) [^] |
| Sel | 1 | 0.1 | 0.07 | 0.18 _(1, 29) |
| Rep (Sel) | 2 | 0.3 | 0.09 | 0.23 _(2, 27) |
| Fam (Rep) | 27 | 19.3 | 0.42 | 1.46 _(27, 132) [^] |
| Sex × Sel | 1 | 3.6 | 2.08 | 5.33 _(1, 29) |
| Sex × Rep (Sel) | 2 | 0.8 | 0.49 | 1.17 _(2, 27) |
| Sex × Fam (Rep) | 22 | 9.4 | 0.25 | 0.87 _(22, 132) |
| Within | 132 | 64.8 | 0.28 | — |

Note. Significance of effects reflects pooling of error terms when significance of lower-order error term was $p > 0.25$. *df*, degrees of freedom; %, percentage of total variance; MS, mean squares; Sel, selection (high vs. low line); Rep, replicate line; Fam, family.

[^] $p \leq 0.10$. * $p \leq 0.05$. ** $p \leq 0.01$. *** $p \leq 0.001$.

TABLE 4. Results of Loglinear Analysis on
Percentage of Hamsters with PI > 0 and
Percentage of Hamsters That Molted by Week 12
in Short-Day Photoperiod

| Effect | df | χ^2 | p |
|-----------------|----|----------|--------|
| % PI > 0 | | | |
| Sex | 1 | 0.00 | 0.997 |
| Sel | 1 | 27.84 | <0.001 |
| Rep (Sel) | 2 | 2.55 | 0.280 |
| Fam (Rep) | 18 | 7.78 | 0.982 |
| Sex × Sel | 1 | 0.04 | 0.840 |
| Sex × Rep (Sel) | 2 | 0.22 | 0.896 |
| Sex × Fam (Rep) | 18 | 9.25 | 0.954 |
| % M12 > 0 | | | |
| Sex | 1 | 0.00 | 0.999 |
| Sel | 1 | 37.02 | <0.001 |
| Rep (Sel) | 2 | 2.75 | 0.241 |
| Fam (Rep) | 18 | 16.50 | 0.558 |
| Sex × Sel | 1 | 0.07 | 0.791 |
| Sex × Rep (Sel) | 2 | 1.63 | 0.443 |
| Sex × Fam (Rep) | 18 | 6.84 | 0.991 |

Note. *df*, degrees of freedom; *p*, significance of effect; Sel, selection (high vs. low line); Rep, replicate line; Fam, family.

study suggesting that PI was heritable (Lynch and Lynch, 1986). Selection on PI also altered the percentage of responding individuals in accordance with expectations from the preliminary study (Fig. 1D). Statistically significant realized heritability estimates, based on the response to selection, were calculated for both sexes (Table 2). Likewise, we estimated several significant heritabilities through parent–offspring regression for molt and decrease in body mass in short-day photoperiod, as well as for PI. In addition, we found significant heritabilities for the molt and photoresponse thresholds (Table 1).

Estimation of the heritability of PI from the two control lines showed, in both lines, a much greater sire–son than dam–daughter regression value; only the male estimates were significantly greater than 0 (Table 1). It is possible that some nongenetic influence on photoresponsiveness exists in females only, so that the proportion of phenotypic variance due to additive genetic variance is decreased relative to that of males. However, this observed sex difference was not apparent in the estimates of realized heritability. It was also generally observed that Control Line 1 heritability estimates were greater than the corresponding estimates from Control Line 2 (Table 1). This difference may be attributable to a slight genetic “bottleneck” in Control Line 2 between the first and second generations of selection, which probably led to some inbreeding and an associated reduction in genetic variance.

Significant heritability estimates suggest that natural selection has not fixed photoresponsiveness in this species. This is not unprecedented. Nonresponsiveness to photoperiod has also been observed in deer mice (*Peromyscus maniculatus*), and artificial bidirectional selection on this character was also successful, providing another example of inherited nonresponsiveness (Desjardins et al., 1986).

One might expect that individuals exhibiting the nonresponsive phenotype should be strongly selected against in the natural habitat of the Djungarian hamster, since harsh winter conditions would seem to favor photoresponsive individuals at northern latitudes, if only because they would have a white pelt. One explanation for the persistence of nonresponsiveness is that there may be sufficiently frequent periods of time during which the phenotype is actually at a selective advantage—that is, there is temporal variation in the direction of selection (see Grant, 1986). This could happen if mild winters occasionally occur. In these conditions, the nonresponding, reproductively competent individuals would be able to produce offspring during the winter, perhaps offsetting the otherwise selective disadvantage of nonresponsiveness. Although we have no knowledge of data on annual variation in weather patterns faced by natural hamster populations, an analogous situation has been reported for an insect species. In photoperiodic populations of the milkweed bug, *Oncopeltus fasciatus*, short-day exposure results in reproductive diapause in females. A heritability estimate of about 70% was measured for extent of reproductive delay (Dingle et al., 1977). Dingle et al. proposed that the high heritability creates a “genetic rheostat” for critical photoperiod, which is maintained by continually reversing selection pressures resulting from variation in the severity of winters and by gene flow within migrant populations.

Other genetic explanations for the persistence of the nonresponsive phenotype may also be valid. Since direct selection on any character is constrained by the effects on individual fitness of genetically correlated characters, it is possible that the nonresponsive phenotype may be maintained in the population by antagonistic pleiotropy or linkage disequilibrium (see Rose, 1982; Lande and Arnold, 1983). In other words, if nonresponsiveness tends to be coupled with traits that benefit the individual, then both responders and nonresponders may continue to coexist in the population.

Another argument is that nonresponsiveness to short-day photoperiod is enhanced under laboratory conditions. Perhaps additional environmental stimuli, such as temperature or food availability, may interact with photoperiod in the wild to bring about winter adaptations. Desjardins and Lopez (1983) found that cold exposure and dietary restriction resulted in gonadal atrophy in deer mice previously nonresponsive to short-day conditions. Cold exposure also accelerated the short-day-induced seasonal molt by 2 weeks in the white-footed mouse (Lynch, 1973). Still, there are no published studies on enhancement of the percentage of photoresponsive Djungarian hamsters by other environmental factors.

Is expression of nonresponsiveness the result of long-term laboratory breeding? Although Nelson (1985) provides convincing data that long-term laboratory breeding may enhance occurrence of nonresponsiveness in the prairie vole, this is probably not the case in *Phodopus sungorus*. We tested a third-generation laboratory stock of hamsters derived from a large wild-caught population trapped by Dr. K. Wynne-Edwards. Nonresponsive individuals frequently occurred in this population (G. R. Lynch, unpublished data). Thus, our present belief is that considerable genetic variation for nonresponsiveness exists in this species. It may be that nonresponsiveness is also expressed in natural populations of *Phodopus*, as has been described for some rodent species (see Nelson, 1987, for a review). Field data are needed to establish whether this occurs.

Photoreponsiveness is heritable in our *Phodopus sungorus* population; however, what specific aspects of the photoresponse are heritable? In our regression analyses, we used five different but related measures of photoreponsiveness. Choosing the most appropriate one is difficult. The characters DBM, M12, and PI reflect both occurrence and degree of response; there is a lower limit to the score for each of these characters (0) that is associated with a lack of response, and a range of positive scores in responders. However, we also considered molt and photoresponse as pure threshold characters, ignoring the degree of response. Data collected from the base population (G_0) of the selection experiment suggested that degree and occurrence of response were related. The distribution of PI appeared Gaussian; 58% of the individuals were responsive, and their PI scores appeared to fill the upper three-fifths of a normal distribution (Lynch et al., 1989). Although 42% of the population had the minimum possible score ($PI = 0$), one could argue that they made up a portion of the normal distribution below the 0 threshold—that is, in the theoretical, but immeasurable, range of negative PI scores. This hypothesis was supported by data from the first generation of selection. In the high lines, both an elevated percentage of responders (defined by $PI > 0$) and an increase in mean PI in responders were observed, relative to those of control lines; similarly, the low lines had a lower incidence of response and a decreased mean PI in responders. This could be explained by a shift of the PI distribution across a fixed threshold for photoreponsiveness.

After four generations, bidirectional selection clearly altered the percentage of individuals responding to short-day conditions, as shown by the loglinear analysis (Table 4). However, there was no significant effect of selection on mean PI in hamsters with $PI > 0$. Furthermore, when the scores of nonmolting hamsters were excluded, selection had no effect on mean M12 (Table 3). Since the degree of response is unaffected by selection, we conclude that although the ability to respond to short-day photoperiod is clearly heritable, this effect is probably genetically independent of the degree of responsiveness.

There is strong evidence that the circadian system controls the threshold aspect of the photoresponse. Earlier studies showed differences in the free-running period of locomotor activity and phase angle of activity to lights-off between responders and nonresponders,

indicating differences in their circadian organization (Puchalski and Lynch, 1986, 1988). However, neither of these circadian characters correlates with the *degree* of photoresponse in responders (Kliman and Lynch, 1991). Further evidence that the circadian system is different in nonresponders can be found in the decreased incidence of rhythm "splitting" in these hamsters (Puchalski and Lynch, 1988, 1991b). Rodents that are placed under constant light often split their activity into two components 180° out of phase; this process is accompanied by a change in free-running period. Splitting is considered a strong argument that the circadian system is composed of two coupled oscillators (Pittendrigh and Daan, 1976). Since "splitting" occurs less readily in nonresponsive Djungarian hamsters, a working hypothesis is that tighter coupling between the component oscillators occurs in this phenotype.

Given the parallel findings from quantitative genetic analysis and the physiological studies, we conclude that the *occurrence* and the *degree* of the photoresponse are physiologically and genetically independent of each other in this species.

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