Effects of food abundance, age, and flea infestation on the body condition and immunological variables of a rodent host, and their consequences for flea survival

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Abstract

Temporal variation in body condition and immunological variables of animals that harbor parasites may explain patterns of variation in infestation, as well as parasite impact on the host. We emulated such variability in Sundevall’s jirds by manipulating food availability and flea infestation in juveniles and adults and examining how these changes affect survival of fleas on their hosts. Body condition of food-restricted jirds deteriorated, but there was no change in their immunological variables. Adult jirds were in better body condition and had higher immunocompetence than juveniles, however there were no significant effects of flea infestation on any of the variables examined. The main effects of flea infestation were a decrease in the response to phytohaemagglutinin injection, and an increase in the negative effects of food restriction on body mass. Flea survival was higher on juveniles, but fleas did not respond to temporal variability in body condition and immunocompetence of the jirds. We concluded that changes in body condition and immune responses due to growth or variability in food abundance are more important than changes caused by the fleas themselves. Flea infestation is more detrimental to jirds when they are not able to compensate for mass loss through increased food consumption.

Keywords: Body mass; Dual energy X-ray absorptiometry; Ectoparasites; Food restriction; Haematocrit; PHA test; Rodents; White blood cells

1. Introduction

Temporal variation in body condition and immunological variables of individual animals that harbor parasites may explain patterns of variation in infestation, as well as the impact of parasites on their hosts. Nevertheless, causes of this variation and its consequences on the mutual responses of hosts and parasites are poorly known (Wilson et al., 2002). It is, however, well known that in any parasite–host association, some host individuals are more susceptible to parasitism than others (reviewed in Wilson et al., 2002). For example, parasite abundance and distribution may be biased depending on host gender (Poulin, 1996; Zuk and McKea, 1996; Schalk and Forbes, 1997; Krasnov et al., 2005c), age (Hudson and Dobson, 1997; Hawlena et al., 2005; Krasnov et al., 2006b), and reproductive status (McLean and Speakman, 1997). Moreover, infestation status of the same host individual may change with time (Krasnov et al., 2006a). On the one hand, a previously
parasitized host may become immunized and lose its parasites. On the other hand, a non-parasitized host may acquire parasites over time. The temporal pattern could result from changes in the parasite attack rate, changes in the host’s environment, physiological changes in the host itself, or changes in all of these (Bize et al., 2008).

Temporal variation in host susceptibility to parasitism can be caused by variability in climate or resource abundance (hereafter, environmental factors; Tschirren et al., 2007; Bize et al., 2008). For instance, in tropical oceans, warmer summers may increase host susceptibility to parasite infections through thermal stress (Harvell et al., 2002). Temporal changes in host susceptibility may take place during the transition of a host from one age, reproductive, or social cohort to another (hereafter, host-related factors; e.g., Christie et al., 1998). Finally, the parasites themselves, by exploiting their hosts may cause physiological and behavioral changes (hereafter, parasite-related factors; e.g., Walker et al., 2003; Mooring et al., 2004).

Temporal changes in the body condition of a host may affect behavior and abundance of its parasites in a manner similar to that in which free-living species respond to changes in resource availability. For instance, mites respond actively to changes in nutritional status of their host bat hosts by choosing the well-fed ones (Christie et al., 1998). However, in contrast to the environment of a free-living species, the environment of a parasite, namely its host, can actively resist its exploiter (Wakelin, 1996; Moore, 2002). Consequently, changes in immune response and/or behavioral defenses against parasites may also alter the survival and reproductive success of parasites on the same host individual at different times (Clayton, 1991; Christie et al., 1998; Singh and Haldar, 2007; Bize et al., 2008).

We investigated the causes and consequences of changes in the body condition and immunological variables of flea-infested rodents. The stochasticity of changes in the temporal pattern of infestation level of rodents by fleas suggests that they are related to changes in the host itself rather than to periodic changes in the environment of the host (Krasnov et al., 2006a). There are indications that environmental, host-, and parasite-related factors affect reciprocal responses of both rodents and fleas (Krasnov et al., 1998, 2002, 2005a; Gouy De Belloco et al., 2006). However, the combined effects of the three types of factors have not been addressed, and there have been few attempts to explore both flea and rodent responses simultaneously (e.g. Gouy De Belloco et al., 2006). Therefore, we examined the separate and combined effects of environmental, host-, and parasite-related factors on variables pertaining to body condition (body mass, mb, absolute fat mass, mf, ml as a fraction of mb (mf/mb), and haematocrit, Hct), and immunological variables (white blood cell count, WBC, and response to phytohaemagglutinin injection, PHA) of rodents, and we quantified flea survival. We emulated temporal variability in the above variables by manipulating food abundance and flea infestation in juvenile and adult rodents.

As food-restricted rodents are, by definition, short of energy, and juvenile, and parasitized rodents have relatively higher energy requirements than adult and non-parasitized animals (Hawlena et al., 2006b), our first hypothesis was that body condition of a rodent, and its immunological variables depend on food availability, age, and its level of flea infestation. Therefore, we predicted that host body condition deteriorates with both food restriction and under flea infestation. We further predicted that food restriction degrades immunological variables, that body condition is poorer and immune responses are weaker in juvenile than in adult rodents, and that flea infestation induces an immune response by rodents that in turn affects the ability of host to resist infection by another parasite (simulated by PHA injection; see below). The way that fleas might affect a rodent’s PHA response is difficult to predict. On the one hand, fleas cause irritation and allergy, damage the host’s skin, inject toxins, induce anemia and cause immunodepression that may confer an advantage on a superimposed infection (Cox, 2001). On the other hand, non-specific immune system cells produced against fleas (e.g., monocytes, neutrophils, and basophils) may be activated against the superimposed infection as well, thus increasing the immune response to PHA injection (Cox, 2001). Moreover, since the three factors may interact, we predicted that juveniles have stronger physiological and immunological responses to flea infestation than adults do. Finally, we assumed that the effects of food regime and parasite load on juvenile host body condition and immunity would be additive. Consequently, we predicted that juvenile food-restricted rodents that are parasitized by fleas would be in the worst body condition.

Our second hypothesis was that a flea’s responses to changes in food abundance, host age, and flea infestation level should depend on its host’s defensive responses. Therefore, we predicted that a stronger immune response by the host decreases flea survival. Conversely, reduction in body condition may decrease the time and energy available for a rodent to groom, and thus increase flea survival. We predicted that these effects are more pronounced in juvenile rodents.

2. Materials and methods

2.1. Experimental animals

As hosts, we used Sundevall’s jird, _Meriones crassus_ (Rodentia, Muridae, Gerbillinae), from our laboratory colony. Progenitors of the colony were captured in the central Negev Desert (30°58′N, 34°79′E) in 1996. Rodents were maintained individually in 20 × 30 × 10 cm3 plastic cages, on a bedding of sand, in our animal facility at an air temperature of 25 °C and photoperiod of 12 h:12 h. The jirds were provided daily with millet seeds _ad libitum_ and alfalfa _Medicago sp._ as an additional food and water source and maintained constant mb (Hawlena, H, unpublished data). Neither the animals we used in experiments, nor their parents had ever been exposed to fleas; this controlled for possible differences due to previous exposure to fleas (Alexander, 1986). We used only healthy animals whose body mass (mb) (adults > 100 g; juveniles > 21 g) was constant over time, and had a healthy blood profile, namely a white blood cell count (WBC) of 5000–25,000 cells mm−3 and a haematocrit (Hct) of 40%–60%, to exclude the possibility that hosts were co-infected by other parasites, which could enfeeble their body condition. To reduce gender-dependent variability in...
susceptibility, and/or in grooming and immune response to fleas, only males were used in experiments (e.g. Moore, 1986; Fuller and Blaustein, 1996). Juvenile rodents were separated from their mothers 30 days post partum, and after 3 days in a cage with other siblings, were placed in individual experimental cages.

Experiments began when juveniles were 34 days old and adults were 116–292 days old and we used five triplets and four pairs of siblings. Individuals from each triplet or pair were randomly assigned to groups of either food-restricted — non-parasitized (n=9), food-restricted — parasitized (n=9), or food ad libitum — non-parasitized (n=5; triplet siblings that were offered millet seeds ad libitum). Twenty adults were randomly assigned to either food-restricted — non-parasitized (n=10), or food-restricted — parasitized groups (n=10). Due to the following practical constraints: 1) a limited number of fleas available to add daily, 2) a limited number of adult-male jirds of age and body mass relevant to our experiments, and 3) a limited number of jird litters with at least four healthy male pups, our experimental design was incomplete. We were unable to have treatment groups of (1) food ad libitum — non-parasitized adults, (2) food ad libitum — parasitized adults, and (3) food ad libitum — parasitized juveniles. The incomplete design prevented us from testing for possible interactions among the three main effects: food restriction, flea infestation, and host age. The experimental protocol met the requirements of the 1994 Law for the Prevention of Cruelty to Animals (Experiments on Animals) of the State of Israel and was approved by the Ben-Gurion University Committee for the Ethical Care and Use of Animals in Experiments.

Sundevall’s jird is naturally parasitized by several flea species, but Xenopsylla conformis predominates (Krasnov et al., 1998 and references therein). We therefore chose to use this species in the present study, and obtained them from our laboratory colony started with specimens collected from wild Sundevall’s jirds. Details on flea maintenance and breeding can be found in Hawlena et al. (2006b). There was no any indication of pathogens in our flea colony that might affect the results reported. Fleas were maintained at 25 °C and 75% relative humidity under a 12 h:12 h photoperiod.

2.2. Experimental design

At the beginning of the experiment (day 0), we weighed each rodent to ±0.01 g (Ohaus CT200-S electronic balance, Ohaus Corporation, USA), took an initial blood sample to measure Hct and WBC, and measured its body composition by dual energy X-ray absorptiometry (DEXA, see below). Subsequently, 50 and 120 newly emerged fleas (3 days old) were placed on each juvenile and adult rodent, respectively, in the parasitized groups. These numbers of fleas are within the natural infestation range of wild Sundevall’s jirds (Krasnov et al., 2003; Shenbrot et al., unpublished data), and resulted in similar flea densities per unit body surface area for both juveniles and adults (0.4±0.009, and 0.4±0.02 fleas cm−2, respectively, where surface area was calculated according to Meeh equation for mammals; Stahl, 1967). On each of the next 14 days, we weighed the rodents, collected surviving fleas, and replaced the fleas that had died with newly emerged ones. Juvenile and adult jirds in the food-restricted treatments were fed seeds at 0.62 and 0.55 g per g (m0)−0.54·days−1, respectively. This restriction was expected to reduce the growth rate of juvenile rodents by about ~20% and represents some 70% of the daily energy requirements for maintenance for adult Sundevall’s jird (Khokhlova et al., 1995). On day 15, after removing fleas from the rodents, we took a second blood sample from each to measure Hct and WBC, measured body composition, and did a PHA skin test. This experimental design, in which, the body conditions and WBC of individuals were measured twice, i.e., before and after treatments were applied, reduced the within-group variance, thereby increasing the power of the statistical tests used. However, we could not apply this approach to the PHA skin tests because (a) pre-treatment tests would have affected the results of the post-treatment tests (reviewed in Kennedy and Nager, 2006), and (b) injection of PHA might have influenced the rodents, thereby increasing within-group variance.

2.3. Body condition in rodents

We measured two indicators of body condition in the rodents, namely, body composition (fat and lean mass and m2/m0), and Hct. Fat and lean mass were measured using dual energy X-ray absorptiometry (DEXA) with a Lunar PIXimus II (General Electric). DEXA is a reasonably accurate, noninvasive method (Nagy and Clair, 2000; Libouban et al., 2002; Gutman et al., 2006). During the measurements, rodents were anesthetized with Isoflurane® using an anesthetizing machine (Cambridge, Tech III Isoflurane Vaporizer). Each rodent was scanned twice and the mean of the two measurements was used as a single datum. Because of the limited scanning platform size of the DEXA and the large size of the adult rodents, we excluded the tail, heads and necks of both juvenile and adults from measurement.

We drew blood samples from the jirds’ infraorbital sinus using heparinized 75 μL capillary tubes. The animals recovered fully within 2 min of blood sampling. The capillaries were centrifuged for 10 min in a standard Hct centrifuge (ALC Haematocrit centrifuge 4203, Milano, Italy) and Hct was measured with digital calipers (Mitutoyo digimatic caliper, 500-12, Japan). Measurements of four capillaries taken from each individual were averaged, and the mean value was used for analysis.

2.4. Immunological variables

To evaluate immunological variables we randomly chose 10 out of 23 juvenile (2 food ad libitum — non-parasitized, 4 food-restricted — non-parasitized, and 4 food-restricted — parasitized) and 10 out of 20 adult rodents (5 food-restricted — non-parasitized, and 5 food-restricted — parasitized). We measured two indicators of immunity, namely non-specific immunity by WBC, and T-cell-mediated immunity by PHA test. To measure WBC, we diluted 4 μL of the whole blood with 96 μL of Türk solution to ensure erythrocyte lysis and counted leucocytes.
under a light microscope at 40× magnification using a Bürker-Türk haemocytometer, and expressed concentration as the number of cells per mm².

To measure cell-mediated immunity, we used the PHA (phytohaemagglutinin) skin test (Smits et al., 1999). The PHA test is commonly used to measure non-specific immunity in birds and mammals and involves subcutaneous injection of vegetal lectin, a phytohaemagglutinin that induces local T-cell stimulation and proliferation and causes swelling (Smits, Bortolotti and Tella, 1999). We measured the left and right footpad thickness with a screw micrometer (Mitutoyo digimatic thickness gauge, 547–301) to ±0.01 mm and immediately injected 0.1 mg of PHA dissolved in 0.03 mL of saline subcutaneously in the middle of the left footpad. The right footpad was injected with 0.03 mL of saline as a control. We measured the footpad thickness again after 6 h. The micrometer exerts a constant pressure on the footpad, therefore to standardize the measurements we took readings 3 s after its application. The PHA response was estimated as the change in thickness of the left footpad subtracted from the change in thickness of the right footpad. We measured the PHA response in triplicate, and used the average for each individual in subsequent analyses. To standardize the measurements of juvenile and adults rodents that have different footpad size, we divided the difference between post- and pre-injection measures of footpad thickness by the average footpad thickness of the left and right feet prior to PHA injection.

2.5. Flea survival

We measured daily flea survival by brushing the fleas from the body of each rodent into a white plastic can, collecting fleas that jumped off spontaneously and counting all the survivors. We estimated flea survival as the ratio of the number of surviving fleas (final flea number) to the initial number of fleas. Then, we replaced the fleas we recovered from each rodent back on their hosts, and supplemented new fleas for the dead ones. This design compensated for the short period of the experiment that did not allow the fleas to produce new offspring (it takes adult X. conformis at least 40 days to produce new imagos; Krasnov et al., 2005b), and hence resembled the natural setting where new fleas emerge daily. Non-parasitized gerbils underwent the same handling procedure. Fleas may die by natural causes as well as due to behavioral or immunological responses of their host. We could not distinguish among the causes of mortality, but as air temperature and humidity remained constant over time and space, and newly emerged fleas were added daily to the different treatment groups, differences in mortality among treatment groups or through time should reflect differences in host responses rather than intrinsic causes.

2.6. Data analysis

We used repeated measures ANOVA (RM-ANOVA) to test for the effects of host age, flea infestation, and food restriction on the measurements of body condition (i.e. mₐ, mₛ, mₒ/mₐ, Hct) and immunological variables (WBC, and PHA response), where time (day 0 vs. day 14) was the repeated variable. We repeated the analysis twice. First, we tested for the effects of host age, and the host age×flea infestation interaction on the dependent variables, and thus included the entire data set except for the group of juvenile, non-parasitized jirds that received food ad libitum. Then, we tested for the effect of food restriction and time (that manifest themselves on growth) on the dependent variables in juvenile rodents, including all three groups of juveniles (food ad libitum, non-parasitized, food-restricted—non-parasitized, and food-restricted—parasitized groups). We did a priori contrasts analyses to test for the predicted effect of time on body condition and immunological variables of (1) adult non-parasitized rodents, and (2) non-parasitized juvenile rodents that were offered food ad libitum. A priori contrasts analyses were also done (1) to test for the effects of time×food interaction on body condition and immunological variables of non-parasitized juvenile rodents, and (2) to compare among the changes in body condition and immunological variables of the three groups of juveniles (e.g. testing for an additive effect of food restriction and flea infestation).

We also used RM-ANOVA to test for the effects of host age (between-group factor) and time (days 1–14; repeated variable) on flea survival. The variables, mₒ/mₐ, and Hct, were arcsine-transformed. We used two-tailed tests to test for the effects of (1) age×infestation interaction on body condition and immunological variables of rodents, (2) flea infestation on immunological variables, and (3) age and time on flea survival. All other statistical tests were one-tailed because we had a priori expectations for the negative effects of food restriction and flea infestation, and for the positive effect of growth on host body condition and immunological variables. Data in tables are untransformed and presented as means±S.E.

3. Results

Body mass and Hct of juvenile-non-parasitized rodents that were offered food ad libitum increased significantly during the 14-day experiment (Tables 1 and 2; prediction 2). However, mₒ, mₒ/mₐ, and WBC in these rodents did not change over time (Tables 1 and 2; prediction 2). The change in WBC in non-parasitized juveniles that were offered food ad libitum was not significant, probably due to the large variance in this variable and our small sample size. However, after including the data for all juveniles in the analysis, the observed pattern of increase in WBC during growth was significant (p<0.005). Juvenile rodents had significantly lower mₒ, mₛ, mₒ/mₐ, and Hct, as well as lower PHA responses than adults did (Tables 1 and 2; prediction 3). There was no significant difference between the number of white blood cells in juvenile and adult rodent blood (Tables 1 and 2; prediction 3).

Adult rodents significantly lost mₒ and mₐ over the experimental period (Tables 1 and 2; prediction 1). The reduction in these variables is most likely a result of food restriction since adult Sundevall’s jirds that are offered food ad libitum maintain constant mₒ and mₐ under the same laboratory conditions that we used in the present study (Khokhlova et al., 1995; Kam et al., 1997). Adult, non-parasitized rodents lost, on
Table 1

Body condition, and immunological variables (mean±SE) on the first (prior to flea infestation) and last days of the experiment of non-parasitized (NP) and parasitized (P) adult and juvenile Sundevall’s jirds that were either offered food ad libitum (AL), or were food-restricted (FR).

<table>
<thead>
<tr>
<th>Host cohort</th>
<th>Food</th>
<th>Day</th>
<th>Host’s body condition</th>
<th>Immunological variables</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mₒ (g)</td>
<td>mᵣ (g)</td>
<td>Lean mass (g)</td>
</tr>
<tr>
<td>Juveniles</td>
<td>AL-NP</td>
<td>0</td>
<td>36.8±4.13</td>
<td>2.03±0.564</td>
<td>22.5±2.63</td>
</tr>
<tr>
<td></td>
<td>FR-NP</td>
<td>14</td>
<td>45.8±6.13</td>
<td>2.59±0.422</td>
<td>28.8±3.45</td>
</tr>
<tr>
<td></td>
<td>FR-P</td>
<td>14</td>
<td>43.3±2.56</td>
<td>1.87±0.212</td>
<td>26.8±1.54</td>
</tr>
<tr>
<td>Adults</td>
<td>FR-NP</td>
<td>14</td>
<td>117±4.30</td>
<td>22.8±2.69</td>
<td>56.7±1.60</td>
</tr>
<tr>
<td></td>
<td>FR-P</td>
<td>14</td>
<td>111±3.68</td>
<td>25.1±2.68</td>
<td>54.6±1.36</td>
</tr>
</tbody>
</table>

N = numbers of rodents in which body condition and immunological variables (within parentheses) were measured.

mₒ = body mass; mᵣ = fat mass; mₒ/mᵣ = fat ratio; Hct = haematocrit; WBC = white blood cell count; PHA = response to phytohaemagglutinin injection.

a Only 3 individuals were sampled for the PHA test.
b Only 4 individuals were sampled for the PHA test.

average, 0.35±0.08% (n = 10) of their initial mₒ per day during the 14 days of food restriction, reaching 95% of their mean initial mₒ during that period. Juvenile rodents gained mass during the experiment. Those that were offered food ad libitum gained an average of 1.62±0.30% (n = 5) of their initial mₒ per day, whereas the food-restricted, non-parasitized juvenile individuals gained 1.32±0.19% (n = 4). However, food restriction did not result in reduced mₒ of juvenile rodents, those that were both food-restricted and parasitized gained less mₒ than juveniles offered food ad libitum, and not exposed to flea infestation (Tables 1 and 2; prediction 4). Although food restriction and flea infestation did not result in reduced mₒ of juvenile rodents, that were both food-restricted and parasitized gained less mₒ than juveniles offered food ad libitum, and not exposed to flea infestation (Tables 1 and 2; prediction 4). Although food restriction and flea infestation did not result in reduced mₒ of juvenile rodents, that were both food-restricted and parasitized gained less mₒ than juveniles offered food ad libitum, and not exposed to flea infestation (Tables 1 and 2; prediction 4). Although food restriction and flea infestation did not result in reduced mₒ of juvenile rodents, that were both food-restricted and parasitized gained less mₒ than juveniles offered food ad libitum, and not exposed to flea infestation (Tables 1 and 2; prediction 4). Although food restriction and flea infestation did not result in reduced mₒ of juvenile rodents, that were both food-restricted and parasitized gained less mₒ than juveniles offered food ad libitum, and not exposed to flea infestation (Tables 1 and 2; prediction 4). Although food restriction and flea infestation did not result in reduced mₒ of juvenile rodents, that were both food-restricted and parasitized gained less mₒ than juveniles offered food ad libitum, and not exposed to flea infestation (Tables 1 and 2; prediction 4).

Table 2

F values of RM-ANOVAs (for all measurements excluding the PHA response) and ANOVA (for the PHA response) testing the effect of food restriction, host age, juvenile growth, flea infestation and their interactions on changes in body condition and immunological variables of adult and juvenile Sundevall’s jirds during the 14-day experiment.

<table>
<thead>
<tr>
<th>Prediction</th>
<th>Rodent group</th>
<th>Specific comparison</th>
<th>mₒ</th>
<th>mᵣ</th>
<th>mₒ/mᵣ</th>
<th>Hct</th>
<th>WBC</th>
<th>PHA</th>
<th>N</th>
</tr>
</thead>
</table>
| 1 — Body condition, growth, and immunological variables of food-restricted jirds deteriorate through time | Adults NP | | 21.8**** | 6.53** | 1.54 | 2.49 | 0.414 | – | 10 (5)
| | Juveniles NP: AL, FR | | | | | | | | |
| | Juveniles AL-NP | | 30.4**** | 2.15 | 0.117 | 13.8**** | 2.54 | | 5 (2)
| 2 — Growth improves body condition and immunological variables | All beside AL-NP | | 498**** | 125**** | 220**** | 14.8**** | 0.0755 | 21.4**** | 38 (18)
| 3 — Juveniles have lower body condition and immunological variables | All beside AL-NP | | 2.06 | 1.06 | 0.01888 | 1.69 | 0.858 | 4.67 | 38 (18)
| 4 — Flea infestation reduces body condition, growth, and immunological variables | All beside AL-NP | | 0.12 | 0.05 | 0.005 | 0.055 | 0.552 | 1.13 | 38 (18)
| 5 — Juveniles suffer more from flea infestation than adults | All beside AL-NP | | 1.16 | 2.90 | 1.75 | 0.0720 | 0.112 | 0.0133 | 14 (6)
| 6 — Negative effects of flea infestation and food restriction are additive | Juveniles AL-NP/FR-NP | | 1.16 | 2.90 | 1.75 | 0.0720 | 0.112 | – | 23 (10)
| | FR-NP/FR-P | | 1.66 | 0.536 | 0.24 | 0.47 | 1.29 | – | |
| | AL-NP/FR-P | | 4.70**** | 1.17 | 1.75 | 0.72 | 0.352 | – | |

Results are summarized according with tested predictions.

**** = p value<0.001; *** = p value<0.005; ** = p value<0.01; * = p value<0.05; exact p values between 0.05 and 0.1 are provided.

RM-ANOVA= Repeated measures ANOVA; mₒ = body mass; mᵣ = fat mass; mₒ/mᵣ = fat ratio; Hct = haematocrit; WBC = white blood cell count; PHA = response to phytohaemagglutinin injection.

a Only 3 individuals were sampled for the PHA test.
b Only 15 individuals were sampled for the PHA test.
period, we found no significant effect of time and the interaction between time and host age on flea survival (RM-ANOVA, $F_{1.38} = 1.38, p=0.169$ for time; $F_{0.804} = 0.804, p=0.656$ for interaction). However, flea survival was significantly higher in juvenile than in adult rodents (RM-ANOVA, $F_{4.42} = 4.42, p=0.05$, Fig. 1).

4. Discussion

We induced temporal variability in the body condition and immunological variables of adult and juvenile Sundevall’s jirds by restricting their food supply and infesting them with fleas. Thereafter, we followed the growth of the juveniles for 14 days. We also quantified existing variability in body condition and immunological variables in juvenile and adult rodents, compared the cohorts, and examined how this variability affects flea survival. In accord with our predictions, body condition of food-restricted juvenile and adult jirds deteriorated between the first and last days of the experiment. Fourteen days of growth improved the body condition and immunocompetence of juvenile hosts, but adults maintained better body condition and higher immunocompetence than juveniles did. However, our prediction that flea infestation would cause changes in body condition and immunological variables of hosts, was only partly supported since we did not find significant effects of flea infestation on any of the relevant variables. The main effects of flea infestation were (1) a decrease in the immune response to injection of PHA, and (2) intensification of the negative effects of food restriction on $m_b$. Moreover, contrary to our prediction, we found no indication that the immunological variables of food-restricted jirds degraded. As predicted, flea survival was affected by the age of the host, being higher in juveniles, the less resistant cohort. However, contrary to our prediction, fleas did not respond to temporal variability in body condition and immunocompetence of the jirds.

4.1. Food restriction

Food availability and quality in desert habitats is spatially and temporally unpredictable, and rodents often face periods of food shortage (Khokhlova et al., 2001). It has been shown that food restriction brings about reductions in fat stores, metabolic rate, Hct, heart rate, immunological variables, and overall activity (Wagland et al., 1984; Steegers et al., 1991; Scott et al., 2005; Gutman et al., 2006; Martin et al., 2007). However, most of these studies were done under extreme food restriction, where $m_b$ loss of the animals was substantial (e.g., Gutman et al., 2006; Martin et al., 2007). In contrast, our study emulated naturally occurring variability in food availability. Adult rodents lost 5% of their initial $m_b$ during the 14-day experiment, which is similar to the $m_b$ loss of this species during autumn in their natural habitat (about 3.77% per two weeks; Khokhlova et al., 2001). The reduction in $m_b$ of adult rodents resulted from their use of both fat stores and lean tissues, as indicated by the decrease in total body fat, but constant $m_f/m_b$ during food restriction. Juvenile rodents responded to food restriction by decreasing body fat accumulation.

Energy restriction often leads to suppression of immune function and may reduce the time and energy that is invested in grooming, thus increasing the risk of parasitism (Norris et al., 1994; Oppliger et al., 1996; Heitman et al., 2003). Nevertheless, in spite of the deterioration in body condition of food-restricted rodents, in the present study it did not affect WBC in either age cohort. This could result from our small sample size and low power of the statistical test ($N=6$ and 5 for juvenile and adult hosts, respectively). However, it is also possible that the energy costs of mounting an immune response are relatively low in Sundevall’s jird. Like its close relative Gerbillus andersoni, this rodent species, may possess a constitutive immune response, and thus have high costs of maintenance but low costs of mounting an immune response (Khokhlova et al., 2004; Hawlena et al., 2006c). Sundevall’s jirds have a high probability of encountering fleas because they inhabit loess planes and sandy habitats characterized by relatively high flea abundance (Krasnov et al., 1998). In fact, flea prevalence on this host species is 100% (Krasnov et al., 1998). Therefore, it may be of advantage for this species always to be prepared for flea attacks.

4.2. Host age and growth

As predicted, during the 14-day experiment, $m_b$ and Hct of juveniles increased as well as did their immunocompetence. We found similar age-dependent differences when comparing between body condition and immunological variables of juvenile and adult jirds, with three main differences. First, 14 days of growth were not enough to cause significant differences in absolute and relative $m_f$. However, comparing juvenile and adult jirds, it appears that these two variables do increase with age. Second, it appears that WBC increased during the 14 days of growth in juveniles, but at 48 days of age, i.e., at the end of the experiment, juveniles retained a WBC similar to that of the adults. Third, comparing juvenile and adult jirds allowed us to test the effect of host age on the PHA response. The PHA response of juveniles is lower than in adults, which further supports our prediction that juveniles are less immunocompetent than are adults. These age-specific differences in non-specific immunity suggest that the costs and
benefits of mounting an immune response for juvenile and adult hosts are different. The PHA skin test response is known to vary with many social and energetic variables, but the relationship between PHA response and age has received little attention (Haussmann et al., 2005). Studies on birds have reported that immature individuals have either lower (Lozano and Lank, 2003; Soler et al., 2003; Haussmann et al., 2005) or higher PHA responses (Moller, 2001; Tella et al., 2002) than adults. It is not yet known whether these differences are due to differences in measurement techniques or due to population and species differences. We are not aware of any other study that evaluated PHA in juvenile and adult rodents, hence we cannot determine whether the reduced PHA response of juvenile Meriones is associated with the energy requirements for growth.

Individuals in better body condition may have more resources to allocate to costly defense mechanisms (Sheldon and Verhulst, 1996), and these mechanisms may reduce the fitness of their parasites (Clayton, 1991; Bize et al., 2008). Despite the improvement in body condition and increase in immunocompetence of juvenile jirds during the experiment, we found no decrease in flea survival on these hosts. It is possible that the increase in WBC, an indicator of non-specific immune response, impairs other components of flea fitness, but does not cause their death. For example, Gouy De Bellocq et al. (2006) found a negative correlation between flea egg production and temporal changes in WBC in adult Sundevall’s jird. Alternatively, it is possible that temporal changes in body condition and immunocompetence over 14 days were too small, hence did not have a significant effect on flea survival. Our data do not suffice to test this hypothesis. However, the fact that flea survival on juvenile rodents (48 days old) was significantly higher than on the older (>194 days old), more immunocompetent, adults, suggests that 146 days of maturation, the minimum age differences between adult and juvenile hosts, may cause vital changes in the host as an environment for its fleas.

4.3. Flea infestation

Potential negative effects of flea infestation on the host are not limited to blood removal alone. Fleas also may damage the skin of hosts, give irritating bites, inject salivary toxins into the wound and inoculate them with pathogens (Nelson et al., 1977; skin of hosts, give irritating bites, inject salivary toxins into the wound and inoculate them with pathogens (Nelson et al., 1977; Marshall, 1981). However, the effect of flea infestation on any of the body condition variables or on WBC was not significant. This suggests that from both host and parasite perspectives, changes in body condition and immune response due to growth, or variability in food abundance are more important than changes caused by fleas themselves. From the host’s perspective, the negative effects of fleas are small relative to the effects of food restriction, or the relatively higher energy requirements of juveniles compared to adults. From the flea’s perspective, host exploitation does not elicit higher resistance by the host, thus does not affect their probability of mortality.

The results suggest that equilibrium natural densities of fleas are at a point at which the negative effect on a host is small enough to be below the sensitivity of our measurements. Several possible direct and indirect density-dependent mechanisms may regulate parasite numbers in nature. These include competition of parasites for limited resources (Patrick, 1991), density-dependent behavioral responses (Edman et al., 1972; Murray, 1987), immune responses of the host (Paterson and Viney, 2002), and density-dependent parasite-induced host damage (i.e. overexploitation of the host) (Jaenike et al., 1995; Ebert et al., 2000; Lowrie et al., 2004). Hawlena et al. (2007) proposed that numbers of X. conformis on their jird hosts are regulated through intraspecific competition among fleas and through grooming by the host. The present study indicates that the immune response of the host and density-dependent parasite-induced host damage may not play an important role in flea control.

The only significant effect of flea infestation that we found was depression of the immune response against a different antigen (simulated by PHA injection). A similar effect was found in X. ramesis that exploits the same host species (Gouy De Bellocq et al., 2006), suggesting that rodents may suffer indirectly from flea infestation while parasitized by other species. Apparent facilitation, in terms of parasite-induced immunodepression, has been reported repeatedly for parasites (reviewed in Cox, 2001). However, we found no evidence that fleas benefit from it. Flea survival did not increase over time during the 14 days of infestation. Furthermore, no relationships were found between the PHA response and blood consumption, egg production, or hatching success of fleas (Gouy De Bellocq et al., 2006). It is still unknown whether immunodepression facilitates other parasite species.

4.4. Combined effects of food restriction, host age, and flea infestation

In spite of the age-dependent differences in body condition and immunocompetence in rodents, we found no indication that juvenile rodents suffer more from flea infestation than adults do. The absence of age-dependent effects of flea infestation is especially intriguing considering previous findings that juvenile Anderson’s gerbil, G. andersoni, suffer higher $m_b$ loss and mortality than adults under similar conditions of food restriction and flea density (Hawlena et al., 2006a,b). Age-dependent differences in $m_b$ and survival in the Anderson’s gerbil were attributed to the larger surface-to-volume ratio of juveniles, which results in higher numbers of fleas per unit of blood volume (Hawlena et al., 2006b). Juvenile Sundevall’s jird are similar in body size to adult G. andersoni (36.8±4.13 and 30.66±1.32 g, respectively). As surface-to-volume ratio decreases exponentially with body size, it is possible that this ratio in juvenile Sundevall’s jird is small enough to result in the loss of blood to fleas per unit $m_b$ to be negligible. Alternatively, the differences between the responses of Sundevall’s jird and juveniles of Anderson’s gerbil to flea infestation may stem from maternal transfer of immunity in the former, but not in the latter, species. There is evidence for maternal immunity transfer in Sundevall’s jird (Khokhlova et al., 2004), but it remains to be tested whether maternal transfer immunity exists in G. andersoni as well.

One of the most striking results of this study is that while food restriction and flea infestation, separately, did not have significant effects on $m_b$ of juvenile jirds, their combined effects
were additive. Parasites do not necessarily induce negative effects if hosts have an energy surplus at the time of infestation (Munger and Karasov, 1989), or are able to compensate for losses through increased food consumption (Tripet and Richner, 1997). Therefore, the effects of parasites should be most significant when the host is food-restricted and compensatory mechanisms are limited. There are other examples where parasites exacerbate the effects of food or nutritional shortage (Keymer et al., 1983; Gulland, 1992; Berven and Boltz, 2001; Thompson et al., 2005), and it is likely that the main negative impact of fleas and other parasites on their hosts will appear during periods of food scarcity.

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