The Journal of Experimental Biology 209, 5029-5030
Published by The Company of Biologists 2006
doi:10.1242/jeb.02601

Schistosome infection in deer mice (*Peromyscus maniculatus*): impacts on host physiology, behavior and energetics

Lisa E. Schwanz

Department of Biology, University of New Mexico, Albuquerque, NM 87109, USA

Present address: Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA 50011, USA
(e-mail: schwanz@iastate.edu)

Accepted 17 October 2006

Summary

Animals routinely encounter environmental stressors and may employ phenotypic plasticity to compensate for the costs of these perturbations. Parasites represent an ecologically important stressor for animals, which may induce host plasticity. The present study examined the effects of a trematode parasite, *Schistosomatium douthitti*, on deer mouse (*Peromyscus maniculatus*) physiology, behavior and energetics. Measures were taken to assess direct parasite pathology as well as potential host plasticity used to reduce the costs of these pathologies. Parasitized mice had increased liver and spleen masses, as well as decreased liver protein synthesis. Parasitism also led to increased gastrointestinal (GI) mass, either directly due to parasite presence or as host compensation for decreased GI function. No additional plasticity was recorded – infected animals did not consume more food, decrease in body mass or reduce their activity. Parasitism led to reduced thermoregulation during short-term cold exposure, indicating that there may be fitness costs of parasitism. There were no changes in the other measures of energetics taken here, namely basal metabolic rate (BMR) and cold-induced maximal metabolic rate (MR_{max}). Together, the results suggest that many costs of parasite infection are largely ameliorated through physiological or morphological compensatory mechanisms.

Key words: rodent, *Schistosomatium douthitti*, metabolic rate, liver, wheel running, phenotypic plasticity.

Introduction

Phenotypic plasticity is an important means through which animals respond to unpredictability and stressors in the environment (Fordyce, 2006; Schlichting and Pigliucci, 1998). Plasticity can occur at many levels, from physiology and morphology to behavior and life history (King and Murphy, 1985). Organ morphology, for example, can be highly flexible in many animals (Piersma and Lindström, 1997; Starck, 1999). The size of gastrointestinal (GI) tract organs changes with energetic and functional demands, increasing during lactation and cold exposure in rodents, decreasing in preparation for migration in birds, and changing over active and hibernation seasons in lizards (e.g. Hammond et al., 1994; Piersma and Lindström, 1997; Tracy and Diamond, 2005). Similarly, metabolic rates can change with season and ambient temperature (e.g. Hill, 1983).

Parasites represent an important stressor for many animals, and their effects on host phenotypes can include direct pathologies and host plasticity. For example, parasitism can cause liver and intestinal damage, anemia and increased thermal conductance (Booth et al., 1993; Holmes and Zohar, 1990; Meagher, 1998; Schall et al., 1982; Tocque, 1993; Wiger, 1977). Parasitism can also lead to changes in the energetics and performance of animals, including reduced feeding and activity, impaired anti-predator and competitive behavior, and altered metabolic rates (Arneberg et al., 1996; Booth et al., 1993; Cunningham et al., 1994; Freeland, 1981; Poirier et al., 1995; Rau, 1983a; Rau, 1983b; Rau and Putter, 1984; Schall et al., 1982; Symons, 1985). Finally, parasitized animals may show shifts in organ size and body tissue composition (Kristan, 2002; Kristan and Hammond, 2000; Kristan and Hammond, 2001; Tocque, 1993). Although it is difficult to distinguish between direct parasite impact and host compensation, host alterations in some systems appear to represent phenotypic plasticity to minimize the fitness costs of infection (e.g. Booth et al., 1993; Kristan and Hammond, 2001; Podesta and Mettrick, 1976). Indeed, parasitism can have strong, negative impacts on host fitness through decreases in survival and reproduction (e.g. Crews and Yoshino, 1989; Fuller and Blaustein, 1996; Goater and Ward, 1992; Neuhaus, 2003; Zuk, 1987). The host–parasite relationship, therefore, provides an interesting and ecologically important stage on which to examine phenotypic plasticity and fitness consequences of stressors. Additionally, understanding the phenotypic response of individual hosts is imperative for understanding population-level effects and coevolution between hosts and parasites

(Anderson and May, 1978; Anderson and May, 1982; Boonstra et al., 1980; Gregory, 1991).

Schistosomes (Trematoda) have indirect life cycles, using many species of birds and mammals as definitive hosts. The adult worms live in the mesenteric and hepatic portal veins of the definitive host (Price, 1931). Sexually produced eggs pass into the lumen of the host intestines and out in the feces. The subsequent life stages develop and reproduce asexually in the intermediate host, freshwater snails. Mobile cercariae are shed from infected snails into surrounding water and, upon encountering the skin of a definitive host, burrow through the skin and migrate to the portal vein where they grow and mature (Price, 1931).

Schistosomatium douthitti is distributed across northern North America and infects a variety of rodents (Malek, 1977). Infection pathology is largely associated with the host's inflammatory response to parasite eggs and with host tissue damage caused directly by the eggs (Kagan and Meranze, 1957; Raiczyk and Hall, 1988; Zajac and Williams, 1981). In addition to being found in the intestinal lining, many eggs become trapped in the liver and spleen of the host. Infection in rodents leads to liver and spleen enlargement, immune cell recruitment to eggs in host tissues, blood in the feces (presumably due to capillary damage during passage of eggs through the intestinal wall) and, in many cases, death (Kagan and Meranze, 1957; Raiczyk and Hall, 1988; Zajac and Williams, 1981). Virtually nothing is known of how these pathologies impact liver or intestinal function, physiology, energetics or behavior (see Raiczyk and Hall, 1988). Additionally, most information on host effects comes from lab mice (*Mus musculus*) (Kagan and Meranze, 1957; Kagan and Meranze, 1958; Raiczyk and Hall, 1988). Natural hosts of *S. douthitti* are not impacted as strongly by infection (Zajac and Williams, 1981), so a great deal of new information can be gained by examining the impacts of infection in natural hosts.

The aim of the present study was to examine the impact of parasitism by *S. douthitti* on deer mouse (*Peromyscus maniculatus*) physiology, behavior and energetics and to assess whether deer mice may employ phenotypic plasticity to compensate for the costs of infection. I examined a suite of host phenotypic traits, with the goal of establishing how these traits may interact to determine the overall impact of infection on hosts. Deer mice are good experimental subjects because they are natural hosts for this parasite (Malek, 1977; Price, 1931) and because there is a wealth of data available on deer mouse physiology and energetics (Chappell et al., 2004; Chappell and Hammond, 2004; Green and Millar, 1987; Hammond and Kristan, 2000; Hayes, 1989; Hayes and Chappell, 1986; Hill, 1983; Koteja, 1996a; Koteja, 1996b; Millar, 1979; Millar, 1985).

Schistosome infection in deer mice is predicted to have direct physiological costs for hosts. These costs are examined here through the primary parasite pathology, specifically by measuring liver and spleen mass and liver damage. Liver damage is assessed with markers of (1) cell damage, including alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and (2) liver protein production (albumin) (Loeb and Quimby, 1989). Liver measures are predicted to reveal typical conditions of schistosomiasis. That is, the liver should be enlarged and liver diagnostics should reveal cell damage (increased ALP, ALT and AST) and reduced organ function (reduced albumin) (Loeb and Quimby, 1989). In addition, I predict that the spleen will be enlarged, as is typically seen in animals infected with schistosomes due to the presence of an inflammatory response to parasite eggs and potentially to increased functional demand on the spleen (Kagan and Meranze, 1958; Zajac and Williams, 1981).

If parasitism carries a physiological cost for hosts, I predict that deer mice adjust their activity or energetics (phenotypic plasticity) to ameliorate the overall costs. I predict that there will be no change in total digestive efficiency in deer mice but an increase in GI mass to compensate for reduced intestinal function per unit mass, as previously seen in parasitized mice (Kristan, 2002; Kristan and Hammond, 2000; Kristan and Hammond, 2001). Compensation for physiological costs may also include increased energy expenditure, resulting in increased food consumption or decreased body mass. However, these mechanisms are not predictably employed by hosts to compensate for parasitic costs, so they are not predicted to change here (Fuller and Blaustein, 1996; Kristan and Hammond, 2000; Raiczyk and Hall, 1988). Reductions in daily activity levels are often seen in parasitized animals (e.g. Poirier et al., 1995; Rau, 1983b). Behavioral activity of deer mice (here, voluntary wheel running) is predicted to decrease in parasitized animals. Finally, metabolic rates representing basal operating costs [basal metabolic rate (BMR)] and the upper limit to sustained activity or thermogenesis [cold-induced maximal metabolic rate (MR_{max})] provide estimates of the energetic costs of *S. douthitti* in deer mice. If mice have compensated for the physiological costs of parasitism, I would predict no change in metabolic measures (Kristan, 2002; Kristan and Hammond, 2000; Meagher and O'Connor, 2001). If mice have not fully compensated for the costs of infection, I would predict changes in metabolic measures that represent either extended costs of infection or further phenotypic plasticity.

Materials and methods

Experimental subjects were 8–10th generation lab-born deer mice (*Peromyscus maniculatus rufinus* Merriam) from a lineage originally collected from New Mexico. The mouse colony was established using culled individuals from a large colony in the Department of Biology at the University of New Mexico (Botten et al., 2001). Experimental mice were housed singly in standard plastic rodent cages (43×27×15 cm, L×W×H) and were provided with rodent chow (Formulab Diet 5008; LabDiet, Richmond, IN, USA) and water *ad libitum*. The animal room was on a 12 h:12 h light:dark cycle throughout the year and was maintained at room temperature (22–24°C). All mice were parasite-naïve at the beginning of the

study. For this experiment, I used 34 virgin, adult females (45–117 days old at initiation of the study), maintained in a non-breeding condition for the duration of the experiment. Three treatment groups were established: control, low dose (LD; exposure to 30–50 cercariae), and high dose (HD; exposure to 100–150 cercariae).

Schistosomatium douthitti and the snail host (*Stagnicola elodes*) were from lines originally collected in Indiana (provided by D. Daniell, Butler University). At the University of New Mexico, the parasite life cycle was maintained through *S. elodes* as the intermediate (snail) host and Djungarian hamsters or deer mice as the definitive hosts. To infect mice, patent snails were placed in artificial spring water (ASW) in the dark for approximately 1.5 h to induce shedding of cercariae. Cercariae were collected under a dissecting microscope by catching a film of contaminated water with a small metal loop (3 mm loop diameter). The film of water was presented to the mouse, which would lick the loop and consume the water and cercariae. This method allowed approximate counts of the number of cercariae to which a mouse was exposed. Control mice were provided uncontaminated ASW for consumption. Host patency, when eggs begin to be passed in the feces and accumulate in the liver, starts around 30 days after infection (DAI) (Kagan and Meranze, 1958; Price, 1931; Zajac and Williams, 1981). All measurements were conducted at $\geqslant$45 DAI to ensure that the immune-inducing phase of the life cycle was present in the hosts.

Behavior

Food and water consumption as well as digestive efficiency were measured for mice between 49 and 52 DAI. Mice were weighed to the nearest 0.1 g and placed in clean cages. Standard rodent chow was weighed to the nearest 0.01 g and provided on the cage lid (food provided in excess). Water was provided in a plastic water bottle (mass to the nearest 0.1 g). After three days, mice, food, feces and water bottles were weighed. Uneaten food was collected from the cage lid and from the cage floor. Food and feces were not dried beyond their state at collection (most fecal pellets were not fresh). Food and water consumption was calculated as the difference in the mass before and after the three-day period. Digestive efficiency [apparent dry matter digestibility (ADMD)] was calculated as: (Food consumed – feces produced)/(food consumed).

Voluntary wheel-running activity was recorded over a single night between 53 and 58 DAI. Mice were placed in tall rodent cages (43×27×20 cm, L×W×H) with bedding, rodent chow on the cage floor, and a hanging water bottle. One end of the cage contained a free-standing metal mesh running wheel (14.8 cm diameter). A cycle computer (Sigma Sport Model BC 1200; Sigma Sport USA, Batavia, IL, USA) was attached to the running wheel to record rotations. Mice were placed in the cages at 19:00 h and allowed to run overnight. Data on the total distance run and the total time of wheel rotation overnight were collected at approximately 8:00 h (7:30–11:00 h) the following morning. Using these data, I calculated the mean speed (km h^{-1}) during the time spent running during the night (12.5–16 h recording period).

Energetics

Basal metabolic rate (BMR) and cold-induced maximal metabolic rate (MR_{max}) were measured once for each animal between 56 and 66 DAI. BMR was measured at least three days after wheel running to ensure at least one night of recovery for the mice between wheel running and fasting (1–4 nights of recovery, randomized between treatments). MR_{max} was measured at least one day after BMR measurement for a given animal. For both, rates of oxygen consumption ($\dot{V}_{O_2}$) were determined using open-flow respirometry. Dry, CO_2-free air was supplied to chambers, with airflow controlled upstream *via* mass flow controllers (OMEGA FMA-2407 Mass Flow Controller and FLT-03ST and -02C Rotameters; OMEGA Engineering, Inc., Stamford, CT, USA). Excurrent air was sampled for CO_2 concentration by a LI-COR CO_2 analyzer (LI-7000; LI-COR; Lincoln, NE, USA), scrubbed of H_2O and CO_2 (using Drierite and ascarite), then sampled for O_2 concentration using a Sable Systems O_2 analyzer (FC-1B; Sable Systems, Henderson, NV, USA). Changes in O_2 and CO_2 concentrations were recorded with Sable Systems DATACAN V data acquisition and analysis software. Oxygen concentrations were not allowed to fall below 20.4%. $\dot{V}_{O_2}$ was calculated according to equation 4a in Withers (Withers, 1977).

For BMR measurements, room air was scrubbed of CO_2 and provided to the animals at a flow rate of 480–1200 ml min^{-1}. Metabolic chambers (plastic; ~680 ml) contained a small amount of bedding and were placed in a warm cabinet maintained at 30±2°C. BMR measurements were recorded during the inactive phase of the animal's daily cycle (between 08:00 h and 12:30 h) after fasting for 10–12 h. Four mice were run simultaneously during a trial. Each chamber was sampled for 5 min at a time (5 s sampling intervals), after which the next chamber was sampled. Baseline air was sampled for 4.5 min every 20 min. Each mouse was recorded over 3.5 h, and the lowest mean O_2 consumption during a 3 min interval was used to calculate BMR. Mice were weighed before and after BMR measurements. Mass at the end of the trial was used in statistical analyses since BMR was typically calculated from sampling sessions late in the trial.

MR_{max} was measured under exposure to cold conditions in heliox (21% oxygen/79% helium), which greatly increases thermal conductance (Rosenmann and Morrison, 1974). Mice were placed in a glass chamber (500 ml) in a cold cabinet maintained at 2–4°C. Trials were conducted between 15:00 h and 19:00 h. At the beginning of the trial, the chamber was flushed with heliox at 1600 ml min^{-1} for 4 min. After flushing, heliox was provided to the chamber at a flow rate of ~980 ml min^{-1} for 15 min. Pure heliox was sampled as a baseline for 1.5 min prior to the trial. Excurrent air from the chamber was then sampled every 5 s during the 15-min trial. MR_{max} was calculated from the highest mean consecutive three minutes of O_2 consumption during the trial. Body mass was measured prior to the trial. Body temperature was recorded

before and after the trial (within 30 s of removing the animal from the chamber) with a rectal thermocouple. Metabolic scope was calculated as MR_{max}/BMR.

Physiology and morphology

Physiological costs of parasitism were assessed at euthanasia (65–68 DAI). Mice were injected with ketamine (0.25 mg g^{-1}) and xylazine (1:3 v/v ketamine:xylazine). Blood was collected *via* heart puncture (250–500 μl), allowed to clot for 30 min in a serum separator tube (Microtainer® brand tube, model SST™; Becton-Dickinson, Franklin Lakes, NJ, USA), then centrifuged at 10 000 ***g*** for 90 s. Blood serum was removed and analyzed by IDEXX Reference Laboratories, Inc., Preclinical Research Division (West Sacramento, CA, USA) for serum levels of ALP, ALT, AST and albumin using photometric assays (Hitachi 747). Immediately following heart puncture, mice were asphyxiated with CO_2. Mice were weighed after exsanguination ('whole body mass') and dissected. Wet liver and spleen masses were recorded, and intestines from the stomach to the rectum, including the cecum, were removed. Carcasses were weighed without livers and intestines but with spleens ['eviscerated body mass' (EBM)]. The liver was homogenized in ASW in a Waring blender and viewed under a dissecting microscope to visually confirm infection status by the presence of parasite eggs.

To assess the potential for plasticity in morphology, the mass of the GI tract (including the cecum) and the food contained therein was calculated as: (whole body mass) – (EBM + wet liver mass). I believe this is an accurate measure of intestinal and food mass because the mice were exsanguinated and, therefore, lost little blood upon dissection. The proportion of the whole body mass that was represented by EBM, wet liver mass and intestinal mass was calculated.

Statistical analyses

Treatments were compared using parametric statistics when data appeared normally distributed, and statistical models revealed no significant differences in variance (ANOVA/ANCOVA, Bonferroni-corrected two-sample *t*-tests). Otherwise, non-parametric comparisons were used as they do not assume normality and are less sensitive to differences in variance among groups (Kruskal-Wallis ANOVA on raw data or mass-residuals, and Bonferroni-corrected Mann–Whitney *U*-tests). In all analyses, the two infection treatments (LD and HD) did not differ, so were combined into one 'Infected' treatment, and differences between treatments in the response variables were again tested using only pairwise comparisons (ANCOVA or *t*-tests/*U*-tests on raw data or mass-residuals). Only the results of the final pairwise comparisons are reported here. For most statistical models, mouse mass was used as a covariate, and an interaction term was included (mass × treatment). If these variables were not significant ($P>0.10$), they were removed from the model sequentially. EBM was used for analyses of liver and spleen variables, whereas mass prior to the variable measurement was used for analyses of behavioral and energetic measurements (except for BMR). Values are presented as means ± s.d.

Results

Four of 11 control mice had 1–2 schistosome eggs in their liver homogenates. However, the livers of these mice visually resembled the livers of uninfected mice (see below), so it is more likely that these eggs were due to contamination at homogenization. These mice were included in the control group for analyses. Infection was not confirmed for four of the 23 infected mice (three of 11 LD mice; one of 12 HD mice), so they were excluded from analyses. One of the remaining 19 infected mice died before 49 DAI, and infection was confirmed following death. Sample sizes were 11 (control) and 18 (infected), unless indicated otherwise.

Physiological impacts

Wet liver mass was significantly greater in infected mice compared with controls (Table 1). Parasitized mice also had larger wet spleen masses than controls (Table 1). These organs also had visual characteristics of disease. Livers of infected mice were darker in color than controls, had a mottled appearance and displayed pitting and pinhead-sized white spots on the lobe surfaces. Spleens were also darker and thicker in appearance in infected mice compared with those of control mice.

Liver diagnostics showed variable results (Table 1; Fig. 1). Contrary to predictions, ALP levels were lower in infected mice compared with control mice. Treatment had no effect on blood serum concentrations of either AST or ALT. Albumin was negatively correlated with mass and was lower for infected mice in an ANCOVA. However, variances differed significantly in this model, so the residuals of albumin on mass were used as the response variable (regression: $r^2=0.09$, $P=0.113$). Albumin residuals were significantly lower in infected mice compared with control mice, as predicted (Table 1). Infected mice also had lower serum albumin when considering raw values ($t=4.56$, $P<0.001$). Raw data are presented in Fig. 1D.

Morphology

Body mass did not differ between treatments pre-infection, but infected mice were heavier than control mice at 49 DAI (Table 1). Additionally, infected mice gained more mass between infection and 49 DAI. Mass differences persisted for whole-body mass at euthanasia but were not seen in eviscerated body mass (EBM; Table 1).

Differences between treatment groups in whole-body mass at euthanasia were largely due to differences in the proportion of this mass contributed by liver and intestinal mass (Table 1; Fig. 2). The proportion of whole-body mass due to EBM was significantly lower in infected mice. As one might expect, wet liver mass made up a greater proportion of mass in infected mice than in control mice. Similarly, the GI tract also made up a greater proportion of whole-body mass in infected mice than

Table 1. *Parasite treatment effects on physiology and morphology in adult female deer mice*

Trait	Control (*N*=11)	Infected (*N*=18)	Statistic	*P*
Liver and spleen				
Liver mass (g)	0.83±0.16	1.20±0.21	*t*=–5.03	**<0.001**
Spleen mass (g)	0.025±0.007	0.058±0.030	*U*=10	**<0.001**
ALP (i.u. l^{-1})	570±307	368±177	*t*=2.26	**0.032**
AST (i.u. l^{-1})	345±226	728±799	*U*=79	0.369
ALT (i.u. l^{-1})	256±166	368±365	*U*=75	0.281
Albumin-mass residuals	0.40±0.14	–0.25±0.36	*U*=8	**<0.001**
Mass				
Mass pre-treatment, 0 DAI (g)	17.6±2.04	18.2±3.56*	*t*=–0.51	0.614
Mass, 49 DAI (g)	19.5±3.7	23.0±3.0	*t*=–2.80	**0.009**
Mass change, 0–49 DAI (g)	1.90±3.24	4.69±2.22	*t*=–2.76	**0.010**
Whole body mass (euthanasia) (g)	18.7±2.5	21.2±2.8	*t*=–2.45	**0.021**
EBM (g)	15.5±2.1	16.0±2.9	*t*=–0.56	0.583
Proportional EBM	0.83±0.03	0.75±0.06	*U*=25	**0.001**
Proportional liver mass	0.044±0.006	0.057±0.011	*t*=–3.61	**<0.001**
Proportional intestine mass	0.13±0.03	0.19±0.05	*U*=25	**0.001**

Values are means ± s.d. (**N*=19). Treatments were control and infected. Bold indicates significance (*P*<0.05).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DAI, days after infection; EBM, eviscerated body mass.

in control mice. The increase in proportional intestinal mass may be due to actual organ mass, food mass, or both.

Behavior

Food consumption over three days was significantly and positively related to mouse mass and change in mass over the three days of consumption measurement but did not differ between treatments (ANCOVA: mass, *P*=0.016; mass change, *P*<0.001; treatment, *P*>0.10). Similarly, water consumption was positively related to mass and mass change, but was not affected by treatment (ANCOVA: mass, *P*=0.005; mass change, *P*<0.001; treatment, *P*>0.10). The relationship between water consumption and food consumption was also analyzed to examine whether there is a difference between treatments in the amount of water consumed per gram of food consumed, as might be expected if parasitism leads to dehydration (ANCOVA: response = water consumption; predictors = food consumption, treatment, food × treatment). The variances were significantly different in this analysis (interaction not significant, so water consumption was regressed against food consumption; r^2=0.77, *P*<0.001). Treatment had no effect on the water consumption residuals (*U*=84.00, *P*=0.50). Apparent dry matter digestibility did not differ between treatments (mass and mass × treatment, not significant; treatment, *U*=82.00, *P*>0.10) and averaged 78.8% (Control) and 76.7% (Infected).

Wheel activity varied greatly among mice. The mean total time spent running on the wheel for all mice during the 12.5–16 h of recording was 3.1±2.7 h and ranged from 74 s to 7.9 h. The mean total distance ran in a night was 6.4±7.8 km (range, 0.02–32.6 km). The mean running speed of a mouse was 1.77±1.1 km h^{-1} (range, 0.57–5.19 km h^{-1}). Total distance, total time of

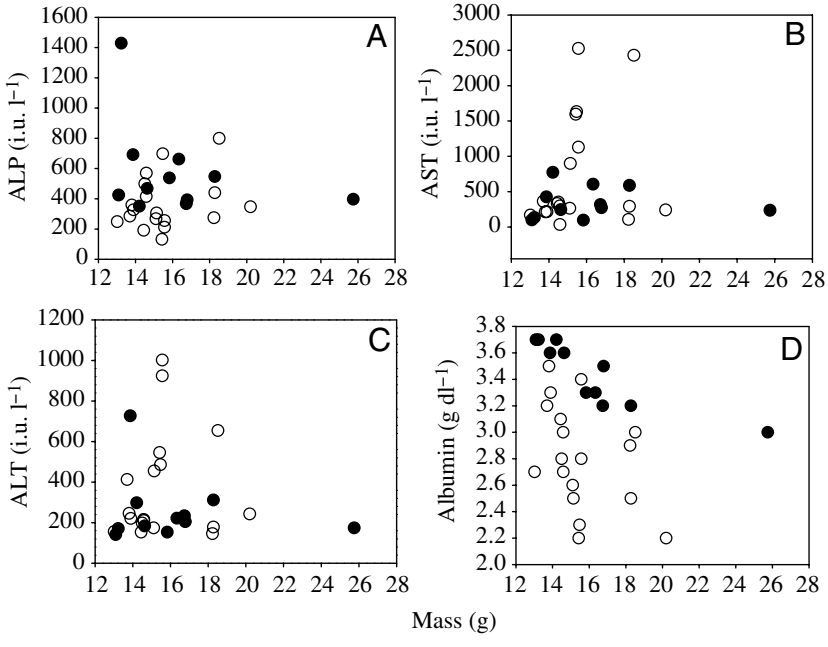

Fig. 1. Liver diagnostics as a function of eviscerated body mass. (A) Alkaline phosphatase (ALP), (B) aspartate aminotransferase (AST), (C) alanine aminotransferase (ALT) and (D) albumin for control (filled circles) and infected (open circles) mice.

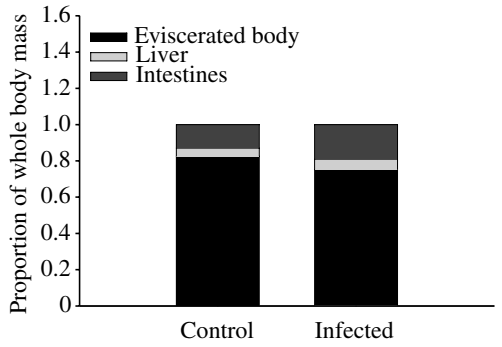

Fig. 2. Proportion of whole body mass composed of eviscerated body, liver and intestinal mass. All components differed significantly between control and infected mice.

activity and mean speed were not predicted by treatment, body mass or mass × treatment (Table 2).

Energetics

One control mouse was excluded from BMR analysis because no prolonged rest period based on CO_2 and O_2 profiles was recorded for this animal. Treatment had no effect on BMR (Fig. 3A; body mass and mass × treatment were non-significant and were removed from final analysis; treatment, t=–0.97, P>0.10). Maximal metabolic rate during cold exposure was correlated with mass, but the ANCOVA model showed significantly different variances. In order to account for mass while using non-parametric statistics, MR_{max} was regressed against mass (Fig. 3B; r^2=0.25, P=0.006), and the residuals were used as the response variable. Treatment had no effect on MR_{max} residuals (U=64.00, P>0.10). Metabolic scope was not predicted by mass, mass × treatment or treatment (Fig. 3C; treatment, t=0.81, P>0.10).

Body temperature averaged 37.8±1.09°C (range 35.4–39.9°C) at the beginning of the cold-exposure trial and 32.8±2.87°C (range 24.8–36.9°C) after the trial. Temperature change was not acquired for one control animal. Infected mice showed a greater loss in body temperature than control mice during the 15-min cold-exposure period (ANCOVA: mass, P=0.030; pre-trial temperature, P=0.036; treatment, P=0.008; Fig. 3D). To examine whether thermogenic capacity (MR_{max}) was related to temperature change during cold exposure (independent of body mass), mass-residuals of both variables were used in a regression (temperature change *versus* mass regression: r^2=0.05, P=0.253). Residuals of temperature change were positively correlated with MR_{max} residuals (Fig. 3E; r^2=0.58, P<0.001; pre-trial temperature was not included in the analysis), indicating that mice with low recorded MR_{max} also had a more negative change in body temperature.

Discussion

Infection of deer mice with *S. douthitti* led to a number of changes in the physiology of hosts. Infected mice showed the classic pathology of schistosomiasis (enlarged livers and spleens) (Zajac and Williams, 1981). The liver diagnostics (ALP, ALT and AST) indicate that liver cell damage was not extensive enough to lead to substantial cell leakage. These results are surprising given that, in other studies, infection of rodents with *S. douthitti* is usually accompanied by lesions in the liver (Kagan and Meranze, 1957; Zajac and Williams, 1981). In addition, previous research on liver-damaging parasites in rodents has demonstrated increases in ALP associated with infection (Raiczyk and Hall, 1988; Meagher, 1998).

Infection in deer mice did lead to reduced serum albumin, typically interpreted as an indication of liver disease (Loeb and Quimby, 1989; Meagher, 1998). Whereas albumin levels in control deer mice were similar to those of healthy lab mice (3.0–4.0 g dl^{-1}) (Loeb and Quimby, 1989), infected mice showed albumin levels reduced by up to 27% compared with control mice. This reduction could be indicative of several potential parasitic effects (Loeb and Quimby, 1989; Symons, 1985). First, and most likely, liver damage caused by infection may have impaired synthesis of albumin. Second, albumin could be lost in feces *via* intestinal lesions caused by parasitic eggs. Third, the adult parasite worms and eggs themselves may absorb and catabolize albumin as a nutrient. Schistosome worms and eggs (which grow and embryonate over ~9 days inside the host) absorb free amino acids (Bruce et al., 1972; Stjernholm and Warren, 1974) and may take up and catabolize albumin (Symons, 1985). Reductions in serum albumin can have a number of consequences for animals (e.g. edema) due to its diverse roles in maintaining physiological homeostasis (e.g. transport of proteins and maintenance of osmotic pressure). Similar reductions (29%) in serum albumin in ill humans dramatically increase the chances of morbidity (by 89%) and mortality (by 137%) (Vincent et al., 2003), which suggests that the albumin reductions recorded in this study for infected deer mice represent a substantial cost.

Despite the obvious liver damage and physiological costs of infection, little change was seen in deer mouse energetics. BMR and MR_{max} measures were similar to those of previous studies for uninfected deer mice (e.g. Hayes, 1989; Hayes and

Table 2. *Wheel running activity in adult female deer mice (control and infected) over 12.5–16 h of recording*

	Control (N=11)	Infected (N=17)	Statistic	P
Total distance (km)	7.85±9.89	5.52±6.35	t=0.765	0.451
Total time (h)	3.68±3.30	2.72±2.23	U=86.00	0.724
Mean speed (km h^{-1})	1.67±1.27	1.83±1.02	t=–0.372	0.713

Values are means ± s.d.

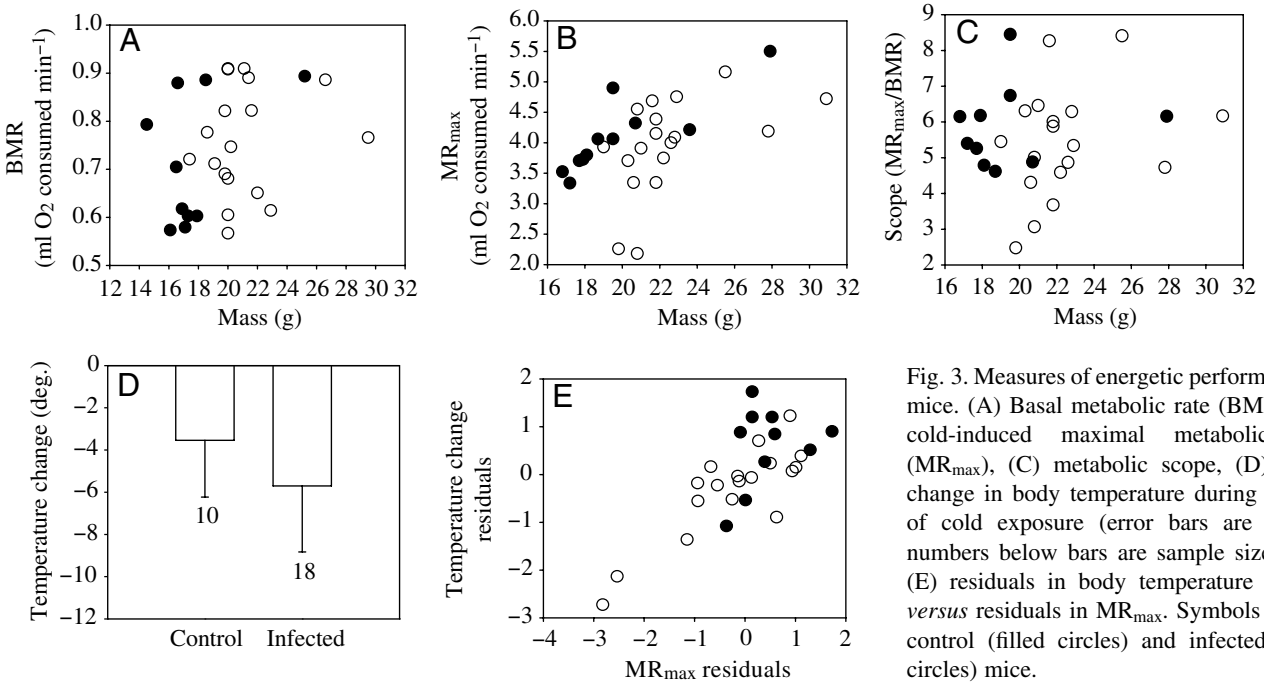

Fig. 3. Measures of energetic performance in mice. (A) Basal metabolic rate (BMR), (B) cold-induced maximal metabolic rate (MR_{max}), (C) metabolic scope, (D) mean change in body temperature during 15 min of cold exposure (error bars are 1 s.d.; numbers below bars are sample sizes) and (E) residuals in body temperature change *versus* residuals in MR_{max}. Symbols are for control (filled circles) and infected (open circles) mice.

Chappell, 1986; Meagher and O'Connor, 2001) but were unaffected by parasitic infection.

In the present study, the only change in energetics recorded was the greater decrease in body temperature by parasitized mice following cold exposure, which indicates altered thermoregulation in infected animals and may be related to fitness reductions. The results may suggest that, despite having equally high metabolic rates in the cold, parasitized mice cannot maintain body heat as well as control mice. This difference may be explained by reduced body fat or fur grooming, both of which have previously been observed in rodents exposed to parasites (Kristan and Hammond, 2000; Kristan and Hammond, 2001; Raiczyk and Hall, 1988; Zajac and Williams, 1981). The temperature loss may also be due to the non-significant tendency (P=0.12) for parasitized mice to have reduced MR_{max}, as suggested by the data shown in Fig. 3E. However, this correlation may also be explained if temperature loss in infected mice during the trial led to inaccurate MR_{max} measures. Finally, the changes in body temperature during cold exposure may indicate that parasitized mice are more willing to enter brief torpor under cold conditions (Hill, 1983; Tannenbaum and Pivorun, 1988). This could be an adaptive plastic behavior employed by infected mice to avoid situations that require sustained high energy output (Hill, 1983; Vogt and Lynch, 1982).

The results seen here mirror those found in many studies of parasitism in rodents. Namely, organ damage often occurs in infected rodents but does not translate to impacts for metabolism or performance (Kristan, 2002; Kristan and Hammond, 2001; Meagher, 1998; Meagher and O'Connor, 2001). The lack of pronounced energetic detriments of parasitism suggests two possible explanations. First, parasitism in rodents in general, and by *S. douthitti* in deer mice in particular, may have small impacts on host energetics, performance and fitness. While non-natural hosts infected with *S. douthitti* suffer high fitness costs (i.e. mortality), some natural hosts tolerate higher parasite loads and show reduced pathology (Zajac and Williams, 1981). The energetic cost of most parasites is largely unknown. Bot flies infesting *Peromyscus leucopus* consume only ~1% of their host's energy budget (Munger and Karasov, 1994). This degree of impact is unlikely to hinder a host. However, measuring the full energetic cost of parasite damage is more difficult (Booth et al., 1993; Holmes and Zohar, 1990). In addition, apparently small effects of parasites may be exacerbated when the host faces additional stressors (i.e. caloric restriction, cold acclimation, multiple parasites) (Kristan and Hammond, 2001; Wiger, 1977; Zuk, 1987). Therefore, it is problematic to conclude from lab-based research that parasites have no impact on host energetics or fitness in the natural environment.

The second potential explanation involves phenotypic plasticity to compensate for the costs of organ damage (King and Murphy, 1985). With respect to the *a priori* predictions for *S. douthitti*-infected deer mice, it appears that mice may largely compensate for the costs of infection through plasticity in physiology and/or morphology, leading to few alterations in metabolic measures. Deer mice have markedly flexible metabolic rates. Cold-induced maximal metabolic rate changes with season and increases during cold acclimation, even after a single exposure to cold (Chappell and Hammond, 2004; Hayes, 1989; Hayes and Chappell, 1986; Heimer and Morrison, 1978; Hill, 1983; Rezende et al., 2004). These increases in MR_{max} are typically correlated with increases in energy consumption (Hammond and Kristan, 2000; Hammond et al., 2001; Koteja, 1996a), indicating that aerobic capacity is not strictly constrained but is tuned to environmental demands. It is possible, therefore,

that the infected deer mice in this study were able to adjust aspects of their physiology or behavior to maintain the same metabolic rates. Altered thermoregulation *via* increased use of torpor (see above) may be a compensatory mechanism used by deer mice to reduce parasitic impacts on metabolic rates.

The costs of parasitism may partly be compensated for by alteration of GI tract morphology. In other studies, infection by *S. douthitti* typically leads to an inflammatory response in the intestines and diarrhea and blood in the feces due to the presence and action of parasite eggs (Zajac and Williams, 1981). The parasitized deer mice in this study also exhibited some degree of diarrhea and blood in feces (L.E.S., personal observation), which may indicate that nutrient and/or water uptake by the intestines were damaged by *S. douthitti* eggs. Despite this potential organ damage, digestive efficiency recorded here did not differ between treatments. Potentially, increases in the mass of the GI tract in infected animals compensated for reduced digestive function in the intestines, ultimately allowing equivalent levels of digestive efficiency to those of uninfected mice. GI flexibility has previously been recorded in response to reduced GI function in lab mice (Kristan, 2002; Kristan and Hammond, 2000; Kristan and Hammond, 2001), providing precedence for such a response. In addition, previous studies have demonstrated that deer mice show marked flexibility in the mass and length of their GI tracts in response to increased energy demands or decreased food quality (Green and Millar, 1987; Hammond and Kristan, 2000; Hammond et al., 2001; Koteja, 1996a).

Differential investment in organs would be an interesting future area of research in this host–parasite system. Primarily, the plasticity suggested by data collected here needs to be confirmed by further study. In addition, increased investment in GI organs may demand decreased investment in other organs. Measurement of multiple organ systems may provide evidence for additional morphological plasticity.

Compensation does not appear to have involved changes in activity or behavior. Deer mice often increase daily energy expenditure when faced with a stressor (e.g. Hammond and Kristan, 2000; Koteja, 1996a; Millar, 1979). There was no indication in this study that parasitized deer mice increased food consumption or used body stores (i.e. mass) for compensation. Lab mice have been shown to have reduced body fat when parasitized, which may be due to use of energy stores (Kristan and Hammond, 2000; Kristan and Hammond, 2001). Body composition was not analyzed in this study, but it may be worth investigating. Finally, parasitized deer mice in this study did not conserve energy by reducing their wheel activity, which was at levels similar to that in other studies of deer mice (Chappell et al., 2004).

It is important to note that the potential compensation recorded here may only be possible in the lab under benign settings, or may be possible in the wild, but only with a survival or reproductive cost. Data on host life histories and data from the field would provide valuable information to consider more fully the results found in this study.

By examining a diversity of interrelated host phenotypes, this study provides a fairly holistic view of the direct and indirect impacts of schistosome infection on deer mice. The results suggest that deer mice may compensate for the costs incurred by parasitism through physiological plasticity, largely alleviating the ultimate fitness impacts. In natural and lab settings, deer mice appear to employ a number of phenotypic plasticities to cope with environmental stressors (e.g. Chappell and Hammond, 2004; Kalcounis-Rueppell et al., 2002; Hammond and Kristan, 2000). Phenotypic plasticity may represent an important mechanism by which animals adjust to changes in the environment. Accordingly, the relationship between changes in an animal's condition due, for example, to infection with a parasite, and the animal's phenotype and fitness may not be straightforward.

Deer mice were provided courtesy of a Material Transfer Agreement with B. Hjelle (University of New Mexico). Animal care was provided by R. Ricci and F. Gurule of UNM's Biology Animal Resource Facility. D. Daniell (Butler University) provided *Stagnicola elodes* and *Schistosomatium douthitti*. Training and resources for the parasitology techniques were generously provided by D. Daniell, E. S. Loker and L. Hertel. D. Kristan, H. Lease, R. Ricci and B. Wolf provided guidance on data collection. Valuable discussion of the research was provided by K. Hammond, J. Hayes, A. Kodric-Brown and D. Kristan. I thank J. Brown, J. Hayes, A. Kodric-Brown, J. Millar and two anonymous reviewers for comments on previous versions of this manuscript. While conducting the research and preparing the manuscript, I was supported by an NSF Graduate Research Fellowship.

References

Anderson, R. M. and May, R. M. (1978). Regulation and stability of host–parasite population interactions. I. Regulatory processes. *J. Anim. Ecol.* **47**, 219-247.

Anderson, R. M. and May, R. M. (1982). Coevolution of hosts and parasites. *Parasitology* **85**, 411-426.

Arneberg, P., Folstad, I. and Karter, A. J. (1996). Gastrointestinal nematodes depress food intake in naturally infected reindeer. *Parasitology* **112**, 213-219.

Boonstra, R., Krebs, C. J. and Beacham, T. D. (1980). Impact of botfly parasitism on *Microtus townsendii* populations. *Can. J. Zool.* **58**, 1683-1692.

Booth, D. T., Clayton, D. H. and Block, B. A. (1993). Experimental demonstration of the energetic cost of parasitism in free-ranging hosts. *Proc. R. Soc. Lond. B Biol. Sci.* **253**, 125-129.

Botten, J., Ricci, R. and Hjelle, B. (2001). Establishment of a deer mouse (*Peromyscus maniculatus rufinus*) breeding colony from wild-caught founders: comparison of reproductive performance of wild-caught and laboratory-reared pairs. *Comp. Med.* **51**, 314-318.

Bruce, J. I., Ruff, M. D., Belusko, R. J. and Werner, J. K. (1972). *Schistosoma mansoni* and *Schistosoma japonicum*: utilization of amino acids. *Int. J. Parasitol.* **2**, 425-430.

Chappell, M. A. and Hammond, K. A. (2004). Maximal aerobic performance of deer mice in combined cold and exercise challenges. *J. Comp. Physiol. B* **174**, 41-48.

Chappell, M. A., Garland, T., Jr, Rezende, E. L. and Gomes, F. R. (2004). Voluntary running in deer mice: speed, distance, energy costs and temperature effects. *J. Exp. Biol.* **207**, 3839-3854.

Crews, A. E. and Yoshino, T. P. (1989). *Schistosoma mansoni*: effect of infection on reproduction and gonadal growth in *Biomphalaria glabrata*. *Exp. Parasitol.* **68**, 326-334.

Cunningham, E. J., Tierney, J. F. and Huntingford, F. A. (1994). Effects of the cestode *Schistocephalus solidus* on food intake and foraging decisions in the 3-spined stickleback, *Gasterosteus aculeatus*. *Ethology* **97**, 65-75.

Fordyce, J. A. (2006). The evolutionary consequences of ecological

interactions mediated through phenotypic plasticity. *J. Exp. Biol.* **209**, 2377-2383.
Freeland, W. J. (1981). Parasitism and behavioural dominance among male mice. *Science* **213**, 461-462.
Fuller, C. A. and Blaustein, A. R. (1996). Effects of the parasite *Eimeria arizonensis* on survival of deer mice (*Peromyscus maniculatus*). *Ecology* **77**, 2196-2202.
Goater, C. P. and Ward, P. I. (1992). Negative effects of *Rhabdias bufonis* (Nematoda) on the growth and survival of toads (*Bufo bufo*). *Oecologia* **89**, 161-165.
Green, D. A. and Millar, J. S. (1987). Changes in gut dimensions and capacity of *Peromyscus maniculatus* relative to diet quality and energy needs. *Can. J. Zool.* **65**, 2159-2162.
Gregory, R. D. (1991). Parasite epidemiology and host population growth: *Heligmosomoides polygyrus* (Nematoda) in enclosed wood mouse populations. *J. Anim. Ecol.* **60**, 805-821.
Hammond, K. A. and Kristan, D. M. (2000). Responses to lactation and cold exposure by deer mice (*Peromyscus maniculatus*). *Physiol. Biochem. Zool.* **73**, 547-556.
Hammond, K. A., Konarzewski, M., Torres, R. M. and Diamond, J. (1994). Metabolic ceilings under a combination of peak energy demands. *Physiol. Zool.* **67**, 1479-1506.
Hammond, K. A., Szewczak, J. and Krol, E. (2001). Effects of altitude and temperature on organ phenotypic plasticity along an altitudinal gradient. *J. Exp. Biol.* **204**, 1991-2000.
Hayes, J. P. (1989). Altitudinal and seasonal effects on aerobic metabolism of deer mice. *J. Comp. Physiol. B* **159**, 453-459.
Hayes, J. P. and Chappell, M. A. (1986). Effects of cold acclimation on maximum oxygen consumption during cold exposure and treadmill exercise in deer mice, *Peromyscus maniculatus*. *Physiol. Zool.* **59**, 473-481.
Heimer, W. and Morrison, P. (1978). Effects of chronic and intermittant cold exposure on metabolic capacity of *Peromyscus* and *Microtus*. *Int. J. Biometeorol.* **22**, 129-134.
Hill, R. W. (1983). Thermal physiology and energetics of *Peromyscus*: ontogeny, body temperature, metabolism, insulation, and microclimatology. *J. Mamm.* **64**, 19-37.
Holmes, J. C. and Zohar, S. (1990). Pathology and host behaviour. In *Parasitism and Host Behaviour* (ed. C. J. Barnard and J. M. Behnke), pp. 34-63. New York: Taylor & Francis.
Kagan, I. G. and Meranze, D. R. (1957). The histopathology of the liver in mice experimentally infected with *Schistosomatium douthitii*. *J. Infect. Dis.* **100**, 32-39.
Kagan, I. G. and Meranze, D. R. (1958). The histopathology of experimental infections in mice with *Schistosomatium douthitti*. *Am. J. Trop. Med. Hyg.* **7**, 285-293.
Kalcounis-Rueppell, M. C., Millar, J. S. and Herdman, E. J. (2002). Beating the odds: effects of weather on a short-season population of deer mice. *Can. J. Zool.* **80**, 1594-1601.
King, J. R. and Murphy, M. E. (1985). Periods of nutritional stress in the annual cycles of endotherms: fact or fiction? *Am. Zool.* **25**, 955-964.
Koteja, P. (1996a). Limits to the energy budget in a rodent, *Peromyscus maniculatus*: does gut capacity set the limit? *Physiol. Zool.* **69**, 994-1020.
Koteja, P. (1996b). Limits to the energy budget in a rodent, *Peromyscus maniculatus*: the central limitation hypothesis. *Physiol. Zool.* **69**, 981-993.
Kristan, D. M. (2002). Effects of intestinal nematodes during lactation: consequences for host morphology, physiology and offspring mass. *J. Exp. Biol.* **205**, 3955-3965.
Kristan, D. M. and Hammond, K. A. (2000). Combined effects of cold exposure and sub-lethal intestinal parasites on host morphology and physiology. *J. Exp. Biol.* **203**, 3495-3504.
Kristan, D. M. and Hammond, K. A. (2001). Parasite infection and caloric restriction induce physiological and morphological plasticity. *Am. J. Physiol.* **281**, R502-R510.
Loeb, W. F. and Quimby, F. W. (1989). *The Clinical Chemistry of Laboratory Animals.* New York: Pergamon Press.
Malek, E. A. (1977). Geographical distribution, hosts, and biology of *Schistosomatium douthitti* (Cort, 1924) Price, 1931. *Can. J. Zool.* **55**, 661-671.
Meagher, S. (1998). Physiological responses of deer mice (*Peromyscus maniculatus*) to infection with *Capillaria hepatica* (Nematoda). *J. Parasitol.* **84**, 1112-1118.
Meagher, S. and O'Connor, T. P. (2001). Population variation in the metabolic response of deer mice to infection with *Capillaria hepatica* (Nematoda). *Can. J. Zool.* **79**, 554-561.
Millar, J. S. (1979). Energetics of lactation in *Peromyscus maniculatus*. *Can. J. Zool.* **57**, 1015-1019.
Millar, J. S. (1985). Life cycle characteristics of *Peromyscus maniculatus nebrascensis. Can. J. Zool.* **63**, 1280-1284.
Munger, J. C. and Karasov, W. H. (1994). Costs of bot fly infection in white-footed mice: energy and mass flow. *Can. J. Zool.* **72**, 166-173.
Neuhaus, P. (2003). Parasite removal and its impact on litter size and body condition in Columbian ground squirrels (*Spermophilus columbianus*). *Proc. R. Soc. Lond. B* **270**, S213-S215.
Piersma, T. and Lindström, A. (1997). Rapid reversible changes in organ size as a component of adaptive behaviour. *Trends Ecol. Evol.* **12**, 134-138.
Podesta, R. B. and Mettrick, D. F. (1976). Pathophysiology and compensatory mechanisms in a compatible host–parasite system. *Can. J. Zool.* **54**, 694-703.
Poirier, S. R., Rau, M. E. and Wang, X. H. (1995). Diel locomotory activity of deer mice (*Peromyscus maniculatus*) infected with *Trichinella nativa* or *Trichinella pseudospiralis*. *Can. J. Zool.* **73**, 1323-1334.
Price, H. F. (1931). Life history of *Schistosomatium douthitti* (Cort). *Am. J. Hyg.* **13**, 685-727.
Raiczyk, G. B. and Hall, J. C. (1988). *Schistosomatium douthitti*: biochemical and morphological effects of an experimental infection in mice. *Exp. Parasit.* **65**, 187-201.
Rau, M. E. (1983a). Establishment and maintenance of behavioural dominance in male mice infected with *Trichinella spiralis*. *Parasitology* **86**, 319-322.
Rau, M. E. (1983b). The open-field behaviour of mice infected with *Trichinella spiralis*. *Parasitology* **86**, 311-318.
Rau, M. E. and Putter, L. (1984). Running responses of *Trichinella spiralis*-infected CD-1 mice. *Parasitology* **89**, 579-583.
Rezende, E. L., Chappell, M. A. and Hammond, K. A. (2004). Cold-acclimation in *Peromyscus*: temporal effects and individual variation in maximum metabolism and ventilatory traits. *J. Exp. Biol.* **207**, 295-305.
Rosenmann, M. and Morrison, P. (1974). Maximum oxygen consumption and heat loss facilitation in small homeotherms by He-O_2. *Am. J. Physiol.* **226**, 490-495.
Schall, J. J., Bennett, A. F. and Putnam, R. W. (1982). Lizards infected with malaria: physiological and behavioral consequences. *Science* **217**, 1057-1059.
Schlichting, C. D. and Pigliucci, M. (1998). *Phenotypic Evolution: A Reaction Norm Perspective.* Sunderland: Sinauer Assoc., Inc.
Starck, J. M. (1999). Structural flexibility of the gastro-intestinal tract of vertebrates – implications for evolutionary morphology. *Zool. Anzeiger* **238**, 87-101.
Stjernholm, R. L. and Warren, K. S. (1974). *Schistosoma mansoni*: utilization of exogenous metabolites by eggs in vitro. *Exp. Parasitol.* **36**, 222-232.
Symons, L. E. A. (1985). Anorexia: occurrence, pathophysiology and possible causes in parasitic infections. *Adv. Parasitol.* **24**, 103-133.
Tannenbaum, M. G. and Pivorun, E. B. (1988). Seasonal study of daily torpor in southeastern *Peromyscus maniculatus* and *Peromyscus leucopus* from mountains and foothills. *Physiol. Zool.* **61**, 10-16.
Tocque, K. (1993). The relationship between parasite burden and host resources in the desert toad (*Scaphiopus couchii*), under natural environmental conditions. *J. Anim. Ecol.* **62**, 683-693.
Tracy, C. R. and Diamond, J. (2005). Regulation of gut function varies with life-history traits in chuckwallas (*Sauromalus obesus*: Iguanidae). *Physiol. Biochem. Zool.* **78**, 469-481.
Vincent, J.-L., Dubois, M.-J., Navickis, R. J. and Wilkes, M. M. (2003). Hypoalbuminemia in acute illness: is there a rationale for intervention? A meta-analysis of cohort studies and controlled trials. *Ann. Surg.* **237**, 319-334.
Vogt, F. D. and Lynch, G. R. (1982). Influence of ambient temperature, nest availability, huddling, and daily torpor on energy expenditure in the white-footed mouse *Peromyscus leucopus*. *Physiol. Zool.* **55**, 56-63.
Wiger, R. (1977). Some pathological effects of endoparasites on rodents with special reference to the population ecology of microtines. *Oikos* **29**, 598-606.
Withers, P. C. (1977). Measurement of $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and evaporative water loss with a flow-through mask. *J. Appl. Physiol.* **42**, 120-123.
Zajac, A. M. and Williams, J. F. (1981). The pathology of infection with *Schistosomatium douthitti* in the laboratory mouse and the meadow vole, *Microtus pennsylvanicus*. *J. Comp. Physiol.* **91**, 1-10.
Zuk, M. (1987). The effects of gregarine parasites on longevity, weight loss, fecundity and developmental time in the field crickets *Gryllus veletis* and *G. pennsylvanicus*. *Ecol. Entomol.* **12**, 349-354.