Latitude affects photoperiod-induced changes in immune response in meadow voles (*Microtus pennsylvanicus*)

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Abstract: Animals use day length (photoperiod) to time seasonal adaptations to annual changes in their environment. Reproductive adjustments in deer mice (*Peromyscus maniculatus* (Wagner, 1845)) from high latitudes are more extensive in response to short days than in deer mice from low latitudes. These adjustments may permit individuals to survive the severe seasonal changes (e.g., temperature and food abundance) in high-latitude environments. Immune function is also affected by photoperiod. Short days were predicted to result in elevated immune and reproductive responses in meadow voles (*Microtus pennsylvanicus* (Ord, 1815)) from the Northwest Territories (NWT), Canada (~62°N), compared with voles from Ohio (OH), USA (~39°N). Male voles from both latitudes were maintained in long or short days for 10 weeks prior to a delayed-type hypersensitivity (DTH) immune challenge. Both populations displayed similar testicular regression and reduction of testosterone concentrations in short days. DTH immune responses, however, diverged between the two populations. DTH immune responses were enhanced in long-day NWT voles and short-day OH voles, but decreased in short-day NWT voles and long-day OH voles. Total and free corticosterone concentrations did not explain the latitudinal differences in immune responses. These results suggest that photoperiod affects reproductive and immune systems differently and that immune responses may reflect other environmental factors.

Résumé : Les animaux utilisent la longueur du jour (photopériode) pour synchroniser leurs adaptations saisonnières avec les changements annuels de l'environnement. En réaction aux jours courts, les ajustements de la reproduction chez les souris sylvestres (*Peromyscus maniculatus* (Wagner, 1845)) des hautes latitudes sont plus importants que ceux des souris des basses latitudes. Ces ajustements permettent vraisemblablement aux individus de survivre aux rudes changements saisonniers (par exemple de température et d'abondance de nourriture) dans les milieux de haute latitude. La fonction immunitaire est aussi influencée par la photopériode. Nous avons posé en hypothèse que les jours cours causeraient des réactions immunitaires et reproductives plus fortes chez les campagnols de Pennsylvanie (*Microtus pennsylvanicus* (Ord, 1815)) des Territoires du Nord-Ouest (NWT), Canada (~62°N) que chez ceux de l'Ohio (OH), USA (~39°N). Nous avons gardé des campagnols mâles des deux latitudes à des jours longs ou courts pendant 10 semaines précédant une épreuve immunologique de type hypersensibilité retardée (DTH). En régime de jours courts, les mâles des deux populations subissent des régressions testiculaires semblables et une réduction des concentrations de testostérone. Les réactions de DTH, cependant, diffèrent dans les deux populations. Les réactions immunitaires DTH sont amplifiées chez les campagnols de NWT en régime de jours longs et les campagnols OH en régime de jours courts; elles sont réduites chez les campagnols NWT en régime de jours courts et les campagnols OH en régime de jours longs. Les concentrations de corticostérone totale et libre n'expliquent pas les différences de réaction immunitaire entre les deux latitudes. Ces résultats laissent croire que la photopériode affecte les systèmes reproducteur et immunitaire de façon différente et que les réactions immunitaires peuvent refléter des facteurs environnementaux différents.

[Traduit par la Rédaction]

Introduction

Changes in photoperiod (day length) trigger modifications in many physiological, morphological, and behavioral processes that are believed to promote survival in seasonally breeding animals (Goldman 2001). The hormone melatonin mediates many of these modifications by communicating night-length information to the rest of the body via its nocturnal secretion from the pineal (Prendergast et al. 2002). The best characterized mammalian seasonal adaptation is reproduction. Exposure to several weeks of short day lengths reduces reproductive tract mass, gametogenesis, and reproductive behavior in long-day breeders (Bronson 1989).

The magnitude of short-day-induced reproductive regression appears to correspond to a latitudinal gradient. For example, adult white-footed mice (*Peromyscus leucopus* (Rafinesque, 1818)) from high latitudes of origin display increased magnitude of adult reproductive regression (Lynch et al. 1981; Gram et al. 1982; Heath and Lynch 1982), and juvenile deer mice (*Peromyscus maniculatus* (Wagner, 1845)) and white-footed mice from high latitudes delay reproductive maturation in response to short days or melatonin supplementation compared with mice from low latitudes

Received 27 April 2005. Accepted 15 August 2005. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 7 October 2005.

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 doi: 10.1139/Z05-121

(Dark et al. 1983; Carlson et al. 1989). A similar gradient in seasonal changes in pelage color and thickness in response to short days or melatonin treatment occurs in white-footed mice (Lynch et al. 1981; Heath and Lynch 1982). These experiments suggest that animals from high latitudes display more extensive photoperiod-induced adjustments than animals from low latitudes. Presumably, this gradient in photoperiodic responses is adaptive because seasonal changes in the environment, to which residents adapt to promote survival and time reproduction, are more extreme at high latitudes (e.g., low temperatures and short durations of light/day). Thus, if reproductive regression conserves energy to promote winter survival, then greater reproductive regression may conserve additional energy.

Immune function is also modulated by photoperiod in seasonally breeding rodents. In general, short days enhance immune responses, although, in contrast to the reproductive system, photoperiod-induced changes in all components of immune function are not uniform. For example, short days (or melatonin treatment) enhance delayed-type hypersensitivity (DTH) responses, leukocyte production, and recovery from endotoxin in cows (*Bos taurus* L., 1758), Siberian hamsters (*Phodopus sungorus* (Pallas, 1773)), several species of *Peromyscus*, sparrows (*Passer domesticus* (L., 1758)), and meadow voles (*Microtus pennsylvanicus* (Ord, 1815)) (Bilbo et al. 2002*a*, 2002*b*; Demas and Nelson 1998*a*; Engeland et al. 2003; Greenman et al. 2005; Pyter et al. 2005), but suppress humoral immunity (as measured by antibody production) in Siberian hamsters and the teleost fish *Sebastiscus marmoratus* (Cuvier in Cuvier and Valenciennes, 1829) (Nakanishi 1986; Hadley et al. 2002). Melatonin mediates many of these photoperiod-induced changes in immune function (Nelson and Drazen 2000; Hotchkiss and Nelson 2002). In contrast to these laboratory studies, field studies report that immune function is reduced during the winter (Nelson and Demas 1996). The decline of food availability and low temperatures may underlie the suppressed immune responses in the field compared with the laboratory (Demas and Nelson 1996; Martin et al. 2004).

We predicted that photoperiod-induced changes in immune responses, along with reproductive responses, would be enhanced in voles from high latitudes compared with voles from mid-latitudes. To test this hypothesis, we examined DTH and reproductive measures, as well as concentrations of the reproductive and immunomodulatory hormones testosterone and corticosterone, in response to long or short days in male meadow voles from both the Northwest Territories, Canada (~62°N; NWT), and Ohio, USA (~39°N; OH). DTH immune challenge was chosen as a general assessment of immune function because it measures antigen-specific immunological memory and T-cell function (Phanuphak et al. 1974). Photoperiod differentially affected DTH responses between voles from different latitudes, despite similar reproductive responses.

Materials and methods

Animals

Fifteen male meadow voles from NWT and 10 male voles from OH were used in this study. The NWT voles were third generation offspring from a breeding colony maintained at Ohio State University. Breeder voles were originally obtained in NWT (~62°N, 114°W). The OH voles were trapped outside of Oxford, Ohio (39°N, 84°W). All voles were maintained in the laboratory for at least 4 weeks under a 16 h light (L) : 8 h dark (D) cycle prior to the experiment. Voles were housed in polypropylene cages (27.8 cm × 7.5 cm × 13 cm) with pine shaving bedding and ad libitum access to food (Teklad 8640 rodent diet; Harlan Teklad, Indianapolis, Indiana) and filtered tap water. Animal housing was kept at constant temperature and humidity of 21 ± 5 °C and 50% ± 10%, respectively. All voles were singly housed in either long photoperiod (long-day — NWT: $n = 6$; OH: $n = 5$) with a reverse 16 h L : 8 h D cycle (lights on at 1000 EST) or in short photoperiod (short-day — NWT: $n = 9$; OH: $n = 5$) with an 8 h L : 16 h D cycle (lights on at 1000 EST). The photoperiods used are sufficient to induce gonadal responses in both populations (B.J. Prendergast and R.J. Nelson, unpublished data) and were maintained for the duration of the 12-week study. Other than experimental procedures, all animals were left undisturbed except for routine cage changing. All procedures were conducted with approval of the Ohio State University Institutional Animal Care and Use Committee and in compliance with all US federal animal welfare requirements.

DTH

After 10 weeks, immune responsiveness was assessed via DTH by sensitizing the mice to 2,4-dinitro-1-flourobenzene (DNFB; Sigma, St. Louis, Missouri). All DTH procedures occurred between 1200 and 1500 EST. For sensitization to DNFB, voles were anesthetized with isoflurane vapors (Minrad Inc., Bethlehem, Pennsylvania), fur on the animal's dorsum was shaved, and 50 μL of DNFB (0.5% (*w*/*v*) in 4:1 ratio of acetone to olive oil) was applied to the skin. Sensitization was repeated the following day. Eight days later, following light anesthetization, baseline thickness of both pinnae were measured with a constant-loading dial micrometer (Mitutoyo America, Aurora, Illinois) and DNFB immune response was challenged by applying 10 μL of DNFB (0.2% (*w*/*v*) in a 4:1 ratio of acetone to olive oil) to the skin of the dorsal surface of the right pinna. Left pinnae were treated with vehicle. Measurement of pinna thickness was repeated every day for 1 week under light anesthesia. Data are presented as a percent change in pinna thickness each day post challenge relative to baseline pinna thickness. Two voles died during the DTH challenge and all data from these individuals were dropped from the analyses.

Tissue collection

Following the final pinnae measurement, voles were weighed, rapidly decapitated, and trunk blood was collected. Blood was allowed to clot at room temperature for at least 30 min, clots were removed, blood was spun at 2500 r/min (1000*g*) for 30 min at 4 °C; serum was stored at –70 °C until testosterone and corticosterone concentration was assayed. Spleens and paired testes were removed and weighed.

RIA procedures

Total serum testosterone, total corticosterone, and free corticosterone concentrations were determined using ^{125}I kits purchased from MP Biomedicals (Costa Mesa, California).

Table 1. Mean (±SE) absolute tissue masses under two photoperiod regimes.

	Paired testes (mg)		Spleen (mg)	
	Long-day	Short-day	Long-day	Short-day
NWT	644.3±123.0	280.0±83.2*	35.1±4.3	33.6±4.4
OH	1329.3±117.6†	688.3±220.3*,†	118.6±79.3†	101.9±51.9†

Note: NWT, Northwest Territories; OH, Ohio.
*Significant difference between photoperiods within latitude.
†Significant difference between latitudes within photoperiod.

Each sample was assessed in duplicate in a single assay according to the manufacturer's protocol. However, because total corticosterone concentrations in voles in general are elevated relative to the concentrations in *Mus musculus* L., 1758 (Klein et al. 1996), serum was diluted 5.2-fold more than recommended for other rodents, and one (testosterone) or two (corticosterone) additional standard dilutions were added to the low end of the standard curve. A portion of sample was filtered through columns (Centrifree Micropartition Devices, Millipore, Bedford, Massachusetts) to separate free hormone from bound hormone for the free corticosterone assay (Taymans et al. 1997) and samples were diluted half as much as recommended for unfiltered serum. Cross-reactivity with other steroid hormones is <3.5% for testosterone and <0.5% for corticosterone. Intra-assay variance was <10% for both assays with minimum detection concentrations of 0.05 ng/mL for testosterone and 5 ng/mL for corticosterone.

Statistical analyses

ANCOVA tests were used to compare DTH data between population and photoperiod treatments across days with corticosterone as a covariable. Within days, multiple pairwise comparisons were planned a priori in the analysis model and were conducted by comparing least square means with Student's *t* tests (Keppel and Wickens 2004) for the DTH data. For example, we predicted that DTH responses would differ on individual days post-DTH challenge. Testosterone and spleen mass data had unequal variances and were compared using the nonparametric Mann–Whitney *U* tests. All comparisons were considered statistically significant when $p < 0.05$. SAS® version 8.2 (SAS Institute Inc. 2001) was used for the DTH data and StatView® version 5.0.1 (SAS Institute Inc. 1998) was used for all other analyses.

Results

Photoperiod responsiveness

Short days decreased paired testes mass relative to body mass (and absolutely; Table 1) in voles from both populations (photoperiod: $F_{[1,20]} = 15.997$, $p < 0.001$; Fig. 1B). Additionally, OH voles had heavier testes (both absolute (see Table 1) and relative to body mass) than NWT voles (population: $F_{[1,20]} = 15.394$, $p < 0.001$) regardless of photoperiod, although there was no interaction between population and photoperiod (population × photoperiod: $F_{[1,20]} = 2.003$, $p = 0.17$). The percent change in relative testis mass, measured as (long days – mean short days)/long days × 100, between photoperiod did not differ by population (data not shown; $p > 0.05$; NWT: 31.2% ± 20.8% and OH: 47.9% ± 11.7%). Photoperiod treatment did not affect absolute or relative spleen mass (absolute: $F_{[1,20]} = 0.069$; relative: $F_{[1,20]} = 0.071$; $p > 0.05$ in both cases). OH voles had significantly heavier absolute spleen mass ($F_{[1,20]} = 4.830$, $p < 0.05$; Table 1) than NWT voles, although after correcting for body mass this difference was not statistically significant ($F_{[1,20]} = 3.782$, $p = 0.06$; Fig. 1C). Body mass did not differ between populations or photoperiod treatments (population: $F_{[1,20]} = 2.173$; photoperiod: $F_{[1,20]} = 1.116$; population × photoperiod: $F_{[1,20]} = 0.582$; $p > 0.05$ in all cases; Fig. 1A). Short days decreased serum testosterone concentrations in NWT voles ($U = 8$, $p < 0.05$; Fig. 1D), but this decrease was not statistically significant in OH voles ($U = 5$, $p > 0.05$). Testosterone concentrations did not differ between NWT and OH voles ($U = 42.5$, $p > 0.05$).

DTH

Short days suppressed the DTH response in NWT voles compared with long days on days 2–7 post challenge ($p < 0.05$ in all cases; Fig. 2A). Conversely, short days enhanced the DTH response in OH voles compared with long days on day 1 post challenge ($p < 0.05$; Fig. 2B). There was a significant interaction between photoperiod and population on days 1–6 ($p < 0.05$ in all cases; Fig. 2C). Specifically, short-day OH voles mounted a greater DTH response compared with short-day NWT voles on days 1–3. Long-day OH voles mounted a greater DTH response compared with long-day NWT voles on day 1, but a decreased relative response on days 4–6 ($p < 0.05$ in all cases; Fig. 2C). Finally, in addition to the previously described photoperiodic differences within population, short-day OH voles mounted a greater DTH response than long-day NWT voles and long-day OH voles mounted a greater DTH response than short-day NWT voles on day 1 ($p < 0.05$ in both cases; Fig. 2C).

Serum corticosterone concentrations

Short days increased serum corticosterone concentrations in both vole populations ($F_{[1,20]} = 12.933$, $p < 0.05$; Fig. 3A). The interaction between population and photoperiod approached significance ($F_{[1,20]} = 1.755$, $p = 0.05$). Presented another way, the percent increase of short-day corticosterone concentrations relative to long-day concentrations was greater in OH voles than in NWT voles (((short days – mean long days)/short days × 100); $t_{[12]} = 2.504$, $p < 0.05$; Fig. 3C). Similarly, free corticosterone concentrations were higher in short-day voles of both populations than in long-day voles ($F_{[1,20]} = 8.671$ $p < 0.05$; Fig. 3B), although this difference was not statistically significant within the NWT population ($p = 0.1$).

Discussion

Despite similarly regressed reproductive systems and comparable hormonal profiles, photoperiodic treatments

Fig. 1. Effects of 12 weeks of long or short days on mean (+SE) (A) body mass, (B) relative mass of paired testes, (C) relative mass of spleen, and (D) serum testosterone concentration in of meadow voles (*Microtus pennsylvanicus*) from the Northwest Territories (NWT; long-day: $n = 6$; short-day: $n = 9$) and Ohio (OH; long-day: $n = 5$; short-day: $n = 5$). *, $p < 0.05$ within population comparison; +, $p < 0.05$ between population comparison.

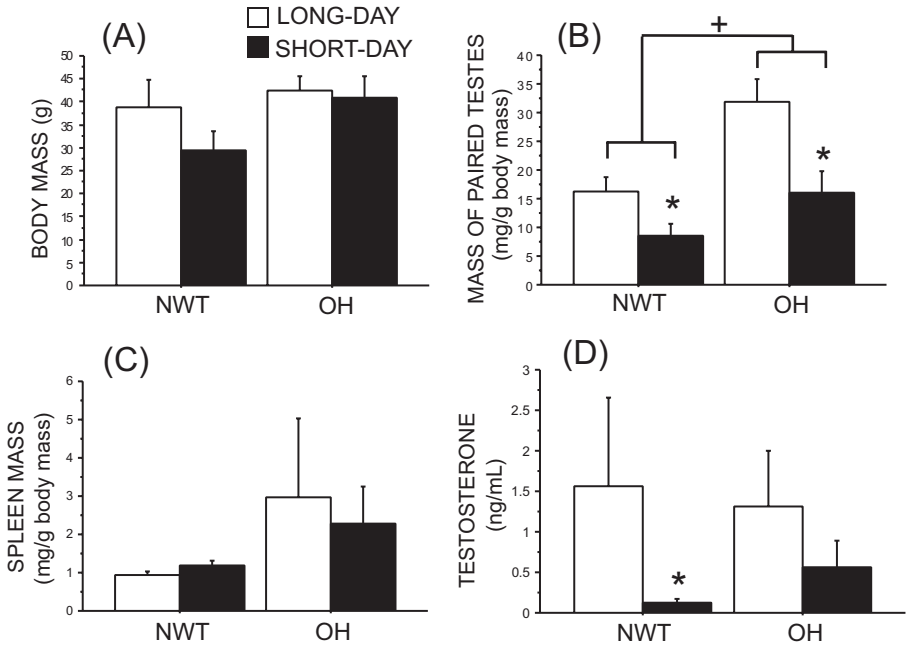


Fig. 2. Effects of 12 weeks of long or short days on the delayed-type hypersensitivity (DTH) response of meadow voles, measured by mean (±SE) percent change from baseline pinna thickness over 7 days after 2,4-dinitro-1-flourobenzene (DNFB) challenge in the pinna of (A) NWT, (B) OH, or (C) both NWT (long-day: $n = 6$; short-day: $n = 9$) and OH (long-day: $n = 5$; short-day: $n = 5$). *, $p < 0.05$ within population comparison; +, $p < 0.05$ between population, but within photoperiod comparison; #, $p < 0.05$ between population and photoperiod comparison.

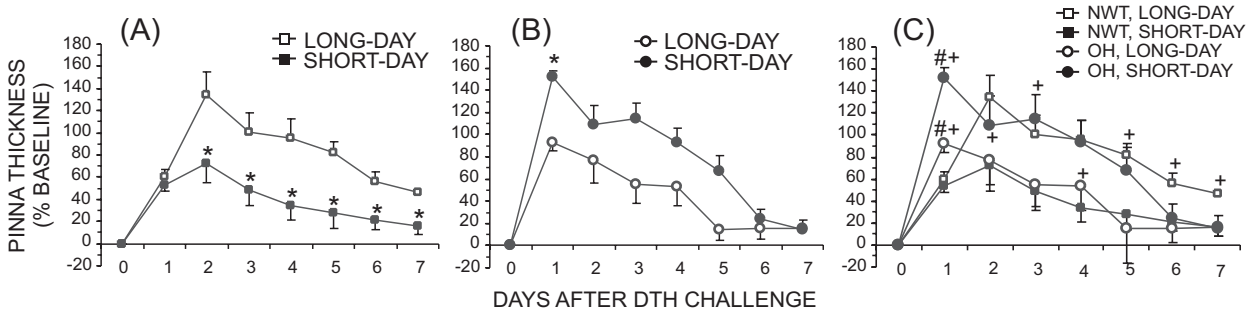
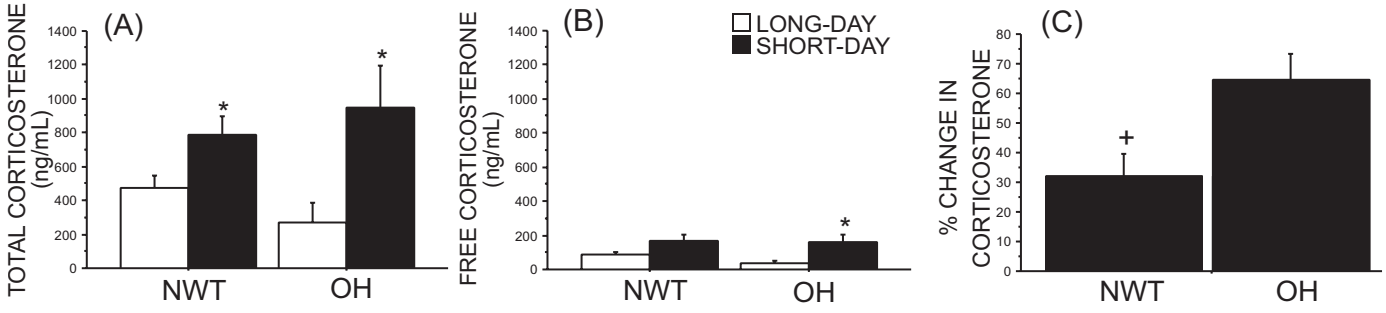


Fig. 3. Effects of 12 weeks of long or short days on mean (+SE) (A) total serum corticosterone, (B) free serum corticosterone, and (C) percent difference ((short days – mean long days)/short days × 100) between photoperiod treatments in total serum corticosterone (C) concentrations in meadow voles from NWT (long-day: $n = 6$; short-day: $n = 9$) and OH (long-day: $n = 5$; short-day: $n = 5$). *, $p < 0.05$ within population comparison; +, $p < 0.05$ between population comparison.



evoked opposite effects on DTH immune responses between male meadow voles from high and mid-latitudes. The results obtained in the present study suggest that, in contrast to our prediction, latitude may have differential effects on reproductive and immune responses.

Most previous studies of photoperiod responsiveness as a function of latitude have focused on reproduction. Our study is the first to test a latitudinal difference in photoperiodic DTH immune response in mammals. Reproductive responsiveness, as measured by the percent change in testis mass between photoperiod for each population, did not differ between latitude. Because previous reports of photoperiodic reproduction gradients are limited to the genus *Peromyscus*, it is possible that the present results reflect a species difference. Contrary to our prediction of an exaggerated photoperiodic difference in immune function in voles from high latitude, the NWT voles decreased DTH responses in short days, whereas voles from the mid-latitude population displayed the predicted short-day DTH enhancement. Although the photoperiodic pattern of the DTH responses in OH voles is consistent with previous studies in the genus *Peromyscus* and in Siberian hamsters (Bilbo et al. 2002*a*; Pyter et al. 2005), the short-day suppression of DTH responses in NWT is novel among mammals (but see birds; Møller et al. 2003). Additionally, the peak DTH response in all OH voles (regardless of photoperiod) occurred the day post immune challenge, whereas the peak response in all NTW voles (regardless of photoperiod) was delayed to 2 days post immune challenge. This difference may represent a shift in the kinetics of the immune response, as well as the direction of the immune response, between these two populations. The cellular mechanisms (e.g., differences in lymphocyte and macrophage production and distribution) underlying this timing difference in peak DTH responses require further investigation.

It is possible that the photoperiod lengths used in this study underlie the contrasting patterns of DTH responses between vole populations from mid- and high latitudes. In the field, the shortest day length during the winter at the NWT latitude is approximately 5 h, whereas the shortest day of the year is approximately 9 h at the OH latitude. Therefore, it is possible that the NWT voles require exposure to a shorter day length than OH voles for appropriate seasonal immune adaptations to occur. However, the 8 h L : 16 h D treatment used in this study was sufficient to trigger comparable reproductive responses in both vole populations. Therefore, either immune response patterns resulting from native photoperiod treatments (e.g., 5 h L : 19 h D for NWT) would be similar to the patterns observed in the present study, or reproductive responses are more strongly linked to photoperiodic cues than to immune responses (i.e., shorter critical day lengths are sufficient to trigger reproductive changes than those necessary for immune changes). The hypothesis that critical day lengths may differ among physiological systems has been previously reviewed (Goldman 2001; Prendergast et al. 2002). Also, critical day length within a physiological system (e.g., reproduction) may differ by latitude of origin (Dark et al. 1983). Perhaps the photoperiods used in this study were interpreted as "intermediate" by the NWT population that would have experienced greater seasonal changes in day length in the field, and therefore resulted in the observed DTH pattern. For example, intermediate levels of photoperiod-induced reproductive regression are achieved by intermediate day lengths in Siberian hamsters (Prendergast et al. 2004). However, a similar, graded response in DTH measures was not observed among the same animals (Prendergast et al. 2004). Thus, it is unlikely that DTH responses are graded, but if they were, the unusual DTH pattern found in the NWT voles would be, in fact, understated in our observed results. Investigation of vole populations from several additional latitudes exposed to multiple photoperiod treatments would address these possibilities.

Alternatively, DTH responses are probably influenced by other local changes in the environment (besides photoperiod), such as temperature, rainfall, social environment, and parasitic abundance. Average temperature and monthly rainfall ranges from approximately –26 to 17 °C and 18 to 44 mm, respectively, in NWT and from –20 °C to 33 °C and 57 to 101 mm, respectively, in OH. Therefore, these additional environmental cues may be necessary for a more complete view of the influence of the environment on immune responses across latitude. For example, social environment, temperature, and food abundance influence seasonal adaptations (including immune function) in deer mice (Demas and Nelson 1996, 1998*b*, 1998*c*). Similarly, a study examining vole population fluctuations that differ by latitude attributed some of the latitudinal differences to local habitat, including local parasite abundance (Mihok et al. 1985). Therefore, it is likely that environmental factors other than photoperiod may impact seasonal immune responses among latitudes.

A latitude-based gradient of body mass has been reported in many species (Litzgus et al. 2004), although the interaction of photoperiod and this gradient have not been studied. In contrast to most species studied (Litzgus et al. 2004), the *Peromyscus* residing in low latitudes have higher body mass than those in high latitudes (MacMillen and Garland 1989). No significant differences in body mass were induced by photoperiod in voles from either latitude, although body masses were within previously reported ranges for this species (Dark and Zucker 1984). However, the NWT population displayed a trend towards reduced body mass in short days that may reach statistical significance with a larger sample size. Additionally, this short-day decrease in body mass in NWT voles is accompanied by reduced immune responses. Reduced body mass during the winter has been reported in many rodent species (Bartness and Wade 1985; Moffatt et al. 1993) and is believed to represent an adaptation that allows for energy conservation during the harsh conditions of winter (Prendergast et al. 2002). Therefore, these data suggest that an energetic trade-off may exist between immune responses (Demas 2004) and body mass similar to that described between humoral immunity and body fat in other rodent species (Demas et al. 2003) so that decreased energetic reserves in short-day NWT voles result in the dampened DTH response.

Our data also revealed that paired testes masses (both absolute and relative to body mass) and spleen masses (absolute only) decreased at high latitude relative to mid-latitude. Similar differences in reproductive tissue masses between latitudes were recorded in subspecies of *Peromyscus* from two latitudes (Demas et al. 1996). High altitude also increased respiratory tissue mass in deer mice, suggesting that

increased functional demands may cause hypertrophy of respective tissues (Hammond et al. 1999, 2001). A similar hypothesis can be proposed for the size and corresponding function of spleen and testes masses observed in the present study.

In contrast to short-day rodents tested in the laboratory, wild rodents display reduced immune parameters in the winter (Newson 1962; Sidky et al. 1972; Sonenshine et al. 1978; Kim et al. 2001). In the present study, the OH population was wild-caught and the NWT population was bred in captivity, which could theoretically, affect immune responses. However, both populations remained in the same laboratory environment for 10 weeks prior to immune challenge during which testing revealed that neither of the populations had parasitic nor serological infections. The wild-caught OH voles displayed enhanced immune responses in short days similar to previously reported laboratory populations of rodents (Bilbo and Nelson 2003; Pyter et al. 2005). In addition, a previous study in sparrows reported that relative photoperiod-induced changes in cell-mediated immune function remain stable between wild and captive animals (Martin et al. 2004).

Immunological testing in wild-caught species is complicated by the effects of mounting a stress response to the laboratory housing conditions. A physiological marker of activation of a stress response is increased circulating glucocorticoid concentrations (Selye 1956). Previous studies in multiple species suggest that corticosterone dampens DTH responses (reviewed in McEwen et al. 1997), although this relation is complicated because the duration of corticosterone exposure yields different responses (Dhabhar and McEwen 1999). Alternatively, corticosterone and DTH responses are uncoupled in other reports (Bilbo and Nelson 2003; Martin et al. 2005; Pyter et al. 2005). After 12 weeks in captivity, wild-caught OH voles displayed similar basal corticosterone concentrations as the NWT voles bred in captivity. The observed corticosterone concentrations were increased in short days relative to long days, consistent with previously reported values in meadow voles (Klein et al. 1997). Taken together, it is unlikely that corticosterone concentrations directly affected the DTH responses observed in this study. Furthermore, the wild-caught OH population did not appear to mount a stress response when held in the laboratory as assessed by corticosterone measurements.

Compared with other rodent species, voles have high basal concentrations of corticosterone, without the detrimental effects associated with chronic corticosterone exposure in other species (Klein et al. 1996). Most circulating corticosterone is sequestered by steroid binding globulins, whereas the "free" corticosterone is thought to be biologically active. Seasonal changes in binding globulins may account for differences in corticosterone-mediated responses, although the total circulating corticosterone remains the same (Deviche et al. 2001). Because corticosterone and DTH may be related, and free corticosterone concentrations may be differentially affected by environmental factors compared with total corticosterone concentrations, free corticosterone concentrations were also assayed; free corticosterone concentrations were similar in voles from the different latitudes. The results observed in this study suggest that photoperiod-induced changes in total and free corticosterone concentrations are uncoupled with DTH response because both OH and NWT voles had similar corticosterone concentrations, but opposite DTH responses.

In summary, our results suggest that photoperiod-mediated changes in reproduction and immune function are differentially influenced by latitude. Specifically, photoperiod affects both the timing and directionality of immune responses, whereas short photoperiods reduce the reproductive system mass of voles from both latitudes. In addition to latitude, other aspects of the local environment may affect immune responses and require further investigation. Corticosterone does not appear to underlie these differences in photoperiodic immune response. Finally, gross tissue masses were lower in high-latitude voles than in mid-latitude voles, which may reflect an energetic constraint on immune responses.

Acknowledgements

The authors thank Nancy Solomon and her laboratory assistants for trapping voles in OH and Brian Prendergast for trapping voles in NWT, Stephanie Bowers and Jessica Trent for technical assistance, and Lynn Martin for helpful comments on the manuscript. This work was supported by the National Institutes of Health (NIH) – National Research Service Award (NRSA) MH 73375, NIH grants MH 57535 and MH 66144, and the National Science Foundation grant IBN 04-16897. Additional support was received from NIH grant P30NS045758.

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