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Local variation in helminth burdens of Egyptian spiny mice (Acomys cahirinus dimidiatus) from ecologically similar sites: relationships with hormone concentrations and social behaviour

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Abstract

Populations of Egyptian spiny mice (Acomys cahirinus dimidiatus) in a fragmented montane wadi system in the Sinai showed significant differences in the abundance of gut helminths. Differences in parasite load between populations were positively associated with measures of androgen activity but showed no significant relationship with glucocorticoid activity. Social discrimination tests with adult males from different wadis showed that those from sites with greater helminth abundance were less likely to investigate odours from other males and were less aggressive when subsequently interacting with the odour donors. Subjects showed markedly more investigation towards the odours of males from distant wadis compared with those from their own or immediately neighbouring wadi, but were less aggressive when confronted with odour donors from distant wadis. Despite this, there was a positive relationship between the amount of investigation towards distant male odour and subsequent aggression towards the male. While aggressiveness was positively associated with measures of androgen and glucocorticoid activity, no significant relationship emerged with individual helminth infection. Thus aggressiveness appeared to relate to overall local population levels of infection rather than individual challenge.

Introduction

The idea that susceptibility to infection, and thus the prevalence and intensity of disease in host populations, reflects adaptive variation in investment in the immune system (so-called ‘ecological immunology’ (Sheldon & Verhulst, 1996)) has gained considerable support in recent years (Behnke et al., 1992; Folstad & Karter, 1992; Sheldon & Verhulst, 1996; Barnard & Behnke, 2001). Evidence from both field and laboratory studies suggests that immunity may be traded off against other components of life history, or, conversely, constrain investment in other components, and that, in birds and mammals, steroid hormones play an important mediating role (Grossman, 1985;
Alexander & Stimson, 1988; Folstad & Karter, 1992; Wedekind & Folstad, 1994; Wilckens & de Rijk, 1997). Such a view has profound implications for the evolution of host–parasite relationships and the epidemiology of parasite-related disease since it suggests that resistance may vary strategically and not simply through chance (genetic or environmental) variation in immunocompetence or exposure to infection.

We have provided extensive experimental evidence for strategic modulation of immunocompetence in relation to social rank in male laboratory mice (e.g. Barnard et al., 1996a,b; 1997a,b, 1998a,b; Barnard & Behnke, 2001). High-ranking males, for example, dissociate secretion of testosterone from measures of current immune responsiveness and incur a testosterone-dependent reduction in resistance to a subsequent infection with Babesia microti. The opposite is the case for low-ranking males. High-ranking males also maintain high levels of aggression, which are associated with reduced resistance to infection (Barnard et al., 1996a; Smith et al., 1996). However, both high- and low-ranking males tend to modulate testosterone secretion and/or behaviours associated with immune responsiveness (such as aggression and sleep (e.g. Barnard et al., 1997a,b; Gasparotto et al., 2002)) when experimentally immunodepressed or subjected to environmental stress (Barnard et al., 1996b, 1997a,b). So far, however, the idea of strategic modulation of immunity has not been tested in the field. Indeed, there are considerable difficulties in doing so, since naturally occurring hosts are likely to vary genetically in susceptibility and resistance to infection, and already to have been exposed to infection, which can itself have profound effects on steroid hormone metabolism and host behaviour (e.g. Edwards & Barnard, 1987; Edwards, 1988; Barnard & Behnke, 1990; Kavaliers & Colwell, 1995; Kavaliers et al., 1998). As a first step, therefore, we have tested whether males of the Egyptian spiny mouse (Acomys cahirinus dimidiatus), show associations between parasite burden, steroid hormone (androgen and glucocorticoid) activity and aggressive behaviour that are consistent with the adaptive immunity modulation. One prediction of the adaptive modulation hypothesis is that the extent and nature of immunity modulation will vary with the selection pressure imposed by parasites during the host’s evolutionary history (Behnke et al., 1992; Sheldon & Verhulst, 1996; Barnard & Behnke, 2001). Local population variation in parasite pressure might be expected to favour different levels of investment in immune responsiveness, either through naturally selected genetic change, or through phenotypic plasticity (reaction norms (Stearns & Koella, 1986)) depending on the magnitude, consistency and timescale of population differences (e.g. Hazel et al., 2000). If so, we should predict a degree of covariance between local parasite burdens, steroid hormone activity and behaviours impacting on immunity. In a parallel study of bank voles (Clethrionomys glareolus) in Poland, high burden populations were characterized by high levels of plasma corticosterone and testosterone and high fluctuations in asymmetry (FA) in hind foot length (a putative measure of developmental instability) (Palmer & Strobeck, 1986; Møller & Swaddle, 1997), but reduced aggressiveness among males (Barnard et al., 2002, 2003). The results of Barnard et al.’s vole studies thus provide correlational evidence that local variation in parasite infection within populations is associated with differences in host life history characteristics, reflected in morphological development and stress physiology, and experimental evidence that it is associated with investment in behaviour (aggression) likely to impact on immunocompetence (Barnard et al., 1996b, 1997a,b). Here, we ask whether similar relationships hold in populations of A. c. dimidiatus in the Sinai. As in C. glareolus, we focus on gut helminth infections, partly because these are the overwhelmingly dominant infections in the subject populations (Behnke et al., 2000), and partly because they are known to be associated with both androgen and glucocorticoid levels in the host (e.g. Wakelin & Selby, 1974; Roberts et al., 1996).

Materials and methods

The study was carried out in the St Katherine’s Protectorate, southern Sinai, an arid montane region characterized by complex systems of dry valleys (wadis) and plains (Hobbs, 1995; Žalat & Gilbert, 1998; Behnke et al., 2000). We chose two pairs of wadis: El Arbaein and Tofaha, close to the Suez Canal University Environmental Research Centre at St Katherine (Behnke et al., 2000), and Abu Seila and Boqeutia, some 5 km to the north across the Plain of El Raha. The wadis were selected as neighbouring pairs (i.e. with neighbouring mouths onto the Plain of El Raha) to test for gene flow between local populations (C.J. Barnard et al., unpublished) and effects of separation on responses to social odours and interactions in A. c. dimidiatus.

Mice were trapped in each wadi during August and September 2001 using Sherman small mammal traps (H.C. Sherman Inc., Tallahassee, USA). Sixty-eight traps were set in the boulder debris in each wadi at sites identified as active from the presence of fresh faeces and disturbed soil. Traps were set each night for 22 consecutive nights and inspected at dawn, whereupon all traps were removed until the following evening. On inspection, animals were weighed, measured and ear punched (samples stored immediately in 20% DMSO in saturated saline) on site, and a maximum of 35% of the catch (excluding lactating or pregnant females) retained for later participation in behavioural tests (adult males only; see later) and/or autopsy. All remaining animals were immediately released at their site of capture. The following morphometric measures were taken from each animal (see Barnard et al., 2002): the maximum length of the skull from the nose to the back of the cranium, the maximum width of the skull at the zygomatic arches, the length of the body from nose to anus, the length of each hind foot (measured twice to control for measurement error in relation to bilateral asymmetry (Palmer & Strobeck, 1986)) and anogenital distance (suggested to correlate with androgenization early in development (Drickamer et al., 1995; Palanza et al., 1995)). Retained animals were returned to the Research Centre at St Katherine, where adult males were housed singly in standard polypropylene laboratory cages (20 × 13 × 10 cm) with sawdust substrate (500 cm³) and
ad libitum food (standard rodent pellet and grain) and water. Males from each wadi were housed in a separate room on a natural light/dark cycle and at ambient temperature with freely-circulating outside air. Owing to variation in capture rate between wadis, and the ceiling on retained animals, a total of 9 (El Arbaein), 9 (Tofaha), 8 (Abu Seila) and 5 (Boqueia) males were eventually retained for testing.

All other retained males and all females were culled and weighed and immediately blood sampled (using 44 μl heparinized capillaries) by cardiac puncture. The entire alimentary canal was removed and placed in vials containing 10% formaldehyde, and liver, lungs and body cavity carefully inspected for helminths, which, when present, were removed and counted. The alimentary canal was carefully dissected and examined later at the University of Nottingham, and all parasites removed, identified and preserved in 70% ethanol. The spleen, kidneys, adrenal glands, thymus gland and, in males, testes and seminal vesicles were removed and weighed. Right and left paired glands were weighed separately. The weight of the seminal vesicles has been shown to be associated with circulating testosterone levels in several species of rodent (Fukazawa & Iguchi, 1999; Desai & Kondaiah, 2000; Jarred et al., 2000), and gonadal hormones generally have been implicated in sex differences in disease resistance (e.g. Klein, 2000). Thymus size and activity reflect immune potential and are influenced by circulating concentrations of steroid hormones (Grossman, 1985), while the size of the spleen can be affected by glucocorticoid levels and haemopoiesis and erythropagocytosis arising from infections (Phillips, 1969; Irvin et al., 1981; Barnard et al., 1993). Blood samples were centrifuged and the resulting serum frozen at −20°C for later hormone assays.

Experimental procedure

Males used in the experiment were allowed a minimum of three days to settle in their cage before a blood sample (pre-test sample) was taken from the tip of the tail in a 44 μl heparinized capillary, centrifuged and the serum stored for later assay as above. All tests were conducted in a separate room in the field station during the evening and water. Males from each wadi were housed in a separate room in the field station during the evening and were allocated as ‘residents’ (see below) for the purposes of testing, and paired with one ‘intruder’ male from each of three wadis, so that each resident experienced an intruder from its own wadi, its neighbouring wadi, and one of the wadis from the distant pair. Pairings were arranged so that males within dyads encountered each other only once. All residents were used as an intruder for another male, but with a minimum of 24 h between tests in the two roles (see also Hurst et al., 1996a).

Twenty-four hours before testing, each resident was established singly in an aluminium arena (30 x 30 x 30 cm) divided into two compartments by an opaque partition with a vertically sliding door. Ad libitum food and water were provided in the front compartment. Three clear Perspex tubes (20 x 4 cm), each end of which could be sealed with a solid or wire mesh stopper, extended from apertures in the front compartment. The resident was allowed free access to both compartments for the 24 h, during which the internal entrances to the tubes were sealed with the solid stoppers.

Odour tests

At the end of this period, the resident was confined to the rear compartment for 5 min during which 50 cm³ of soiled sawdust (taking care to exclude fragments of food) from the cage of one of the resident’s allocated intruders was spread inside each of the tubes. Which tube received the cheese, home, or distant wadi intruder’s sawdust was randomized across residents. Each tube was fitted with a wire mesh stopper at the distal end but was open inside the arena. A small fan blew a gentle current of air through the tubes from outside the arena. After 5 min, the resident was allowed to enter the front compartment and the number of times it sniffed at, or entered, each of the tubes, along with the duration and extent (e.g. head only, entire body and tail) of each entry was dictated in real time onto magnetic tape for a further 5 min. The resident was then confined once again in the rear compartment, and the tubes were removed, washed thoroughly in detergent, dried and replaced in the apertures with solid stoppers.

Intruder exploration and interaction tests

Immediately following the odour tests, the first donor intruder was introduced into the front compartment (while the resident was confined in the rear) and allowed to explore the compartment for 5 min. During this time the number of sniffs directed by the intruder at the floor, walls and ends of the tubes, and the occurrence and location of rearing up the walls (a behaviour associated with escape tendency in other rodents (Hurst et al., 1996b); table 1), was recorded onto tape. After the period of exploration, the door between the two compartments was raised and the resident and intruder allowed to interact for a further 5 min, during which all aggressive

| Behaviour categories recorded during social interaction tests. See text. |

| Anogenital sniffing – olfactory investigation of the anogenital region of the opponent. |
| Following – the subject followed a retreating opponent with the nose maintained close to the opponent’s anogenital region. |
| Chasing – the subject pursued the opponent as it moved away. |
| Biting – the subject bit the opponent. |
| Fleeing - the subject retreated rapidly from the opponent. |
| Defensive upright/sideways – the subject stood upright on hind legs with head facing away from the opponent (Defensive upright), or maintained all four feet on the substrate, presenting its flank to the opponent with the body curving away (Defensive sideways). |
| Rearing – the subject reared up against the wall of the arena while standing on its hind legs. |

Intruder exploration and interaction tests
interactions between the two were recorded (see below). Following interaction, the arena was wiped with detergent to remove any pools or streaks of urine left by the intruder, but it was not cleaned completely in order to ensure the resident’s odour was present on the substrate for returning tests. The procedure was then repeated for the second intruder, and finally the third. Intruders were returned to their home cage immediately after their interaction test. The order in which home, neighbouring and distant intruders were introduced followed the left-right order of the three odour samples in the tubes and was thus randomized across residents.

Six categories of aggressive and defensive behaviour were recognized, broadly concurring with those in wild (Mus domesticus) and laboratory (Mus musculus) house mice (Mackintosh, 1981; Hurst et al., 1994; Barnard et al., 1997a,b; table 1). Most interactions involved anogenital sniffing, following and chasing. Biting was very rare, and interactions were stopped immediately if aggression became escalated, or either individual was persistently harried. Only five out of 84 interactions had to be terminated for these reasons. No individual was injured in any way as a result of interactions during tests. Following their last intruder test, a second blood sample (post-test sample) was taken from each resident and stored as for the pre-test sample. Residents were then returned to their home cage and all cages returned to the room allocated to their wadi. When all tests had been completed, experimental animals were weighed again, then culled, measured and autopsied as above, and a final sample of blood (terminal sample) taken by cardiac puncture. All serum samples were later analysed for testosterone and cortisol (the major glucocorticoid in Mus domesticus puncture. All serum samples were later analysed for testosterone and cortisol (the major glucocorticoid in

In every case the control sample was thus randomized across residents.

To ensure the resident’s odour was present on the substrate and did not differ from normality (Kolmogorov-Smirnov one sample test, Dmax = 0.11, NS), the sample across wadis showed a small (0.17 ± 0.04 SE mm), but significant (t125 = 4.76, P < 0.0001) left bias, implying directional, rather than fluctuating, asymmetry in foot length (e.g. Palmer & Strobeck, 1986; Møller & Swaddle, 1997). There was no significant difference in measurement error between the left and right feet (paired t125 = 0.68, NS), which suggests the directional difference was not attributable to a side bias in measurement accuracy.

The first two components of the PCA accounted for 75.8% of the variance, the first correlating positively with all the measures of size (r125 = 0.64–0.93, P < 0.0001 in all cases), but not with hind foot asymmetry (r125 = –0.21, NS), the second negatively with head length (r125 = –0.26, P < 0.01) and positively with hind foot length (r125 = 0.36, P < 0.001) and asymmetry (r125 = 0.87, P < 0.0001). A similar contrast between body size and foot asymmetry emerged in a parallel study of bank voles (Barnard et al., 2003). We therefore refer to the first component as SIZE and the second as ASYMM.

Kruskal-Wallis analysis of variance (ANOVA) by wadi revealed no difference between wadis in trap sample sex ratio (H = 0.31, N = 138, NS). There was a significant difference in age category overall (H = 8.64, N = 134, P < 0.05), but this was due to a slight bias towards weanlings from El Arbaein and Abu Seila, and older pregnant or lactating females from Tofaha, none of which were autopsied or used in behavioural tests, and so did not contribute to the analyses in the present paper. There was no difference between wadis in the age distribution analysed here (H = 2.12, N = 60, NS). A parametric three-way ANOVA by wadi, sex and age category showed that SIZE differed significantly (and unsurprisingly) between sexes (F1,97 = 12.19, P < 0.001) and age categories (F2,111 = 56.49, P < 0.0001), but did not differ between wadis (F3,97 = 2.56, NS). A similar analysis of ASYMM showed no significant difference between wadis (F3,96 = 0.64, NS), age categories (F2,96 = 0.45, NS), or with sex (F1,96 = 3.06, NS). The differences remained significant or non-significant as above in all cases in the autopsied samples.

**Helminth infection**

Table 2 summarizes the prevalence and abundance of infection with helminth parasites in the study. The component parasite community structure was the subject of a separate analysis (J.M. Behnke et al., unpublished) and will not be discussed further here.

**Statistical analysis**

The results were analysed using Statgraphics Plus v 7 (Manugistics Inc., Maryland, USA). Parametric statistics were used where data (transformed as log10 x, log10 x + 1 or square root x where necessary) conformed to a normal distribution. Where data could not be normalized, non-parametric tests were used.

**Results**

The study tested the idea that differences in parasite abundance between local host populations are associated with differences in potentially immunomodulatory steroid hormone activity and aggressive behaviour (Folstad & Karter, 1992; Sheldon & Verhulst, 1996; Barnard & Behnke, 2001). Before comparing populations in this way, however, we checked for any biases in age, sex ratio and body size between sites that might affect comparisons.

**Size, age and sex**

Principal components analysis (PCA) was carried out on the weight and morphometric measures taken (initial body weight, nose–anus length, length and width of the skull and the mean length of the hind feet) to derive a composite measure of body size (Borkowska, 1999; Barnard et al., 2002). Since the lengths of the left and right feet showed a significant unsigned asymmetry when compared with measurement error (the difference between the two successive measures of each foot; paired t125 = 4.86, P < 0.0001), and hind foot asymmetry has been shown to be associated with local population differences in parasite burdens and behaviour in other rodent species (Barnard et al., 2002), we also included hind foot asymmetry in the PCA. However, while the distribution of the signed left minus right difference did not differ from normality (Kolmogorov-Smirnov one sample test, Dmax = 0.11, NS), the sample across wadis showed a small (0.17 ± 0.04 SE mm), but significant (t125 = 4.76, P < 0.0001) left bias, implying directional, rather than fluctuating, asymmetry in foot length (e.g. Palmer & Strobeck, 1986; Møller & Swaddle, 1997). There was no significant difference in measurement error between the left and right feet (paired t125 = 0.68, NS), which suggests the directional difference was not attributable to a side bias in measurement accuracy.

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In the present study, we are concerned with variation in abundance and its relationship with endocrine activity and host behaviour as these reflect the predictions of the adaptive immunity modulation hypothesis (see also Barnard et al., 2002). As in our previous study of A. c. dimidiatus in the region (Behnke et al., 2000), ANOVA, with SIZE as a covariate, revealed a significant difference in overall helminth abundance between wadis (F<sub>1,49</sub> = 3.69, P < 0.01), with abundances being greater in El Arbaein and Abu Seila, than in Tofaha and Boqueia (fig. 1). The peak abundance in Abu Seila (although a different site from previously) relative to El Arbaein and Tofaha concurred with those for spirurid worms in 1997 (Behnke et al., 2000), but the relative abundances in El Arbaein and Tofaha were reversed. There was no significant difference between the sexes and no interaction. Of course, the relative abundances of the different helminth groups are very different (table 1). Thus some further analyses were carried out to see whether any subset of infections had a disproportionate influence. These analyses suggested the site differences resided mainly in the total abundance of nematodes (F<sub>1,49</sub> = 3.10, P = 0.04). Although spirurid abundance was significant at the 10% level (F<sub>1,49</sub> = 2.29, P = 0.09), neither oxyurid (F<sub>1,49</sub> = 0.66, NS) nor cestode (F<sub>1,49</sub> = 1.41, NS) abundance on their own varied significantly with wadi.

Organ weights and hormone concentrations

From our previous studies of rodents, both in the laboratory (see Barnard & Behnke, 2001) and in the field (Barnard et al., 2002), we expected differences in parasite abundance across wadis to be reflected by differences in measures of androgen and glucocorticoid activity. However, the prediction is complicated by the fact that most animals in the sample were already infected. If differences in infection are a consequence of immunodepressive hormone activity, then evidence for activity should be greater in wadis with higher levels of infection (i.e. El Arbaein and Abu Seila; fig. 1). On the other hand, if once infected, or exposed to other infected individuals, animals downregulate immunodepressive hormone activity, and/or modulate it in relation to current immunocompetence, we should expect to see less evidence of androgen activity in wadis where infection levels are high (Barnard et al., 1996a,b, 1997a,b).

To see whether hormone activity differed between wadis, we first carried out two further PCAs: one on terminal testosterone concentrations and organ weight (testes, seminal vesicles) and morphometric measures (anogenital distance) relating to androgen activity in males, and one on terminal cortisol concentrations and organ weights (adrenal weight and weight asymmetry) relating to glucocorticoid activity across both sexes. While terminal testosterone concentrations were generally low (mean ± SE = 0.43 ± 0.02 ng ml⁻¹, range = 0.33–0.83 ng ml⁻¹, N = 37), the first component derived from the PCA for androgen activity (referred to hereafter as TEST) accounted for 61% of the variance and

<table>
<thead>
<tr>
<th>Wadi</th>
<th>El Arbaein</th>
<th>Tofaha</th>
<th>Abu Seila</th>
<th>Boqueia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirurida*</td>
<td>3.92 ± 0.99</td>
<td>3.57 ± 0.85</td>
<td>5.67 ± 1.05</td>
<td>2.20 ± 0.58</td>
</tr>
<tr>
<td>(63.2)[0–12]</td>
<td>(82.3)[0–11]</td>
<td>(80.0)[0–13]</td>
<td>(50.0)[0–4]</td>
<td></td>
</tr>
<tr>
<td>Oxyuroidea†</td>
<td>5.45 ± 2.36</td>
<td>1.53 ± 1.23</td>
<td>30.13 ± 25.69</td>
<td>1.50 ± 1.00</td>
</tr>
<tr>
<td>(40.0)[0–41]</td>
<td>(23.5)[0–21]</td>
<td>(60.0)[0–39]</td>
<td>(25.0)[0–7]</td>
<td></td>
</tr>
<tr>
<td>Total nematodes</td>
<td>10.55 ± 2.59</td>
<td>8.12 ± 2.26</td>
<td>38.30 ± 20.4</td>
<td>3.75 ± 1.45</td>
</tr>
<tr>
<td>(85.0)[0–53]</td>
<td>(82.3)[0–32]</td>
<td>(93.3)[0–402]</td>
<td>(50.0)[0–11]</td>
<td></td>
</tr>
<tr>
<td>Cestoidea (adult)</td>
<td>0.45 ± 0.19</td>
<td>0.35 ± 0.26</td>
<td>1.07 ± 0.76</td>
<td>0.13 ± 0.13</td>
</tr>
<tr>
<td>(25.0)[0–3]</td>
<td>(11.8)[0–4]</td>
<td>(33.3)[0–11]</td>
<td>(12.5)[0–1]</td>
<td></td>
</tr>
<tr>
<td>Cestoidea (cysts)</td>
<td>3.90 ± 2.38</td>
<td>0.88 ± 0.82</td>
<td>9.27 ± 6.89</td>
<td>0</td>
</tr>
<tr>
<td>(16.5)[0–43]</td>
<td>(11.1)[0–14]</td>
<td>(18.8)[0–100]</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>N (hosts)</td>
<td>20</td>
<td>18</td>
<td>16</td>
<td>10</td>
</tr>
</tbody>
</table>

* Protospirura muricola, Mastophorus muris, Streptopharagus kuntzi.
† Dentostomella kuntzi, Syphacia minuta, Aspiculuris africana.
was significantly positively correlated with the weights of both organs, anogenital distance and terminal testosterone concentration \( (r_{27} = 0.59-0.89, \ P < 0.01 \) in all cases). The PCA for glucocorticoid activity yielded three components, all correlating significantly positively with terminal cortisol concentration \((\text{mean} \pm \text{SE} = 738.16 \pm 52.83 \text{ ng ml}^{-1}, \text{range} = 52.1-1586.4 \text{ ng ml}^{-1}, \text{N} = 36)\). The third component (accounting for 22% of the variance, and referred to hereafter as CORT), however, yielded the expected association between positive correlations with size \( (r_{25} = 0.54, \ P < 0.001) \) and adrenal gland weight \( (r_{25} = 0.53, \ P < 0.001) \), and a negative correlation with adrenal gland asymmetry \( (r_{25} = -0.35, \ P < 0.05) \). As in mice \((\text{Mus musculus})\) \((\text{Abramov et al., 1996})\) and voles \((\text{Barnard et al., 2002})\; \text{M. Moshkin, personal communication})\, adrenal gland asymmetry in \( \text{A. c. dimidiatus} \) showed a significant left bias \( (t_{34} = 2.49, \ P < 0.02) \) \((\text{see also Szigethy et al., 1994}; \text{Gerendai & Halasz, 1997})\). In keeping with the well-recognized association between androgen and glucocorticoid concentrations \( (\text{e.g. Folstad & Karter, 1992}; \text{Poiani et al., 2000})\, \text{TEST} \text{and} \text{CORT} \) were significantly positively correlated \( (r_{27} = 0.66, \ P < 0.001) \). Each was therefore taken into account in analyses relating to the other.

ANOVA by wadi, controlling for SIZE and CORT, revealed a significant difference in TEST \( (F_{3,21} = 3.12, \ P < 0.05) \), with the differences in value between wadis reflecting those in helminth abundance \((\text{fig. 2, cf. fig. 1})\). Thus, mice from sites with the highest infections were those showing evidence of greater androgen activity. No differences emerged for CORT, however \( (F_{3,21} = 0.17, \text{NS}) \). ANOVA of thymus weight, controlling for SIZE, initially revealed no significant difference between wadis \( (F_{3,40} = 0.61, \text{NS}) \). However, sex steroids are known to have a negative feedback effect on thymus activity and size \((\text{Grossman, 1985})\), and stepwise partial regression with SIZE, CORT and total helminth abundance as additional independent variables showed a strong negative association between thymus weight and TEST \( (F_{3,18} = -3.22, \ P < 0.01) \). There was also a significant positive relationship with CORT \( (t_{34} = 2.56, \ P < 0.02) \) but no significant relationship with helminth abundance. When the relationships with TEST and CORT were taken into account as additional covariates in the ANOVA by wadi, residual thymus weight differed significantly between wadis \( (F_{3,18} = 3.39, \ P < 0.05; \text{fig. 3})\), being greater in El Arbaein and Abu Seila, where helminth burdens were higher. No significant differences between wadis, or relationships with TEST or CORT emerged for spleen weight.

**Response of residents to intruder odours**

To analyse the response to odours from different intruders, we carried out a further ANOVA with resident home wadi and the origin of the intruder with respect to the resident \((\text{home, neighbouring or distant wadi})\) as factors. Individual residents and intruders and tube position \( (\text{left, centre, right}) \) were also included as factors to control for pseudoreplication and any positional bias.

The analysis revealed a significant tendency for residents to investigate the outer two tubes more than the centre tube \( (F_{2,19} = 6.13, \ P < 0.01) \). Taking this into account, there were significant effects of both resident home wadi \( (F_{3,18} = 10.31, \ P < 0.001) \) and the wadi of origin of the intruder \( (F_{2,18} = 4.93, \ P < 0.02) \) on investigation. Residents from the wadis with the higher infections \((\text{El Arbaein and Abu Seila})\) investigated tubes less than those from the other wadis \((\text{fig. 4})\). While residents in general investigated the odours of distant intruders more than those from home or neighbouring wadis \((\text{fig. 5a})\). The effects of resident wadi and the wadi of origin of the intruder remained significant when helminth burden, TEST and CORT for residents and intruders were included as covariates. None of the
were significant. There was no significant effect of resident or intruder wadi on the number of entries to tubes.

Response of intruders to resident odour

A similar ANOVA for exploratory sniffing by intruders when introduced into the resident’s arena prior to interaction revealed a significant effect of resident home wadi \( (F_{3,23} = 3.36, P < 0.05) \), with intruders tending to explore the arena of residents from El Arbaein more than those of animals from other wadis. There was no significant effect of the intruder’s wadi of origin (home, neighbouring, distant; \( F_{2,23} = 1.14, \text{NS} \)) and no effect of the order (first, second or third) in which intruders were introduced in each resident’s sequence of encounters \( (F_{2,23} = 2.21, \text{NS}) \). There was no significant effect of any factor on the tendency for intruders to rear up the walls of the arena during the exploratory phase.

Aggressive interactions

ANOVA for the number of aggressive acts (chasing, following and biting) initiated by residents showed a difference between resident home wadis in the predicted direction of fewer initiations by residents from the higher infection sites of El Arbaein and Abu Seila. While the difference just failed to reach significance \( (F_{3,19} = 2.97, 0.05 < P < 0.1) \) when wadis were considered separately, there was a significant difference when high (El Arbaein and Abu Seila) and low (Tofaha and Boqueia) burden wadis were combined \( (F_{2,23} = 2.21, \text{NS}) \). There was no significant effect of any factor on the tendency for intruders to rear up the walls of their home wadi and least towards those from a distant wadi \( (F_{2,19} = 4.26, P < 0.03, \text{fig. 5b}) \). Initiations by intruders showed no effect of either resident home wadi \( (F_{3,23} = 1.81, \text{NS}) \) or whether intruders came from the resident’s home, neighbouring or distant wadi of \( (F_{2,23} = 0.87, \text{NS}) \), and there was no significant order effect for either residents \( (F_{2,19} = 1.18, \text{NS}) \) or intruders \( (F_{2,23} = 0.11, \text{NS}) \).

Stepwise partial regression analysis, controlling for helminth burden, SIZE, ASYMM, TEST and CORT, showed no significant association between the amount of investigation of an intruder’s odour and subsequent aggression against the intruder when home, neighbouring and distant intruders were combined, or when home or neighbouring intruders were considered separately. However, a significant positive association emerged for distant intruders \( (t_{21} = 2.93, P < 0.01; \text{fig. 6}) \), with residents showing more aggression towards distant intruders whose odour they had spent more time investigating. Interestingly, CORT also showed a significant positive association with aggression towards distant intruders \( (t_{21} = 2.94, P < 0.01; \text{fig. 7a}) \), but not with aggression towards home or neighbouring intruders, or all intruders combined. While no significant associations with TEST emerged, regression analysis with terminal hormone concentrations instead of PCs revealed a significant positive relationship between overall aggression by residents and terminal testosterone concentration \( (t_{21} = 2.81, P < 0.02; \text{fig. 7b}) \). Interestingly, no significant relationships emerged between aggression and the helminth burden of individual hosts, either overall or when high and low abundance wadis were analysed separately. Thus the association between helminth abundance and aggression across wadis

![Fig. 4](image1.png)

![Fig. 5](image2.png)
appeared to reflect population-level differences, rather than effects of infection on individual behaviour.

**Discussion**

The results provide broad support for the idea of local covariation in helminth infection, steroid hormone activity and aggressive behaviour in *A. c. dimidiatus*. However, the interpretation of this covariation is not straightforward.

Population-level comparisons between wadis showed that local differences in the intensity of helminth infection were associated positively with a composite index of androgen activity. This is consistent with other studies of rodents demonstrating negative relationships with androgen activity and resistance to infection (e.g. Klein, 2000; Barnard & Behnke, 2001; Barnard et al., 2002), and the negative relationship between androgen measures and thymus weight in the present study. While measures of androgen and glucocorticoid activity were positively correlated, again as found in many other studies (e.g. Folstad & Karter, 1992; Poiani et al., 2000), there was no evidence of any association between local differences in helminth intensity and glucocorticoid activity. Coupled with the lack of association between morphological asymmetry and helminth infection, the results suggest that variation in infection does not reflect differences in stress between local populations, an outcome that contrasts with our findings in bank voles, where both putative stress indicators (glucocorticoid activity and hind foot asymmetry) covaried with helminth intensity (Barnard et al., 2002). One reason for the difference between the two host species may lie in the temporal stability of selection pressures acting on local populations. In the voles, local differences in helminth intensity, androgen and glucocorticoid activity and morphological asymmetry were consistent in samples separated by three years, suggesting chronic differences in selection pressure (Barnard et al., 2003). While relative infection levels showed some temporal consistency across wadis in spiny mice, it is clear that some wadis differed between years. Thus local conditions facing spiny mice in the St Katherine's region may be temporally more variable than those facing voles in different forest fragments in Poland.

Of course, the cause and effect reasons for the association between helminth infection and androgen activity cannot be gleaned from the present results, though one possibility is that the timing or magnitude of
investment in reproductive activity differed between local populations, perhaps driven by differences in resource distribution or availability and leading to differences in exposure or susceptibility to infection. That testosterone concentration in experimental males correlated positively with aggressiveness in tests suggests that androgen levels may underpin competitive relationships between males. Paradoxically, however, aggressiveness overall was lower in populations from high infection/high androgen activity sites, as was interest in social odours. While a similar negative association has been found in voles, this could be interpreted in terms of differences in apparent stress between local populations (Barnard et al., 2003). No such association with stress was evident here (see above). Interestingly, in view of these differences between host species, relationships between aggressiveness and circulating (terminal) corticosterone (voles; Barnard et al., 2003) and cortisol (spiny mice) concentrations were in opposite directions: negative in the first case and positive in the second. Thus cortisol concentration appears to rise in relation to the number of attacks initiated by male spiny mice, while male voles are more likely to initiate attacks when corticosterone levels are low. This difference may be related to the different apparent impact of glucocorticoid activity on infection in the two species.

An important finding was that aggression in experimental males was not influenced by the helminth burden of the individuals themselves. Rather it was associated with the average intensity of infection in their source population. While caution in relation to the unavoidably small sample sizes is necessary, this is consistent with potentially immunodepressive behaviours being modulated in relation to perceived infection risk in the environment rather than in response to individual challenge. It is well known that parasites can affect odours and behaviour in infected hosts, and that this can provide social information to which other, non-infected, individuals respond, either behaviourally (Edwards & Barnard, 1987; Edwards, 1988) or physiologically (Kavaliers & Colwell, 1995; Kavaliers et al., 1998; Penn & Potts, 1998; Fernandes, 2000; Ehman & Scott, 2001). An interesting possibility, therefore, is that animals from high risk sites downregulate potentially immunodepressive activity in response to social information about risk, rather than, or as well as, their own current infection status. Animals at the various sites might thus be trading off investment in costly reproductive behaviours against future survival (reproductive opportunity) differently according to the infection risks to survival in their respective environments (Sheldon & Verhulst, 1996; Barnard & Behnke, 2001).

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