

Maternal and direct effects of the intestinal nematode *Heligmosomoides polygyrus* on offspring growth and susceptibility to infection

Deborah M. Kristan

Department of Biology, University of California, Riverside, CA 92521, USA

Present address: Department of Biological Sciences, University of Idaho, Moscow, ID 83844, USA (e-mail: kristand@uidaho.edu)

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Summary

The laboratory mouse (*Mus musculus*) has a naturally occurring intestinal nematode (*Heligmosomoides polygyrus*) that induces an immune response, causes phenotypic plasticity in metabolism and in organ structure and function, and results in changes in host reproductive output. The objectives of the present study were to determine (1) whether pups infected with parasites at weaning grew differently and had a different body composition at adulthood compared with uninfected pups, (2) whether offspring from parasitized mothers grew differently and had a different body composition at adulthood compared with offspring from unparasitized mothers, (3) whether parasite effects on body composition of pups varied under different infection intensities and (4) whether maternal parasite infection affected susceptibility, duration and intensity of offspring parasite infection. *H. polygyrus* had direct and maternal effects on offspring growth, but final adult mass was not affected by

parasites. Parasite infection in offspring had no effect on overall fat mass, but mass changes for some organs were greater for mice that had a high infection intensity compared with mice that had a low infection intensity. Only offspring from parasitized mothers cleared their parasite infection; however, if the infection was not cleared, the final infection intensity was greater for offspring born to parasitized mothers than to unparasitized mothers. This study shows that chronic, sublethal parasite infection with *H. polygyrus* has both maternal and direct effects that induce physiological changes in growing mice sufficient to alter host growth trajectories, morphology and susceptibility to parasite infection.

Key words: parasites, maternal effects, offspring growth, susceptibility, morphology, phenotypic plasticity, *Heligmosomoides polygyrus*, nematode.

Introduction

Young growing animals may respond to demands in their environment by using energy that would otherwise be allocated to growth. Such demands may include harsh weather, predation risk, difficult access to food or parasitic infection (Møller, 1993; Merino and Potti, 1995; Saino et al., 1997; Merino et al., 2000). Size at weaning may affect how an individual copes with such demands because of differences in the absolute amount of energy reserves potentially available (e.g. fat and protein body stores) and because demands may be different for animals of different sizes. Therefore, both host size at weaning and environmental conditions during growth may directly affect the host growth trajectory and final adult size and reproduction (Solomon, 1994).

Maternal condition can also affect offspring growth, immunocompetence and survival (Sorci et al., 1994; Sorci and Clobert, 1995; Merino et al., 1996; Saino et al., 1997; Hörak et al., 1999). For example, maternal ectoparasite load in the lizard *Lacerta vivipara* was positively correlated with offspring growth rate in the first year but negatively correlated with offspring growth rate in the second year

(Sorci and Clobert, 1995) and differentially affected the survival of male and female offspring (Sorci et al., 1994). Most research on the effects of parasitism on host life history has studied the effects of ectoparasites, but fewer studies have examined large endoparasites such as helminths (Kyriazakis et al., 1996). Many rodents encounter helminth parasites as young, growing animals but the effects on their growth are unknown. Importantly, some rodents have compensatory growth after weaning [e.g. eastern woodrats (*Neotoma floridana*) and northern grasshopper mice (*Onychomys leucogaster*); Sikes 1996, 1998], whereas mass at weaning for other rodent species can influence adult size and affect reproduction [e.g. deer mouse (*Peromyscus maniculatus*); Myers and Master, 1983), Levant vole (*Microtus guentheri*); German, 1993) and prairie voles (*Microtus ochrogaster*); Solomon, 1994]. Therefore, perturbations of the growth process may have far-reaching implications for some rodent species.

Laboratory mice (*Mus musculus*) reallocate resources during a sublethal infection with *Heligmosomoides polygyrus* (an

intestinal nematode), as shown by increased metabolic rate associated with increased lean mass and by changes in organ size and function (Kristan and Hammond, 2000, 2001). Chronic sublethal parasite infection with *H. polygyrus* was also associated with changes in host reproductive output, whereby parasitized mothers produced 6% smaller female offspring at weaning than unparasitized mothers (Kristan, 2002). Although female offspring of infected mothers were smaller at weaning, the effects of parasites (both direct and maternal) on subsequent offspring growth rates and adult size and body composition are unknown.

In the present study, I measured direct effects of parasite infection on offspring growth by experimentally infecting pups at weaning with *H. polygyrus*. I also examined the consequences of maternal infection on offspring growth by measuring uninfected pups from either parasitized or unparasitized mothers. In both cases, I examined differences in growth between male and female offspring to account for potential sex differences in parasite susceptibility (e.g. Dobson, 1961; Dobson and Owen, 1978; Zuk and McKean, 1996) and growth rates. I first predicted that parasitized mice would grow more slowly than unparasitized mice because resources normally used for growth would be used to respond to parasites. Second, I predicted that maternal parasite infection would not affect offspring growth trajectories but would affect adult size (based on smaller size of pups from parasitized mothers; Kristan, 2002), assuming no compensatory growth. Third, I predicted that greater infection intensity would differentially affect body composition and that mice with more parasites would have greater increases in organ mass associated with parasitism than mice with fewer worms. Finally, I predicted that the offspring of parasitized mothers would have a greater infection intensity than the offspring of unparasitized mothers because offspring would be in a poorer condition at weaning (assuming smaller size as an index of overall quality).

Materials and methods

Animals and experimental design

To control for possible effects of parity, all pups used in this experiment were from the second litter of continuously mated pairs of *Mus musculus* L. Swiss-Webster mice where the mother was either parasitized ($N=13$) with *Heligmosomoides polygyrus* Dujardin 1845 or unparasitized ($N=13$). For all mated pairs, the adult males were not parasitized. Every mated pair was checked daily to determine the date of birth, and the number of pups in the litter was counted on the day of birth. Beginning from 15 days after birth, each pup was marked with a unique color combination on the neck or back using permanent markers (Sharpie®, Sanford, Bellwood, IL, USA). Mice were housed in 27 cm×21 cm×14 cm polypropylene cages at 14 h:10 h L:D and 23°C and were given standard laboratory chow (LabDiet® 5001, Purina Mills, Inc., St Louis, MO, USA) and water *ad libitum*.

On days 15, 17 and 20, and every 5 days thereafter until the

pups were 60 days old, mass (measured in g) and two variables of size – right hindfoot length (from the heel to tip of the claw on the longest toe; measured in mm) and tail length (from the base to the tip of the tail; measured in mm) – were measured. At 60 days, growth rates have begun to plateau (Poiley, 1972) and mice are sexually mature; therefore, measurements were stopped at this time.

At 20 days of age, pups were weaned (i.e. removed from the parental cage) and individually marked with an ear punch. From each litter (if litter size and sex ratio permitted), two male and two female pups were infected with parasites (see below) and two male and two female pups were given tapwater. For smaller litters or litters with uneven sex ratios, approximately half of the offspring were parasitized and half were unparasitized for each sex. This design made it possible to discern maternal effects (by examining unparasitized pups from parasitized mothers) and direct effects (by examining parasitized pups from unparasitized mothers) of parasitism.

Mouse infection procedures

H. polygyrus infective-stage larvae (L_3) were cultivated from non-experimental mice. For adult females used in mated pairs (50–90 days old), mice were given 300 ± 1 to 300 ± 11 L_3 (number of worms ± 1 S.D.; range of S.D. reflects different parasite cultures used during the experiment) by anesthetizing mice and administering the L_3 with a feeding tube (Kristan and Hammond, 2000). Females were mated 14 days post-infection (PI) after *H. polygyrus* eggs were detected in the feces, indicating that adult worms occupied the small intestine lumen.

For pups, on the day of weaning parasitized mice were infected either with 90 ± 1 L_3 (low-infection-intensity group) or 200 ± 1 to 200 ± 7 L_3 (high-infection-intensity group) suspended in tapwater. Parasites were administered using a 200 μ l pipette tip (Fisherbrand Redi-Tip on a Pipetman) that was placed at the back of the mouse's throat. Larvae that were suspended in water were dispensed into the throat, and mice swallowed to complete the infection procedure. This method eliminated the use of anesthesia and feeding tubes and produced a similar final infection intensity to the more invasive method. All unparasitized mice were given an approximately equal volume of tapwater only. These two infection intensities (high and low) resulted in final worm burdens that approximated either a naturally occurring infection intensity (approximately 80–100 L_3) for mice at their age at the end of the experiment for the high-infection-intensity group or resulted in half of the naturally occurring worm burden (Scott, 1988). The infection status of all pups was checked using a modified McMaster technique (Bowman, 1995) when they were 35 days old (15 days PI).

Body composition, organ morphology and hematocrit measures of offspring

On days 60–62, mice were anesthetized by intraperitoneal injection of 0.07 ml sodium pentobarbital (65 mg ml^{-1}). Two 25–50 μ l blood samples were collected using retro-orbital puncture with a 75 μ l heparinized capillary tube. Samples were

spun for 10 min on a microhematocrit centrifuge, and the average hematocrit of the two samples was used in the analysis. Mice were then euthanized by cutting the diaphragm, and the small intestine, stomach, cecum, large intestine, heart, liver, spleen, kidneys and lungs were subsequently removed. Excess fat and connective tissue were removed from each organ and returned to the mouse carcass. The pancreas and associated mesentery were also removed for another experiment, and data for this organ were not included in the calculation of fat and lean body mass.

For stomach, cecum and large intestine, each organ was weighed with and without contents (flushed clean with mammalian Ringer's solution: for composition, see Karasov and Diamond, 1983). The small intestine was divided 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 small intestine mass (corrected for mass of the parasites as described below). Mucosal and submucosal tissue (hereafter called 'mucosa') was separated from muscularis and serosal tissue (hereafter called 'serosa') for two 1.5-cm segments per region (Diamond and Karasov, 1984). The dry mass:wet mass ratio was calculated for each segment, and the mean of these ratios was used to calculate the mucosal and serosal wet mass and dry mass of the entire small intestine (Diamond and Karasov, 1984).

Small intestine mass of infected mice was corrected for the mass of the parasites. All adult *H. polygyrus* found in the small intestine during rinsing, from intestinal segments used for mucosa and serosa measures, and from remaining unused tissue were collected and counted. The number of worms was counted using a stereoscope to determine the final infection intensity. The wet mass of *H. polygyrus* (Kristan and Hammond, 2001) was subtracted from the small intestine wet mass so that calculations of small intestine dry mass used in analyses did not include worm mass. The dry mass of all organs and the carcass after drying to a constant mass at 55–60°C for 2 days and 2 weeks, respectively, was measured.

The dried carcass was ground and lipids were extracted using petroleum ether (Goldfische apparatus; Labconco, Kansas City, MO, USA) and the percentage fat and mass of fat were calculated for each mouse. The fat content of all organs, except the small intestine, was measured. 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 the mass of fat (in g) and percentage fat were calculated from mass loss. Total fat mass of each mouse was the sum of the masses of body and organ fat. Therefore, lean mass was calculated as the initial whole body mass minus the total fat mass.

Statistics

This experiment consists of three independent variables [maternal parasite status (parasitized, unparasitized), pup parasite status (parasitized-low-intensity, parasitized-high-intensity, unparasitized) and pup sex] and numerous dependent variables (body growth, body mass and composition, organ

masses, hematocrit and infection intensity). Data were analyzed with a split-plot general linear model [either analysis of variance (ANOVA) or multivariate analysis of variance (MANOVA)] where the main plot was maternal parasite status and the subplot was pup parasite status.

Because the Gompertz growth equation:

$$Y = A \times e^{-e^{-(b-ct)}}$$

fits the data well, this equation was used to examine the growth of both mass and size variables (tail and foot length) to generate a curve for each individual, where Y is size (mass, tail length or foot length), A is the upper asymptote, b is the intercept, c is the slope, t is time, and e is a constant (the base of the natural log). The coefficients generated from each curve (A , b and c) were examined in a MANOVA to determine whether overall curve shape differed among treatment groups. Three variables of the Gompertz growth curve for mass, foot length and tail length were then calculated: maximum rate of change (determined as the maximum slope of the curve), size of variable at the maximum rate of change (determined as the y -intercept from the maximum slope) and the age of the animal at maximal growth (determined as the x -intercept at the maximum slope; Fig. 1). It was determined whether these variables were significantly associated with natal litter size, and litter size was used as a covariate in analyses when necessary. The three calculated variables were used in a multivariate analysis of covariance (MANCOVA) to test for overall differences among treatment groups for each growth variable (mass, foot length and tail length). The MANCOVA was not significant for foot length, but, because the MANCOVA was significant for mass and tail length ($P < 0.05$), *post-hoc* univariate ANOVAs or ANCOVAs were performed [factors: pup sex, pup parasite status (parasitized, unparasitized), maternal parasite status (parasitized, unparasitized); covariate: litter size] for these two growth measures to determine which components of the curve differed among treatment groups.

To examine morphological variables of pups at day 60, a MANOVA was first used to test for significant differences between treatments [pup parasite status (unparasitized, low infection intensity, high infection intensity), maternal parasite status and pup sex] for all dependent variables together (except mucosa and serosa variables because of missing data for some individuals). Because this MANOVA was significant ($P < 0.05$), independent *post-hoc* ANOVAs were used to determine which treatments and which dependent variables were statistically significant. For all ANOVAs of organ masses, effects of body mass were tested for. If the relationship between the dependent variable and body mass was significant, ANCOVA and present least-squares means ± 1 S.E.M. were used; otherwise, ANOVA and present arithmetic means ± 1 S.E.M. were used. For infection-intensity data, the low- and high-infection-intensity levels were examined separately, and the effects of body mass on this variable were examined as described above.

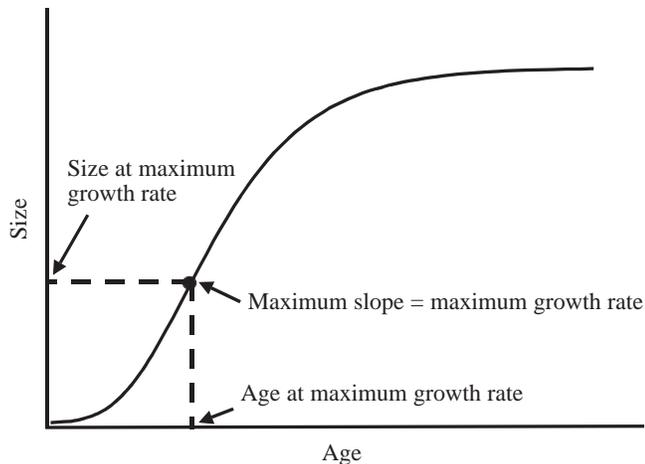


Fig. 1. Theoretical Gompertz growth curve showing the three calculated variables used in analyses of offspring growth.

Results

All mice that were given parasites produced a mature infection, as evidenced by the presence of *H. polygyrus* eggs in host feces, and no control mice became infected. Sample sizes varied among treatment groups due to variation in litter size and sex ratio (Table 1).

Offspring growth rate

Growth curve coefficients.

Overall, when examining the three coefficients of the Gompertz growth curve together, body mass growth differed with pup parasite status ($F_{1,18}=5.6$, $P=0.008$) and pup sex ($F_{1,18}=8.2$, $P=0.002$) but not with maternal parasite status (Fig. 2), and tail length growth varied with pup parasite status ($F_{1,18}=3.6$, $P=0.037$) but not pup sex or maternal parasite status (Fig. 3). There was a significant interaction between pup and maternal parasite status ($F_{1,18}=3.6$, $P=0.037$) for tail length because the slope of the curves differed with maternal parasite status but not pup parasite status. Coefficients for the growth curves of foot length did not differ among treatment groups (Fig. 4).

Body mass growth

An overall examination of the three calculated variables of

Table 1. Sample size among treatment groups for the offspring growth experiment

Offspring	Maternal infection status	
	Unparasitized mother	Parasitized mother
Unparasitized		
Male	25	21
Female	23	17
Parasitized		
Male	25	17
Female	22	18

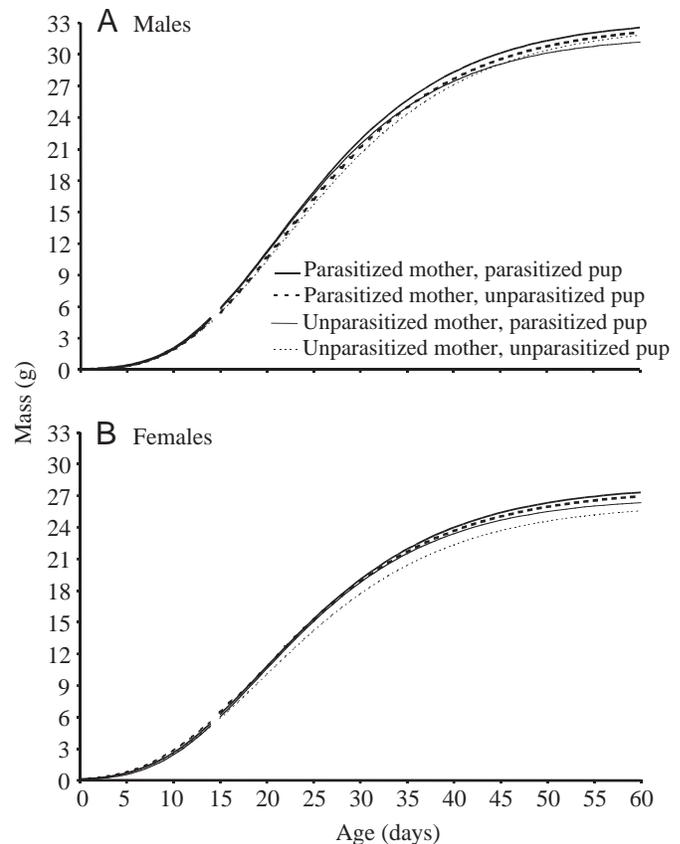


Fig. 2. Body mass growth of parasitized and unparasitized mice that were born to either parasitized or unparasitized mothers. Portions of curves from days 15–60 are empirical and portions of curves from days 0–15 are theoretical extrapolations of curves.

the growth curve (maximum growth rate, size at maximum growth rate and age at maximum growth rate) showed that body mass growth varied with pup sex ($F_{1,19}=6.4$, $P=0.004$) and pup parasite status ($F_{1,19}=4.9$, $P=0.012$) and that litter size was a significant covariate ($F_{1,17}=5.7$, $P=0.008$). Maternal parasite treatment ($F_{1,17}=2.6$, $P=0.091$) and the interaction between pup and maternal parasite treatments ($F_{1,19}=2.9$, $P=0.063$) were marginally significant.

When I examined the three dependent variables separately, I found that parasitized pups grew 5% faster than unparasitized pups ($F_{1,19}=4.5$, $P=0.048$; parasitized, 1.041 ± 0.018 g day⁻¹; unparasitized, 0.988 ± 0.017 g day⁻¹; least squares-means \pm 1 S.E.M.) and males grew 23% faster than females ($F_{1,19}=4.3$, $P=0.053$; male, 1.121 ± 0.018 g day⁻¹; female, 0.908 ± 0.017 g day⁻¹) with litter size as a significant covariate ($F_{1,17}=7.7$, $P=0.013$). There was no maternal effect on the maximum rate of body mass gain.

When pups were growing at their fastest, males were 20% heavier than females ($F_{1,19}=8.5$, $P=0.009$; male, 12.03 ± 0.092 g; female, 10.00 ± 0.096 g) and pups from parasitized mothers were 4% heavier than pups from unparasitized mothers ($F_{1,17}=6.9$, $P=0.018$; parasitized mother, 11.10 ± 0.102 g; unparasitized mother, 10.93 ± 0.089 g) with

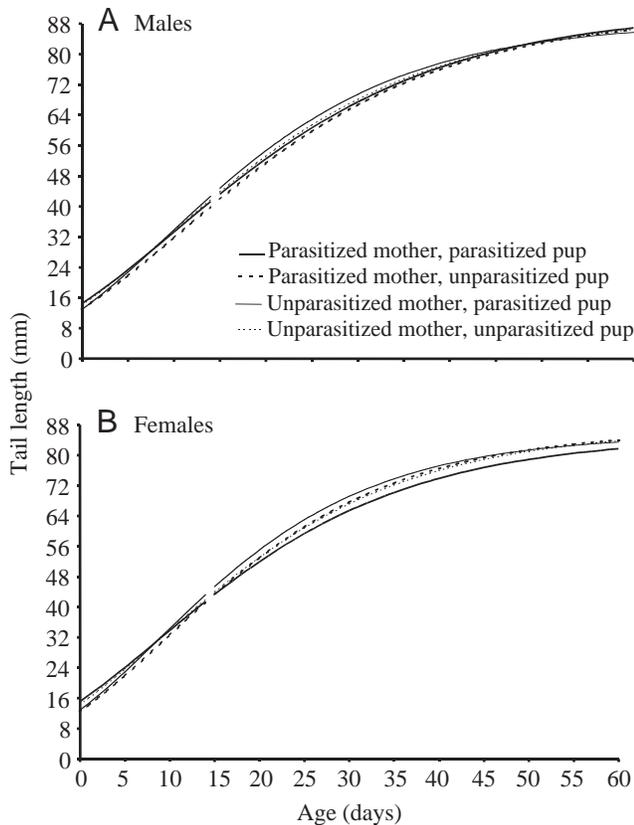


Fig. 3. Tail growth of parasitized and unparasitized mice that were born to either parasitized or unparasitized mothers. Portions of curves from days 15–60 are empirical and portions of curves from days 0–15 are theoretical extrapolations of curves.

litter size as a significant covariate ($F_{1,17}=17.7$, $P=0.0006$). There was no effect of pup parasite status on the mass of a pup during maximum body mass growth.

Parasitized pups reached their maximum growth rate 0.5 days earlier than unparasitized pups ($F_{1,19}=13.5$, $P=0.002$; parasitized, 20.2 ± 0.183 days; unparasitized, 20.6 ± 0.179 days) and females reached their maximum growth rate 1.7 days earlier than males ($F_{1,19}=7.6$, $P=0.013$; males, 21.3 ± 0.177 days; females, 19.6 ± 0.185 days) with litter size as a significant covariate ($F_{1,17}=5.4$, $P=0.033$). There was a significant interaction between maternal parasite status and pup parasite status ($F_{1,19}=8.8$, $P=0.008$) because the age at which parasitized and unparasitized pups achieved maximal growth was similar for parasitized mothers but differed for unparasitized mothers. There was also a marginally significant interaction between maternal parasite status and offspring sex ($F_{1,19}=3.7$, $P=0.070$) because the difference between males and females was greater for unparasitized mothers than for parasitized mothers.

Tail growth

When I examined all three calculated variables together, pups differed in tail length according to their parasite status

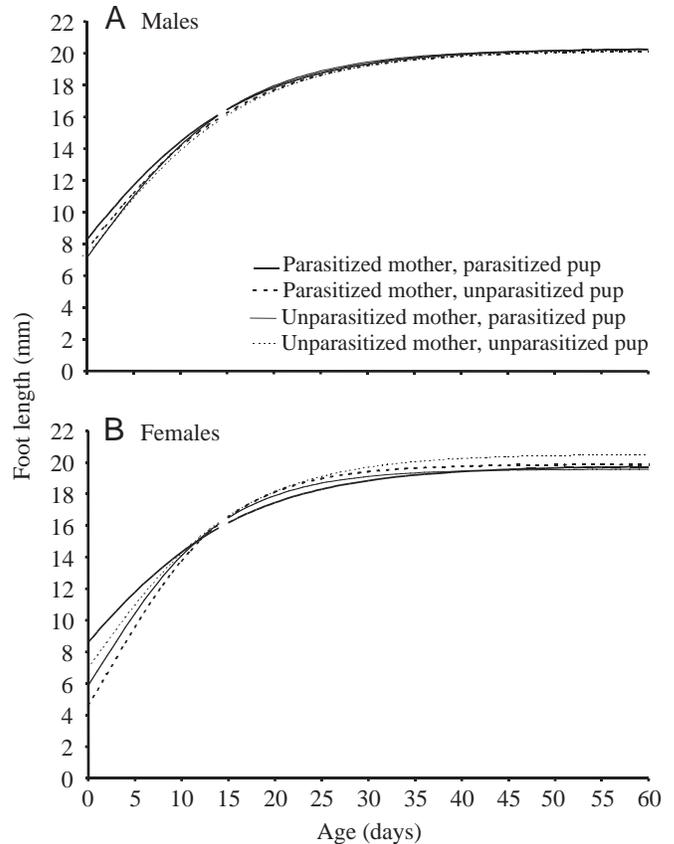


Fig. 4. Foot growth of parasitized and unparasitized mice that were born to either parasitized or unparasitized mothers. Portions of curves from days 15–60 are empirical and portions of curves from days 0–15 are theoretical extrapolations of curves.

($F_{1,17}=4.7$, $P=0.015$) but not as a function of maternal parasite status or sex. Litter size was a significant covariate ($F_{1,17}=5.2$, $P=0.011$) and there was a significant interaction between maternal and pup parasite status ($F_{1,17}=4.2$, $P=0.022$). If the mother was unparasitized, her parasitized offspring had tails that grew 9% faster than her unparasitized offspring. However, if the mother was parasitized, her parasitized offspring had tails that grew 6% slower than her unparasitized offspring.

Post-hoc analyses revealed no effect of pup parasite status, maternal parasite status or pup sex on maximal rate of tail growth, but litter size was a significant covariate ($F_{1,17}=14.2$, $P=0.002$). Parasitized pups had 1% shorter tails at their time of maximal growth compared with unparasitized pups ($F_{1,19}=6.3$, $P=0.022$; parasitized, 32.2 ± 0.17 mm; unparasitized, 32.6 ± 0.17 mm), but there was no effect of maternal parasite status or pup sex on the size of the tail when it was at maximum growth rate. Females reached their maximum tail growth rate 1 day earlier than males, which was marginally significant ($F_{1,19}=4.3$, $P=0.053$; males, 10.0 ± 0.284 days; females: 9.0 ± 0.297 days), and parasitized pups reached their maximum tail growth rate 1 day earlier than unparasitized pups ($F_{1,19}=10.1$, $P=0.005$; parasitized, 9.1 ± 0.294 days; unparasitized, 9.9 ± 0.287 days). There was a significant

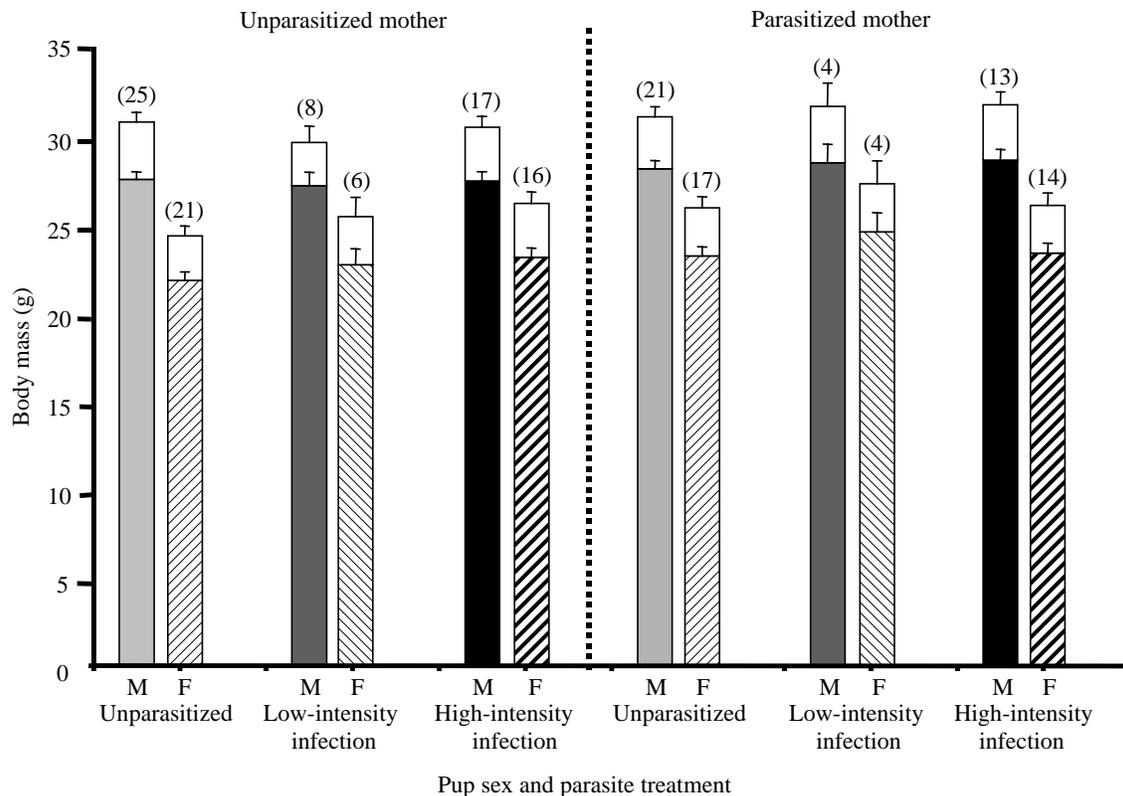


Fig. 5. Body mass (g) of parasitized and unparasitized mice that were born to either parasitized or unparasitized mothers, showing body composition (lean mass in bottom portion of bars and fat mass in top portion of bars). Error bars are +1 S.E.M. of lean mass or total body mass, and sample size (N) is in parentheses above each bar. M, male; F, female.

interaction between maternal parasite status and pup sex ($F_{1,19}=4.5$, $P=0.047$) because the difference between males and females was greater for parasitized than for unparasitized mothers. The age when a pup achieved maximum tail growth did not differ with maternal parasite treatment alone or with litter size.

Foot growth

There were no overall differences in foot growth among treatment groups, but there was a significant interaction between maternal and pup parasite status ($F_{1,17}=7.2$, $P=0.003$). Univariate *post-hoc* analyses showed that this occurred because maximum growth rate differed, but only marginally so ($F_{1,19}=4.1$, $P=0.058$). For parasitized mothers, maximum foot growth was greater for their unparasitized than their parasitized pups, but, for unparasitized mothers, foot growth was less for their unparasitized than parasitized pups.

Morphology and hematocrit

Body mass regressions were significant for all morphological variables but not for hematocrit. Therefore, I present least-squares means ± 1 S.E.M. for all morphological variables (to show the effects of treatments after body mass effects were removed) except hematocrit, where I present the arithmetic mean ± 1 S.E.M. The MANOVA for morphology variables showed significant effects of pup parasite status

(using all three levels of unparasitized, low-intensity and high-intensity infection; $F_{2,13}=6.4$, $P=0.042$) and pup sex ($F_{1,13}=127.6$, $P=0.008$) and there was a significant interaction between pup and maternal parasite status ($F_{2,13}=5.4$, $P=0.026$).

Body mass and composition

At 60 days old, males were 19% heavier than females ($F_{1,15}=6.2$, $P=0.025$; males, 30.86 ± 0.34 g; females, 25.830 ± 0.35 g) but body mass did not differ either with pup or maternal parasite treatment (Fig. 5). Mass differences between males and females reflect differences in lean mass, whereby males had 21% greater lean mass ($F_{1,15}=6.32$, $P=0.024$) but similar total fat mass and percentage fat mass as females. There were no maternal effects or direct effects of parasites on body composition (fat *versus* lean mass) and all mice had approximately 10% fat mass (range 8–12%).

Organ masses and hematocrit

Because fat was not extracted separately from each organ, I used whole organ dry masses (including fat content) to examine treatment effects. Compared with pups from unparasitized mothers, pups from parasitized mothers had 2% larger livers ($F_{1,13}=5.6$, $P=0.035$), 5% larger stomachs ($F_{1,13}=13.8$, $P=0.003$), marginally heavier small intestines (by 4%; $F_{1,13}=4.5$, $P=0.055$), longer small intestines (by 2%; $F_{1,13}=3.9$, $P=0.069$) and heavier serosa (by 8%; $F_{1,11}=4.1$,

Table 2. Organ dry mass ($g \pm 1$ S.E.M.) of male and female offspring born to parasitized or unparasitized mothers

Pup parasite status	Unparasitized mother			Parasitized mother		
	Unparasitized	Low intensity	High intensity	Unparasitized	Low intensity	High intensity
Males						
Stomach	0.045±0.001	0.042±0.001	0.043±0.001	0.045±0.001	0.048±0.002	0.046±0.001
Small intestine	0.299±0.015	0.319±0.029	0.363±0.017	0.330±0.017	0.259±0.051	0.407±0.021
Mucosa	0.225±0.012	0.221±0.022	0.295±0.014	0.261±0.014	0.157±0.040	0.314±0.016
Serosa	0.072±0.005	0.106±0.010	0.096±0.006	0.077±0.006	0.099±0.017	0.105±0.007
Cecum	0.026±0.001	0.027±0.001	0.027±0.001	0.024±0.001	0.030±0.002	0.024±0.001
Large intestine	0.063±0.002	0.066±0.003	0.064±0.002	0.063±0.002	0.067±0.004	0.062±0.002
Spleen	0.018±0.001	0.021±0.001	0.023±0.001	0.019±0.001	0.019±0.002	0.025±0.001
Kidneys	0.115±0.002	0.119±0.004	0.112±0.003	0.115±0.002	0.120±0.005	0.112±0.003
Liver	0.487±0.008	0.493±0.013	0.517±0.009	0.485±0.009	0.497±0.019	0.546±0.011
Heart	0.032±0.001	0.035±0.001	0.033±0.001	0.031±0.001	0.035±0.002	0.031±0.001
Lung	0.036±0.001	0.038±0.001	0.037±0.001	0.036±0.001	0.037±0.001	0.036±0.001
Females						
Stomach	0.051±0.001	0.048±0.002	0.050±0.001	0.052±0.001	0.049±0.002	0.052±0.001
Small intestine	0.324±0.019	0.360±0.035	0.397±0.018	0.355±0.018	0.352±0.049	0.447±0.019
Mucosa	0.280±0.015	0.276±0.027	0.320±0.014	0.291±0.016	0.304±0.054	0.338±0.015
Serosa	0.069±0.007	0.098±0.012	0.099±0.006	0.078±0.007	0.095±0.024	0.128±0.007
Cecum	0.028±0.001	0.029±0.002	0.029±0.001	0.029±0.001	0.032±0.002	0.026±0.001
Large intestine	0.071±0.002	0.075±0.003	0.069±0.002	0.071±0.002	0.074±0.004	0.070±0.002
Spleen	0.023±0.001	0.025±0.002	0.027±0.001	0.022±0.001	0.025±0.002	0.032±0.001
Kidneys	0.096±0.003	0.092±0.004	0.091±0.003	0.096±0.003	0.099±0.005	0.091±0.003
Liver	0.480±0.009	0.475±0.015	0.498±0.010	0.479±0.009	0.506±0.018	0.498±0.010
Heart	0.032±0.001	0.031±0.001	0.032±0.001	0.032±0.001	0.033±0.001	0.031±0.001
Lung	0.039±0.001	0.037±0.001	0.037±0.001	0.039±0.001	0.037±0.001	0.039±0.001

$P=0.067$; Table 2). Maternal parasite treatment did not affect the masses of heart, lung, kidney, spleen, cecum or large intestine.

Males had larger livers (by 3%; $F_{1,14}=13.9$, $P=0.002$) and kidneys (by 21%; $F_{1,14}=19.5$, $P=0.0006$) but marginally smaller spleens (by 19%; $F_{1,14}=4.3$, $P=0.057$) than females (Table 2). There was a significant interaction between pup sex and pup parasite status for cecum mass ($F_{2,14}=4.4$, $P=0.032$) because males had a 12% smaller cecum than females if they were unparasitized, but, if mice had parasites (regardless of infection intensity), males had only a 7% smaller cecum than females. Males and females had similar organ masses for stomach, small intestine, large intestine, heart and lung.

Overall, parasitized pups had 5% larger livers ($F_{2,14}=12.6$, $P=0.0008$) than unparasitized pups (Table 2), but the difference between unparasitized and parasitized pups is significant only for the high-intensity-infection treatment (based on 95% confidence interval around the least-squares mean). Mice with high infection intensity had kidneys that were 5% smaller than those of unparasitized mice ($F_{2,14}=4.0$, $P=0.043$), but the kidneys of parasitized mice in the low-infection-intensity group did not differ from those of uninfected mice. Spleen mass varied with pup parasite infection ($F_{2,14}=42.2$, $P<0.0001$) and was smallest for unparasitized mice, larger for low-infection-intensity mice and largest for high-infection-intensity mice. Small intestine length increased with pup parasite status ($F_{2,14}=17.1$, $P=0.0002$)

because pups in the high-infection-intensity group had longer small intestines than those of pups in either the low-infection-intensity group or unparasitized pups, which did not differ from each other. Similarly, small intestine mass differed with parasite treatment ($F_{2,12}=35.3$ $P<0.0001$), such that mice in the high-infection-intensity group had a greater small intestine mass than either unparasitized or low-infection-intensity pups, which did not differ from each other. The increase in small intestine mass was a result of increases in both mucosa ($F_{2,12}=32.6$, $P<0.0001$) and serosa ($F_{2,12}=17.3$, $P=0.0003$; Table 2). Mucosa mass was greater for the high-intensity-infection group compared with the others but did not differ between unparasitized and low-infection-intensity treatments. Serosa mass, however, did not differ between the two infection intensity groups, although parasitized mice had a greater serosa mass than unparasitized mice. Pup parasite treatment did not affect the masses of heart, lung, stomach, cecum or large intestine.

Hematocrit was recorded only for pups in the high-intensity group and unparasitized mice; therefore, pup parasite status has two levels for this analysis (parasitized, unparasitized). Pups from parasitized mothers had 3% lower hematocrit than pups from unparasitized mothers, which was marginally significant ($F_{1,7}=5.5$, $P=0.051$), and parasitized pups had a 2% greater hematocrit than unparasitized pups ($F_{1,9}=5.6$, $P=0.043$; parasitized pup, parasitized mother: $48.8\pm0.428\%$; parasitized pup, unparasitized mother: $50.2\pm0.363\%$; unparasitized pup,

parasitized mother: 47.8 ± 0.436 units; unparasitized pup, unparasitized mother: 49.7 ± 0.430 units).

Parasite infection intensity

The number of adult worms averaged 52% of the number of larvae administered for the low-intensity group and 49% for the high-intensity group. The regressions of infection intensity with body mass were not significant for mice in either the high- or low-infection-intensity groups; therefore, I present arithmetic means (number of worms) ± 1 S.E.M. of final infection intensity. Nine pups that were confirmed to be infected at 35 days old cleared their infection by 60 days old. All of these pups were born to parasitized mothers [1 pup (female) had received low-intensity treatment, and eight pups (three males and five females) had received high-intensity treatment; Fig. 6]. I excluded mice that cleared their infection from the analysis of final infection intensity.

Low-intensity treatment

For the low-intensity treatment, one female had 10 worms, whereas all other pups ($N=20$) had 23–91 worms (46 ± 4 worms; mean ± 1 S.E.M.). Therefore, I analyzed the effect of pup sex and maternal parasite status first for all individuals and then only for mice with >10 worms to determine if the outlier affected the results. Final infection intensity did not differ with pup sex or maternal parasite treatment when all infected mice were included or when only mice with >10 worms were included (Fig. 6).

High-intensity treatment

For mice in the high-intensity treatment, three pups had <10 worms (two worms, $N=1$ female; nine worms, $N=2$ males), whereas all other pups ($N=49$) had 54–201 worms (110 ± 6 worms; mean ± 1 S.E.M.). When all pups were included in the analysis, there was no effect of either pup sex or maternal parasite treatment. However, when only pups with >10 worms were included, pups born to parasitized mothers had a 36% greater infection intensity than pups born to unparasitized mothers ($F_{1,12}=4.8$, $P=0.049$; parasitized mother, 131.8 ± 9.1 worms; unparasitized mother, 99.2 ± 6.3 worms; Fig. 6).

Discussion

Overview

Parasitized mice generally showed faster and earlier growth than unparasitized mice but the differences were very slight. Although female mice born to parasitized mothers are smaller at weaning (Kristan, 2002), female pups showed compensatory growth such that there were no differences in adult mass either with direct parasite infection or maternal parasite infection by the time pups were 60 days old. Although final

adult body mass did not differ with parasite treatment for mice fed *ad libitum* in benign laboratory conditions, the effects of the observed differences in growth trajectories may reveal more long-lasting effects for wild mice. Maternal effects of infection with *H. polygyrus* for susceptibility of offspring to this parasite were varied. On the one hand, there appeared to be some maternal protection because only offspring from parasitized mothers were able to clear their infection during the experiment. On the other hand, if offspring born to parasitized mothers did not clear their infection, they had a greater infection intensity compared with pups born to unparasitized mothers. Taken as a whole, these data add to the body of evidence that sublethal parasites can have both direct and maternal effects on host life history.

Direct effects of parasites on growth and morphology

Growth

Overall growth curves of mass and tail length differed with parasitism, but foot length was similar between parasitized and unparasitized pups. Unlike other growth measures, foot length may not have varied with parasite treatment because it already averaged 80% of adult size when pups were first measured (compared with tail length, which was 63% of adult size, and body mass, which was 31% of adult size). Therefore, there may have been little distinction among treatment groups because there was relatively little growth left for this structure. Final adult body mass did not vary with pup parasite infection, indicating that the slight differences in growth trajectories with parasitism were not biologically significant under laboratory conditions.

Morphology

Parasitized pups had similar body mass to unparasitized pups but different body composition, which may suggest a

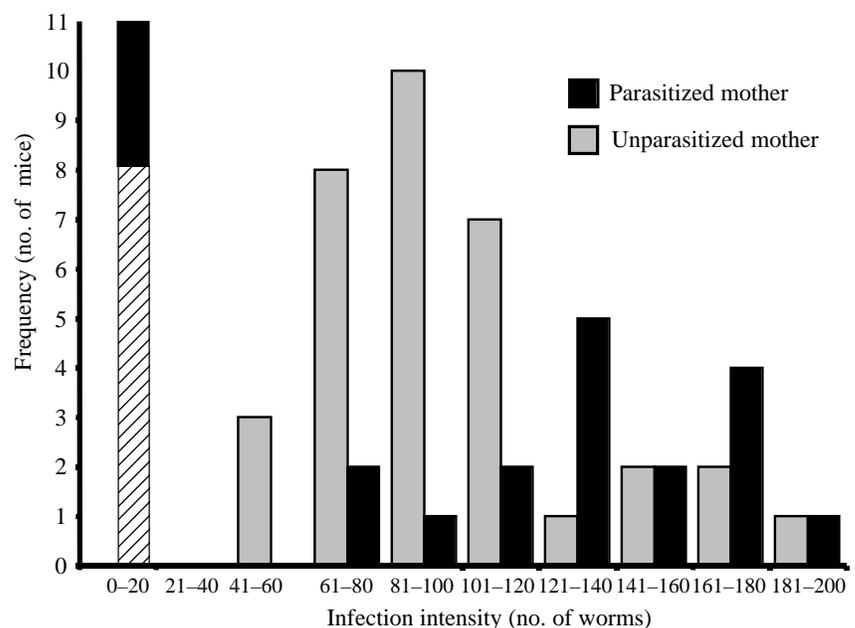


Fig. 6. Infection intensities for offspring in the high-infection-intensity group (ranging from 0 to 200 worms, shown in intervals of 20 worms) for mice born to parasitized or unparasitized mothers. The hatched portion of the first bar shows mice with 0 worms.

change in energy allocation to organs during growth or may simply reflect systemic morphological changes owing to parasite pathology. Specifically, parasitized pups had larger livers, kidneys, spleens and small intestines. Mice have both a cell- and antibody-mediated immune response to *H. polygyrus* that involves both the spleen and small intestine (Liu, 1965; Panter, 1969; Dehlawi and Wakelin, 1988; Scott and Koski, 2000), and presumably changes in size of these organs may, at least partially, be a result of their function in the immune response. Some intestinal parasites elicit complement responses by their hosts (Schmidt and Ruppel, 1988; Shin et al., 2001) and it may be possible that *H. polygyrus* also elicits this response in *M. musculus*. An increased liver size may result from increased function during complement production while the mouse is still growing. Other organs show an increased size during times of increased function (e.g. small intestine, Hammond et al., 1994; kidney, Hammond and Janes, 1998) and, although kidney size can increase with increased protein intake rate (Hammond and Janes, 1998), it is puzzling that kidney size changed with parasitism, especially because previous studies of this host-parasite system do not show changes in kidney mass for infected mice (Kristan and Hammond, 2000, 2001). These previous studies used adult mice, however, and allocation of resources to kidneys may differ for growing mice and adult mice.

Differences between morphological responses of the low- and high-infection-intensity groups indicate that infection intensity modulated the phenotypic plasticity of organ sizes in growing mice such that a greater infection intensity produced a greater increase in organ mass. Hematocrit (one indicator of the blood's capacity to carry oxygen) was slightly greater for parasitized mice than for unparasitized mice. Parasitized mice may use greater hematocrit to enhance oxygen delivery to enlarged organs or to help supply oxygen during greater resting metabolism, which can occur with *H. polygyrus* infection (Kristan and Hammond, 2000, 2001).

Maternal effects of parasites on offspring growth and morphology

Growth

Growth curves of body mass but not size measures (tail and foot length) were affected for offspring from parasitized compared with unparasitized mothers. Although maternal parasite status did not affect growth trajectories directly for size measures, maternal effects did modulate the direct effects of parasites on growth. Previous research has shown that parasitized mothers wean smaller female pups (Kristan, 2002), but data in the present study show no effects of maternal parasite status on final adult size of male or female offspring, indicating that laboratory mice female pups fed *ad libitum* have some compensatory growth. Pups may use energy for fast growth potentially to reach reproductive ability early and thereby modulate possible negative effects of parasites (Sorci and Clobert, 1995). This use of energy for fast growth may come at a cost of using energy for other purposes, such as activity or immune response.

Morphology

A change in energy allocation by offspring during growth occurred as a result of maternal parasite infection, as indicated by offspring of parasitized mothers having similar overall body mass to offspring of unparasitized mothers, despite some greater organ masses (stomach, small intestines and livers). Importantly, the greater small intestine mass resulted from an increase in length and also an increase in the mass of the serosal layer, but not the mucosal layer. The greater mass of the serosal layer may result from increases in muscularis layers or layers of connective tissue, which could be determined by histological analysis. Why such morphological changes occur in small intestines of offspring born to infected mothers is unclear but may suggest altered digestive functions related to peristalsis (which utilizes the muscularis layers). Finally, pups born to parasitized mothers had lower hematocrit values than pups born to unparasitized mothers, indicating that maternal parasitism infection affects blood characteristics of offspring, which may influence overall offspring health.

Effects of sex on growth and morphology

In general, males grew faster, weighed more at their time of maximal growth, and reached their maximal growth rate earlier than females, but overall differences in growth between males and females were mainly related to body mass and not size (foot or tail length). Sex differences in body morphology were modified by parasite infection for only one organ, the cecum. The difference in caecae size between males and females was more when mice were unparasitized than when they had parasites. This is surprising because parasite infection alone did not affect cecum size. It is apparent, however, that *H. polygyrus* infection produces variable effects in the cecum, because some studies show no effect of parasites (Kristan and Hammond, 2000; present results) whereas another study showed greater caecae size for infected mice compared with uninfected mice (Kristan and Hammond, 2001). Changes in nutrient composition and the density of ingesta with parasitism may affect cecal function and warrant future investigation.

Infection intensity as a function of sex and maternal parasite status

Host sex

Although male mice are expected to be more susceptible to infection with *H. polygyrus* than female mice (Dobson, 1961, 1962b; Dobson and Owen, 1978), infection intensity did not differ between males and females for either the low-infection-intensity or high-infection-intensity groups. Importantly, a number of researchers have shown that host susceptibility to *H. polygyrus* is variable for *M. musculus*. For example, susceptibility varies with host age and infection duration such that young mice with 5-day-old infections showed no sex differences in worm burden but both young and mature males with 10-day-old infections had more worms than similar-aged females (Dobson, 1962a). Given that many aspects of this host-parasite system vary with mouse strain (Liu, 1965; Monroy and Enriquez, 1992; Scott and Tanguay, 1994; Su and Dobson,

1997), it is not altogether surprising that susceptibility of Swiss-Webster mice to *H. polygyrus* is similar for males and females given the infection intensities and duration used in my study.

Maternal effects

Only pups from parasitized mothers cleared their previously mature infections by 60 days of age. Moreover, whereas some infected mothers had all their infected pups clear the infection, other mothers had only some of their infected pups clear the infection. To try to understand this result, consider the host response to *H. polygyrus*. In an initial infection, the infective-stage larvae first enter the small intestine muscularis layer and elicit a cell- and antibody-mediated immune response (Liu, 1965; Panter, 1969; Monroy and Enriquez, 1992). However, before the host's immune response is fully effective, some worms migrate to the intestinal lumen and mature into adults that release an immunosuppressive factor (Dehlawi and Wakelin, 1988; Monroy et al., 1989; Monroy and Enriquez, 1992; Scott and Koski, 2000). This immunosuppressive factor protects the adult worms and, presumably, any additional larvae that emerge into the intestinal lumen. In a second infection, mice mount a rapid cell-mediated immune response that attacks larvae and delays larval development, and any larvae that do reach maturity to enter the small intestine lumen are expelled within several weeks (Monroy and Enriquez, 1992; Scott and Koski, 2000).

Because of the initial immune response, mothers with *H. polygyrus* infections have circulating levels of antibodies [e.g. immunoglobulin G (IgG) and immunoglobulin E (IgE); Scott and Koski, 2000] that can be transferred to pups *via* milk (Greenberg, 1971; Jansen et al., 1994 and references therein). This transfer of antibodies may act similarly to a secondary infection where parasites could easily be expelled by 60 days of age (i.e. 5 weeks PI). In this scenario, the antibodies passed from mother to pup act in place of the typical cell-mediated response of secondary infections.

So why don't all pups of a litter from parasitized mothers clear their infections? The effects of the host immune response in secondary infections with *H. polygyrus* are variable among mouse strains (Scott and Tanguay, 1994), and presumably among individuals, and are at least partially genetically based (Scott and Tanguay, 1994). Therefore, different parasitized mothers potentially have variations in circulating antibody levels that can be transferred to pups, who will also show different capacities to respond to infection. Furthermore, at 20 days old (when pups were experimentally infected), some pups may have a lot of antibodies from milk whereas others may have relatively little, depending both on circulating maternal levels and on the amount of nursing done by pups. There may be a threshold level of antibodies needed before an infection can be cleared, given that this is not the typical pathway of the immune response to secondary infections, and this threshold may differ among individual pups. These proposed mechanisms of the effects of maternal antibodies on offspring susceptibility to *H. polygyrus* remain to be tested.

When high-infection-intensity pups that cleared their

infections were excluded from the analysis, offspring from parasitized mothers had greater infection intensities than offspring from unparasitized mothers, suggesting that the overall immune capacity of pups from parasitized mothers was compromised. Therefore, maternal condition and, possibly, subsequent differences in maternal effort (e.g. nursing, brooding) may change the susceptibility of offspring to *H. polygyrus* and possibly other parasite species as well.

Ecological and evolutionary implications

Although research has linked parasite infection with empirical and theoretical changes in host and parasite life history (Minchella, 1985; Hochberg et al., 1992; Forbes, 1993; Richner and Heeb, 1995; Koella et al., 1998; Gustafsson et al., 1994; Perrin and Christe, 1996; Thomas et al., 2000), current models of host-parasite associations do not adequately account for the cost of a long-term demand of chronic, sublethal infections that may ultimately affect host reproduction, metabolism and life history (Roberts et al., 1995). Measuring the effects of parasites on adult physiology and reproduction and the direct and maternal effects of parasites on juvenile growth will provide a better picture of the importance and time course of life history traits affected by these types of parasites. We still need to know if offspring of parasitized mothers have different reproductive success than offspring of unparasitized mothers to determine if *H. polygyrus* has evolutionary implications for its host.

Conclusions

This study examined how a host responded to sublethal parasite infection as a young, growing animal and how an infected mother can affect her offspring growth and final body condition even after weaning. I provided evidence of limited effects of both direct and maternal effects of parasite infection on offspring growth and adult body condition in a controlled setting, which can provide the basis for future comparisons in nature. Because observations of captive animals can show different growth patterns compared with their wild counterparts (Morrison et al., 1977), it will be important to determine whether the patterns exhibited by these laboratory mice can be extrapolated to wild house mice.

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