Photoperiod and Temperature Interact to Affect Immune Parameters in Adult Male Deer Mice *(Peromyscus maniculatus)*

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Abstract Nontropical rodents often experience large seasonal fluctuations in both food availability and energy demands. The energy required for thermoregulation is highest during the winter when food availability may be at an annual minimum. Failure to cope with winter probably accounts, in part, for the increased prevalence of disease and death relative to that in summer. Winter conditions may elevate circulating glucocorticosteroid levels, which can compromise immune function. To increase the odds of surviving the energetic demands of winter, individuals of some rodent species appear to enhance immune function before conditions deteriorate. Previous laboratory studies suggest that immune enhancement can be induced by short days. These findings contrast with the results of several field studies reporting suppressed immune function during the winter. To resolve this conflict, the authors hypothesized that winter stressors present in field studies counteracted the short-day enhancement of immune function reported in laboratory studies. If true, then immune function of captive mice in short days should be compromised by low temperature or reduced food availability. Both ambient temperature and photoperiod were manipulated in the present study to assess their effects on immune parameters in male deer mice (*Peromyscus maniculatus*). Animals in short days regressed their reproductive systems and also displayed significantly higher immunoglobulin G (IgG) levels than did those in long days. Deer mice maintained in low temperatures had significantly reduced splenic masses and basal IgG levels independent of day length. Animals maintained in both short days and low temperatures displayed IgG levels comparable to those of mice in long-day/mild-temperature conditions. Animals maintained in long days and low temperatures had significantly higher serum corticosterone levels than did animals maintained in long days at mild temperatures. These data are consistent with the hypothesis that immune parameters are enhanced in short days to counteract stress-mediated immune suppression occurring during the winter.

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INTRODUCTION

Animals inhabiting nontropical latitudes must adapt to potentially large fluctuations of environmental energy availability and physiological energy expenditures (Bronson, 1989; Goldman and Nelson, 1993). For instance, the energy required for thermogenesis peaks during the winter, a time when energy availability typically is low (Heldmaier and Ruf, 1992). Breeding is an energetically demanding activity, and breeding at inappropriate times can compromise survival of both parents and offspring. Consequently, individuals of many species have evolved to restrict breeding to specific periods of the year to ensure production of young when energy availability is high (Bronson, 1989). Animals that restrict breeding activities to times when environmental conditions are optimal increase their inclusive fitness relative to that of animals that breed at inappropriate times. Thus curtailment of breeding is central among the suite of winter coping strategies among nontropical rodents (Blank, 1992; Wunder, 1992).

Individuals of most animal species studied to date rely mainly on photoperiod as a precise temporal cue to estimate the time of year. Photoperiodic information is used to phase energetically expensive activities to coincide with adequate energy availability (reviewed in Nelson et al., 1990). Virtually all previous studies of seasonal changes in mammals have placed considerable emphasis on seasonal fluctuations of reproduction and energy balance (Bronson and Heideman, 1994). Many studies have demonstrated striking seasonal patterns of mating and birth (Bronson, 1995; Bronson and Heideman, 1994); although less commonly studied, there also exist salient cycles of disease and death (John, 1994; Lochmiller et al., 1994). Disease and death rates typically increase during the winter as compared to summer for individuals of many vertebrate species (John, 1994; Lochmiller et al., 1994). Many of these animals presumably become sick and die from exposure to extreme weather or starvation; in other words, premature mortality results from a failure to balance their energy needs successfully. However, many animals die from opportunistic diseases that seem to overwhelm immunological defenses, presumably at times when these defenses are low. Several epidemiological studies have provided evidence for reduced immune function and increased death rates from infectious disease during the winter (Afoke et al., 1993; Boctor, et al., 1989; John, 1994; Lochmiller et al., 1994).

Most field studies examining immune parameters across seasons have reported reductions in lymphatic tissue size and immune activity during the winter (reviewed in Nelson and Demas, in press). For example, in short-tailed voles (*Microtus agrestis*), both splenic mass and splenic reticular cell counts are reduced during the winter as compared to those during spring and summer (Newson, 1962). Reductions in white blood cells in cotton rats (*Sigmodon hispidus*) (Lochmiller et al., 1994) and splenic lymphoid tissue in European ground squirrels (*Citellus citellus*) (Shivatcheva and Hadjioloff, 1987) occur during the winter compared to summer values. Ground squirrels also display lower levels of hemagglutinins raised against sheep red blood cells during the winter (Sidky et al., 1972). Beagle dogs (*Canis familiaris*) display reduced lymphocyte response to concanavalin A during the winter (Shifrine et al., 1980). Lastly, cattle raise fewer antibodies in response to an antigen (J substance) during the winter than they do during the summer (Stone, 1956).

Birds and ectothermic animals also show a similar seasonal pattern of immune function (Zapata et al., 1992; John, 1994). Mallard ducks (*Anas platyrhynchos*) undergo regression of thymic tissue at the end of the mid-summer breeding season (Höhn, 1947). Similar observations have been made for house sparrows (*Passer domesticus*) and robins (*Turdus migratorius*) (Höhn, 1956). In turtles (*Clemnys leprosa, Testudo mauritonica*), thymic mass is reduced during winter estivation but regenerates in the spring (Aimé, 1912). A similar pattern of thymic involution occurs during the winter in lizards (*Scincus scincus*) (Hussein et al., 1979) and Colubrid snakes (*Psammophis schokari*) (El Ridi et al., 1981).

Photoperiod plays a critical role in seasonal changes in immune status. Laboratory studies have reported photoperiodic changes in splenic mass in deer mice (*Peromyscus maniculatus*) (Vriend and Lauber, 1973), Syrian hamsters (*Mesocricetus auratus*) (Brainard et al., 1987, 1988; Vaughan et al., 1987), and laboratory strains of rats (*Rattus norvegicus*) (Wurtman and Weisel, 1969). The photoperiodic effects on splenic size seen in laboratory rats are particularly intriguing given that this species traditionally has been considered to be unre-

sponsive to photoperiod (Nelson et al., 1994). In contrast to field studies, virtually all studies of "seasonal" changes in immune parameters conducted within a laboratory setting demonstrate enhanced immune function in short (i.e., winter) day lengths. Increased lymphoproliferative activity and changes in spleen morphology occur in hamsters maintained on short day lengths (Champney and McMurray, 1991). In addition, deer mice significantly increase lymphocyte, neutrophil, and white blood cell counts in short photoperiods (Blom et al., 1994). Deer mice maintained in short days and subsequently injected with the chemical carcinogen 9,10-dimethyl-1-2-benzanthracene (DMBA) failed to develop any tumors (Nelson and Blom, 1994). Nearly 90% of the DMBA-treated animals maintained in long days, however, developed squamous cell carcinomas (Nelson and Blom, 1994). Further, small lesions developed at the site of injection in all animals. Short-day mice had less severe lesions and faster healing rates than did long-day animals.

The present experiment was designed to address the discrepancy between field studies of seasonal alterations of immune function and laboratory studies of photoperiodic effects on immune parameters—that is, to determine why wild-trapped animals often display reduced immune function in winter, whereas exposure to simulated winter photoperiods appears to enhance immune function. Although photoperiod is a primary cue for seasonally breeding animal species, many other environmental factors—including temperature, humidity, and food availability—also vary on a seasonal basis (Bronson, 1989).

Some of the seasonal alterations in environmental conditions may be perceived as stressful. Stress is operationally defined here as sustained activation of the hypothalamic-pituitary-adrenal axis (Knol, 1991; Selye, 1955). For example, acute and chronic exposure to low ambient temperatures evokes increased glucocorticosteroid secretion in many species of animals (Shu et al., 1993; Spinu and Degen, 1993). The stress experienced by an animal exposed to low temperatures could compromise immune function; elevated circulating blood levels of adrenocortical steroids suppress immune parameters in the laboratory (Ader and Cohen, 1993; Berczi, 1986; Black, 1994). If low ambient temperatures act as an environmental stressor and if stress suppresses immune function, then deer mice maintained in low temperatures should show reduced immunoglobulin G (IgG) levels compared to those of animals maintained in mild temperatures. If, however, short photoperiods provide a seasonal cue for immune enhancement, then animals maintained in short days should demonstrate higher basal levels of IgG compared to those of their long-day cohorts. It is predicted that deer mice maintained in both low temperatures and short days will have intermediate basal IgG levels, similar to animals maintained in long days and mild temperatures (i.e., operationally defined as "baseline" IgG levels).

MATERIALS AND METHODS

Animals

A total of 47 adult (> 60 days of age) male deer mice (*Peromyscus maniculatus bairdii*) were obtained from our laboratory breeding colony. This colony is derived from animals from the *Peromyscus* Genetic Stock Center at the University of South Carolina. These animals are descendants of animals originally trapped near East Lansing, Michigan.

Housing Conditions

Deer mice were weaned at 21 days of age and housed with same-sex siblings. Two weeks prior to the initiation of the experiment, all animals were individually housed in polypropylene cages in colony rooms with a 24-h (16L:8D) light cycle (lights on at 0600 h Eastern Standard Time [EST]). Relative humidity was maintained at 50 ± 5%. Food (Agway Prolab 1000, Syracuse, NY) and tap water were provided ad libitum throughout the experiment.

Experimental Conditions

All of the animals in the experiment were randomly assigned to one of four experimental groups. LD/20° animals (n = 12) were housed in a long-day photoperiod (16L:8D) with colony room temperature kept constant at 20 ± 1°C. LD/8° animals (n = 10) were also housed in long days, but the ambient temperature of the colony room was maintained at 8 ± 1°C. SD/20° animals (n = 15) were housed in a short-day photoperiod (8L:16D) with the colony room temperature set at 20 ± 1°C. Finally, SD/8° animals (n = 10) were housed in short days, but in a room with temperature kept constant at 8 ± 1°C.

Blood Sampling

After 10 weeks, animals were brought into the surgery room one at a time and lightly anesthetized with methoxyflurane vapors (Metofane, Pitman-Moore, Mundelein, IL). Handling time was kept consistent and to a minimum; the time from initial removal from the cage to the end of bleeding was less than 3 min. Blood samples (500 µl) were drawn into capillary tubes via retro-orbital bleeding (Riley, 1960) between 1000 and 1200 h EST, a time when corticosterone values in this species have been shown to be consistently low (Carter et al., in press). All samples were allowed to clot for 1 h, the clot was removed, and then the samples were centrifuged (at 4°C) for 1 h at 3500 rpm. Serum aliquots were extracted and stored in sealable polypropylene microcentrifuge tubes at –80°C until assayed.

Autopsy Procedures

After blood sampling, all animals were killed by cervical dislocation. Paired testes, epididymides, and seminal vesicles, as well as adrenal glands and spleens, were removed and cleaned of fat and connective tissue. Seminal vesicles were compressed with a glass vial to remove seminal fluid. Testicular dimensions (length × width) were determined using handheld calipers. All organs were weighed by laboratory assistants naive to the experimental hypotheses and treatment assignments.

Immunological Assay

Total IgG levels in the blood serum samples were determined using a sandwich ELISA. Ninety-six-well immunoplates (Nunc®, MaxiSorp) were incubated overnight at 4°C 100 µl/well of a goat polyclonal antibody against mouse IgG (Cappel) diluted 1:3000 in a carbonate/bicarbonate buffer (0.1 M, pH 9.6). The following day, the plates were washed four times with phosphate-buffered saline (PBS [0.05 M, pH 7.4]) containing 0.05% Tween-20 and 0.001% NaN_3 using an automatic microplate washer (BioRad, Model 1550). A standard curve (upper limit: 1000 µg/ml; lower limit: 0.001 µg/ml) was prepared using purified mouse IgG (Sigma) diluted in standard diluent (PBS [0.05 M, pH 7.4] containing 0.05% Tween-20). The standards (100 µl/well in triplicate) and samples of deer mouse serum (100 µL/well in duplicate), diluted 1:100 with the standard diluent, were then placed in wells on the plates. The plates were incubated overnight at 4°C. The following day, the plates were washed and 100 µL of alkaline phosphatase-conjugated sheep-anti-mouse IgG diluted 1:2500 in the standard diluent was added to each well of the plates. The plates were again incubated overnight at 4°C. The following day, the plates were washed and 100 µl of substrate buffer (0.1 M p-nitrophenyl phosphate in diethanolamine buffer [0.1 M, pH 9.5] containing 5 mM $MgCl_2$) was added to each well. The plates were incubated for 30 min, and the optical density of the resulting colored product in each well was measured at 405 nm using a microplate reader (BioRad, Model 450). The concentrations of IgG in the samples were determined relative to the standard curve.

Serum Corticosterone Assay

Blood serum corticosterone levels were assayed by radioimmunoassay (RIA) using the ICN Biomedicals (Costa Mesa, CA) ^{125}I kit. The deer mouse dilution was prepared according to the guidelines furnished by ICN. The corticosterone assay was highly specific, cross-reacting at less than 3% with other steroid hormones. Serum corticosterone values were determined in a single RIA. Intra-assay variation was less than 1%.

Data Analyses

Serum IgG and corticosterone concentrations, as well as reproductive organ weights and other tissue weights, were analyzed using a 2 (photoperiod) × 2 (temperature) analysis of variance. All pairwise comparisons of mean differences were conducted using Tukey HSD comparisons. Differences between group means were considered statistically significant if $p < .05$.

RESULTS

Testis size, as well as paired testes (Figure 1), epididymides, seminal vesicle, and epididymal fat pad masses (Table 1) were lower in short-day deer mice than they were in long-day animals ($p < .05$). Temperature did not significantly affect paired testes, epididymides, or epididymal fat pad masses ($p > .05$ in all cases) (Table 1). LD/20° animals had seminal vesicle masses significantly above those of animals kept in short days ($p < .05$) but significantly below those of LD/8° animals ($p < .05$). Photoperiod had no effect on splenic mass ($p > .05$). Adrenal mass remained unaf-

Table 1. Mean (± *SEM*) body mass, testis size, and tissue masses.

	Body Mass (g)	*Testis Size (mm^2)*	*Seminal Vesicle (mg)*	*Epididymis (mg)*	*Epididymal Fat (mg)*	*Paired Adrenal (mg)*
LD/20°	18.0 ± 0.51	54.7 ± 2.0*	85.9 ± 12*	106.6 ± 7.0*	94.9 ± 16.0*	3.4 ± 0.4
LD/8°	19.2 ± 0.29	54.2 ± 2.2*	129.8 ± 14*	92.6 ± 20.0*	107.5 ± 7.5*	3.4 ± 0.3
SD/20°	18.0 ± 0.39	38.8 ± 2.4	54.9 ± 9	73.4 ± 6.0	52.9 ± 7.8	3.3 ± 0.3
SD/8°	20.3 ± 0.54	35.8 ± 2.7	43.9 ± 5	51.2 ± 0.4	100.3 ± 38.0	3.6 ± 0.4

NOTE: LD = long day; SD = short day.
*Statistically significant difference from treatment groups lacking a symbol.

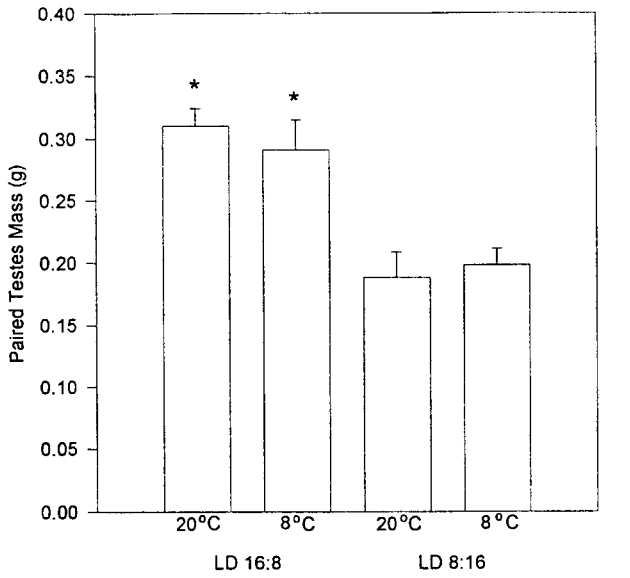

Figure 1. Mean (± *SEM*) paired testes mass (g) of male deer mice housed in long (LD 16:8) or short (LD 8:16) days and mild (20°C) or low (8°C) temperatures. Columns with no symbol or sharing the same symbol are statistically equivalent. Columns with different symbols are significantly different at $p < .05$.

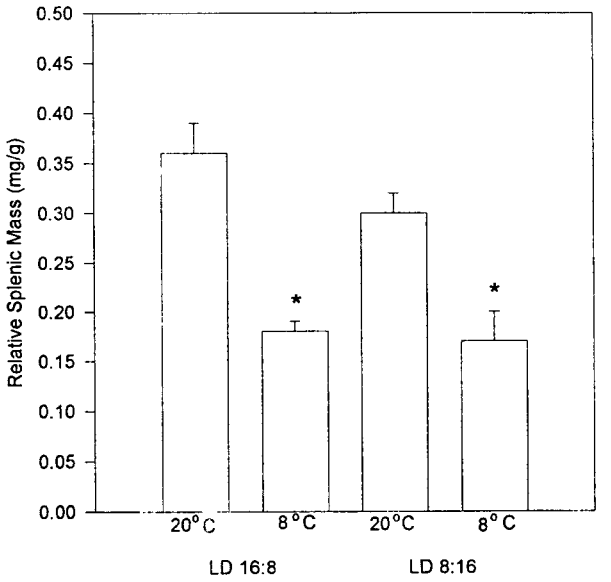

Figure 2. Mean (± *SEM*) relative splenic mass (mg/g) of male deer mice housed in long (LD 16:8) or short (LD 8:16) days and mild (20°C) or low (8°C) temperatures. Other symbols and conventions are as in Figure 1.

fected by either photoperiod or temperature ($p > .05$ in all cases) (Table 1).

Deer mice maintained in low temperatures had significantly reduced splenic masses compared to those of animals maintained in mild temperatures ($p < .05$) (Figure 2). There were no significant differences in body mass between any of the experimental groups ($p > .05$) (Table 1).

Serum corticosterone values were significantly elevated in SD/8° animals compared to those in SD/20° animals ($p < .01$) (Figure 3). No other pairwise comparisons were significantly different ($p > .05$ in all cases). IgG levels were significantly elevated in SD/20° animals compared to those of animals kept in long days ($p < .05$ in both cases) (Figure 4). LD/8° deer mice showed reduced IgG levels compared to those of LD/20° or short-day animals ($p < .05$). SD/8° animals displayed serum IgG levels comparable to those of LD/20° animals ($p > .05$).

DISCUSSION

Deer mice in mild temperatures on short days exhibited higher basal IgG levels than did animals maintained on long days. These results confirm and extend several previous studies demonstrating enhancement of immune parameters in short photoperiods (e.g., Blom et al., 1994; Brainard et al., 1987, 1988; Champney and McMurray, 1991; Nelson and Blom, 1994). Overall, low temperatures were associated with depressed basal IgG levels and elevated corticosterone

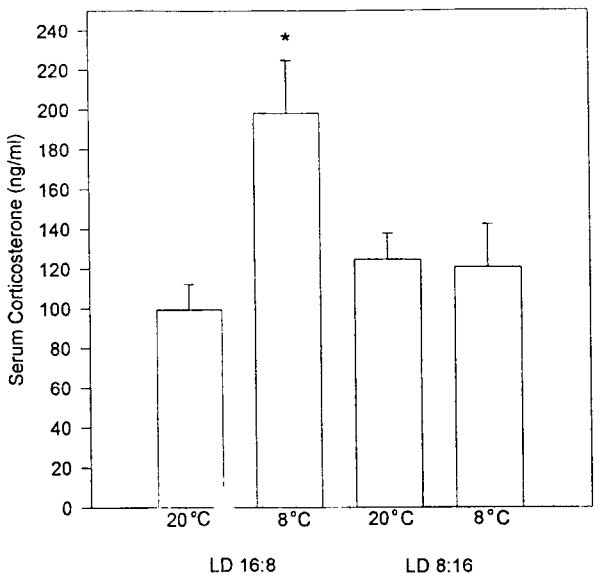

Figure 3. Mean (± *SEM*) Immunoglobulin G levels (μg/ml) of male deer mice housed in long (LD 16:8) or short (LD 8:16) days and mild (20°C) or low (8°C) temperatures. Other symbols and conventions are as in Figure 1.

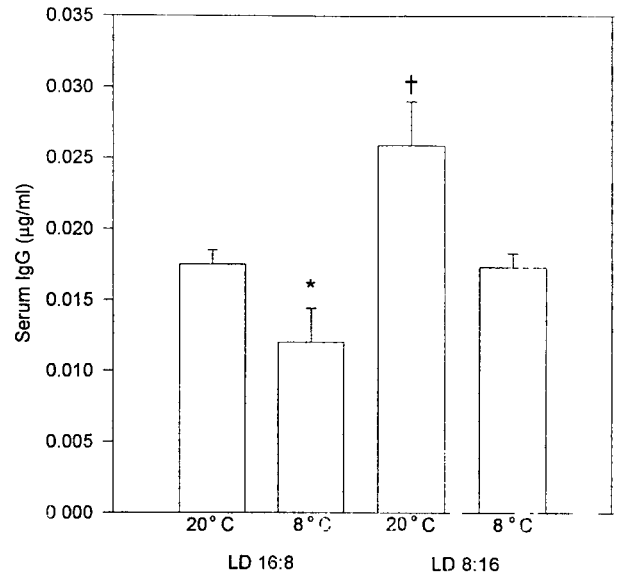

Figure 4. Mean (± *SEM*) corticosterone levels (ng/ml) of male deer mice housed in long (LD 16:8) or short (LD 8:16) days and mild (20°C) or low (8°C) temperatures. Other symbols and conventions are as in Figure 1.

levels in the present study. Short days caused winter-coping adaptations that may have modulated corticosterone secretion. Deer mice housed in long days and exposed to low ambient temperatures had markedly suppressed basal IgG levels but elevated corticosterone levels as compared to long-day mice exposed to typical room temperatures. Exposure to short day lengths ameliorated the temperature-induced reduction in IgG values. Serum IgG levels of short-day deer mice maintained in low temperatures were comparable to IgG levels of long-day mice housed in mild temperature conditions. These results provide a resolution of the inconsistencies in immune responses between laboratory studies using simulated winter photoperiods (e.g., Blom et al., 1994; Brainard et al., 1987, 1988; Champney and McMurray, 1991; Nelson and Blom, 1994) and field studies (e.g., John, 1994; Lochmiller et al., 1994; Newson, 1962). Male adult deer mice regressed reproductive organ size in short days. There were no photoperiod-mediated changes in splenic, adrenal, or body mass in the present study. Splenic mass was significantly smaller in low-temperature deer mice than it was in those in mild ambient temperatures. Taken together, these results have important physiological and adaptive functional significance.

Animals require a balanced energy budget (i.e., energy intake ≥ energy expended) to maximize survival (Wunder, 1992). Similarly, a "balanced" immune system (i.e., short-day immune enhancement ≥ stress-mediated immune suppression) may be an important requirement for survival as well. Energetics can certainly affect immune function (Henken and Brandsma, 1982), but the extent to which immune function affects energetics remains unspecified. If enhanced immune function maximizes survival, then why has the enhancement of immune function not evolved to occur during the breeding season when interactions with mates occur often and parasite loads are high? One potential reason is that immune enhancement is energetically incompatible with breeding or other energetically costly activities (John, 1994; Lochmiller et al., 1994). Cytokine activation elevates body temperature, and the intense energy requirements of inflammation and acute phase immune response likely drains energy reserves (Maier et al., 1994). No study to date, however, has examined precisely the metabolic costs of increased immunity in small mammals.

The present results partially resolve the apparent discrepancy between field studies of seasonal changes in immune function and those studies manipulating photoperiod in the laboratory. Although short photoperiods enhance immune function (Blom et al., 1994; Champney and McMurray, 1991), these laboratory studies were conducted with ad libitum access to food and water, mild ambient temperatures, reduced social

interactions, and lack of predatory pressures. All of these other environmental factors can act as seasonal stressors and potentially affect immune function in the field (Spinu and Degen, 1993). Reduced ambient temperature is one specific stressor that compromises immune function (Shu et al., 1993). The present finding that low temperatures reduce IgG levels in deer mice provides preliminary evidence that ambient temperature is one of several possible environmental stressors that mediate seasonal changes in immune function in the wild. The lack of elevated corticosterone levels in deer mice maintained on short days and low ambient temperatures is most likely due to short-photoperiod-induced improvement in thermogenic function (Blank and Ruf, 1992). Improved thermoregulation in short days may allow individuals to adapt more readily to the environmental challenge of low ambient temperatures. It is possible that other environmental factors (e.g., predator avoidance, lack of shelter, food availability) affect immune function to a similar degree. The cumulative effect of all these environmental factors may account for the winter reduction in immune function reported in most field studies.

Individuals of some animal populations increase immune function during the winter (Dobrowolska and Gromadzka-Ostrowska, 1984; Dobrowolska et al., 1974). If the winter-associated challenges evoke immune suppression, then animals experiencing mild winters (i.e., mild ambient temperatures and relatively moderate food abundance) will be subjected to less stress and presumably sustain normal immune function. In contrast to other environmental factors, seasonal changes of photoperiod do not vary from year to year. Consequently, animals experiencing mild winters may exhibit a short-day immune enhancement in the absence of any substantial stress-induced suppression. The net effect, similar to photoperiodic studies conducted in the laboratory, is an enhancement of immune indexes and presumably decreased morbidity and mortality in the population. Long-term field studies are necessary to assess this hypothesis.

Basal IgG levels and gross splenic mass were used as indexes of immune suppression in this study. Increased basal IgG levels can represent either a state of immunological "readiness" or a state of immunological activation (i.e., increased antibody levels in response to illness). Because manipulations of photoperiod alone sufficiently increased IgG levels, and because none of the animals was ill, the data support the former interpretation of elevated IgG levels. Further, "sentinel" animals maintained in our rooms failed to display any clinical signs of parasites or pathogens at necropsy. Changes in gross splenic mass were also observed in this study. The mammalian spleen is an important immune organ that provides disease resistance through filtration of pathogens, lymphocyte differentiation, and rapid antibody synthesis, among other immune functions (Kopp, 1990). Environmentally induced changes in these parameters, although suggestive, do not necessarily reflect seasonal changes in immune *function* per se because the immune system remains unchallenged. Because of this limitation, future studies will assess changes in immune function using more sophisticated immunological techniques that measure antibody responses and lymphocyte proliferation rates to specific antigens.

These data suggest an important physiological and adaptive functional role for photoperiod-mediated enhancement of immune parameters. The net effect of elevated immune status in short days is to counteract the suppressive immune effects of environmental stressors such as low ambient temperatures or food restriction. The dynamic relationship between immune function and energetics must be considered to understand the seasonal adaptations that boost winter survival and subsequent reproductive fitness.

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