

Fever and sickness behaviour vary among congeneric rodents

Lynn B. Martin II*, Zachary M. Weil and Randy J. Nelson

Departments of Psychology, Neuroscience, and Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, OH 43210, USA

Summary

1. Fever and sickness behaviour are immune defences that most organisms engage to control bacterial and viral infections. Although generally beneficial, these defences can be energetically expensive, which may lead to variation within and among species. Here, we asked whether fever and sickness behaviour differ among five species of mice in the genus, *Peromyscus*.

2. This comparison was motivated by our previous discovery of extensive, but systematic, immunological variation among many of these same rodent species. Some species were adept at controlling gram-negative bacteria whereas others were proficient at generating antibodies; no species was strongly capable of both. Such discrete variation suggested a continuum of immune defence strategies. We therefore predicted that variation in fever and sickness behaviour would mirror variation in bacterial killing capacity, as these defences are mediated by some of the same molecular pathways.

3. To test this hypothesis, we characterized responses to lipopolysaccharide (LPS), a component of gram-negative bacteria that activates febrile responses without causing infection. *Peromyscus* species that showed little sickness behaviour post-LPS engaged fever; species that engaged sickness behaviour, however, either did not mount fevers or became hypothermic post-LPS. As predicted, species that were adept at killing bacteria *in vitro* mounted the largest fevers; species that were not as proficient at killing bacteria did not engage fever.

4. These results further indicate a continuum of immunological strategies among *Peromyscus* species, which we expect applies to other taxa. We propose a few possible reasons for why species occupy specific points along an immune continuum; life-history orientation appears the most viable alternative at present.

Key-words: acute phase response, immune, lipopolysaccharide, *Peromyscus*, trade-off

Introduction

Acute phase responses (APRs) are behavioural and physiological mechanisms that organisms use to defend themselves during the early stages of bacterial and viral infection (Kluger *et al.* 1997; Blatteis 2003; Dantzer 2004). APRs consist of regulated adjustments of body temperature and elevated production of specific proteins by the liver (Blatteis 2006). Behaviourally, APRs entail decreased food intake (anorexia) and pain sensitivity (analgesia), reticence to engage in pleasurable activities (anhedonia), and reductions in motivated behaviours (e.g. sex and aggression), a syndrome collectively

referred to as sickness behaviour (Hart 1988). Physiological adjustments during APRs inhibit pathogen proliferation and simultaneously restrict other physiological processes of the host, including growth and reproduction (Dantzer 2004). Sickness behaviours promote these physiological adjustments by limiting availability of critical nutrients (e.g. iron) to infectious organisms and minimizing energy expenditure on activities not immediately conducive to recovery from infection (Hart 1988). APRs are broadly and rapidly effective at pathogen control (Blatteis 2006), which may explain their persistence across ancient and derived vertebrate lineages (Kluger *et al.* 1997). APRs have rarely been studied in non-domesticated organisms, however (Muchlinski, Baldwin & Gramajo 2000; Owen-Ashley *et al.* 2006), but in the few cases they have been considered, they appear important mediators of trade-offs between immunity and reproduction (Bonneau *et al.* 2003; Lee, Martin & Wikelski 2005).

*Correspondence author. Division of Integrative Biology, University of South Florida, 4202 East Fowler Avenue, Tampa, FL 33620, USA. E-mail: lmartin@cas.usf.edu

APRs likely mandate trade-offs because some aspects of APRs are quite costly. The hallmark of APRs, fever, is very expensive energetically. A 1 °C elevation in body temperature in an endotherm requires *c.* 10% increase in metabolic rate (Kluger 1991). Other aspects of APRs are costly in different ways. Essential amino acid (e.g. lysine) demands during APRs are greater than all other physiological processes in which vertebrates engage, except growth during maturation (Klasing 2004). Finally, although sickness behaviours do not entail resource costs (and may actually produce resource savings over short-terms), a negative energy balance can ensue if an animal remains anorectic/lethargic for a long period of time. In these cases, individuals would experience opportunity costs [i.e. reduced reproductive or growth prospects (Aubert *et al.* 1997)]. Altogether then, the high costs of APRs should often force organisms to sacrifice, or trade-off, other physiological activities especially when resources are limited (Lochmiller & Deerenberg 2000).

The high costs of APRs have been proposed to explain why they vary within species (Klasing & Leshchinsky 1999; Martin, Weil & Nelson 2006b; Martin, Weil & Nelson 2007a). At low ambient temperatures, fevers are modest or absent in response to the same stimuli that induce fever in thermoneutral conditions (Rudaya *et al.* 2005). Further, pregnant rats reduce fever (Martin *et al.* 1995) and male song sparrows (*Melospiza morphna*) do not employ sickness behaviour during the breeding season (Owen-Ashley & Wingfield 2006). In response to food restriction, components of APRs are also decreased or absent in Siberian hamsters (*Phodopus sungorus*) (Conn *et al.* 1995). The costs of APRs may also explain their variability among species (Klasing & Leshchinsky 1999; Lochmiller & Deerenberg 2000; Klasing 2004). Domesticated chicken lines selected for rapid growth and protein accretion into body mass mount weak APRs whereas other lines selected for egg-laying mount robust responses (Leshchinsky & Klasing 2001). Rigorous comparisons among free-living species have yet to be conducted.

Recently, we asked whether a cost–benefit construct could predict variation in other aspects of the immune systems, besides APRs, among *Peromyscus* mice (Martin, Weil & Nelson 2007b). Some species of *Peromyscus* breed prolifically and mature rapidly (i.e. fast-living), but others spread modest reproductive efforts over long periods of time (i.e. slow-living; Table 1) (Promislow & Harvey 1990; Martin *et al.* in press-a). The fast-living species were expected to favour (developmentally) inexpensive types of immune defence (e.g. capacity to kill bacteria) due to their prioritization of reproduction over survival (Klasing & Leshchinsky 1999; Martin, Hasselquist & Wikelski 2006a). This hypothesis was not strongly supported, perhaps because of low phylogenetic coverage, but species were adept at killing bacteria (*in vitro*) or generating antibodies, but not both. As APRs are mediated by some of the same molecular pathways as bacterial killing (i.e. both are facets of innate immunity), we expected that *Peromyscus* that were most competent at controlling bacterial infections would also mount the strongest APRs (Blatteis 2006). We tested this

Table 1. Life-history traits and distribution of five *Peromyscus* species

Species	Life-history traits				Habitat†
	Adult mass (g)	Litter size* (no.)	Species distribution†	Population origin*	
<i>P. leucopus</i> (white-footed mouse)	18.4	3–6	Most of eastern USA	North Carolina, USA	Mature hardwood
<i>P. maniculatus</i> (deer mouse)	18.1	4–0	Most of USA, Canada and Mexico	Michigan, USA	Mature hardwood
<i>P. aztecus</i> (Aztec mouse)	38.9	2–2	Eastern Mexico	Michoacan, Mexico	Montane cloud forests
<i>P. melanophrys</i> (plateau mouse)	39.3	3–0	Central to southern Mexico	Zacatecas, Mexico	Desert grasslands
<i>P. californicus</i> (cactus mouse)	38.0	1–9	Central and southern California	California, USA	Chapparal mesic woodlands

*Data from the *Peromyscus* Genetic Stock Center.

†Data from Hooper (1968).

hypothesis by comparing the effects of lipopolysaccharide (LPS), a component of the same gram-negative bacteria used in the previous study and a substance commonly used to induce APRs in vertebrates (Dantzer 2004; Owen-Ashley & Wingfield 2006), among five species of *Peromyscus*. The advantage of using LPS to compare APRs instead of a replicating pathogen is that the immune response itself (and in particular its cost) can be studied independent of the influence of an infectious organism on the host.

Materials and methods

ANIMAL CARE

Mice were purchased from the *Peromyscus* Genetic Stock Center (University of South Carolina, Columbia, SC, USA). All species were initially captured from the wild many years ago (Table 1) and out-bred since capture under standard conditions (see <http://stkctr.biol.sc.edu/> for details). The *Peromyscus* Genetic Stock Center screens mice every 4 months for murine pneumonia virus, minute virus, and parvovirus, lymphocyte choriomeningitis, Theiler's murine encephalomyelitis virus, murine hepatitis, *Mycoplasma pulmonis*, cilia-associated respiratory bacillus and hantavirus. No mouse has ever tested positive for these pathogens. *Peromyscus* are also screened for endo- and ectoparasites. Only *Giardia* sp. has ever been found, and only in a very few individuals during one screening. In addition to these screenings, sentinel mice (*Mus musculus*) were maintained in our animal rooms throughout the study; all sentinel mice also tested negative for the above pathogens. In the previous study (Martin *et al.* in press-a), two species (*Peromyscus leucopus* and *P. maniculatus*) were most proficient at killing gram-negative bacteria *in vitro* but least competent at generating antibodies against a novel protein. The two largest species (*P. aztecus* and *P. melanophrys*) exhibited the opposite pattern. *Peromyscus californicus* was not included in the original study, but was included here because of its similar body size and lifestyle as the other two large-bodied species.

Prior to and during experiments, mice (all virgin adult males) were housed singly in rodent cages (27 × 7.5 × 13 cm), provided with bedding material, food (Harlan TekLad 8640) and filtered, chlorinated tap water *ad libitum*, and housed at 22.5 °C (± 3) in long (16 h light, 8 h dark) photoperiods. Mice remained in these conditions for the duration of the study, and were given 1 week to adjust to these new conditions before telemeter implantation (see below). Four of the five species from the previous study [*P. leucopus* (*n* = 6), *P. maniculatus* (*n* = 6), *P. melanophrys* (*n* = 9) and *P. aztecus* (*n* = 8)] were included in the present study; one species was not in the previous study (*P. californicus*, *n* = 4). Other species from the previous study were unavailable from the *Peromyscus* Stock Center or were too small to implant with telemeters (e.g. *P. polionotus*). All procedures were conducted following NIH guidelines and were approved by The Ohio State University Institutional Animal Care and Use Committee (IACUC).

FEVER AND SICKNESS BEHAVIOUR CHARACTERIZATION

Mice were implanted intraperitoneally (i.p.; under sterile conditions) with telemeters (PDT-4000; Minimitter, Bend, OR) while under isoflurane anaesthesia. Surgical wounds were topically treated

with Betadine (Sigma Chemical, St. Louis, MO) to discourage infection, and individuals were injected (i.p.) with buprenorphine (0.75 µg; Sigma Chemical) in sterile saline to alleviate pain during recovery. Immediately after surgery, mice were returned to their home cages, which were placed on receivers (Minimitter) connected to a computer. Receivers collated emitted body temperature and movement activity frequencies continuously over 30 min intervals and converted them to raw data based on pre-programmed calibration curves for each transmitter. Cages were not changed from the time of surgery until the end of the experiment, and mice were allowed three days to recover from surgery before baseline data were collected.

For the 4 days prior to LPS injections, baseline body temperature, activity and food consumption were recorded; for the last 2 days of this 4-day period, sweetened condensed milk (Kroger brand, diluted 1 : 1 with tap water) intake was also quantified. This milk is a desired substance by rodents; lack of consumption is indicative of a reluctance to engage in hedonic behaviour. For 4 h each night (15.00–19.00 h), food and water bottles were removed, and a sterile syringe barrel containing 10 mL diluted milk was placed in the cage. Milk intake was quantified by subtracting the mass of the syringe at the end of the session from its initial filled mass. One week after telemeter implantation, mice were injected (i.p.) with 1 mg kg⁻¹ LPS (from *Escherichia coli* serotype 026:B6, Sigma Chemical) dissolved in sterile 0.9% saline. Injections occurred at 14.00 h, just prior to lights out. Body temperature and activity were monitored over the next 24 h, and food and milk intake over the next 48 h. Body mass was recorded at the time of implantation and just prior to LPS injection to ensure appropriate LPS dosage. Body mass was not measured continuously, as individuals of some species are difficult to handle (Martin *et al.* 2007), which may have induced stress and thus affected APRs. Mice were killed 48 h post-LPS via decapitation while under deep isoflurane anaesthesia, and blood and tissues collected for a separate study.

DATA ANALYSIS

Data met the assumptions of parametric statistics, so repeated-measures ANOVA and univariate ANOVA in conjunction with Tukey post-hoc tests were used. In addition to these analyses, percentage departure from normothermia and percentage change in activity post-LPS were also compared among species, but results were not qualitatively different from analyses of raw data and are thus not reported here. Body mass (at the time of telemeter implantation) was included as a covariate in analyses of changes in body temperature or activity, but it had no significant effects on these variables and was excluded from final analyses. For analyses of anorexia and anhedonia, daily food and milk intake data were divided by body mass, and these mass-adjusted values were used in ANOVA. To assess whether phylogeny influenced APR, tests for serial independence (Abouheif 1999) were employed on aggregate fever, sickness behaviour, and food and milk consumption variables. No comparisons were significant, thus phylogeny was not considered further. To detect relationships among physiological and behavioural variables, Pearson's correlation analysis was used. Food and milk intake were inadvertently not recorded for one individual (*P. aztecus*), but behavioural and thermal data for this individual were collected and included in all other analyses. To calculate costs of fever, published mass-adjusted basal metabolic rates (BMRs) (Mueller & Diamond 2001) were first multiplied by 10%, as this magnitude elevation is

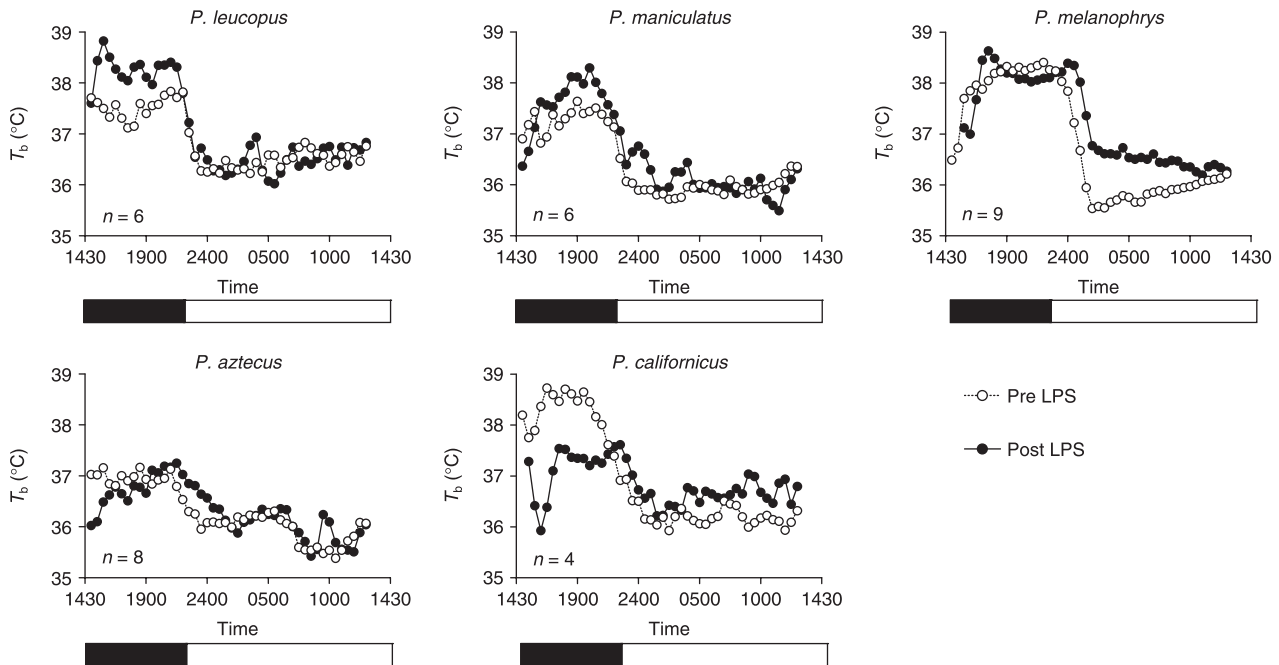


Fig. 1. Effects of lipopolysaccharide (LPS) on body temperature (T_b) in *Peromyscus*. Open symbols depict T_b prior to LPS (average of 3 days at each time point); closed symbols depict T_b after LPS. Numbers on x-axis are time of day, and open (illuminated) and solid (darkness) rectangles below each figure represent light conditions. Numbers in lower left corner of each figure indicate sample size for each species.

necessary to increase body temperature 1 °C in vertebrates (Kluger 1991). Then, these values were multiplied by the maximum departure from normothermia during the 7.5 h post-LPS (see below) and divided by the total time each individual required to reach this maximum. This approach provided us the most conservative estimate of the cost of fever; addition of the period post-thermal maximum may have overestimated the cost of fever to each individual because body temperature could have been passively maintained at that point via decreases in conductance and/or evaporative water loss. As BMR was unavailable for *P. aztecus*, it was extrapolated from a linear regression between body mass and BMR using data from the other four species [$BMR = (-0.02 \times \text{mass}) + 1.77$, $R^2 = 0.74$], and cost of fever calculated as above. Significance was indicated when $P \leq 0.05$.

Results

BODY TEMPERATURE

Pre- and post-LPS body temperatures varied extensively among *Peromyscus* species (Fig. 1). To compare body temperature responses to LPS among species, pre-LPS values were subtracted from post-LPS values of each individual and repeated-measures ANOVA was performed. Species body temperatures were differently affected by LPS in the 24 h post-challenge (time \times species: $F_{172,1204} = 4.2$, $P < 0.001$; Fig. 1). Some species (*P. leucopus* and *P. maniculatus*) rapidly became hyperthermic then returned to normothermia. One showed little change in body temperature (*P. aztecus*) whereas another (*P. californicus*) displayed prolonged hypothermia. The fifth species (*P. melanophrys*) showed

little change in body temperature until a sustained hyperthermia appeared for several hours after the onset of the inactive phase.

The most marked differences in body temperature among species manifested in the first 7.5 h post-LPS (Fig. 1). As this period is the window considered to be the most important as a defence mechanism (Blatteis 2003), this period was compared among species in detail. LPS affected body temperature differently among species during this window (time \times species: $F_{56,392} = 2.82$, $P < 0.001$), and three different patterns of change in body temperature were indicated by post-hoc analyses (Fig. 2). *Peromyscus leucopus* exhibited marked, rapid onset hyperthermia, which persisted for most of the 7.5 h post-LPS. *Peromyscus melanophrys* showed modest hypothermia followed by brief hyperthermia. *Peromyscus maniculatus* and *P. aztecus* responses were intermediate of *P. leucopus* and *P. melanophrys*. *Peromyscus californicus* was unique among species, as LPS induced dramatic hypothermia, which persisted for the entire 7.5 h period. Hyperthermia was statistically significant for *P. leucopus* ($F_{1,5} = 8.6$, $P = 0.03$) and hypothermia was significant for *P. californicus* ($F_{1,3} = 63.7$, $P = 0.004$), in that change in body temperature during the 7.5 h post-LPS was significantly different than zero in both species. Similar analyses indicated that hyperthermia was non-significant in *P. maniculatus* ($F_{1,5} = 4.1$, $P = 0.10$), *P. aztecus* ($F_{1,7} = 0.12$, $P = 0.74$) and *P. melanophrys* ($F_{1,8} = 0.64$, $P = 0.45$).

To contrast overall changes in body temperature in response to LPS, departures from normothermia were integrated over the 7.5 h period post-LPS and compared. Aggregate departure in body temperature ($^{\circ}\text{C h}^{-1}$) differed significantly

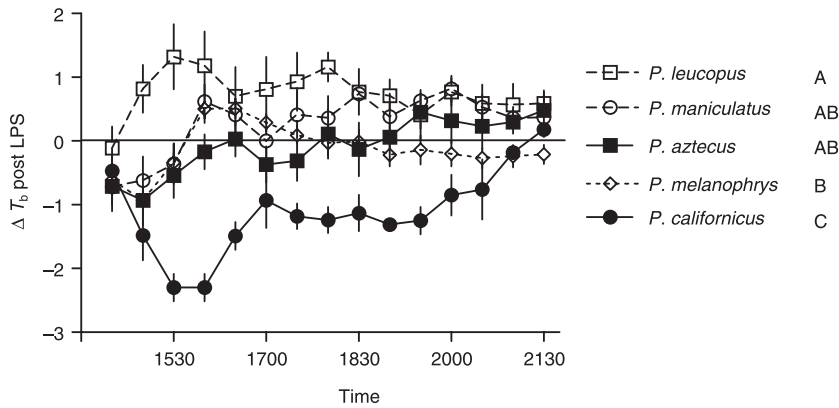


Fig. 2. Changes in T_b from normothermia post-LPS in five species of *Peromyscus*. Individual symbols represent means of (post-LPS T_b)–(pre-LPS T_b) at each time point for each individual in the first 7.5 h after LPS. Numbers on x-axis are time of day, and letters to right of labels in legend indicate significant species differences by Tukey post-hoc tests. Error bars are ± 1 SEM.

among species ($F_{4,28} = 8.68$, $P < 0.001$). *Peromyscus leucopus*, and less so *P. maniculatus*, remained hyperthermic, but *P. melanophrys*, *P. aztecus*, and most dramatically *P. californicus* remained hypothermic post-LPS (Fig. 3a). Percentage of hyperthermic intervals during the 7.5 h period post-LPS was also distinct among species ($F_{4,28} = 5.3$, $P = 0.003$). *Peromyscus leucopus* and *P. maniculatus* were most frequently hyperthermic followed by *P. melanophrys* and *P. aztecus*, and finally *P. californicus* (Fig. 3b). Estimated energetic investment in fever was also different among species ($F_{4,27} = 6.1$, $P = 0.001$). Kilojoules expended on fever was greatest in *P. leucopus*, but not significantly more so than *P. maniculatus* and *P. melanophrys*; *P. aztecus* expenditure was less than *P. leucopus*, but not *P. maniculatus* or *P. melanophrys*. Estimated cost of fever in *P. californicus* was significantly lower than most other species (Fig. 3c).

ACTIVITY

LPS also affected locomotor activity differently in the five *Peromyscus* species (Fig. 4). As with body temperature data, pre-LPS activity values were subtracted from post-LPS values and these data compared. Effects of LPS on behaviour over the 24 h after injection varied among species (time \times species: $F_{172,1204} = 7.1$, $P < 0.001$). Activity was dramatically decreased in some species (*P. californicus* and *P. melanophrys*), modestly decreased in others (*P. aztecus* and *P. maniculatus*) and minimally affected in one (*P. leucopus*). LPS decreased activity during the 7.5 h following injection (time \times species: $F_{56,392} = 4.3$, $P < 0.001$), but only *P. californicus* was unique among species (Fig. 5). When change in activity relative to baseline was examined in each species, LPS was found to decrease activity in *P. melanophrys* ($F_{1,8} = 81.2$, $P < 0.001$), *P. californicus* ($F_{1,3} = 49.7$, $P = 0.006$) and *P. aztecus* ($F_{1,7} = 5.5$, $P = 0.05$), but had marginally non-significant effects on *P. maniculatus* ($F_{1,5} = 5.6$, $P = 0.07$), and did not affect activity in *P. leucopus* ($F_{1,5} = 0.46$, $P = 0.53$).

ANOREXIA AND ANHEDONIA

LPS injection affected body mass-adjusted food intake differently among species (time \times species: $F_{8,54} = 5.6$, $P < 0.001$).

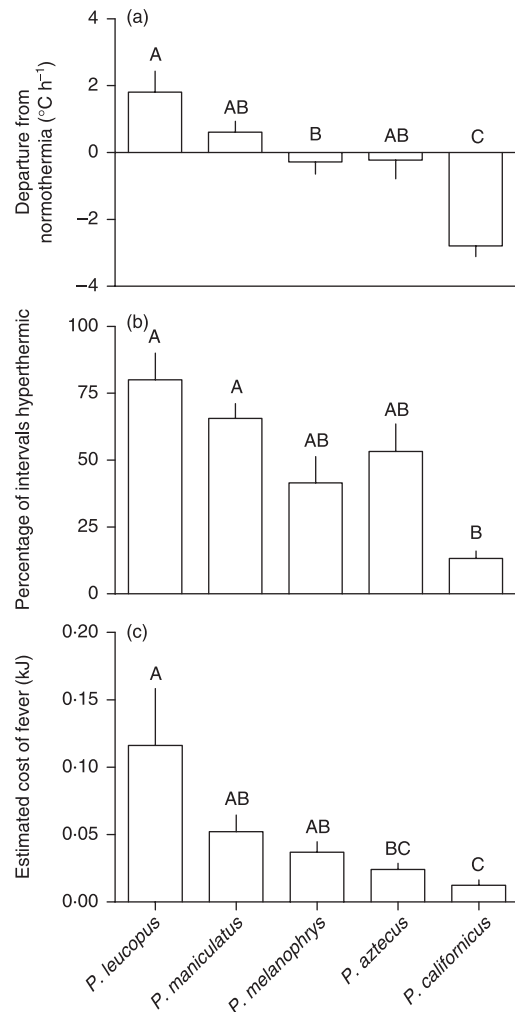


Fig. 3. Aggregate effects of LPS on T_b during 7.5 h post-injection in *Peromyscus*. (a) Mean departure from normothermia per hour, (b) % of 0.5 h intervals hyperthermic, and (c) cost of fever in kilojoules (see text for calculation). Letters above bars indicate significant species differences by Tukey post-hoc tests. Bars are means ± 1 SEM.

Peromyscus aztecus and *P. californicus* food intake decreased post-LPS, whereas *P. maniculatus* and *P. leucopus* were unchanged (Fig. 6a). The feeding profile of *P. melanophrys* was different from *P. maniculatus* and *P. leucopus*, although

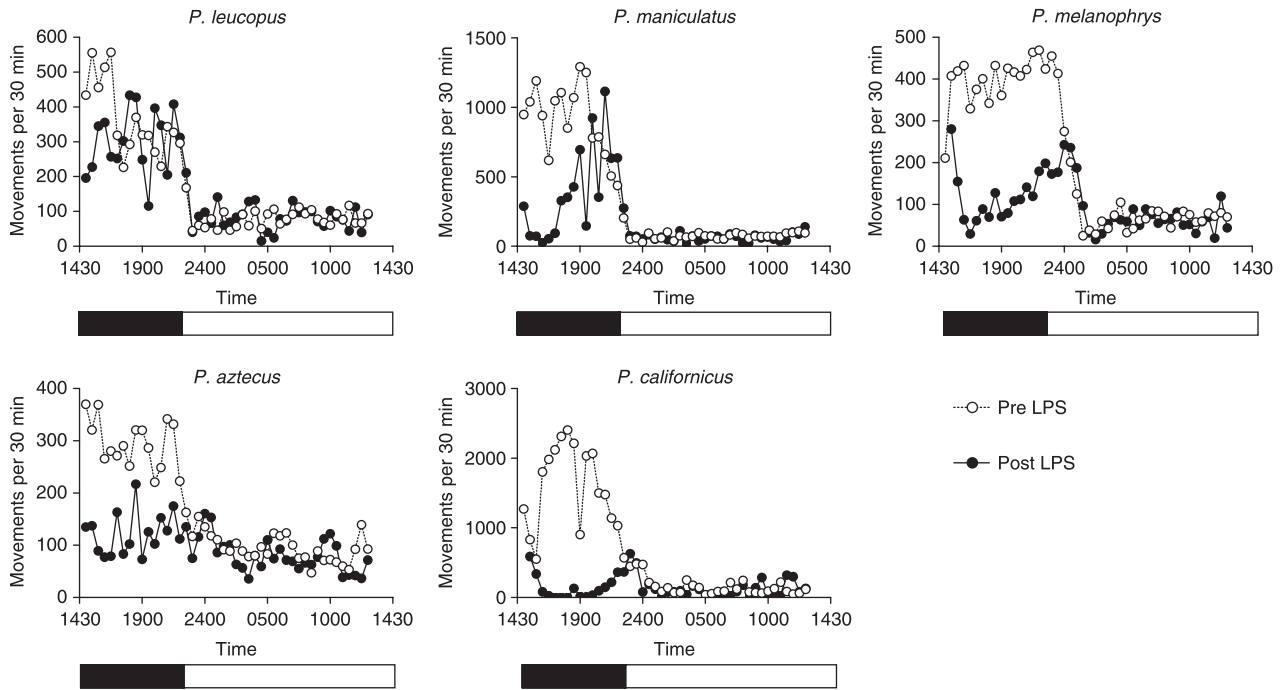
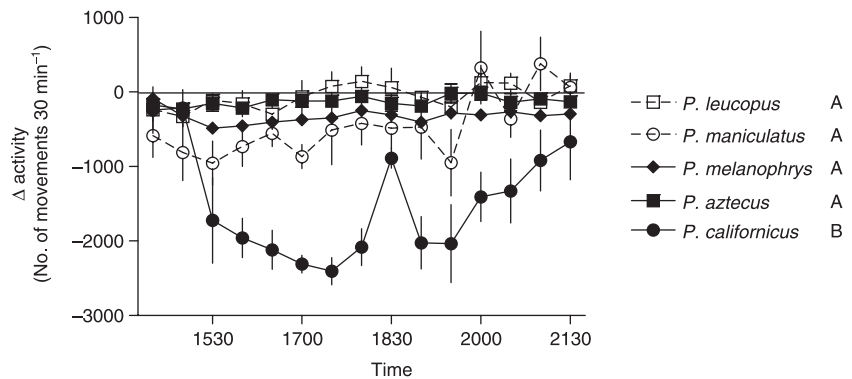


Fig. 4. Effects of LPS on movement activity in five species of *Peromyscus*. Open symbols depict activity (movements 30 min⁻¹) prior to LPS (average of 3 days at each time point); closed symbols depict activity after LPS. Numbers on x-axis are time of day, and open (illuminated) and solid (darkness) rectangles below each figure represent light conditions. Note the different scales on y-axes.

Fig. 5. Change in locomotor activity in *Peromyscus* post-LPS. Individual symbols represent means of (post-LPS activity) – (pre-LPS activity) at each time point for each individual in the first 7.5 h after LPS. Numbers on x-axis are time of day, and letters to right of labels in legend indicate significant species differences by Tukey post-hoc tests. Error bars are ± 1 SEM.



this outcome was partly due to the lower feeding rates prior to LPS in the three large species ($F_{4,27} = 7.8$, $P < 0.001$). Indeed, when aggregate change in food intake in the 2 days post-LPS was compared, *P. californicus* and less so *P. aztecus* exhibited anorexia; the other three species increased in food intake ($F_{4,27} = 3.5$, $P = 0.02$; Fig. 6b). The only statistically significant pairwise difference in food intake during this period, however, was between *P. californicus* and *P. maniculatus*.

LPS also induced anhedonia in all species, as indicated by a significant decrease in sweetened condensed milk intake post-LPS ($F_{2,54} = 23.8$, $P < 0.001$). This anhedonic response was similarly strong among species ($F_{8,54} = 1.5$, $P = 0.20$). Comparison of the aggregate decrease in milk intake 2 days post-LPS among species reinforced this result ($F_{4,27} = 1.8$, $P = 0.16$; Fig. 6c).

CORRELATIONS BETWEEN BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES TO LPS

Only two relationships between fever and sickness behaviour variables were detected. Daily rate of food consumption during the period prior to LPS was positively related to thermal response to LPS ($\rho = 0.39$, $P = 0.03$). Individuals with higher rates of food intake prior to LPS exhibited more marked hyperthermia post-LPS (Fig. 7a). Also, change in body temperature post-LPS was positively correlated to change in activity ($\rho = 0.63$, $P < 0.001$). Individuals exhibiting hypothermia also decreased locomotor activity (Fig. 7b). When these analyses were conducted within species, correlations were not significant or consistent in direction.

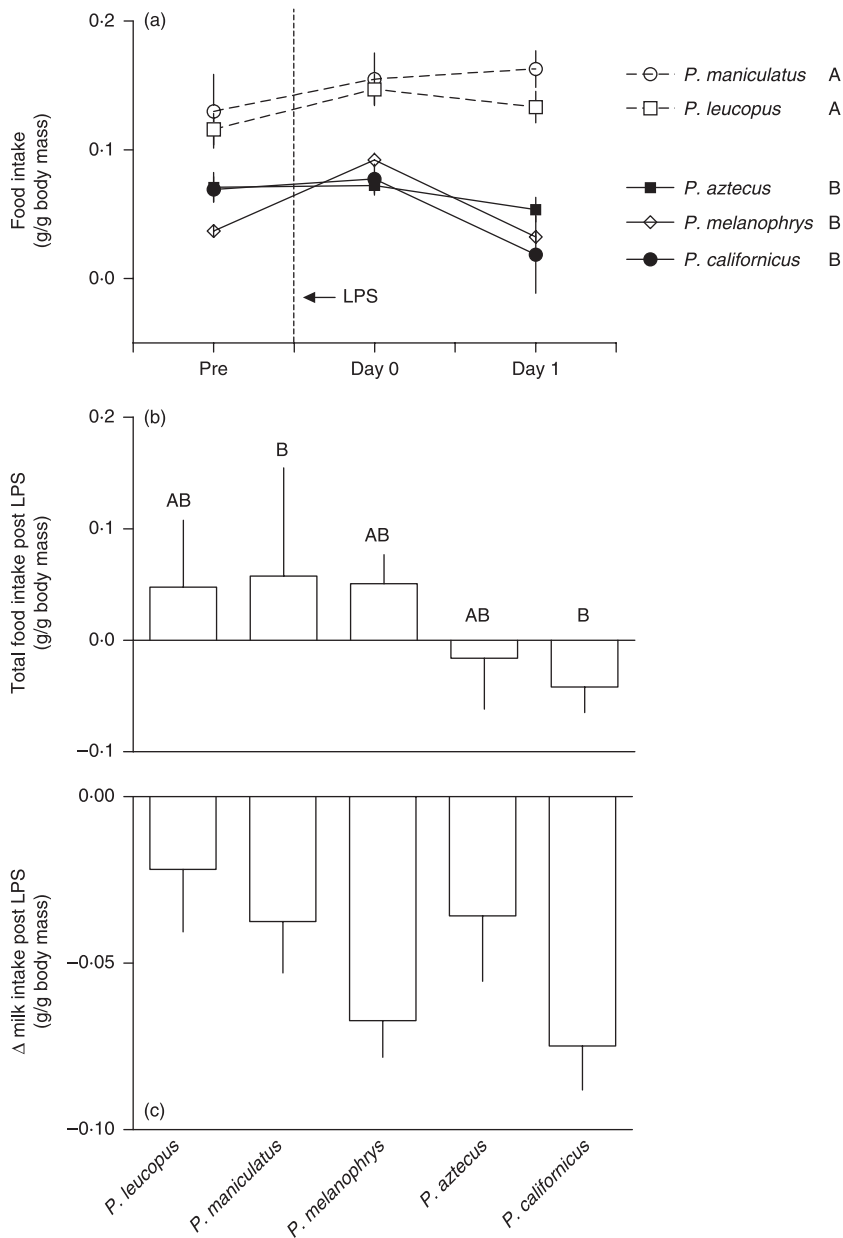


Fig. 6. LPS induction of anorexia and anhedonia in *Peromyscus*. (a) Food intake (per gram body mass) before, and 1 and 2 days after LPS. Letters to right of labels in legend indicate significant species differences by Tukey post-hoc tests, and bars on symbols (means) are ± 1 SEM. (b) Aggregate changes in food intake post-LPS (adjusted for body mass). Letters above bars indicate significant species differences by Tukey post-hoc tests. (c) Aggregate change in sweetened-condensed milk intake (body mass adjusted) post-LPS. In both (b) and (c), bars are means ± 1 SEM.

Discussion

Closely-related species of mice responded differently to simulated bacterial infection. Some species exhibited strong fever, but weak or no reductions in activity and food intake (*P. leucopus* and less so *P. maniculatus*). Other species mounted little (*P. aztecus*) or no fever (*P. melanophrys*), but showed robust sickness behaviours. Still another species became hypothermic, almost completely inactive, and anorectic (*P. californicus*). Tests for serial independence (Abouheif 1999) did not indicate a significant influence of phylogeny on any APR variable. Further, methodologies (e.g. surgical implantation and monitoring of physiological variables) precluded large sample sizes, so important within- and between-species variation may yet exist but were undetectable. Still, other forces besides phylogeny and statistical power cannot explain

the patterns detected in the present study, and it is those we discuss below.

ECOLOGICAL DETERMINANTS OF APR VARIATION

One obvious possible determinant of the APR variation among *Peromyscus* could be the pathogen environment experienced, either in recent times or in previous generations. The strong fevers and minimal sickness behaviour in *P. maniculatus* and *P. leucopus* may be related to their similar broad distributions (Hooper 1968). Broadly distributed species may encounter diverse pathogens, which require robust non-specific defences including strong febrile responses. As the ranges of both species overlap the ranges of *P. aztecus* and *P. melanophrys*, however, one would expect some cross-over of pathogens. Thus, common disease environment would seem to be an

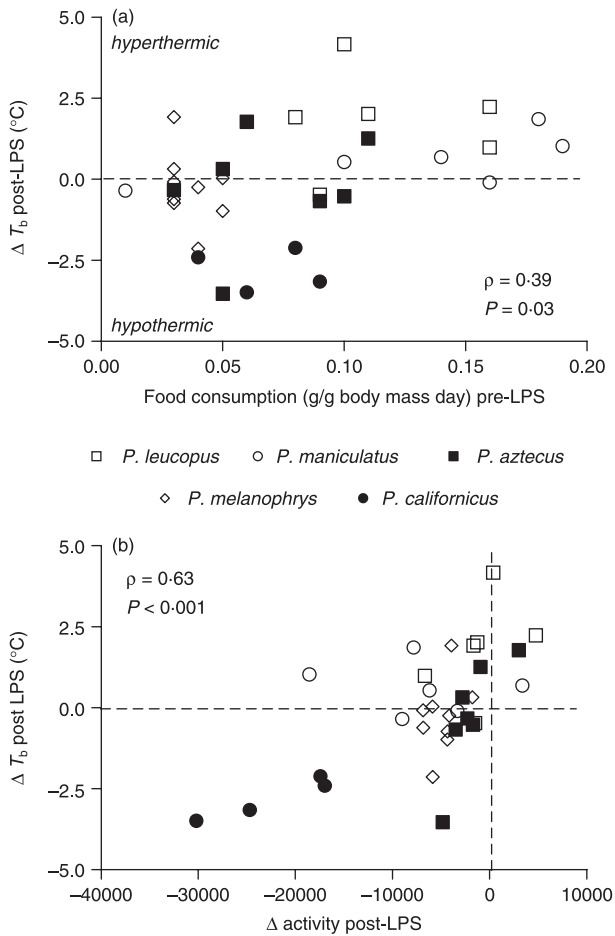


Fig. 7. Correlations between (a) food consumption (mass adjusted), and (b) change in activity on T_b post-LPS in *Peromyscus*. Numbers in bottom-right of both figures depict Pearson's correlation coefficients and respective P -values. Symbols denote species identity defined by legend between figures. Note that statistical results are species-independent.

unlikely driver of APR variation among species. This observation is reinforced by the experience of all individuals in the study over the past several generations. All mice in this experiment were reared under identical conditions for (at least) the past 20 years. Still, because the number of species in the study is low and because the most common (prevalent) pathogens can be widespread, it is possible by chance that the disease environments of some species are (or were) sufficiently similar to have favoured similarity in APRs.

A more viable explanation of the particular pattern of APR variation, however, is the life history context that motivated our previous study (Martin *et al.* in press-a). In that study reproductive pace of life did not predict immune defence strategy, but fast-living species were more competent at *in vitro* bacterial killing. The manifestation of fever in the same two fast-living species in this study complements those results and further indicates a continuum of immune defence among species. Specifically, *P. leucopus* and less so *P. maniculatus* invested more energy into fever than the other species, and they were better able to kill *E. coli* bacteria *in vitro* than

P. aztecus and *P. melanophrys*. Altogether, these results plus similar findings in domestic (Leshchinsky & Klasing 2001) and wild birds (Tella, Scheuerlein & Ricklefs 2002; Ardia 2005; Martin *et al.* 2006a) indicate that further consideration of life history influences on immune defences is warranted (Lee 2006). Interestingly, however, both species that engaged fever minimally expressed sickness behaviour. Further study is necessary to reconcile how and why this decoupling occurred. One possibility is the need to power a vigorous febrile response. Another is a reticence to sacrifice activity to combat infection in fast-lived species. If survival probability in such species is indeed low, fast-lived species may be reluctant to expose themselves to opportunity costs (e.g. sacrifice copulations) as opportunities may not present themselves again in a short lifetime (Weil *et al.* 2006).

SPECIES-SPECIFIC APR STRATEGIES AND THEIR POTENTIAL COSTS AND BENEFITS

Life-history orientation, parasite environment and phylogenetic history may explain broad patterns of immune variation in *Peromyscus*, but other factors may be more important to explain nuances of the APRs of particular species. One of the most striking discoveries in this study was the dramatic hypothermic response to LPS by *P. californicus*. In most species, low doses of pyrogens (i.e. exogenous or endogenous fever-inducing substances) induce hyperthermia (Blatteis 2006), but large doses can induce hypothermia (Kluger 1991). Furthermore, fever can be induced in rabbits with a little as 5 ng kg^{-1} LPS whereas three orders of magnitude larger doses are required for mice and rats (Kluger 1991), and even more for birds (Leshchinsky & Klasing 2001; Owen-Ashley *et al.* 2006). Hypothermia in *P. californicus* may represent heightened sensitivity to bacterial components in this species, but this possibility would have to be tested (at a minimum) by quantifying TLR4 (the main cellular receptor for LPS) expression on leukocytes in this species (Blatteis 2006).

A second explanation for hypothermia involves the environmental conditions at the time of challenge. Under thermoneutrality, most rodents become febrile, but at low ambient temperatures, fever is depressed and sometimes hypothermia is induced (Rudaya *et al.* 2005). The thermoneutral zone for *P. californicus* is presently unknown, but as this species derives from coastal scrub habitats (King 1968), it is possible that the temperatures in which individuals were maintained in the present study were below the low critical temperature for this species. Based on other observations, however, it would seem more likely that hypothermia is adaptive, or at least representative of responses among free-living individuals. Body temperature was maintained prior to LPS, circadian rhythms in body temperature persisted throughout the study, and no individual died post-LPS (due to septic shock). It has been proposed that hypothermia might be a viable anti-pathogen defence under conditions of low resource availability (Romanovsky & Szekely 1998). Perhaps the low productivity habitat where *P. californicus* occurs (Mueller & Diamond 2001) selected for this particular

response to bacterial infections. An indirect way to test this hypothesis would be to inject LPS and assess whether mice move to low ambient temperatures when allowed to thermoregulate behaviourally.

If hypothermia is an adaptive response to bacterial infections in rodents in habitats of low productivity, then it is surprising that another species from a similar habitat type, *P. melanophrys*, responded to LPS differently. *Peromyscus melanophrys* did not become strongly febrile or hypothermic post-LPS although this species also comes from low resource habitats. Indeed to the contrary, individuals exhibited high body temperatures before LPS injection and engaged modest hyperthermia after LPS during the light (inactive) phase. Perhaps the high pre-LPS body temperature during the night (active) phase in *P. melanophrys* prevented further hyperthermia because of an already high set point for body temperature. Alternatively, all three large-bodied species exhibited some hyperthermia during the inactive period, so this may be a strategy common to larger *Peromyscus*, although the benefits of this strategy are unknown. Finally, high baseline body temperature in *P. melanophrys* could serve as a constitutive bacterial defence itself. This argument was offered to explain the higher baseline body temperatures of pyrogen-challenged, free-living California ground squirrels (*Spermophilus beecheyi*) compared to captive individuals (Muchlinski *et al.* 2000). Further, rats treated with antibiotics that decreased the size of their gut flora population had lower baseline body temperatures than controls (Kluger *et al.* 1990). The converse of this argument has also been proposed to explain why body temperature in most organisms is maintained below a threshold that promotes optimal immune cell function (Blatteis 2006). Organisms are thought to restrict their body temperature to lower-than-optimal levels to minimize senescence through reactive oxygen species damage (Sohnle & Gambert 1982). This hypothesis cannot explain the hyperthermia during the inactive phase in this species or others, but it may explain the low body temperature and weak to absent fevers in *P. aztecus* and *P. californicus*. These species may promote their slow pace-of-life (Layne 1968) by avoiding self-damaging defences in spite of the protective benefits fevers could provide (Lee 2006).

RELATIONSHIPS AMONG COMPONENTS OF THE APR

A final result in our study was the relationships between sickness behaviour and body temperature variables independent of species identity. Although these analyses are not rigorous statistically by nature, they reveal important phenomena for future consideration. For example, a positive correlation was detected between rate of food consumption pre-LPS and the degree of hyperthermia post-LPS among all individuals. Although this relationship (and the previous one) was not detectable within species, a permissive effect of food intake on manifestation of fever is intriguing. Food intake rate in *Peromyscus* is strongly positively correlated with BMR (Mueller & Diamond 2001). This correlation may thus imply that only species living in areas where a threshold amount of food can be obtained would be able to mount fever

endogenously. If organisms living in resource depauperate habitats were to mount a fever, then they would have to do so behaviourally. Behavioural thermoregulation in response to pyrogens is pervasive in ectotherms (Vaughn, Bernheim & Kluger 1974; Kluger *et al.* 1997). When prevented from behaviourally thermoregulating or treated with anti-inflammatory drugs, ectotherms can die if the challenge is infectious (Bernheim & Kluger 1976). It would be informative to determine whether some *Peromyscus* behaviourally thermoregulate post-LPS, what consequences these actions have on recovery from infection, and how much of an energy savings this strategy imparts relative to endogenously generated fever.

Another positive correlation was detected between degree of change in body temperature and decrement in activity among individuals. This relationship may indicate that hypothermia post-LPS in some *Peromyscus* may be achieved simply by decreases in locomotor activity. Mammals predominantly increase body temperature by shivering, panting, catabolizing brown adipose tissue, or decreasing thermal conductance and evaporative water loss (Blatteis 2006). However, increases in body temperature ≤ 3 °C can occur in rodents through movement alone (Kluger 1991). Hypothermia in *P. californicus*, therefore, may be as much a consequence of LPS-induced lethargy as a decrease in physiological thermogenesis.

Conclusion

In sum, five *Peromyscus* species responded differently to simulated infection with gram-negative bacteria. All species decreased hedonic behaviour (i.e. sweetened, condensed milk consumption), and most decreased locomotor activity and altered food intake post-LPS. Most also adjusted body temperature although in different directions and to different degrees. Further studies should identify the physiological mediators of this variation [e.g. cytokine production and toll-like and prostaglandin receptor distributions in the brain and periphery (Blatteis 2006; Schnare, Rollinghoff & Qureshi 2006)], especially as multiple steroid hormones differ among *Peromyscus* and influence the immune system extensively (Trainor *et al.* 2006; Martin *et al.* 2007). Also, it is critical to ascertain what forces (i.e. pathogens, climate and food availability) shaped these distinct responses among species, and whether they are present in other taxa. Only work on free-living populations can provide this information. Life-history orientation appears to be an important influence in these species. Finally, the consequences of these different APRs for infection warrant consideration. Perhaps more modest APRs in some species exist to promote survival through decreased oxidative damage (Walford 1969; Sohnle & Gambert 1982; Finch & Crimmins 2004), as these species are protected against infection by other types of potentially less self-damaging immune defences (Martin *et al.* in press-a). Alternatively, as fever is mechanism to speed recovery from infection (Mackowiak 1994), some species may favour fever to expedite pathogen clearance and thus return rapidly to breeding, despite the cost this strategy would have for survival.

Acknowledgements

The authors thank S.L. Kidder for help with animal care and surgeries, and C.R. Hutch and J. Workman for assistance monitoring APRs in mice, and R. Beattie and J. Crossland at the *Peromyscus* Genetic Stock Center. The authors also thank K.A. Lee for comments on a previous draft, and K.J. Navara and B.C. Trainor for help with data interpretation and analysis. This work was funded by NIH Grants: MH 57535 and MH 66144, and NSF Grant: IBN 04-16897.

References

- Abouheif, E. (1999) A method to test the assumption of phylogenetic independence in comparative data. *Evolutionary Ecology Research*, **1**, 895–909.
- Ardia, D.R. (2005) Tree swallows trade off immune function and reproductive effort differently across their range. *Ecology*, **86**, 2040–2046.
- Aubert, A., Goodall, G., Dantzer, R. & Gheusi, G. (1997) Differential effects of lipopolysaccharide on pup retrieving and nest building in lactating mice. *Brain Behavior and Immunity*, **11**, 107–118.
- Bernheim, H.A. & Kluger, M.J. (1976) Fever: effect of drug-induced antipyresis on survival. *Science*, **193**, 237–239.
- Blatteis, C.M. (2003) Fever: pathological or physiological, injurious or beneficial? *Journal of Thermal Biology*, **28**, 1–13.
- Blatteis, C.M. (2006) Endotoxic fever: new concepts of its regulation suggest new approaches to its management. *Pharmacology and Therapeutics* **111**, 194–223.
- Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B. & Sorci, G. (2003) Assessing the cost of mounting an immune response. *American Naturalist*, **161**, 367–379.
- Conn, C.A., Kozak, W.E., Tooten, P.C.J., Gruys, E., Borer, K.T. & Kluger, M.J. (1995) Effect of voluntary exercise and food restriction in response to lipopolysaccharide in hamsters. *Journal of Applied Physiology*, **78**, 466–477.
- Dantzer, R. (2004) Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. *European Journal of Pharmacology*, **500**, 399–411.
- Finch, C.E. & Crimmins, E.M. (2004) Inflammatory exposure and historical changes in human life-spans. *Science*, **305**, 1736–1739.
- Hart, B.L. (1988) Biological basis of the behavior of sick animals. *Neuroscience and Biobehavioral Reviews* **12**, 123–137.
- Hooper, E.T. (1968) Classification. *Biology of Peromyscus (Rodentia)* (ed. J.A. King), pp. 27–70. The American Society of Mammalogists, Stillwater, OK.
- King, J. (ed.) (1968) *Biology of Peromyscus (Rodentia)*. Special Publication No. 2. The American Society of Mammalogists, Stillwater, OK.
- Klasing, K. (2004) The costs of immunity. *Acta Zoologica Sinica* **50**, 961–969.
- Klasing, K.C. & Leshchinsky, T.V. (1999) Functions, costs, and benefits of the immune system during development and growth. *Proceedings of the 22th International Ornithological Congress* (ed. N. Adams & R. Slotow), pp. 2817–2832. Johannesburg, South Africa.
- Kluger, M.J. (1991) Fever-role of pyrogens and cryogens. *Physiological Reviews*, **71**, 93–127.
- Kluger, M.J., Conn, C.A., Franklin, B., Freter, R. & Abrams, G.D. (1990) Effect of gastrointestinal flora on body-temperature of rats and mice. *American Journal of Physiology*, **258**, R552–R557.
- Kluger, M.J., Kozak, W., Conn, C.A., Leon, L.R. & Soszynski, D. (1997) The adaptive value of fever. *Fever: Basic Mechanisms and Management* (ed. P.A. Mackowiak), pp. 255–266. Lippincott-Raven, Philadelphia, PA.
- Layne, J. (1968) Ontogeny. *Biology of Peromyscus (Rodentia)* (ed. J. King), pp. 148–199. American Society of Mammalogists, Stillwater, OK.
- Lee, K.A. (2006) Linking immune defenses and life history at the levels of the individual and species. *Integrative and Comparative Biology*, **46**, 1000–1015.
- Lee, K.A., Martin, L.B. & Wikelski, M.C. (2005) Responding to inflammatory challenges is less costly for a successful avian invader, the house sparrow (*Passer domesticus*), than its less-invasive congener. *Oecologia*, **145**, 244–251.
- Leshchinsky, T.V. & Klasing, K.C. (2001) Divergence of the inflammatory response in two types of chickens. *Developmental and Comparative Immunology*, **25**, 629–638.
- Lochmiller, R.L. & Deerenberg, C. (2000) Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos*, **88**, 87–98.
- Mackowiak, P.A. (1994) Fever: blessing or curse? A unifying hypothesis. *Annals of Internal Medicine*, **120**, 1037–1040.
- Martin, L.B., Hasselquist, D. & Wikelski, M. (2006a) Immune investments are linked to pace of life in house sparrows. *Oecologia*, **147**, 565–575.
- Martin, L.B., Weil, Z.M. & Nelson, R.J. (2006b) Refining approaches and diversifying directions in ecoimmunology. *Integrative and Comparative Biology*, **46**, 1030–1039.
- Martin, L.B., Trainor, B.C., Finy, M.S. & Nelson, R.J. (2007) HPA activity and neotenic and anxiety-like behavior vary among *Peromyscus* species. *General and Comparative Endocrinology*, **141**, 342–350.
- Martin, L.B., Weil, Z.M. & Nelson, R.J. (2007a) Immune defense and reproductive pace of life in *Peromyscus* mice. *Ecology*, **88**, 2516–2528.
- Martin, L.B., Weil, Z.M. & Nelson, R.J. (2007b) Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. *Philosophical Transactions of the Royal Society of London B Biological Sciences*, doi: 10.1098/rstb.2007.2142.
- Martin, S.M., Malkinson, T.J., Veale, W.L. & Pittman, Q.J. (1995) Fever in pregnant, parturient, and lactating rats. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology*, **37**, R919–R923.
- Muchlinski, A.E., Baldwin, B.C. & Gramajo, R. (2000) Endotoxin elicits a febrile response in laboratory-maintained but not free-living California ground squirrels. *Journal of Mammalogy*, **81**, 701–708.
- Mueller, P. & Diamond, J. (2001) Metabolic rate and environmental productivity: Well-provisioned animals evolved to run and idle fast. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 12550–12554.
- Owen-Ashley, N.T. & Wingfield, J.C. (2006) Seasonal modulation of sickness behavior in free-living northwestern song sparrows (*Melospiza melodia morphna*). *Journal of Experimental Biology*, **209**, 3062–3070.
- Owen-Ashley, N.T., Turner, M., Hahn, T.P. & Wingfield, J.C. (2006) Hormonal, behavioral, and thermoregulatory responses to bacterial lipopolysaccharide in captive and free-living white-crowned sparrows (*Zonotrichia leucophrys gambelii*). *Hormones and Behavior*, **49**, 15–29.
- Promislow, D.E.L. & Harvey, P.H. (1990) Living fast and dying young – a comparative-analysis of life-history variation among mammals. *Journal of Zoology*, **220**, 417–437.
- Romanovsky, A.A. & Szekely, M. (1998) Fever and hypothermia: two adaptive thermoregulatory responses to systemic inflammation. *Medical Hypotheses*, **50**, 219–226.
- Rudaya, A.Y., Steiner, A.A., Robbins, J.R., Dragic, A.S. & Romanovsky, A.A. (2005) Thermoregulatory responses to lipopolysaccharide in the mouse: dependence on the dose and ambient temperature. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology*, **289**, R1244–R1252.
- Schnare, M., Rollinghoff, M. & Qureshi, S. (2006) Toll-like receptors: sentinels of host defence against bacterial infection. *International Archives of Allergy and Immunology*, **139**, 75–85.
- Sohnle, P.G. & Gambert, S.R. (1982) Thermoneutrality: an evolutionary advantage against aging? *Lancet*, **1**, 1099–1100.
- Tella, J.L., Scheuerlein, A. & Ricklefs, R.E. (2002) Is cell-mediated immunity related to the evolution of life-history strategies in birds? *Proceedings of the Royal Society of London Series B – Biological Sciences*, **269**, 1059–1066.
- Trainor, B.C., Martin, L.B., Greiwe, K., Kuhlman, J.R. & Nelson, R.J. (2006) Social and photoperiod effects on reproduction in 5 *Peromyscus* species. *General and Comparative Endocrinology*, **148**, 252–259.
- Vaughn, L.K., Bernheim, H.A. & Kluger, M.J. (1974) Fever in the lizard, *Dipsosaurus dorsalis*. *Nature*, **252**, 473–474.
- Walford, R.L. (1969) *The Immunologic Theory of Aging*. Munksgaard, Copenhagen.
- Weil, Z.M., Bowers, S.L., Pyter, L.M. & Nelson, R.J. (2006) Social interactions alter proinflammatory cytokine gene expression and behavior following endotoxin administration. *Brain Behavior and Immunity*, **20**, 72–79.

Received 27 April 2007; accepted 19 September 2007
Handling Editor: Peeter Horak