The Journal of Experimental Biology 205, 3955–3965 (2002)
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JEB4325

Effects of intestinal nematodes during lactation: consequences for host morphology, physiology and offspring mass

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Accepted 16 September 2002

Summary

Sublethal parasites are often assumed to have no detrimental effects on their host. However, the sublethal intestinal nematode *Heligmosomoides polygyrus* affects both the morphology and the physiology of its laboratory mouse (*Mus musculus*) host and therefore has the potential to affect host life history. The objectives of the present study were to determine (1) whether lactating and non-lactating mice responded similarly to experimental infection with *H. polygyrus* and (2) whether the changes in morphology and physiology that occurred with parasite infection affected host reproductive performance. Parasitized mice had greater whole body mass as a result of greater lean mass compared with unparasitized mice. Parasitized mice had larger organs (spleen, stomach, cecum and small intestine) and a diminished rate of glucose transport by the small intestine compared with unparasitized mice. Lactating mice had larger organs (liver, kidney, spleen, heart, stomach, large intestine, cecum and small intestine), lean mass and whole body mass, but a similar rate of glucose transport compared with virgin mice. Resting metabolism increased with lactation but not with parasitism. Lactating and non-lactating mice responded similarly to parasite infection for most measured variables. Production of large litters was followed by production of small litters for parasitized but not unparasitized females. After adjusting for parity and litter size, parasitized mothers produced female pups that were 6% smaller at weaning than female pups from unparasitized mothers, but there was no effect of maternal parasite infection on mass at weaning for male pups. Other measures of reproductive output were not affected by parasite infection.

Key words: lactation, phenotypic plasticity, parasites, host reproduction, offspring mass, *Heligmosomoides polygyrus*, nematode.

Introduction

Demands such as lactation and parasitism can elicit phenotypic plasticity of organ structures and metabolism (Piersma and Lindström, 1997; Hammond et al., 1996a,b; Kristan and Hammond, 2000), and animals can use phenotypically plastic responses, along with their physiological reserves such as body fat or extra capacity of organs, to endure demanding conditions. Lactation in laboratory mice (*Mus musculus*, Swiss-Webster strain) induces changes in organ morphology, small intestine function, resting metabolic rate and body composition (Hammond et al., 1994, 1996b). Laboratory mice also adjust several physiological and morphological variables when infected with the sublethal intestinal nematode *Heligmosomoides polygyrus* (Liu, 1965; Cypess et al., 1988; Kristan and Hammond, 2000, 2001). For example, parasitized mice exhibited no change in digestible food intake but used physiological reserves (as shown by decreased fat content) and had a greater resting metabolism compared with unparasitized mice (Kristan and Hammond, 2000). Effects of parasitism on lactation by mammalian hosts is not well studied despite the fact that pregnant and lactating mice have decreased immunocompetence compared with that of non-reproducing mice (e.g. Ferguson et al., 1982; Sulila and Mattsson, 1990; Medina et al., 1993).

It is useful to understand how multiple simultaneous demands affect morphological and physiological responses. Although some demands occur serially, animals typically face multiple, simultaneous demands in nature; for example, parasite infection and reproduction. Some potential consequences of changes in resource allocation within an individual resulting from concurrent parasitism and lactation include increased morbidity or mortality, behavioral changes, changed organ morphology and function, or a shift in life-history traits such as host reproductive output. Because many wild animals encounter parasites during their lives, it is important to know if there is a fitness consequence of parasitism during host response. Also, because response to parasitism can affect the host's response to a second demand (Carlomagno et al., 1987; Banerjee et al., 1999; Kristan and Hammond, 2001), it is also important to understand if the demands of parasitism and lactation act independently or synergistically.

Effects of ectoparasites on host reproduction have been demonstrated for a number of species (e.g. Richner and Heeb, 1995; Van Vuren, 1996; Richner and Tripet, 1999), and, more recently, the physiological costs of sublethal endoparasite infection, and the consequences of these costs to host reproduction and other life-history traits, have also been addressed (Kyriazakis et al., 1996). Some researchers found no effect of sublethal endoparasites on host reproduction (Munger and Karasov, 1991), whereas others revealed negative effects on host reproduction (Feore et al., 1997); for example, small rodents with a naturally occurring infection of cowpox virus increased the time to first-litter production (Feore et al., 1997), which may affect lifetime reproductive success in short-lived rodents.

The objective of my study was to compare how infection with *H. polygyrus* affected host morphology and physiology in the laboratory mouse during lactation and non-reproduction, and, for reproducing mice, to determine if there were consequences for host reproductive output. I hypothesized that (1) the effects of parasitism would be greater during lactation than for virgin mice and (2) parasitized mice would experience differential resource allocation (evidenced by changes in morphology and physiology) that would result in changes in reproductive output compared with unparasitized mice. To examine these hypotheses, I conducted two experiments. First, I designed a lactation experiment to examine changes in resting metabolism, organ morphology, body composition and small intestine function during lactation. I measured these variables at peak lactation, approximately 15 days post-partum, when the energetic demand to the mother is at its greatest (Hammond et al., 1994). Based on previous studies (Kristan and Hammond, 2000, 2001), I predicted that parasitized lactating mice would have a greater decrease in small intestine function, greater resting metabolism, less body fat and greater lean mass than lactating mothers without parasites. Second, I conducted a reproductive-output experiment where I used continuously mated pairs of mice (some with parasitized females and some with unparasitized females) to examine reproductive output (e.g. litter size and offspring mass). I predicted that parasitized mothers would have an increased time to first-litter production (Feore et al., 1997), longer inter-litter intervals, more pup loss from birth to weaning, more unsuccessful litters, and smaller offspring than unparasitized mothers, because energy normally invested in offspring would be used in response to parasite infection or, possibly, less energy would be absorbed when infected with intestinal parasites.

Materials and methods

Two experiments were performed to test the effects of parasitism on (1) lactation performance and (2) reproductive output. For the lactation experiment, 20 virgin 50–90-day-old Swiss-Webster female *Mus musculus* L. mice (unparasitized, N=10; parasitized, N=10) and 22 reproductive female mice (unparasitized, N=11; parasitized, N=11) were used. Twenty-two male mice were each housed individually in the same cage as one female for 19 days and were then removed from the cage. For the reproductive-output experiment, both unparasitized (N=15) and parasitized (N=19) females were continuously housed with males. Mated pairs remained together for 8 months, the approximate maximum duration of infection with *Heligmosomoides polygyrus* (Dujardin 1845) in laboratory mice (Ehrenford, 1954). Females were checked bi-monthly for the presence of *H. polygyrus* eggs in their feces (Bowman, 1995) to ensure that they remained infected. For pairs with parasitized females, some females cleared the infection prior to 8 months, at which time the pair was removed from the experiment. For both experiments, mice were housed in 27 cm×21 cm×14 cm polypropylene cages and were given food and water *ad libitum*.

Mouse infection procedures

H. polygyrus infective-stage larvae (L_3) were cultivated from non-experimental mice. Parasitized mice from both experiments were infected with 300±11 L_3 suspended in tapwater as described by Kristan and Hammond (2000). Unparasitized mice were given an approximately equal volume of tapwater. For both experiments, parasitized females were checked for parasite eggs using a modified McMaster technique (Bowman, 1995) at 14 days post-infection (PI) to be sure that they were infected prior to mating, and unparasitized females were checked to be sure they were not infected. Thereafter, females from the reproductive-output experiment were checked for the presence of infection at the weaning of each litter or every 3 weeks if litters were not successfully produced or weaned. Males from mated pairs were also checked every 2–6 weeks to determine if the infection was transferred from the female to the male. At weaning, a subset of pups was also checked for the presence of parasites.

Lactation experiment

Digestive efficiency measures

Mice were maintained at 14 h:10 h L:D, 23°C and initially fed standard rodent diet (LabDiet® 5001, Purina Mills, Inc., St Louis, MO, USA). On day 33 PI, mice were switched to a high carbohydrate diet (Custom Karasov diet, ICN Nutritional Biochemicals, Cleveland, OH, USA: 55% sucrose, 15% casein, 7% cottonseed oil, 2% brewer's yeast, 4% salt mix, 1% vitamin and 16% non-nutritive bulk; Diamond and Kasarov, 1984) to determine maximal glucose-transport capacity. On day 47–49 PI, body mass, food-intake rate (I; measured in g day^{-1}) and fecal output rate (O; measured in g day^{-1}) were measured (Hammond and Diamond, 1992). Average percentage apparent dry-matter digestibility (i.e. digestive efficiency) was calculated as $[(I–O)I^{-1}]\times100$ for days 47–49 PI.

Females from the reproductive-output experiment were maintained on standard laboratory diet for the first 14 days PI. On day 14, a male was added to their cage and the diet was switched to the Custom Karasov diet to allow comparisons between reproductive output measures for mated pairs and physiological and morphological measures obtained from mice in the lactation experiment.

Resting metabolic rate

Resting metabolic rate (RMR) of non-postabsorptive mice was measured as oxygen consumption ($\dot{V}_{O_2}$; measured in ml min^{-1}) using open-flow respirometry starting between 08.00 h and 09.30 h on day 49 PI. Dried air (Drierite, W. A. Hammond Drierite Co., Xenia, OH, USA) entered a 600 ml Plexiglas chamber housed in a dark cabinet (30±1°C; within the *M. musculus* thermoneutral zone; Hart, 1971) at 650–700 ml min^{-1} from mass-flow controllers (Porter Instrument Company, Inc., Hatfield, PA, USA). Air leaving the chamber was dried, scrubbed of CO_2 (soda lime) and re-dried before entering an Applied Electrochemistry S-3A/II oxygen analyzer (AEI Technologies, Pittsburgh, PA, USA) that was connected to a Macintosh computer. $\dot{V}_{O_2}$ was measured [using equation 4a of Withers (1977)] for 3 h, recorded every 5 s, and data was analyzed using customized software (WartHog Systems, M. A. Chappell, Riverside, CA, USA; custom software available at warthog.ucr.edu). Each mouse was sampled for approximately 2.5 h. RMR was calculated as the lowest 5-min interval of $\dot{V}_{O_2}$.

Organ morphology and body fat measures

Between 08.30 h and 11.30 h on day 50 PI, mice were anesthetized by intraperitoneal injection of 0.07 ml sodium pentobarbital (65 mg ml^{-1}). The small intestine was removed (see below) while mice were alive. Mice were euthanized by cutting the diaphragm and then the stomach, cecum, large intestine, heart, liver, spleen, pancreas and associated mesentery (for another experiment), kidneys and lungs were removed. Excess fat and connective tissue were trimmed from each organ and returned to the carcass. For the stomach, cecum and large intestine, each organ was weighed with and without contents (flushed with mammalian Ringer's solution; for composition, see Karasov and Diamond, 1983). The dry mass of the organs and the carcass after drying at 55–60°C (for 2 days for organs and for 2 weeks for the carcass) was measured.

The dried carcass was ground and lipids were extracted using petroleum ether (Goldfische apparatus; Labconco, Kansas City, MD, USA), and absolute fat mass and fat mass as a percentage of body mass were calculated. The fat content of all organs, except the small intestine, were measured together. Organ fat was removed by soaking organs in 10 ml aliquots of petroleum ether for six 24-h periods (pouring off ether at the end of 24 h and replacing it with fresh ether), and absolute fat mass and percentage organ fat were calculated. Total absolute fat mass was the sum of the masses of body and organ fat. Lean mass was calculated as initial whole body mass minus total absolute fat mass.

Small intestine morphology and glucose-uptake measurements

While the mouse was anesthetized, the small intestine was rinsed *in situ* with cold Ringer's solution, then excised and placed in cold oxygenated Ringer's solution (bubbled with 5% CO_2:95% O_2 at 2–3 l min^{-1}). The small intestine was cut into three regions of equal length (proximal, mid and distal), the wet mass of each region was measured and the three masses were added together to determine the total intestinal mass (corrected for mass of the parasites, as described below). Mucosal/submucosal tissue (hereafter called 'mucosa') was separated from muscularis/serosal tissue (hereafter called 'serosa') for two 1.5-cm sleeves per region (Diamond and Karasov, 1984). The dry mass:wet mass ratio was calculated for each sleeve, and the average of these ratios was used to calculate mucosal and serosal wet mass and dry mass of the entire small intestine (Diamond and Karasov, 1984; Hammond and Diamond, 1992). For parasitized mice, all adult *H. polygyrus* from the small intestine during rinsing, from intestinal sleeves used for mucosa/serosa measures and from remaining tissue were collected and counted (Kristan and Hammond, 2001). The number of worms on the sleeves used for glucose uptake was assumed to be the same as for adjacent sleeves used for mucosal scrapes, and this number was added to the number of worms directly counted to determine the final infection intensity. The wet mass of *H. polygyrus* (0.0002 g × the number of worms; Kristan and Hammond, 2001) was subtracted from the wet mass of the small intestine prior to the calculation of small intestine dry mass used in analyses. When each intestinal region was examined separately, parasite mass was subtracted only from the mass of the proximal region, because adult parasites occupy that portion of the small intestine almost exclusively (Bansemir and Sukhdeo, 1996).

The everted sleeve technique was used to measure carrier-mediated (sodium glucose transporter I, SGLT1) glucose uptake by the small intestine (Diamond and Karasov, 1984; Karasov and Diamond, 1983). Each region of the small intestine was everted so that the mucosa faced outwards. From each region, four 1.5-cm-long sleeves immediately adjacent to each other were cut: two sleeves for measuring relative mucosal and serosal mass, as described above, and two sleeves for measuring glucose uptake. To measure carrier-mediated (SGLT1) glucose uptake, everted sleeves were mounted on stainless steel rods, incubated for 2 min in 36°C Ringer's solution containing 50 mmol l^{-1} D-glucose and trace amounts of [^{14}C]D-glucose. The incubating solution also contained trace amounts of [^{3}H]L-glucose, which was used to correct for glucose in the adherent mucosal fluid and for passive uptake of D-glucose. The amount of isotope taken up by each sleeve was measured using liquid scintillation counting (LS 6500 scintillation system, Beckman, Fullerton, CA, USA) to determine glucose-uptake rates (mmol day^{-1} g wet mucosal tissue^{-1}). The average uptake rate of the two sleeves from each region was then calculated. The glucose-uptake capacity for each region of the small intestine was calculated by multiplying the mean glucose-uptake rate by the wet mucosal mass (g) of the region. The products of each region were added together to determine total glucose-uptake capacity for the entire small intestine.

Reproductive-output experiment

Litter production

Pairs were checked daily, beginning at 18 days post-mating, and seven variables were recorded: (1) the date that each litter

was produced, (2) the number of pups on the day the litter was born (natal litter size), (3) inter-litter intervals, calculated as the number of days between the birth of one litter and the birth of the next, (4) whether or not a litter was successfully weaned (litter success was designated as either '1', if at least one pup survived to 20 days old, or '0', if no pups survived to 20 days old), (5) the number of pups that died (either eaten by parents or found dead) between the first observation on the day of birth and weaning at 20 days old, (6) the number of pups weaned in each litter (weaning litter size) and (7) the sex ratio at weaning.

Litter and individual pup masses

Litters were weighed and pups were counted on days 5, 10, 15 and 20 after birth. On day 20 after birth, pups were removed from the parental cage and each pup was individually weighed and sexed. Pups from the second litter were checked for parasites to determine if pups contracted the infection from their mother.

Statistics

Lactation experiment

This experiment consists of two independent [parasite infection (parasitized, unparasitized) and reproductive status (lactating, virgin)] and numerous dependent variables (food intake, digestive efficiency, body mass, small intestine morphological variables, organ masses, body fat, glucose-uptake rate and capacity, and RMR). First, a multivariate analysis of variance (MANOVA), which tested for significant differences between treatments for all dependent variables together, was used. Because this MANOVA was significant (P<0.0001), the results from subsequent independent analyses of variance (ANOVAs) were used to determine which treatment and which dependent variables were statistically significant. For RMR data, because virgin mice had smaller body mass than lactating mice, analysis of covariance (ANCOVA), with whole body mass as a covariate, was used. Also, because parasitized mice had greater lean body mass than unparasitized mice, and lactating mice had a greater lean mass than virgin mice, ANCOVA with lean mass as the covariate was also used for RMR data.

Reproductive-output experiment

For this experiment, repeated measures ANOVA (RM ANOVA; Wilk's Lambda) was used to examine the effects of maternal parasite infection on reproductive output (inter-litter interval, success of weaning a litter, number of pups lost from birth to weaning, and sex ratio). Individual pup mass at weaning was analyzed with a nested ANCOVA (pups nested by maternal pair ID; independent variables: pup sex and maternal parasite treatment; covariates: parity and litter size, with factors tested against the between-pairs error term). The time to first litter production was tested with a t-test comparing parasitized pairs with unparasitized pairs. Regression was used to test for relationships between the first litter size and the subsequent inter-litter interval, between first litter size and second litter size, and for food intake and litter size separately for parasitized and unparasitized mice.

Results

Lactation experiment

All mice given parasites produced a mature infection, as evidenced by the presence of *H. polygyrus* eggs in host feces. However, of the 10 parasitized virgin mice, six cleared their infection prior to experimental measures and were therefore not included in data analysis. Unparasitized mice had litters of 6–12 pups (sample size in parentheses): six pups (1), nine pups (2), 10 pups (5), 11 pups (2) and 12 pups (1). Parasitized mice had litters of 6–11 pups: six pups (1), seven pups (1), eight pups (1), nine pups (1), 10 pups (4) and 11 pups (3).

Body mass and composition and organ masses

Whole body mass was 18% heavier for lactating mice than for virgin mice ($F_{1,32}$=39.9, P<0.0001) and 8% heavier for parasitized mice than for unparasitized mice ($F_{1,32}$=4.3, P=0.046; Fig. 1). *Post-hoc* analysis showed that the effect of parasites on whole body mass was significant for virgin but not for lactating mice. Total body fat did not differ with parasite treatment, but lean mass of parasitized mice was 10% heavier than for unparasitized mice ($F_{1,32}$=4.6, P=0.041). Total body fat was 53% less for lactating than for virgin mice ($F_{1,29}$=131.4, P<0.0001), and lean mass was 36% more for lactating than for virgin mice ($F_{1,29}$=23.1, P<0.0001). Percentage body fat was similar for parasitized and unparasitized mice (18%) but was 55% less for lactating than for virgin mice (11% and 25%, respectively; $F_{1,30}$=253.1, P<0.0001). Because body mass differed with both reproductive and parasite treatments, I used analysis of covariance (ANCOVA) to determine which dependent variables were affected. Body mass was a significant covariate for mass of liver, kidney, spleen, heart, lung, mucosa and small intestine, and for food intake, RMR and total glucose-uptake capacity. Therefore, I present least-squares means ± 1 S.E.M. for these variables. Body mass was not a significant covariate for mass of stomach, large intestine, cecum or serosa or for percentage mucosa, small intestine length, digestive efficiency or glucose-uptake rate, so I present the arithmetic mean ± 1 S.E.M. for these variables.

I used data for organ dry masses (including fat content) to examine the effects of each treatment on organ mass. Parasitized mice had heavier cecae (22%) and spleens (30%) but lighter stomachs (8%) than unparasitized mice (Table 1). Lactating mice had heavier stomachs (47%), cecae (47%), large intestines (60%), kidneys (8%), liver (22%) and hearts (22%), but lighter spleens (28%) and marginally lighter lungs (7%) than virgin mice (Table 1). Small intestine length was 5% longer for parasitized compared with unparasitized mice ($F_{1,32}$=5.5, P=0.026) and 11% longer for lactating compared with virgin mice ($F_{1,32}$=21.6, P<0.0001; Fig. 2). Similarly, small intestine dry mass (after adjusting for body mass and mass of the parasites) was 24% heavier for parasitized than

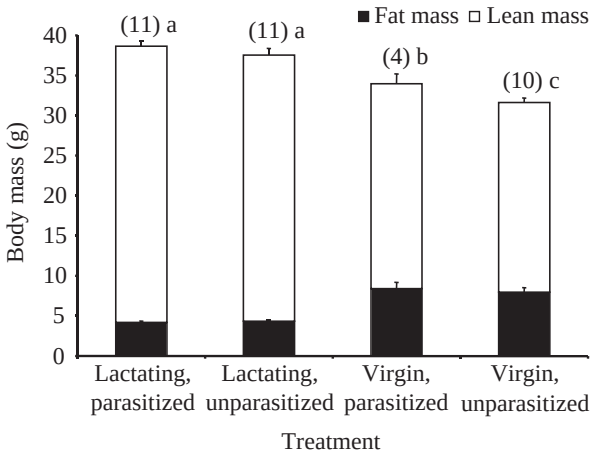

Fig. 1. Body mass and composition (fat *versus* lean mass) of parasitized and unparasitized mice that were either lactating or virgin. Error bars are 1 S.E.M. of whole body mass; sample sizes (*N*) are in parentheses above each bar; and similar letters indicate no significant difference at P=0.05.

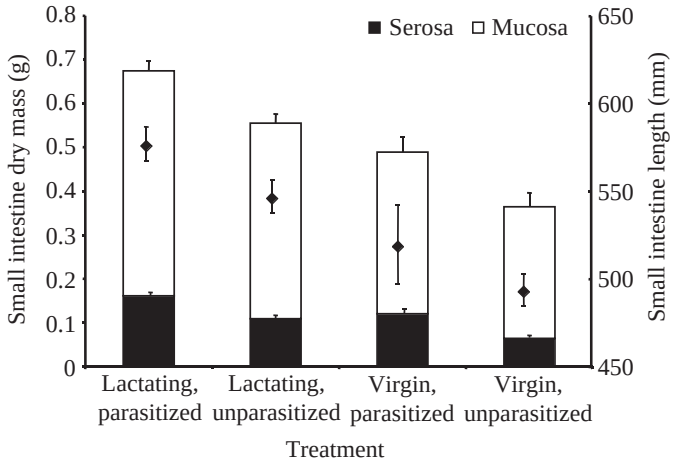

Fig. 2. Small intestine dry mass (+1 S.E.M.; on left *y*-axis shown by bars; partitioned into mucosal and serosal dry mass) and small intestine length (±1 S.E.M.; on right *y*-axis shown by diamonds) for parasitized and unparasitized mice that were either lactating or virgin. Sample sizes are as in Fig. 1.

unparasitized mice ($F_{1,29}$=17.8, P<0.0001) and 38% heavier for lactating compared with virgin mice ($F_{1,29}$=20.9, P<0.0001; Fig. 2). Dry mass per unit length of small intestine yielded qualitatively the same results as absolute dry mass, so I used absolute dry mass when examining each region of the small intestine.

Parasitized mice had 18% more mucosal and 61% more serosal tissue than did unparasitized mice ($F_{1,29}$=8.4, P=0.007 and $F_{1,30}$=28.8, P<0.0001, respectively; Fig. 2). Because parasitized mice had a disproportionate increase in serosal compared with mucosal tissue, the percentage mucosa was 6% less in parasitized than unparasitized mice ($F_{1,30}$=11.5, P=0.002). Mucosal and serosal dry mass both increased by approximately 45% with lactation ($F_{1,29}$=19.5, P<0.0001 and $F_{1,30}$=18.3, P<0.0001, respectively), but percentage mucosal mass was unchanged. When examining intestinal regions separately, parasitized mice had 38% heavier proximal and 15% heavier mid regions ($F_{1,31}$=16.7, P<0.0001 and $F_{1,31}$=9.2, P=0.005, respectively) but had a similar mass for the distal region compared with those of unparasitized mice (Fig. 3). Lactating mice had 45–65% heavier masses of all three small intestine regions compared with virgin mice (proximal: $F_{1,31}$=31.1, P<0.0001; mid: $F_{1,31}$=35.2, P<0.0001; distal: $F_{1,31}$=20.3, P<0.0001; Fig. 3). There were no significant interactions among treatments for mass of any intestinal region. Changes in the serosal and mucosal components of the regional masses also varied with treatment. Mucosal mass was heavier for lactating compared with virgin mice for each region (proximal by 68%: $F_{1,30}$=35.8, P<0.0001; mid by 81%: $F_{1,30}$=28.6, P<0.0001; distal by 37%: $F_{1,30}$=5.2, P=0.029), but parasite treatment did not affect mucosal mass of any region. For the parasite treatment, serosal mass increased by 219% in the proximal region ($F_{1,30}$=117.4, P<0.0001), 39% in the mid region ($F_{1,30}$=6.9, P=0.014) and 8% in the distal region (P>0.05). There were no significant interactions among treatments for components of regional masses.

Food intake and digestive efficiency

Parasitism had no effect on food intake, and digestive

Table 1. *Effects of parasitism and reproduction on organ dry mass of lactating and virgin mice either with or without parasites*

	Parasitized		Unparasitized		Parasite	Lactation
Organ	Lactating	Virgin	Lactating	Virgin	*P*	*P*
Stomach	0.060±0.002	0.043±0.003	0.069±0.002	0.046±0.002	0.041	<0.0001
Cecum	0.027±0.002	0.021±0.003	0.026±0.002	0.014±0.002	0.029	<0.0001
Large intestine	0.082±0.002	0.058±0.004	0.086±0.003	0.047±0.003	0.173	<0.0001
Spleen	0.025±0.002	0.038±0.003	0.022±0.002	0.027±0.003	0.002	0.004
Kidneys	0.118±0.003	0.108±0.004	0.113±0.002	0.104±0.004	0.164	0.040
Liver	0.795±0.026	0.669±0.038	0.787±0.023	0.795±0.026	0.382	0.001
Heart	0.041±0.001	0.034±0.001	0.041±0.001	0.034±0.001	0.986	<0.0001
Lung	0.041±0.001	0.045±0.002	0.040±0.001	0.042±0.001	0.138	0.056

Values are arithmetic or least-squares means ± 1 S.E.M. (see text for type of mean used for each variable).

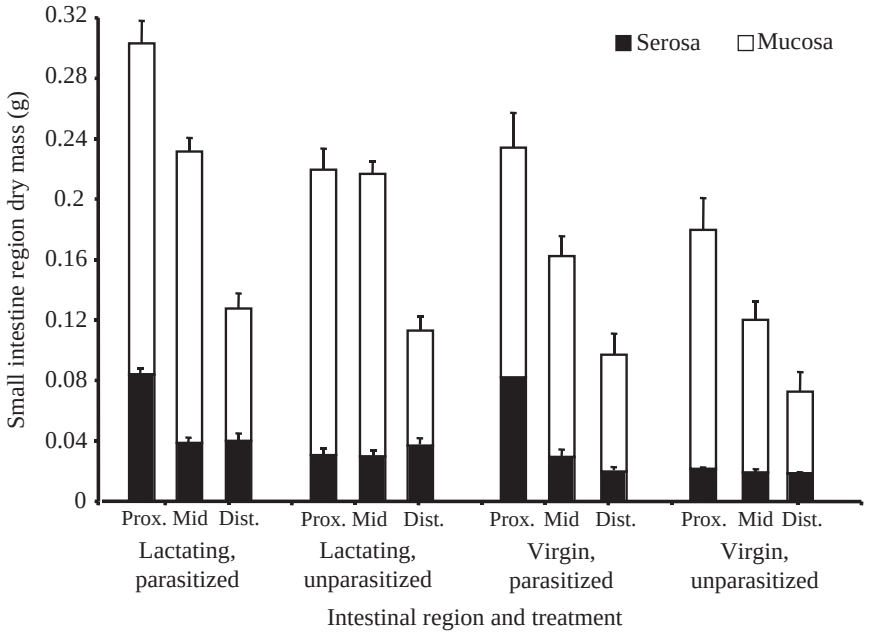

Fig. 3. Total, serosal and mucosal dry masses of three small intestine regions for parasitized and unparasitized mice that were either lactating or virgin. Error bars are +1 S.E.M. of total region dry mass, and sample sizes are as in Fig. 1.

efficiency was 79±0.4% for all mice regardless of treatment. After adjusting for body size, females at peak lactation (15 days post-partum) ate 174% more food than did virgin mice (virgin, parasitized=5.98±0.79 g day^{-1}; virgin, unparasitized=6.17±0.74 g day^{-1}; lactating, parasitized=16.29±0.54 g day^{-1}, lactating, unparasitized=16.98±0.49 g day^{-1}; $F_{1,29}$=160.8, P<0.0001). Food intake increased with larger litter sizes for unparasitized mice (r^2=0.364, P=0.049, N=11) and marginally for parasitized mice (r^2=0.360, P=0.051, N=11; Fig. 4).

Resting metabolic rate

Of the 22 lactating mice measured, four (three unparasitized and one parasitized) did not attain RMR conditions (i.e. did not show a lowered, steady rate of oxygen consumption) within 3 h of measuring and were thus excluded from analyses. Because whole body mass and lean body mass increased with infection and lactation, I examined the relationship among both mass variables and RMR. There was a significant relationship for whole body mass (r^2=0.67, P<0.0001, N=32) and lean body mass (r^2=0.64, P<0.0001, N=32) with RMR (Fig. 5). RMR did not change with parasite infection when corrected for either whole body mass or lean body mass (ANCOVA). RMR was 27% greater for lactating compared with virgin mice ($F_{1,27}$=4.9, P=0.036) when whole body mass was used as the covariate (least-square-adjusted mean ± 1 S.E.M.: virgin, parasitized=35.7±4.4 kJ day^{-1}; virgin, unparasitized=36.1±3.3 kJ day^{-1}; lactating, parasitized=47.4±3.1 kJ day^{-1}; lactating, unparasitized=43.8±3.2 kJ day^{-1}), but RMR did not differ between lactating and virgin mice when lean body mass was used as the covariate. For lactating mice, RMR was not affected by litter size or litter mass.

Glucose transport

Intestinal glucose-transport rate was normalized to wet mucosal mass of the small intestine. Average mass-specific glucose-uptake rate (mmol g^{-1} day^{-1}) was 17% lower for parasitized than for unparasitized mice ($F_{1,30}$=5.3, P=0.028) but did not differ between lactating and virgin mice (Fig. 6).

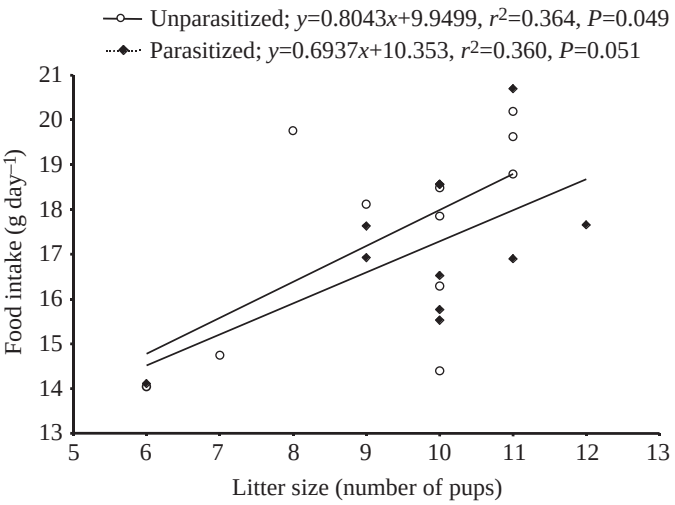

Fig. 4. Food intake for parasitized and unparasitized lactating mice for litter sizes ranging from 6 pups to 12 pups; each data point represents one individual.

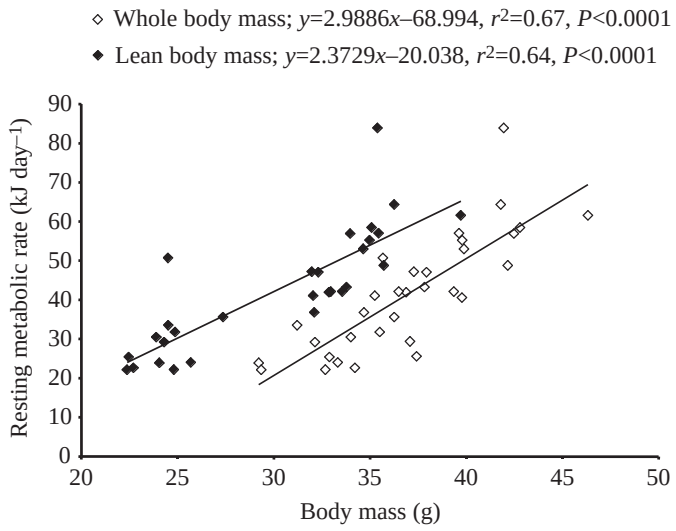

Fig. 5. Relationship between whole body mass and lean body mass with resting metabolic rate.

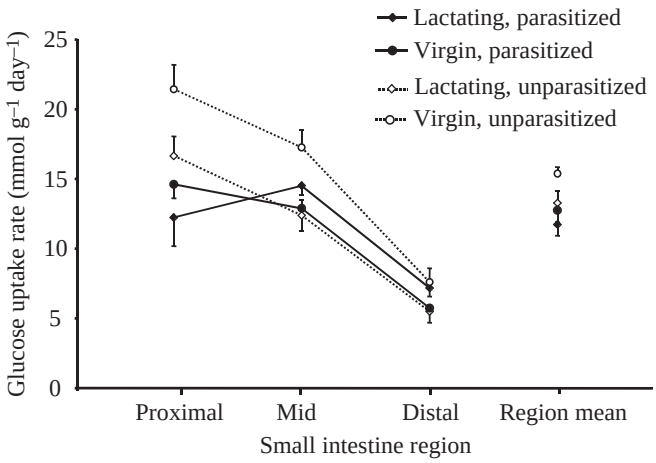

Fig. 6. Rate of glucose uptake for three small intestine regions and the mean rate for the entire small intestine for parasitized and unparasitized mice that were either lactating or virgin. Error bars are ±1 S.E.M., and sample sizes are as in Fig. 1.

However, because of increases in mucosal mass with parasitism, total glucose-uptake capacity (mmol day^{-1}) summed for the entire small intestine (adjusted to whole body mass) did not differ between parasitized and unparasitized mice (Fig. 7). There was no significant difference in total glucose-transport capacity between lactating and virgin mice.

I next examined each region separately to determine if parasites had only a localized effect in the proximal region and to determine if lactation affected the small intestine uniformly. Glucose-transport rate was 28% greater for parasitized than unparasitized mice in the proximal region ($F_{1,30}$=7.9, P=0.009), but there was no effect of parasite infection on glucose-transport rate of the mid or distal regions and no effect of lactation for any region (Fig. 6). There was a significant interaction for glucose-transport rate in the mid and distal regions ($F_{1,30}$=7.3, P=0.011 and $F_{1,30}$=5.4, P=0.027, respectively) because, for both regions, unparasitized lactating mice had lower glucose-transport rates than virgins but parasitized lactating mice had higher glucose-transport rates than virgins. Parasites had marginal effects on total glucose-transport capacity in the proximal (a 28% decrease; $F_{1,29}$=3.5, P=0.071) and distal regions (a 24% increase; $F_{1,30}$=3.6, P=0.068), and lactating mice had a 78% greater glucose-transport capacity in the distal region ($F_{1,30}$=24.9, P<0.0001) but no effect on other small intestine regions (Fig. 7). There was a significant interaction among treatments for the mid region because, when mice were parasitized, lactating mice had a greater glucose-transport capacity than virgin mice but the opposite was true for unparasitized mice ($F_{1,29}$=6.1, P=0.019).

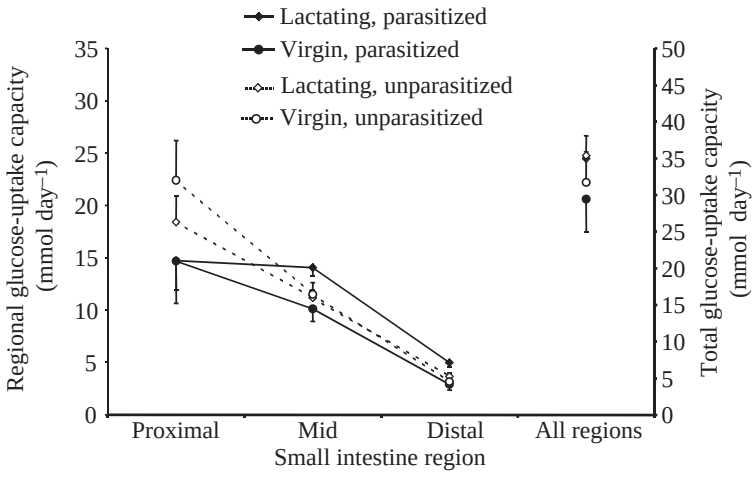

Fig. 7. Total glucose-uptake capacity adjusted for whole body mass for each small intestine region (left y-axis) and the entire small intestine (right y-axis) for parasitized and unparasitized mice that were either lactating or virgin. Error bars are +1 S.E.M., and sample sizes are as in Fig. 1.

Reproductive-output experiment

One female that was given parasites cleared the infection prior to producing her first litter, otherwise all infected females (N=18) had a mature parasite infection while producing at least one litter. The male from one pair became infected, but this pair was not significantly different from other pairs with uninfected males for any measured variable and so was included in analyses. None of the pups checked for *H. polygyrus* that were born to parasitized mothers were infected at weaning.

Litter production and success

Numbers of pairs included in repeated measures analyses varied because not all data were recorded for all pairs for every litter, and pairs produced different numbers of litters (Table 2). Maternal parasite infection did not affect the time to first litter production (N=18 parasitized, 15 unparasitized) or any of the variables in Table 2. Numbers of litters produced by parasitized pairs ranged from 1 to 8 (sample size in parentheses): one litter (2), two litters (2), three litters (3), four litters (3), five litters (3), seven litters (3) and eight litters (2). Numbers of litters produced by unparasitized pairs ranged from 2 to 10: two litters (1), three litters (1), six litters (1), seven litters (5), eight litters (2), nine litters (3) and 10 litters (1). Natal litter size decreased with increasing parity, starting at litter number 5 ($F_{5,10}$=4.1, P=0.028; Table 2) for both parasitized and unparasitized pairs. When the time to first litter production was included in the inter-litter interval analysis, mice produced their first litter more quickly than they produced subsequent litters ($F_{5,12}$=7.4, P=0.002; Table 2) but there were no other differences in inter-litter intervals with parity.

Pup mass

A total of 1243 pups were measured, of which 1091 were sexed and used in the analysis. The covariates litter size and parity were significant ($F_{1,22}$=5.7, P=0.027 and $F_{1,22}$=4.9, P=0.037, respectively). Pups from large litters were smaller than pups from small litters for both

Table 2. *Reproductive output from breeding pairs with either parasitized or unparasitized females*

Variable	No. of pairs	Litter number 1	2	3	4	5	6
Unparasitized							
Inter-litter interval	12	21.8±0.35	26.2±1.12	25.2±1.21	28.0±2.13	24.4±0.89	28.5±3.92
Natal litter size	11	9.2±0.83	10.3±0.94	10.2±1.19	10.1±0.93	9.0±1.18	7.0±1.07
Weaning litter size	13	8.8±0.83	9.8±0.79	7.9±1.01	8.5±1.32	6.5±1.24	6.9±1.17
Pup loss	3	1.5±0.32	0.7±0.42	1.5±0.75	1.2±0.65	1.2±0.57	0.7±0.44
Weaning success	13	1.0±0.06	1.0±0.00	0.9±0.09	0.9±0.12	0.8±0.12	0.8±0.12
Sex ratio	12	1.72±0.37	1.34±0.35	2.42±1.15	0.91±0.23	0.79±0.31	
Parasitized							
Inter-litter interval	6	21.3±0.50	24.2±1.58	26.2±1.72	26.2±3.01	25.5±1.25	34.5±5.55
Natal litter size	5	10.2±1.23	11.2±1.40	10.0±1.76	10.8±1.38	7.2±1.75	5.8±1.58
Weaning litter size	5	8.0±1.34	9.2±1.28	9.4±1.62	6.4±2.12	8.4±2.00	5.6±1.88
Pup loss	6	0.3±0.45	1.0±0.59	2.0±1.06	0.3±0.93	0.3±0.80	0.3±0.62
Weaning success	5	0.8±0.12	1.0±0.00	1.0±0.15	0.6±0.19	0.8±0.20	0.8±0.20
Sex ratio	5	1.07±0.27	1.28±0.26	2.52±0.85	1.09±0.17	1.14±0.23	

Values are least-squares means ± 1 S.E.M.

sexes combined (Fig. 8A,B) and for males and females separately (males: $F_{1,24}$=7.6, P=0.011; females: $F_{1,24}$=12.4, P=0.002; Fig. 8A,B). Pup mass increased with increasing parity, up to litter number 5 for all pups combined (Fig. 9), which was due to the effects of males ($F_{1,24}$=5.3, P=0.030) but not females.

After accounting for effects of litter size and parity, parasitized mothers had pups that were 4% smaller at weaning (20 days after birth) compared with pups from unparasitized mothers ($F_{1,22}$=4.4, P=0.049; Fig. 8A,B), and male pups were 2% larger than female pups ($F_{1,22}$=6.5, P=0.018; least-square-adjusted mean ± 1 S.E.M.: male pups, parasitized mother=8.8±0.1 g; male pups, unparasitized mother=9.1±0.1 g; female pups, parasitized mother=8.6±0.1 g; female pups, unparasitized mother=9.0±0.1 g). As a *post-hoc* analysis, I examined the effect of maternal parasite status for male and female pups separately. Parasitized females produced 6% smaller female pups than did unparasitized mothers ($F_{1,24}$=7.0, P=0.014), but maternal parasite status did not affect male pup mass at weaning ($F_{1,24}$=1.9, P=0.185).

To examine whether pups from parasitized mothers were smaller at ages 5 days, 10 days and 15 days, I calculated average pup mass (litter mass/litter size) for the first litter produced by mated pairs (parasitized mothers, N=15; unparasitized mothers, N=13). Average pup mass did not differ with maternal parasite treatment for pups at ages 5 days, 10 days or 15 days ($F_{1,26}$=3.0, P=0.095) using an RM ANOVA. However, the percentage differences in pup mass from parasitized and unparasitized mothers increased from day 5 (6%) to day 10 (7%) to day 15 (9%; day 5, parasitized mother: 2.95±0.12 g; day 5, unparasitized mother: 3.14±0.13 g; day 10, parasitized mother: 5.27±0.18 g; day 10, unparasitized mother: 5.65±0.19 g; day 15, parasitized mother: 6.91±0.24 g; day 15, unparasitized mother: 7.61±0.26 g).

Litter size and inter-litter intervals

Based on the first and second litters produced, pairs with larger litters had a longer subsequent inter-litter interval than did pairs with smaller litters for both parasitized (r^2=0.58, P=0.004, N=12) and unparasitized females (r^2=0.41, P=0.019, N=13). Current litter size was not affected by previous litter size for unparasitized mice, but parasitized mice that produced large first litters had small second litters, or, conversely, parasitized mice that produced small first litters had large second litters (r^2=0.42, P=0.022, N=12).

Discussion

Parasitism produced changes in morphology and physiology that were associated with decreased size of female offspring at weaning and effects of the first litter size on the second litter size. My study provides evidence of a link between parasite infection, phenotypic plasticity of morphology and physiology, and life history. Given that this experiment occurred in a laboratory setting with benign ambient temperature and food and water *ad libitum*, effects of maternal parasitism on offspring size may be even more prominent and important in nature.

Parasite infection during lactation

A change in resource allocation with parasitism, as evidenced by a change in body composition, suggests that infection with *H. polygyrus* affects energy use in the laboratory mouse despite no changes in energy input (food intake did not vary with parasite infection). Part of the change in body composition with parasitism resulted from an increase in mucosal mass of the small intestine, which is important because the mucosal layer is responsible for nutrient digestion and absorption. Unlike previous studies (Kristan and

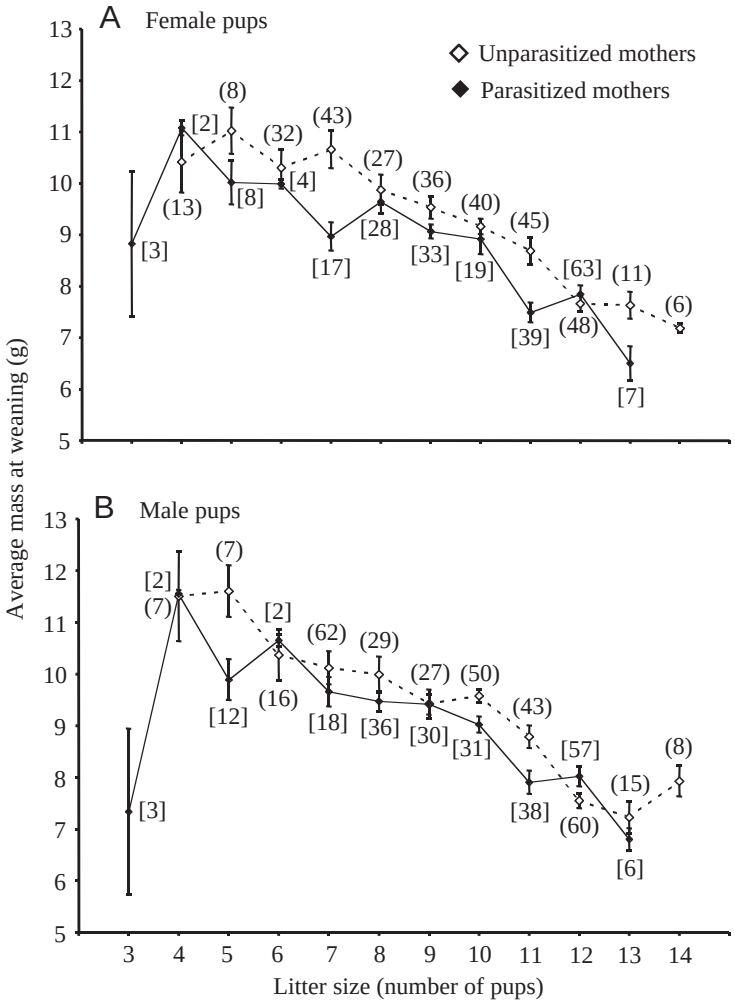

Fig. 8. Average pup mass at weaning (±1 S.E.M.) for litter sizes ranging from 3 pups to 14 pups for female (A) and male (B) pups of parasitized or unparasitized mothers. Number of pups (*N*) is shown in square brackets for parasitized mothers or in parentheses for unparasitized mothers.

Hammond, 2000, 2001), parasitized mice in this study were able to attain similar total glucose-transport capacity, despite a decreased rate of glucose transport per gram of tissue, by increasing the total mass of mucosal tissue. One explanation for these contradictory results may be that mice in this study were parasitized for approximately 26 days longer than in previous studies (Kristan and Hammond, 2000, 2001), and the potential negative effects of larval-stage parasites on mucosal tissue function may have been overcome. For example, if immune responses to larval parasites affect the function of small intestine tissue [perhaps by the proliferation of cells in the small intestine (Symons, 1965) yielding an accumulation of immature enterocytes rather than functional mucosal tissue] then the more time that has passed since the larval stages, the less *H. polygyrus* should impact on total glucose acquisition. As in previous studies (Kristan and Hammond, 2000, 2001), the effects of *H. polygyrus* on small intestine function reached beyond the site of worm occupation to other regions of the small intestine. This is likely to be an indirect effect of parasitism, possibly resulting from changes in the nutrient density of the ingesta, which results in 'nutrient spilling' into more distal parts of the small intestine that will then change the capacity of these regions for nutrient uptake.

In contrast to previous studies (Kristan and Hammond, 2000, 2001), after accounting for mass, resting metabolism did not change with parasite infection. This difference in response to parasitism again may reflect different experimental protocols. In previous work (Kristan and Hammond, 2000, 2001), mice were parasitized for 23 days prior to metabolism measures, whereas in the present study mice were infected on average for 49 days. It is well documented that *H. polygyrus* elicits an immune response by the host during larval stages (e.g. Monroy and Enriquez, 1992). If this immune response is partly responsible for the increased resting metabolism of mice then the more time that has passed since the larval stages of the infection, the more likely it is that resting metabolism will return to levels similar to those of uninfected mice. Differences between the present study and our previous work suggest that at least part of the increased resting metabolism shown with parasitism may reflect the time-course of the infection and the related immune response to *H. polygyrus* by *M. musculus*.

Energy acquisition and metabolism during lactation

Both energy intake and allocation changed with lactation and, for lactating mice, larger litter sizes explained approximately 36% of the variation in increased food intake for both parasitized and unparasitized mice (Fig. 4). As with parasitism, lactation was associated with increases in mucosal mass of the small intestine. Similar to previous studies (Hammond et al., 1994, 1996a,b), maximal

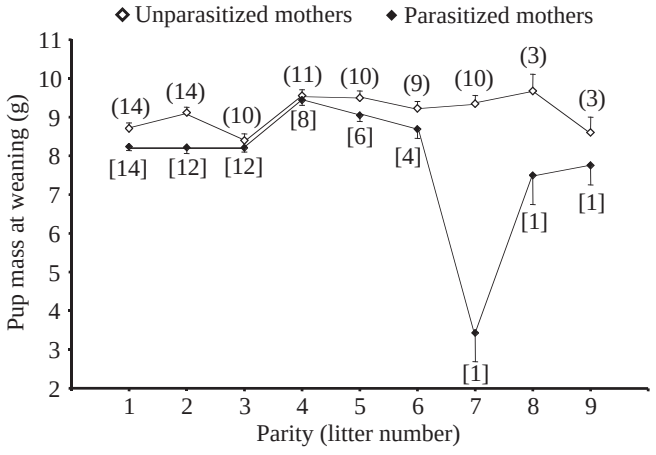

Fig. 9. Average pup mass at weaning (±1 S.E.M.), adjusted for litter size, as a function of parity ranging from litter number 1 to litter number 9 for parasitized or unparasitized mothers. Numbers of litters are shown in brackets for parasitized mothers or in parentheses for unparasitized mothers.

glucose-transport rate did not differ between lactating and virgin mice. However, in contrast to previous studies, lactating mice in my experiment did not have greater total glucose-transport capacity than virgin mice, despite their increased small intestine mucosal mass. This discrepancy may reflect differences in calculations of glucose-transport capacity (using mucosal mass *versus* whole small intestine mass), mass relationships (changes in intestine mass with lactation) or mammary pressure (amount of suckling per teat). For example, one previous study showed that body mass was a significant covariate with total glucose-transport capacity (Hammond et al., 1996b), whereby capacity did not differ between virgins and lactating mice having five teats but only differed between virgins and lactating mice with 10 teats (after experimental manipulation of teat number and litter sizes). In two other studies (Hammond et al., 1994, 1996a), body mass was not a significant covariate with glucose-transport capacity; therefore, body mass effects on glucose-uptake capacity remain somewhat elusive.

After accounting for changes in whole body mass, lactating mice had a greater resting metabolism than virgin mice, which was partially due to the increased lean mass (metabolically active tissue) because, when the effects of lean mass were removed (ANCOVA with lean mass as the covariate), there were no effects of lactation on resting metabolism. Regardless of the cause of increased metabolism during lactation, this response must be fueled either with increased food intake (as seen in laboratory mice) or possibly by decreased activity or catabolism of body fat stores, which may occur in nature if extra food is unavailable.

Response to simultaneous demands

In this study, physiological or morphological responses to one demand were not affected by the presence of a second demand. This is similar to the finding that simultaneous demands of cold exposure and *H. polygyrus* did not interact with each other (Kristan and Hammond, 2000) but is in contrast to the finding that simultaneous demands of caloric restriction and *H. polygyrus* did significantly interact (Kristan and Hammond, 2001). Whether demands interact (Carlomagno et al., 1987; Kristan and Hammond, 2001) or remain independent (Kristan and Hammond, 2000; the present study) varies with combinations of demands presented to the mice; therefore, general conclusions about effects of simultaneous demands on physiological and morphological responses are not forthcoming.

It is important to note that some simultaneous demands elicit similar responses (e.g. both cold exposure and parasitism generally result in greater organ sizes and resting metabolism) whereas others elicit contradictory responses (e.g. short-term caloric restriction results in decreased organ masses and resting metabolism, and parasites elicit increased organ masses and resting metabolism). When multiple demands elicit responses that occur in the same direction (either increases or decreases), the animal may be able to respond to each demand relatively independently, but, when demands require conflicting responses by the animal, a response to one demand may compromise an animal's ability to respond to the second demand.

Reproductive output and parasitism

Despite changes in morphology and physiology that occurred during parasite infection, parasitized mice showed no change in reproductive output except for differences in offspring mass and effects of current litter size on subsequent litter size. I found that female pup mass at weaning, a potential measure of pup quality as related to adult reproductive performance (Solomon, 1994), was significantly affected by maternal parasite infection. Interestingly, this was not true for male offspring.

Sex-biased effects

Differential investment in male *versus* female offspring between reproductive events has been examined both theoretically and empirically (e.g. Trivers and Willard, 1973; Clutton-Brock, 1991); however, differential investment in male and female offspring within a reproductive event is not so well studied. Differential investment in males *versus* females within a reproductive event may reflect differences in maternal behavior. Helminth parasite infection can affect maternal behavior as shown by female Sprague-Dawley rats (*Rattus norwegicus*), which, when infected with a tapeworm, retrieved their offspring more quickly than did unparasitized rats (Willis and Poulin, 1999). If maternal behavior of laboratory mice infected with *H. polygyrus* differs towards female and male offspring (e.g. access to teats, brooding behavior) then differential mass at weaning may result. Moreover, if parasitized mothers cannot produce pups of the same mass as unparasitized mothers then there may be selection for sex-biased investment in offspring. Given that male mice must compete for mates and thereby potentially have more variable reproductive success than do female mice, it would be predicted that mothers invest more in sons than in daughters (Willson and Pianka, 1963; Trivers and Willard, 1973; Clutton-Brock, 1991) under certain circumstances. In this experiment, parasitized mothers had male offspring of similar mass to those of unparasitized mothers (i.e. no effect of parasites on male offspring mass) but had smaller female offspring (i.e. decreased investment in female offspring when parasitized). Alternatively, sex-biased effects of maternal parasite infection on offspring mass may reflect differences in offspring behavior. For example, adult mice can detect the presence of *H. polygyrus* in a conspecific based on odor cues in the urine (Kavaliers and Colwell, 1995). It is possible that male and female pups differentially detect or respond to these odor cues in maternal urine, which may affect how pups interact with their mother. Sex-biased parental investment within a reproductive event warrants further investigation in general for this and other host–parasite systems.

Current versus *future reproduction*

In contrast to unparasitized females, infected females that

produced large first litters had relatively smaller second litters, which implies that the cost of producing a large litter may be more for parasitized females than for unparasitized females. Importantly, parasitized females that produced small first litters tended to have larger second litters. A shift in optimal allocation of energy between current and future offspring associated with parasitism can occur (Richner and Tripet, 1999), and further exploration of relative changes in reproductive effort over the course of numerous reproductive events will provide valuable insight into how parasites may influence this life-history parameter.

Conclusions

This study adds to the body of literature showing that simultaneous demands that include parasitism can either remain independent of each other (Kristan and Hammond, 2000) or interact with each other (Carlomagno et al., 1987; Banerjee et al., 1999; Kristan and Hammond, 2001) depending on the combination of demands presented. Parasite infection affected host morphology and physiology, the size of female offspring at weaning, and the relationship between litter sizes of two adjacent reproductive events but not other measures of reproductive output. Future studies to determine why female pups from parasitized mice are smaller (e.g. due to maternal care, milk output, milk quality, pup behavior), whether female pups show compensatory growth and whether pup reproduction at adulthood is ultimately affected will help elucidate whether *H. polygyrus* could have important evolutionary consequences to its host.

Helpful comments were provided by K. Hammond, M. Chappell, E. Platzer, M. Zuk and two anonymous reviewers. I would like to thank Mi Kyong Kim for assistance in weighing mice, and Stacey Leder and Joanne Narksompong for assistance with glucose-uptake measurements. This project was supported by a Dissertation Research Grant, Graduate Division, University of California, Riverside and NSF Doctoral Dissertation Improvement Grant (IBN-0073229) and by DBI-9987605 to Kimberly A. Hammond.

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