Journal of Helminthology (2003) **77**, 185–195 DOI: 10.1079/JOH2003194

Local variation in helminth burdens of bank voles (*Clethrionomys glareolus*) from ecologically similar sites: temporal stability and relationships with hormone concentrations and social behaviour

C.J. Barnard[1]*, K. Kulis[3], J.M. Behnke[2], A. Bajer[3], J. Gromadzka-Ostrowska[4], M. Stachon[4] and E. Sinski[3]

[1]Animal Behaviour and Ecology Research Group, and [2]Infection and Immunity Research Group, School of Life and Environmental Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, UK: [3]Department of Parasitology, Institute of Zoology, University of Warszawa, ul. Miecznikowa 1, 02-096 Warszawa, Poland: [4]Department of Dietetics and Functional Foods, Faculty of Human Nutrition and Consumer Services, Warszawa Agricultural University, Nowoursynowska 166, 02-787 Warszawa, Poland

Abstract

Populations of bank voles (*Clethrionomys glareolus*) in a fragmented forest habitat in north-east Poland showed local differences in helminth infection intensity, morphometric measures and organ weights that were consistent with differences at the same locations two years previously. Although overall intensities of infection were lower than previously, and there were some differences in the relative intensities of individual helminth species, site differences remained significant and were consistent across replicated subsites. In keeping with site differences in helminth infection and adrenal gland weight and asymmetry, voles at site 1 (high intensity infection) had higher circulating concentrations of corticosterone than those at site 2 (low intensity infection). Since males were sampled outside the breeding season, and thus non-scrotal, testosterone levels were low and did not differ between sites. As previously, voles at site 1 also showed greater hind foot asymmetry. Dyadic interactions between males from the same and different sites in the laboratory showed that males from site 1 were significantly less aggressive, especially when confronted with intruder males from site 2. There was no relationship between aggressiveness and intensity of infection overall or at site 1, but a significant negative relationship emerged at site 2. Aggression thus appeared to be downregulated at the higher intensity site independently of individual levels of infection. Terminal corticosterone concentrations were greater at site 1 and lower among residents that initiated more aggression. While corticosterone concentrations rose over the period of testing, they did not correlate with the amount of aggression initiated or received.

*Fax: (++44) 115 9513251
E-mail: christopher.barnard@nottingham.ac.uk

Introduction

Variation in parasite intensities within and between host populations (e.g. Crofton, 1971; Shaw & Dobson, 1995), and the processes resulting in the aggregation of parasites in certain individuals (Gregory & Woolhouse, 1993; Haukisalmi & Henttonen, 1999), are of central interest in the study of host–parasite community ecology. This is particularly so when populations with very different infection intensities inhabit ecologically similar habitats (Kennedy *et al.*, 1991; Hartvigsen & Kennedy, 1993; Sire *et al.*, 2001; Behnke *et al.*, 2000, 2001; Barnard *et al.*, 2002). While much is now understood about underlying ecological, immunological and demographic factors (Anderson & May, 1978, 1991; Anderson & Gordon, 1982; Quinnell & Keymer, 1990; Wakelin & Blackwell, 1993), recent interest has focused on differences in individual host life history strategy and the behavioural and physiological mechanisms underpinning them (Folstad & Karter, 1992; Sheldon & Verhulst, 1996; Nunn *et al.*, 2000; Barnard & Behnke, 2001). In particular, there is accumulating evidence that steroid hormones associated with stress and reproduction may mediate trade-offs between behaviour and physiology on the one hand and immune function on the other (e.g. Grossman, 1985; Folstad & Karter, 1992; Maier *et al.*, 1994; Sheldon & Verhulst, 1996; Barnard & Behnke, 2001), with immunocompetence being conserved or traded off in the face of incentives for investment elsewhere, for instance in territorial aggression or sexual behaviour (Folstad & Karter, 1992; Barnard *et al.*, 1997a,b; Hillgarth & Wingfield, 1997).

Two factors that are likely to influence such trade-offs are the risk and reproductive costs of infection and the value of current versus future reproductive opportunity. While Barnard *et al.* (e.g. 1993, 1994, 1996a,b, 1998), have provided experimental evidence for the latter as differences in the tendency to modulate testosterone and aggression in relation to current immunocompetence in male laboratory mice (*Mus musculus*) of different social rank, there is as yet little information as to the importance of either in natural populations in the field. In two recent papers, however, we have shown that bank voles (*Clethrionomys glareolus*), inhabiting ecologically similar but mutually isolated mixed woodland habitats in the Mazury region of north-east Poland, differed in the component community structure and intensity of helminth parasite infections (Behnke *et al.*, 2001), and that these differences were associated with anatomical and morphometric measures relating to endocrine function and apparent developmental constraint (Barnard *et al.*, 2002). In general, sites with greater parasite burdens were those in which male and female *C. glareolus* had significantly larger adrenal glands, and males larger testes and seminal vesicles for their age and body size. Voles from high intensity sites also showed greater fluctuating asymmetry (FA) in hind foot length, and thus, potentially, underlying developmental instability (Palmer & Strobeck, 1986; Møller & Swaddle, 1997; Barnard *et al.*, 2002).

These associations with parasite intensity are consistent with site-specific relationships between immunocompetence, steroid hormones and life history variation. However, in our previous study, we did not measure hormone concentrations directly; neither did we test whether animals modulated potentially immunodepressive activities in relation to site differences in infection. From our earlier work with mice (e.g. Barnard *et al.*, 1996a,b, 1997a,b), the obvious candidates to measure are testosterone and corticosterone concentrations and aggressive behaviour, since all have been shown to affect immunocompetence (e.g. Grossman, 1985; Brinkman & Kristofic, 1995; Klein *et al.*, 1997; Barnard *et al.*, 1996b) and to be modulated in relation to current immune status (e.g. Smith *et al.*, 1996; Wilckens & de Rijk, 1997; Barnard *et al.*, 1997a). Since our results suggested that voles at high infection sites were more stressed and showed evidence of developmental constraint, we might expect them to show higher concentrations of the stress hormone corticosterone and reduced aggressiveness compared with voles from other sites. In the breeding season, we might also expect greater investment in current reproduction (Barnard *et al.*, 2002), and thus generally higher concentrations of testosterone, but with concentrations being modulated in relation to current immune responsiveness (Barnard & Behnke, 2001).

Since these predictions assume that site differences in infection intensity and anatomical and morphometric measures are relatively stable, and not just chance sampling artefacts, it is also important to confirm that the differences are maintained over time (see Hazel *et al.*, 2000). We therefore conducted a further study at two of the three sites used by Behnke *et al.* (2001) and Barnard *et al.* (2002) two years after their initial study (September 1999). This time, however, we sampled two widely-separated areas at each site, both to replicate within sites and to look at the geographical extent of local differences (see e.g. Gerlach & Musolf, 2000). As well as sampling animals as previously (see below), we took blood samples to assay testosterone and corticosterone concentrations and tested for site differences in aggressive behaviour between males. Male *C. glareolus* in the field appear to establish aggressive dominance relationships on the basis of their sequence of recruitment to the breeding population (Gliwicz & Rajska-Jurgiel, 1983) and form stable hierarchies when maintained in groups in the laboratory (Gustafsson *et al.*, 1980; Hoffmeyer, 1982). Aggressive relationships are underpinned by a chemical communication system involving urinary and faecal odour cues denoting, among other things, prior residence and social status (e.g. Rozenfeld & Rasmont, 1991). The animals are thus well-suited to the kind of dyadic resident-intruder tests carried out here (see below).

The study was carried out in October in order first to test males outside their normal breeding season (May–September (Alibhai & Gipps, 1985)), when reproductive activity and testosterone concentrations were expected to be low. A second, replicate, study compares test males during the peak of the breeding season (June 2002) when the two measures are expected to be high (C.J. Barnard *et al.*, unpublished).

Materials and methods

The two sites chosen from our previous studies were those (Urwitalt and Pilchy) that had shown the most

https://doi.org/10.1079/JOH2003194 Published online by Cambridge University Press

marked differences in the various measures in our previous studies (Behnke *et al.*, 2001; Barnard *et al.*, 2002). Urwitalt is located east (long. 21° 39.6′, lat. 53° 47.7′) of a nature reserve surrounding Lake Luknajo to the north of the largest lake in the region, Lake Śniardwy, and Pilchy 11 km to the south-east (long. 21° 48.8′, lat. 53° 42.3′) at Leśnistwo Lisiej Jamy. Further details of the locations, and full habitat descriptions of the sites, can be found in Behnke *et al.* (2001). Urwitalt and Pilchy will be referred to as sites 1 and 2 respectively, but it should be noted that Pilchy was site 3 in our previous two studies.

Voles were trapped at each site during October 2001 using locally made wooden rodent traps (see Pawelczyk & Sinski, 2000; Bajer *et al.*, 2001; Behnke *et al.*, 2001). Each site was divided into two subsites approximately 500 m apart and 80 traps set in pairs (2–3 m apart) at 20 m intervals 10 m to the side of tracks running through the wood. Traps were set over 16 consecutive nights and inspected at dawn and dusk each day. Any traps containing animals were replaced with fresh traps and the animals brought back to the University of Warsaw's field station at Urwitalt (site 1) or Pilchy (site 2). On inspection at the field station, adult males (up to a maximum of 12 per subsite) were weighed and immediately housed singly in standard polypropylene laboratory cages ($30 \times 22.5 \times 10$ cm) with sawdust substrate (500 cm^3), shredded paper nesting material and *ad libitum* food (standard rodent pellet supplemented with fresh apple and carrot) and water for later use in experimental tests (see below). Males from each subsite were housed in a separate room at Urwitalt on a natural light/dark cycle and at ambient temperature with freely-circulating outside air. Owing to variation in capture rate between sites, and two mortalities, a total of 7 (site 1, subsite 1), 10 (site 1, subsite 2), 11 (site 2, subsite 3) and 10 (site 2, subsite 4) males were eventually retained for testing.

All other males and all females were culled and weighed, then exsanguinated by cardiac puncture and the following morphometric measurements taken: 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)). Animals were then autopsied. 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, counted and preserved in vials containing 70% ethanol. The intestines were carefully dissected, examined later at the University of Warsaw, 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), including *C. glareolus* (Tahka *et al.*, 1997) and gonadal hormones generally have been implicated in sex differences in disease resistance (e.g. Klein, 2000). We have already established that adrenal gland, testis and seminal vesicle weights are associated with parasite burdens and morphological differences between voles at the sites (Barnard *et al.*, 2002). The thymus gland had not been weighed in our previous studies, but thymus size and activity reflect immune response and are influenced by circulating concentrations of steroid hormones (Grossman, 1985). The lenses were also removed from the eyes and dried and later used to calculate the age of each individual (Morris, 1972; Kozakiewicz, 1976; Behnke *et al.*, 2001). 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 two 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. Following sampling, and to increase the likelihood of interaction between males (e.g. Hurst, 1993; Barnard *et al.*, 1997b), 25 cm^3 of soiled sawdust mixed in equal proportions from three cages of three grouped females from a distant third site was sprinkled over the substrate of each male's cage (see Barnard *et al.*, 1997b). The procedure was repeated with fresh soiled sawdust every night for the duration (10 days) of the experiment.

All tests were conducted in a separate room in the field station at Urwitalt during the early morning (approx. 0700–0930 h) and late afternoon and evening (approx. 1630–2200 h), when voles in the cages were most active. The test area was illuminated by a 40 W red lamp throughout. The test procedure was developed from the method of Hurst *et al.* (1996) for resident-intruder dyads of aboriginal house mice (*Mus spretus*). Thirty-six males (7, 9, 10 and 10 from subsites 1–4 respectively) were allocated as 'residents' (see below) for the purposes of testing and paired with one 'intruder' male from each of three subsites, so that each resident experienced an intruder from its own subsite, the other subsite at its own location and one of the subsites at the other location. 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.*, 1996). Six categories of aggressive behaviour between residents and intruders were recognized (e.g. Rutovskaya, 1968):

1. Boxing – the animal sat back on its haunches and directed rapid paddling motions of the forepaws towards the opponent, usually with the eyes closed and mouth open.
2. Lungeing – the animal launched itself towards the opponent, usually while 'boxing', but without biting.
3. Pushing – the animal bodily pushed the opponent along in front of it.

https://doi.org/10.1079/JOH2003194 Published online by Cambridge University Press

4. Vocalization – the animal emitted high-pitched, chattering vocalizations, either while 'boxing' or while sitting facing the opponent.
5. Chasing – the animal pursued the opponent as it moved away.
6. Biting – the animal bit the opponent.

Like *M. spretus* in Hurst *et al.*'s (1996) study, male voles in the present experiment showed little escalated aggression (chasing, biting). Instead, disputes were generally settled by a combination of vocalization and stylized 'boxing' in which winners and losers were identified by which retreated in response to the other. In some cases, aggressive interactions simply involved one animal pushing the other back. We therefore adapted Hurst *et al.*'s procedure of testing dyads in a clear Perspex tube in which advances and retreats could readily be measured.

Each resident was tested with each of its three intruders in a succession of 5 min tests. For each series of tests, the 'resident' was gently introduced into a clear Perspex tube (20 × 4 cm (length × internal diameter)) with a wire mesh stopper at the far end and into which soiled sawdust from the resident's home cage had been introduced 5 min previously. The tube was sealed with a second wire mesh stopper and the resident was allowed to settle for 5 min. After a brief period of investigation, residents quickly settled, usually against one of the end stoppers and facing along the tube. After 5 min, an intruder was gently introduced at the opposite end of the tube to the resident and the tube re-sealed. Interactions between resident and intruder were then recorded on a dictaphone for a further 5 min. Aggression was recorded as boxing actions and lunges by one animal towards the other, and corresponding responses (retaliation, no response or retreat) of the recipient. Two instances of biting were recorded, but neither resulted in injury. No injuries of any kind occurred during tests and no encounters had to be stopped because of excessive or persistent aggression. The position of each individual with respect to a midpoint marker on the tube was noted throughout. After the period of encounter, the intruder was returned to its home cage, the resident's tube emptied and replenished with fresh soiled sawdust from its home cage, and the resident allowed to settle again for a further 5 min. The procedure was then repeated for the second and finally the third intruder. Residents from all subsites were tested in each observation period to ensure a balanced distribution through the experiment, and intruders were tested in random order across residents with respect to the resident's own, neighbouring or distant subsite. 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 subsite. 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 corticosterone concentration at Warsaw Agricultural University using the radioimmunoassay method of Stachon *et al.* (2001).

Statistical analysis

Parametric statistics were used where data (transformed as $\log_{10} x$, $\log_{10} x + 1$ or square root x where necessary) conformed to a normal distribution. Where data could not be normalized, non-parametric tests were used.

Results

Differences between sites

The study was based on the assumption that voles from sites 1 and 2 in 2001 would show similar differences in the intensity of helminth infection to those from the same sites in 1999 (Behnke *et al.*, 2001; Barnard *et al.*, 2002). We therefore tested this after first checking for any site biases in age, body size and/or sex ratio.

Size, age and sex

Following Barnard *et al.* (2002; see also Borkowska, 1999), we used principal components analysis (PCA) to derive a composite measure of body size from the morphometric measures (skull length and width, nose–anus length, length of hind feet). Since Barnard *et al.* (2002) had also shown a significant difference between sites in fluctuating asymmetry in hind foot length, unsigned hind foot asymmetry was included in the analysis to take account of any relationships between character size and asymmetry (Palmer & Strobeck, 1986). Once again, the unsigned hind foot asymmetry did not depart from normality (Kolmogorov-Smirnov one-sample $D_{max} = 0.09$, N = 119, NS) and the signed asymmetry significantly exceeded measurement error ($t_{118} = 6.48$, $P < 0.0001$), but this time the unsigned asymmetry showed a small (mean ± SE = 0.16 ± 0.04 mm), but significant ($t_{118} = 3.76$, $P < 0.001$) departure from zero towards the left (cf. Barnard *et al.*, 2002). Unlike our previous study, therefore, we cannot rule out a directional component to the asymmetry in the present sample.

The first two derived components of the PCA accounted for 55% of the variance, the first correlating positively with all measures of size ($r_{116} = 0.51$–0.75, $P < 0.0001$ in all cases), but not with hind foot asymmetry ($r_{116} = 0.12$, NS), the second negatively with nose–anus length and hind foot length ($r_{116} = -0.31$, $P < 0.001$ and -0.24, $P < 0.02$ respectively), but positively with hind foot asymmetry ($r_{116} = 0.92$, $P < 0.0001$). The first component (here called SIZE) was thus taken as a measure of overall body size, and the second (here called ASYMM) as an index of constraint on morphological development (short body, short and asymmetric feet).

Multifactor ANOVA revealed no significant difference in morphometric measures, organ weights or helminth infection between males used in the behavioural experiment and males autopsied straight from the field. All animals were therefore used in analyses of site differences in these measures.

ANOVA revealed no significant differences between sites 1 and 2 in age distribution or sex ratio (Kruskal-Wallis test), or SIZE (parametric one-way ANOVA). Neither were there any differences in these variables between subsites within each site. In keeping with the results of Barnard

https://doi.org/10.1079/JOH2003194 Published online by Cambridge University Press

et al. (2002), however, there was a significant difference in ASYMM ($F_{1,114} = 5.38$, $P < 0.03$), with animals from site 1 showing greater hind foot asymmetry than those from site 2 (table 1). There was no difference in ASYMM between the sexes and no interaction between site and sex. There was also no difference between animals from the two subsites at each location.

Helminth infection

Table 2 summarizes the prevalence and intensity of infection with helminth parasites in the study compared with the data from the same sites in 1999. While at a qualitative level, the component community of helminths showed no change except for the absence of *Trichuris muris* in 2001, the prevalence and relative intensity of the different species changed in both directions between years, and overall intensity was lower in 2001, perhaps partly due to being sampled slightly later in the year. However, when analysed by site, animals from site 1 once again emerged with significantly higher intensities overall than those from site 2 (table 1), confirming the differences recorded in 1999 (Barnard *et al.*, 2002). There was no significant effect of sex ($F_{1,111} = 2.03$, NS) and no interaction between site and sex ($F_{1,111} = 0.39$, NS). Again, no differences emerged between subsites at either location ($F_{1,57} = 0.73$, NS for site 1; $F_{1,52} = 0.41$, NS for site 2).

Organ weights and hormone concentrations

In keeping with the site difference in infection, and our previous findings (Barnard *et al.*, 2002), adrenal weights were significantly greater at site 1 (table 1), with no difference between subsites. However, this time (cf. Barnard *et al.*, 2002), there was also a site difference in adrenal gland asymmetry (controlling for SIZE and adrenal weight as covariates), with the expected left bias being lower at site 1 (table 1). A significant sex difference emerged in adrenal weight (controlling for SIZE), with females having larger adrenals for their body weight than males (mean ± SE paired gland weight in males = 0.0074 ± 0.0003 g, in females = 0.0110 ± 0.0007 g; $F_{1,108} = 15.52$, $P < 0.001$), but there was no difference in adrenal asymmetry.

As might be expected from the above, terminal corticosterone concentrations were significantly higher among voles from site 1, both when animals used in the experiment were excluded (see below) ($F_{1,60} = 5.15$, $P < 0.03$) and when they were included (table 1), but with no significant difference between the sexes. Stepwise partial regression analysis (forward inclusion) of all animals, with SIZE, ASYMM, adrenal gland weight and weight asymmetry and intensity of helminth infection as independent variables, showed that adrenal weight was positively associated ($t_{43} = 2.33$, $P < 0.03$) with terminal corticosterone concentration in males (no other variables entering the equation) but not females.

All males caught in the study were non-scrotal, as expected for the time of year. No difference in testis or seminal vesicle weights emerged between sites across the sample of males as a whole ($F_{1,52} = 2.39$, NS; $F_{1,42} = 0.49$, NS respectively, controlling for SIZE; cf. Barnard *et al.*, 2002), but, among experimental males, those from site

Table 1. Means ± SEs from multifactor analyses of variance of morphology, helminth burden, organ weights and aggressive behaviour in voles from sites 1 (Urwitalt) and 2 (Pilchy). See text.

	Site 1 (Urwitalt)	Site 2 (Pilchy)	
ASYMM (PC)	0.20 ± 0.13	−0.24 ± 0.14	$F_{1,114} = 5.38$, $P < 0.03$
Total helminth burden ($\log_{10} x + 1$ no. worms)	0.99 ± 0.07	0.74 ± 0.07	$F_{1,111} = 6.33$, $P < 0.02$
Paired adrenal weight (mg)	0.01 ± 0.0005	0.008 ± 0.0005	$F_{1,108} = 5.76$, $P < 0.02$
Adrenal gland asymmetry (mg)	0.0008 ± 0.0003	0.0017 ± 0.0003	$F_{1,107} = 8.24$, $P < 0.01$
Terminal corticosterone concentration (ng ml^{-1})	102.29 ± 7.97	77.35 ± 5.00	$F_{1,93} = 4.12$, $P < 0.05$
Thymus weight ($\log_{10} x$ mg)	−1.80 ± 0.04	−1.63 ± 0.04	$F_{1,99} = 8.72$, $P < 0.01$
Aggression as resident or intruder ($\log_{10} x$ no. attacks)	0.72 ± 0.12	1.19 ± 0.11	$F_{1,35} = 7.12$, $P < 0.02$

Table 2. The prevalence and mean ± SE and range (in square brackets) of intensities of infection with higher taxa and individual species of parasites in October 2001.

	Prevalence	Intensity	
Nematodes	90.7 (85.6)	21.7 ± 6.5 [0–510]	(74.6 ± 35.9 [0–4029])
Heligmosomum mixtum	50.4 (40.3)	4.4 ± 0.7 [0–37]	(1.9 ± 0.3 [0–13])
Heligmosomoides glareoli	22.7 (36.0)	1.2 ± 0.3 [0–22]	(3.8 ± 0.3 [0–18])
Syphacia petrusewiczi	16.0 (13.7)	15.0 ± 6.5 [0–501]	(63.6 ± 35.9 [0–4026])
Aspiculuris tetraptera	14.3 (28.8)	0.8 ± 0.2 [0–16]	(6.9 ± 2.4 [0–240])
Mastophorus muris	8.4 (10.1)	0.2 ± 0.1 [0–14]	(0.2 ± 0.08 [0–9])
Trichuris muris	0 (0.70)	0	(0.007 ± 0.007 [0–1])
Cestodes[1]	10.9 (12.9)	0.1 ± 0.03 [0–2]	(1.2 ± 0.7 [0–93])

[1] *Catenotaenia henttoneni*, *Paranoplocephala gracilis*, *Mesocestoides lineatus*, *Taenia martis*, *Taenia mustelae*.

N = 119 in all cases. Numbers in parentheses are corresponding data for the same sites in September 1999. See text.

https://doi.org/10.1079/JOH2003194 Published online by Cambridge University Press

1 had, as in our previous study, significantly larger testes than those from site 2 ($F_{1,33} = 5.39$, $P < 0.03$). Once again, there was no site difference in anogenital distance. In keeping with values for non-scrotal male *C. glareolus* in other studies (e.g. Hughes & Randolph, 2001), testosterone concentrations were extremely low (mean ± SE ng ml^{-1} = 0.06 ± 0.014, N = 52) and could be estimated only by extrapolation from the equation for the calibration line of the assay. The small volumes of serum available for intercurrent samples also limited assays almost entirely to terminal samples. No significant difference in terminal testosterone concentrations emerged between sites across either all males ($F_{1,38} = 0.098$, NS; controlling for SIZE, and terminal corticosterone concentration) or those used in the experiment ($F_{1,28} = 0.06$, NS). However, regression analysis, with SIZE, ASYMM, terminal corticosterone concentration and adrenal gland asymmetry as other independent variables, showed that estimated terminal concentrations increased with testis weight in males from site 1 ($t_{19} = 2.54$, $P < 0.03$). No relationships emerged from site 2 or across all males combined.

Further evidence that differences in helminth infection were associated with underlying differences in endocrine and immune system activity came from thymus weights, which were not recorded in our previous study. ANOVA showed that thymus glands were significantly smaller at site 1 (table 1), with no difference between the sexes or subsites.

Aggressive behaviour

From the differences between sites in morphometric measures, infection intensity and organ weights, both in this and our previous study, we should predict that males from site 1 would be less aggressive, both as residents and intruders, than those from site 2, since they show more evidence of physical compromise and stress-related infection.

While males used in the experiment and those autopsied from the field did not differ in weight, morphometric measures or organ weights (see above), experimental males had significantly lower terminal corticosterone concentrations than their non-experimental counterparts (mean ± SE ng ml^{-1} for experimental males = 77.99 ± 5.12, for non-experimental males = 131.47 ± 25.25; $F_{1,34} = 5.85$, $P < 0.03$), probably as a result of the period of settling after trapping. No difference emerged for estimated terminal testosterone concentration ($F_{1,38} = 0.23$, NS). The average number of aggressive acts (lungeing, boxing and vocalizing) initiated by males from each of the sites when residents in their own tube and when intruders in the tubes of other males were significantly lower among animals from site 1 ($F_{1,33} = 4.67$, $P < 0.05$ when residents; $F_{1,34} = 7.15$, $P < 0.02$ when intruders, controlling for SIZE). Overall, therefore, males from site 1 were significantly less aggressive than those from site 2 (table 1). While the time spent by residents and intruders in each other's half of the tube was, respectively, positively ($t_{33} = 2.09$, $P < 0.05$) and negatively ($t_{34} = -2.68$, $P < 0.02$) related to the number of attacks by the resident, there was no tendency for residents or intruders from either site to spend more time there ($F_{1,32}$, NS in both cases). No significant differences in aggressive behaviour or time in the other individual's half of the tube emerged between subsites at either site.

When aggression by residents was analysed by intruder home site, residents from site 1 were less likely to attack intruders from either site, as expected from table 1, but the difference between residents from the two sites was significant only when they were confronted with intruders from site 2 ($F_{1,32} = 2.86$, NS for site 1 intruders; $F_{1,31} = 5.86$, $P < 0.03$ for site 2 intruders). No significant difference emerged between subsites at site 1, but males from sites 1 and 2 differed in their aggressiveness towards intruders from the two subsites at site 2 ($F_{1,15} = 5.42$, $P < 0.05$).

Analysis of resident and intruder aggression according to whether intruders came from the resident's home, neighbouring subsite, or more distant subsite (controlling for individual resident and intruder to avoid pseudoreplication) revealed no effect of intruder origin with respect to the resident's home location ($F_{2,32}$, NS for both residents and intruders).

Effects of parasite infection, morphometrics and organ weights on hormone concentrations and behaviour

In our previous study (Barnard *et al.*, 2002), we showed that the intensity of helminth infection in male voles increased with adrenal gland and seminal vesicle weights, but that the relationships were restricted to site 1. Stepwise partial regression analysis, with intensity of helminth infection as the dependent variable and the weight and asymmetry of adrenal glands, SIZE, ASYMM and thymus weight as independent variables showed that the site-specific positive relationship with adrenal weight remained in the present study, but only when both sexes were combined ($t_{47} = 2.30$, $P < 0.03$). Only adrenal weight entered the equation, and no significant relationships emerged with testis or seminal vesicle weight when these were included as further independent variables in an analysis of males only.

If differences in infection intensity between sites reflect chronic differences in stress, as suggested by the morphometric and organ weight analyses in our two studies, we might expect these also to predict differences in corticosterone concentration and aggression, since studies of other rodents have shown that these can be modulated in response to current immunocompetence (e.g. Barnard *et al.*, 1996a, 1998; Smith *et al.*, 1996). Our predictions are that (i) aggressiveness should decline with increased infection and evidence of morphological stress, while (ii) adrenal weight (relative to body size) and corticosterone concentration should decline with aggression initiated but increase with aggression received during tests (since stress might be expected to be greater in less competitive individuals). Since testosterone values were extrapolated estimates, we omitted them from this part of the analysis. Instead we included testis weight, which correlated with testosterone concentration at site 1 and showed a significant site difference in our previous study (Barnard *et al.*, 2002). We tested the predictions in a series of stepwise partial regression analyses. In line with expectation, overall aggression

https://doi.org/10.1079/JOH2003194 Published online by Cambridge University Press

decreased with increasing ASYMM ($t_{32} = -1.80$, one-tailed $P < 0.05$; fig. 1), though separate analyses of behaviour as resident and intruder revealed that the relationship applied only when males were intruders ($t_{34} = -1.76$, one-tailed $P < 0.05$). While there was no significant effect of infection intensity on aggression when sites were combined, analysis of sites individually showed that aggression by residents at site 2 declined with increasing intensity of infection ($t_{16} = -3.02$, one-tailed $P < 0.01$; fig. 2). No independent relationships with ASYMM emerged when sites were analysed separately. Analysis of the effects of aggression initiated by residents on terminal corticosterone concentration (with SIZE, ASYMM, testis weight, adrenal weight and asymmetry as other independent variables) showed a significant negative relationship with both corticosterone ($t_{29} = -2.31$, $P < 0.05$; fig. 3) and testis weight ($t_{29} = -2.07$, $P < 0.05$). The relationships were not significant when each site was analysed separately. There were no significant relationships between attacks initiated as an intruder or attacks received as a resident on terminal corticosterone concentration, and no significant effects of aggression with respect to resident or intruder emerged for adrenal gland weight. While corticosterone concentration (post-test minus pre-test concentration) increased significantly within individuals over the period of testing (paired $t_{13} = 2.66$, $P < 0.02$), implying a general increase in stress, there were again no significant relationships between the magnitude of change and aggression initiated or received by voles, and no difference in the magnitude of change between sites.

Discussion

Two important conclusions emerge from this study. Firstly, the differences between sites 1 and 2 in intensity of helminth infection, adrenal gland weight and morphological asymmetry reported by Barnard *et al.* (2002) have been confirmed two years later, suggesting long term differences in parasite burdens, stress endocrinology and morphological development between ecologically similar (Behnke *et al.*, 2001) sites. As previously, all measures were greater at site 1. In addition, voles from site 1 had lower adrenal gland asymmetries and thymus weights and higher levels of

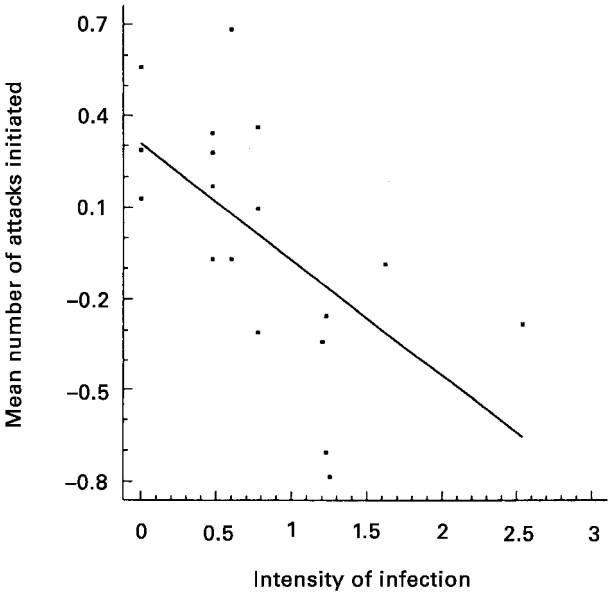

Fig. 2. Component effect from stepwise partial regression analysis for the relationship between the number of attacks initiated by residents and infection intensity at site 2 (Pilchy). See text.

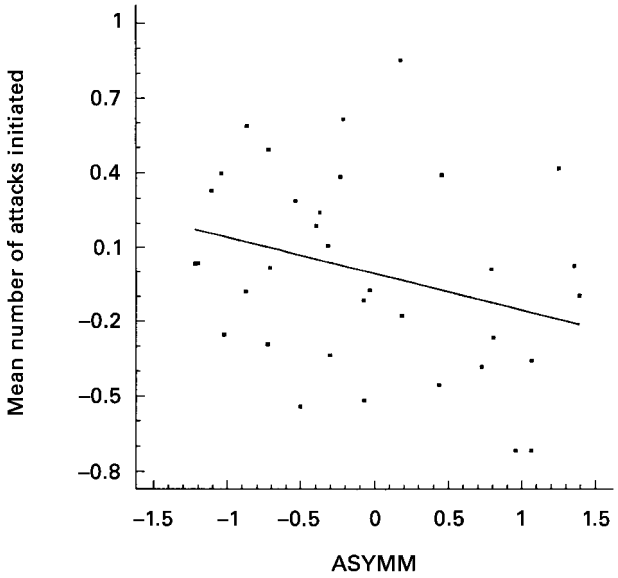

Fig. 1. Component effect from stepwise partial regression analysis for the relationship between the number of attacks initiated by voles as both residents and intruders in tests, and the principal component ASYMM. See text.

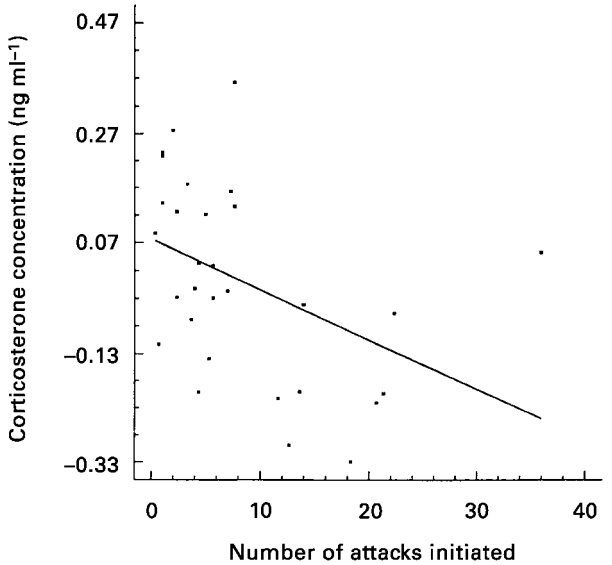

Fig. 3. Component effect from stepwise partial regression analysis for the relationship between the number of attacks initiated by residents and (log 10x) terminal corticosterone concentration (ng ml^{-1}).

https://doi.org/10.1079/JOH2003194 Published online by Cambridge University Press

corticosterone than those at site 2, differences consistent with stress-related reduction in immunocompetence at site 1 (Szigethy *et al.*, 1994; Abramov *et al.*, 1996; Barnard *et al.*, 1996a; Smith *et al.*, 1996; Gerendai & Halasz, 1997). No differences in any measure emerged between subsites at either location, implying our site differences are locally extensive and not chance sampling artefacts.

Secondly, the study shows that these site differences are associated with differences in aggressive behaviour among males that are consistent with the covariation between aggression, steroid hormones, immunocompetence and/or infection reported in other studies (e.g. Alexander & Stimson, 1988; Barnard *et al.*, 1994, 1996a,b; Wilckens & de Rijk, 1997). Our expectation was that voles from site 1 would show less aggression as a result of their greater levels of infection and physiological and morphological stress. This was borne out by: (i) reduced aggression among site 1 residents and intruders; (ii) the fact that site differences in aggression by residents was most pronounced when confronting intruders from site 2; and (iii) the significant relationships between aggression, infection intensity and corticosterone concentration among experimental animals. That a negative relationship between aggression and intensity of infection emerged only at site 2 is interesting. While this may simply reflect a floor effect imposed by the chronically higher infection and apparent stress levels at site 1 (i.e. sick animals don't fight), it is also consistent with animals at site 1 downregulating aggression independently of their present individual infection status. This is as might be expected if potentially immunodepressive behaviours were modulated in relation to perceived infection risk in the current environment rather than in response to infection itself. 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 (see also Barnard *et al.*, 2003). Animals at the two 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). In this view, higher levels of morphological asymmetry and corticosterone and reduced aggressiveness at site 1 reflect this trade-off rather than higher levels of stress predisposing animals to greater infection.

Taken together, therefore, the results of this and Barnard *et al.*'s (2002) earlier study provide correlational evidence that local variation in parasite infection within populations is associated with differences in host life history characteristics, as reflected in morphological development and stress physiology, and experimental evidence that it is associated with investment in behaviour (aggression) likely to impact on immunocompetence. Of course, caution is necessary here because, replication within sites notwithstanding, practical considerations limited our samples to only two sites. There may thus be other factors associated with the sites that confound our interpretation. However, the fact that similar covariation between helminth burden, hormone concentrations and behaviour has emerged from local populations in an entirely different rodent host–parasite system (Barnard *et al.*, 2003) increases confidence in our conclusions.

Other studies have shown that ecologically similar sites can differ in parasite burden, invoking stochastic immigration and extinction events, or local host–parasite adaptation, as explanations for patterns of local variation (Kennedy *et al.*, 1991; Hartvigsen & Kennedy, 1993; Sire *et al.*, 2001). Our results strongly suggest long term local covariation between infection intensities and host development, physiology, and behaviour, and, while we are not yet in a position to infer cause and effect, imply that local variation in parasite infection can reflect fundamental differences in the physiological and behavioural ecology of host subpopulations.

Acknowledgements

We thank Professor M. Kozakiewicz and Dr R. Halba for use of the field station at Urwitalt (Department of Ecology, Institute of Zoology), Drs A Kowalczewski and A. Jachner-Miskiewicz for use of the field station at Pilchy (Department of Hydrobiology, Institute of Zoology), Professor M. Kozakiewicz for generously providing a field vehicle for the study, Dr M. Brzezinski for advice and assistance in the field, and the forestry departments responsible for permission to work in the woodland sites used in our study (Nadlesnictwa Gizycko, Mikolajki and Orzysz). The work was generously supported by the University of Warsaw and a Research Fellowship from the Leverhulme Trust to CJB.

References

Abramov, V.V., Karmatskikh, O.L., Kozlov, V.A. & Oskina, I.N. (1996) Functional asymmetry of adrenal glands in CBA × C57BL/6 F-1 mice. *Doklady Akademii Nauk* **347**, 831–833.

Alexander, J. & Stimson, W.H. (1988) Sex hormones and the course of parasitic infection. *Parasitology Today* **4**, 189–193.

Alibhai, S.K. & Gipps, J.H. (1985) The population dynamics of bank voles. pp. 120–134 *in* Flowerdew, J.R., Gurnell, J. & Gipps, J.H. (*Eds*) *The ecology of woodland rodents: bank voles and wood mice*. Oxford, Clarendon Press.

Anderson, R.M. & Gordon, D.M. (1982) Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**, 373–398.

Anderson, R.M. & May, R.M. (1978) Regulation and stability of host-parasite population interactions.

https://doi.org/10.1079/JOH2003194 Published online by Cambridge University Press

I. Regulatory processes. *Journal of Animal Ecology* **47**, 219–247.

Anderson, R.M. & May, R.M. (1991) *Infectious diseases of humans: dynamics and control*. Oxford, Oxford University Press.

Bajer, A., Pawelczyk, A., Behnke, J.M., Gilbert, F.S. & Sinski, E. (2001) Factors affecting the component community structure of haemoparasites in bank voles (*Clethrionomys glareolus*) from the Mazury Lake District region of Poland. *Parasitology* **122**, 43–54.

Barnard, C.J. & Behnke, J.M. (2001) From psychoneuroimmunology to ecological immunology: life history strategies and immunity trade-offs. pp. 35–47 *in* Ader, R., Felten, D. & Cohen, N. (*Eds*) *Psychoneuroimmunology*. 3rd edn, San Diego, Academic Press.

Barnard, C.J., Behnke, J.M. & Sewell, J. (1993) Social behaviour, stress and susceptibility to infection in house mice (*Mus musculus*): effects of duration of grouping and aggressive behaviour prior to infection on susceptibility to *Babesia microti*. *Parasitology* **107**, 183–192.

Barnard, C.J., Behnke, J.M. & Sewell, J. (1994) Social behaviour and susceptibility to infection in house mice (*Mus musculus*): effects of group size, aggressive behaviour and status-related hormonal responses prior to infection on resistance to *Babesia microti*. *Parasitology* **108**, 487–496.

Barnard, C.J., Behnke, J.M. & Sewell, J. (1996a) Social status and resistance to disease in house mice (*Mus musculus*): status-related modulation of hormonal responses in relation to immunity costs in different social and physical environments. *Ethology* **102**, 63–84.

Barnard, C.J., Behnke, J.M. & Sewell, J. (1996b) Environmental enrichment, immunocompetence and resistance to *Babesia microti* in male laboratory mice. *Physiology and Behaviour* **60**, 1223–1231.

Barnard, C.J., Behnke, J.M., Gage, A.R., Brown, H. & Smithurst, P.R. (1997a) Modulation of behaviour and testosterone concentration in immunodepressed male laboratory mice (*Mus musculus*). *Physiology and Behaviour* **61**, 907–917.

Barnard, C.J., Behnke, J.M., Gage, A.R., Brown, H. & Smithurst, P.R. (1997b) Immunity costs and behavioural modulation in male laboratory mice (*Mus musculus*) exposed to the odour of females. *Physiology and Behaviour* **62**, 857–866.

Barnard, C.J., Behnke, J.M., Gage, A.R., Brown, H. & Smithurst, P.R. (1998) Maternal effects on the development of social rank and immunity trade-offs in male laboratory mice (*Mus musculus*). *Proceedings of the Royal Society of London Series B* **265**, 2087–2093.

Barnard, C.J., Behnke, J.M., Bajer, A., Bray, D., Race, T., Frake, K., Osmond, J., Dinmore, J. & Sinski, E. (2002) Local variation in endoparasite burdens of bank voles (*Clethrionomys glareolus*) from ecologically similar sites: morphometric and endocrine correlates. *Journal of Helminthology* **76**, 103–112.

Barnard, C.J., Sayed, E., Barnard, L.E., Behnke, J.M., Abdel Nabi, I., Sherif, N., Shutt, A. & Zalat, S. (2003) Local variation in helminth burdens of Egyptian spiny mice (*Acomys cahirinus dimidiatus*) from ecologically similar sites: relationships with hormone concentrations and social behaviour. *Journal of Helminthology* **77**, 197–207.

Behnke, J.M., Barnard, C.J., Mason, N., Harris, P.D., Sherif, N.E., Zalat, S. & Gilbert, F.S. (2000) Intestinal helminths of spiny mice (*Acomys cahirinus dimidiatus*) from St Katherine's Protectorate in the Sinai, Egypt. *Journal of Helminthology* **74**, 31–43.

Behnke, J.M., Barnard, C.J., Bajer, A., Bray, D., Dinmore, J., Frake, K., Osmond, J., Race, T. & Sinski, E. (2001) Variation in the helminth community structure in bank voles (*Clethrionomys glareolus*) from three comparable localities in the Mazury Lake District region of Poland. *Parasitology* **123**, 401–414.

Borkowska, A. (1999) Genetic and morphological variation among populations of the bank vole *Clethrionomys glareolus* from north-eastern Poland: the seasonal aspect. *Zeitschrift für Saugetierkunde* **64**, 285–297.

Brinkmann, V. & Kristofic, C. (1995) Regulation by corticosteroids of Th1 and Th2 cytokine production in human $CD4^+$ effector T cells generated from $CD45RO^-$ and $CD45RO^+$ subsets. *Journal of Immunology* **152**, 3322–3328.

Crofton, H.D. (1971) A quantitative approach to parasitism. *Parasitology* **62**, 179–193.

Desai, K.V. & Kondaiah, P. (2000) Androgen ablation results in differential regulation of transforming growth factor beta isoforms in rat male accessory sex organs and epididimys. *Journal of Molecular Endocrinology* **24**, 253–260.

Drickamer, L.C., Saal, F.S., vom, Marriner, L.M. & Mossman, C.A. (1995) Anogenital distance and dominance status in male house mice (*Mus domesticus*). *Aggressive Behaviour* **21**, 301–309.

Edwards, J.C. (1988) The effects of *Trichinella spiralis* infection on social interactions in mixed groups of infected and uninfected male mice. *Animal Behaviour* **36**, 529–540.

Edwards, J.C. & Barnard, C.J. (1987) The effects of *Trichinella* infection on intersexual interactions between mice. *Animal Behaviour* **35**, 533–540.

Ehman, K.D. & Scott, M.E. (2001) Urinary odour preferences of MHC congenic female mice, *Mus domesticus*: implications for kin recognition and detection of parasitized males. *Animal Behaviour* **62**, 781–789.

Fernandes, G.A. (2000) Immunological stress in rats induces bodily alterations in saline-treated conspecifics. *Physiology and Behaviour* **69**, 221–230.

Folstad, I. & Karter, A.J. (1992) Parasites, bright males and the immunocompetence handicap. *American Naturalist* **139**, 603–622.

Fukazawa, Y. & Iguchi, T. (1999) Effects of hormones and growth factors on the development of the male mouse reproductive tract in vitro. *Zoological Science* **16**, 153–160.

Gerendai, I. & Halasz, B. (1997) Neuroendocrine asymmetry. *Frontiers of Neuroendocrinology* **18**, 354–381.

Gerlach, G. & Musolf, K. (2000) Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conservation Biology* **14**, 1066–1074.

https://doi.org/10.1079/JOH2003194 Published online by Cambridge University Press

Gliwicz, J. & Rajska-Jurgiel, E. (1983) Social organization. *Acta Theriologica* **28** (Suppl.), 134–140.
Gregory, R.D. & Woolhouse, M.E.J. (1993) Quantification of parasite aggregation: a simulation study. *Acta Tropica* **54**, 131–139.
Grossman, C.J. (1985) Interactions between the gonadal steroids and the immune system. *Science* **227**, 257–261.
Gustafsson, T., Andersson, B. & Meurling, P. (1980) Effect of rank on the growth of the preputial glands in male bank voles, *Clethrionomys glareolus*. *Physiology and Behaviour* **24**, 689–692.
Hartvigsen, R. & Kennedy, C.R. (1993) Patterns in the composition and richness of helminth communities in brown trout, *Salmo trutta*, in a group of reservoirs. *Journal of Fish Biology* **43**, 603–615.
Haukisalmi, V. & Henttonen, H. (1999) Determinants of helminth aggregation in natural host populations: individual differences or spatial heterogeneity? *Ecography* **22**, 629–636.
Hazel, S.M., Bennett, M., Chantrey, J., Bown, K., Cavanagh, R., Jones, T.R., Baxby, D. & Begon, M. (2000) A longitudinal study of an endemic disease in its wildlife reservoir: cowpox and wild rodents. *Epidemiology and Infection* **124**, 551–562.
Hillgarth, N. & Wingfield, J.C. (1997) Testosterone and immunosuppression in vertebrates: implications for parasite-mediated sexual selection. pp. 143–155 *in* Beckage, N.E. (*Ed.*) *Parasites and pathogens; effects on host hormones and behavior*. London, Chapman and Hall.
Hoffmeyer, I. (1982) Responses of female bank voles (*Clethrionomys glareolus*) to dominant vs. subordinate conspecific males and to urine odors from dominant vs. subordinate males. *Behavioral Neural Biology* **36**, 178–188.
Hughes, V.L. & Randolph, S.E. (2001) Testosterone depresses innate and acquired resistance to ticks in natural rodent hosts: a force for aggregated distributions of parasites. *Journal of Parasitology* **87**, 49–54.
Hurst, J.L. (1993) The priming effects of urine substrate marks on interactions between house mice, *Mus musculus domesticus*, Schwarz and Schwarz. *Animal Behaviour* **45**, 55–81.
Hurst, J.L., Hall, S., Roberts, R. & Christian, C. (1996) Social organization in the aboriginal house mouse, *Mus spretus* Lataste: behavioural mechanisms underlying the spatial dispersion of competitors. *Animal Behaviour* **51**, 327–344.
Jarred, R.A., Cancilla, B., Prins, G.S., Thayer, K.A., Cunha, G.R. & Ridbridger, G.P. (2000) Evidence that estrogens directly alter androgen-regulated prostate development. *Endocrinology* **141**, 3471–3477.
Kavaliers, M. & Colwell, D.D. (1995) Discrimination by female mice between the odours of parasitized and non-parasitized males. *Proceedings of the Royal Society of London Series B* **261**, 31–35.
Kavaliers, M., Colwell, D.D. & Choleris, E. (1998) Analgesic responses of male mice exposed to the odors of parasitized females: effects of male sexual experience and infection status. *Behavioral Neuroscience* **112**, 1001–1011.
Kennedy, C.R., Hartvigsen, R. & Halvorsen, O. (1991) The importance of fish stocking in the dissemination of parasites throughout a group of reservoirs. *Journal of Fish Biology* **38**, 541–552.
Klein, S.L. (2000) Hormones and mating system affect sex and species differences in immune function among vertebrates. *Behavioural Processes* **51**, 149–166.
Klein, S.L., Hairston, J.E., DeVries, A.C. & Nelson, R.J. (1997) Social environment and steroid hormones affect species and sex differences in immune function in voles. *Hormones and Behavior* **32**, 30–39.
Kozakiewicz, M. (1976) The weight of the eye lens as a proposed age indicator of the bank vole. *Acta Theriologia* **21**, 314–316.
Maier, S.F., Watkins, L.R. & Fleshner, M. (1994) Psychoneuroimmunology: the interface between behavior, brain and immunology. *American Psychologist* **49**, 1004–1017.
Moller, A.P. & Swaddle, J. (1997) *Asymmetry, developmental stability and evolution*. Oxford, Oxford University Press.
Morris, P. (1972) A review of mammalian age determination methods. *Mammal Review* **2**, 69–104.
Nunn, C.L., Gittelman, J.L. & Antonovics, J. (2000) Promiscuity and the primate immune system. *Science* **290**, 1168–1170.
Palanza, P., Parmigiani, S. & Saal, F.S. vom (1995) Urine marking and maternal aggression of wild female mice in relation to anogenital distance at birth. *Physiology and Behaviour* **58**, 827–835.
Palmer, A.R. & Strobeck, C. (1986) Fluctuating asymmetry: measurement, analysis, pattern. *Annual Review of Ecology and Systematics* **17**, 391–421.
Pawelczyk, A. & Sinski, E. (2000) Prevalence of IgG antibodies response to *Borrelia burgdorferi* in populations of wild rodents from the Mazury Lake District region of Poland. *Annals of Agricultural and Environmental Medicine* **7**, 79–83.
Penn, D. & Potts, W.K. (1998) Untrained mice discriminate MHC-determined odors. *Physiology and Behaviour* **64**, 235–243.
Quinnell, R.J. & Keymer, A.E. (1990) Acquired immunity and epidemiology. pp. 317–343 *in* Behnke, J.M. (*Ed.*) *Parasites: immunity and pathology*. London, Taylor and Francis.
Rozenfeld, F.M. & Rasmont, R. (1991) Odour cue recognition by dominant male bank voles, *Clethrionomys glareolus*. *Animal Behaviour* **41**, 839–850.
Rutovskaya, V. (1968) Acoustic activity and social behaviour of the bank vole. pp. 177–188 *in* Sokolov, V.E., Rozhnov, V.V. & Serbenyuk, M.A. (*Eds*) *Behaviour, communication and behaviour in mammals*. V.E. IEE RAS Moskva.
Shaw, D.J. & Dobson, A.P. (1995) Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* **111** (Suppl.), S111–S133.
Sheldon, B.C. & Verhulst, S. (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology and Evolution* **11**, 317–321.
Sire, C., Langland, J., Barral, V. & Théron, A. (2001) Parasite (*Schistosoma mansoni*) and host (*Biomphalaria glabrata*) genetic diversity: population structure in a fragmented landscape. *Parasitology* **122**, 545–554.

Smith, F.V., Barnard, C.J. & Behnke, J.M. (1996) Social odours, hormone modulation and resistance to disease in male laboratory mice. *Animal Behaviour* **52**, 141–153.

Stachon, M., Furstenburg, E., Gromadzka-Ostrowska, J. & Romanowicz, K. (2001) The influence of different dietary fat sources on tissue corticosterone concentration in rats. *Journal of Animal and Feed Sciences* **10**, 341–354.

Szigethy, E., Conwell, Y., Forbes, N.T., Cox, C. & Caine, E.D. (1994) Adrenal weight and morphology in victims of completed suicide. *Biological Psychology* **36**, 374–380.

Tahka, K.M., Zhuang, Y.H., Tahka, S. & Tuohimaa, P. (1997) Photoperiod-induced changes in androgen receptor expression in testes and accessory sex glands of the bank vole, *Clethrionomys glareolus*. *Biology of Reproduction* **56**, 898–908.

Wakelin, D. & Blackwell, J.M. (1993) Genetic variation in immunity to parasitic infections. pp. 3–32 *in* Warren, K.S. (*Ed.*) *Immunology and molecular biology of parasitic infections*. Boston, Blackwell Scientific Publications.

Wilckens, T. & Rijk, R. de (1997) Glucocorticoids and immune function: unknown dimensions and new frontiers. *Immunology Today* **18**, 418–424.

(Accepted 1 May 2003)