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*

C. J. BARNARD[1], J. M. BEHNKE[2] *and* J. SEWELL[1,2]

[1] *Behaviour and Ecology Research Group and* [2] *Experimental Parasitology Research Group, Department of Life Science, University of Nottingham, University Park, Nottingham NG7 2RD, UK*

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SUMMARY

Associations between social rank, immunodepression and resistance to *Babesia microti* infection within single-sex groups of male house mice suggest rank-dependent suites of response involving different hormonal and immune changes in relation to aggressive behaviour and group size prior to infection. Reduced resistance among high-ranking males was associated with increased serum testosterone and corticosterone concentration and reduced serum immunoglobulin, but was independent of group size. Among low-ranking males, hormonal changes were not associated with resistance to *B. microti* but changes in corticosterone concentration and measures of immunodepression increased with group size and aggressive behaviour. The results concur with earlier findings suggesting differences between high- and low-ranking mice in their physiological responses to social experience and consequently reduced resistance to *B. microti* infection among high-ranking individuals.

Key words: house mouse, *Mus musculus*, *Babesia microti*, social status, aggression, group size, immunity, testosterone, corticosterone.

INTRODUCTION

Recent interest in the relationship between host social and sexual behaviour and parasitic infection (Hamilton & Zuk, 1982; Edwards, 1988; Milinski & Bakker, 1990; Moore & Gotelli, 1991) has highlighted the importance of attributing cause and effect in associations between parasite burdens and host behavioural phenotypes (Read, 1991; Barnard, Behnke & Sewell, 1993). Evidence suggests that associations between parasite burdens and behaviour can arise because host behaviour predisposes certain individuals to infection through stress-related immunodepression (Vessey, 1964; Sapolsky, 1983; Peng *et al.* 1989; Mormede *et al.* 1990) or increased exposure to sources of infection (Bundy & Blumenthal, 1991), or because parasites themselves cause changes in the behaviour of the host species (Rau, 1983; Milinski & Bakker, 1990; Moore & Gotelli, 1991). Correlations between burdens and behaviour by themselves are thus not very informative. Furthermore, where social responses are associated with parasite burdens, relationships with social status and social environment (e.g. group size and density) vary within and between species (e.g. Sassenrath, 1970; Hausfater & Watson, 1976; Halvorsen, 1986; Henry, Stephens & Ely, 1986; Barnard *et al.* 1993).

There is evidence for associations between parasitic infection and host behaviour in both cause and effect directions among house mice (Davis & Read, 1958; Jackson & Farmer, 1970; Edwards & Barnard, 1987). In a recent experiment, Barnard *et al.* (1993) investigated the consequences of social responses among male CFLP mice for resistance to a subsequent infection of *Babesia microti*. Initially unfamiliar mice, maintained for different periods in groups of 6, differed in the time taken to reach peak parasitaemia and in their rate of clearance of the infection in relation to their aggressive behaviour within groups. Males that initiated more aggressive interactions and/or had higher social status based on their ratio of attacks initiated:received reached a peak of infection sooner and were slower to clear infection. Serum IgG and corticosterone analysis showed that, while low-ranking males experienced the greatest increase in serum corticosterone over the period of grouping, immunodepression was greatest among high-ranking, aggressive males. This suggested that immunodepression and subsequent reduced resistance to *B. microti* was not a direct consequence of stress but was mediated by some other physiological change associated with aggression. An obvious candidate is testosterone which is both associated with aggressive behaviour and known to have a range of immunodepressive effects (Grossman, 1985; Schuurs & Verheul, 1990; Folstad & Karter, 1992). The immunodepressive properties

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of androgens such as testosterone are well established and are linked with gender-dependent differences in the course of parasitic and other infections (Goble & Konopka, 1973; Alexander & Stimpson, 1988; Brabin & Brabin, 1992). *Babesia microti* and *B. rodhaini* infections peak at a higher percentage in male compared with female mice (Irvin *et al.* 1981; Goble, 1966 respectively), one interpretation being that sex hormones affect the capacity to resist infection. Although the basis of this difference between the sexes has not been investigated further in *Babesia*, similar gender-dependent patterns of infection with *Plasmodium chabaudi* in mice, with males sustaining more intense infections, have been attributed to the immunodepressive consequences of testosterone (Wunderlich *et al.* 1988). Orchidectomized males showed stronger resistance, comparable to that in females, but this was reduced when testosterone levels were restored by injection. Similarly, treatment of females with testosterone reduced their resistance to infection. *P. chabaudi*, like *B. microti*, is an intra-erythrocytic parasite and is probably cleared from the host by a related mechanism (Clark & Howell, 1990).

Here, we investigate the relative roles of corticosterone and testosterone secretion in the relationship between aggressive behaviour, immunodepression and status-related resistance to *B. microti* infection in male CFLP mice. In this case, mice were maintained in groups for the same period, but groups differed in size because aggressive behaviour is known to vary with group size (Poole & Morgan, 1973) and because there is evidence that group size influences immunocompetence and resistance to infection in caged mice (Vessey, 1964; Brayton & Brain, 1974; Rabin *et al.* 1987; Peng *et al.* 1989). Following the report of Barnard *et al.* (1993), the experiment took into account both pre-existing individual variation in physiological measures and the effects of social experience on the measures prior to infection with *B. microti*.

MATERIALS AND METHODS

Mice and husbandry

The subjects of the experiment were males of the randomly-bred CFLP strain of laboratory mice used extensively in immunological and behavioural studies (Behnke, Wakelin & Wilson, 1978; Williams & Behnke, 1983; Barnard, Hurst & Aldhous, 1991, Barnard *et al.* 1993). All experimental animals were maintained on a 12 h:12 h reversed light:dark cycle (lights on at 20.00 h, lights off at 08.00 h) from conception to the end of the experimental period. Mice were weaned at 21 days *post-partum*, separated at 27 days into single-sex natal litters and maintained in their natal litters in polypropylene cages (45 × 28 × 13 cm, 8 mice maximum) until 24 h before being assigned to experimental groups as adults at 70 days. Twenty-four hours before grouping (see Barnard *et al.* 1993), males were separated and housed individually in smaller polypropylene cages (48 × 15 × 13 cm) to standardize their social experience immediately prior to group establishment.

The parasite

Following the method of Barnard *et al.* (1993), the King's 67 strain of *B. microti* was used. The parasite had been passaged in excess of 20 times through CFLP mice (both sexes) until parasitaemia peaked consistently in excess of 60%. Several mice were then infected to provide a large, uniform stock of infectious material.

Collection of serum samples

Two retro-orbital blood samples were taken from each mouse in the experiment, one two weeks before the establishment of experimental groups and the second immediately after the period of grouping and prior to infection with *B. microti*. Anaesthesia (Trilene (BDH)), transport of mice between experimental and sampling rooms and the timing of blood sampling followed the procedures of Barnard *et al.* (1993) to minimize disturbance to animals. Samples of blood (88 μl) were collected in heparinized capillary tubes within 1 min of each mouse being removed from its experimental cage. The blood was centrifuged for 5 min in a haematocrit centrifuge and the serum stored at −20 °C prior to analysis.

Measurement of the concentration of serum corticosterone

The concentration of corticosterone was measured using 6 μl samples of undiluted serum and a Gamma-B ^{125}I-corticosterone-Immunodiagnostic Systems Ltd kit based on double antibody radioimmunoassay (RIA), as advised by the manufacturers. Serum corticosterone concentrations were calculated by reference to standards provided with the kit.

Measurement of the concentration of serum testosterone

The concentration of testosterone was measured using a Coat-a-Count solid-phase ^{125}I total testosterone RIA kit (Diagnostic Products Corporation, Los Angeles) using 25 μl samples for experimental and calibration assays. Testosterone concentrations were calculated by reference to the calibration curves.

Measurement of total serum IgG

Total serum IgG was determined by the method of Mancini, Carbonara & Heremans (1965) using radial

immunodiffusion (RID) kits (The Binding Site, Birmingham). Ring diameters were measured in two directions at 90° and the mean was used to calculate the concentrations of immunoglobulins from a calibration curve obtained using appropriate standards.

In a small number of cases, limited serum volumes meant it was not possible to obtain a reliable estimate of corticosterone, testosterone and/or IgG from a particular sample. As a result, sample sizes in some subsequent analyses vary (see below).

Concurrent pinworm infections

To eliminate potentially confounding effects of pinworm (*Syphacia obvelata* and *Aspiculuris tetraptera*) infections (see Barnard *et al.* 1993), all mice were treated with piperazine at approximately 28 days of age. Autopsies of simultaneously treated non-experimental mice at the end of the experimental period revealed no evidence of pinworm infection. Experimental mice were thus not autopsied for pinworm burden.

Experimental procedure

Males were arbitrarily assigned to experimental groups so that no two groups shared more than 20% of individuals from the same natal litters. Three different group sizes were established comprising 3, 6 and 10 individuals housed in a plywood and glass observation cage (30 × 60 × 30 cm) fitted with a 60 W red light bulb and 2 food and water dispensers that were replenished *ad libitum*. Ten replicates of each group size were established. All mice had previously (3 weeks) been individually marked with black hair dye to allow identification during behavioural observations. At the time of group establishment, mice were weighed and an arbitrary index of fur condition (fur score) ranging from 1 (good) to 5 (poor) (see Barnard *et al.* 1993) was recorded. All groups were established between 09.00 and 10.00 h to control for circadian variation in hormone secretion at the time of establishment.

All behavioural observations were made in the dark phase under dim red light. Each group was observed for 2 randomly distributed 15-min periods daily for 8 days during which all social investigatory, aggressive and defensive behaviours (see Barnard *et al.* 1993 for detailed descriptions) initiated and received by each individual was recorded on audio tape for later analysis. The individuals in the vicinity (within 5 cm) of the food and water dispensers were noted on 6 occasions/day.

After 8 days of grouping, all mice were weighed and their fur score recorded. The second 88 μl sample of blood was taken and each mouse was infected with 4×10^8 *B. microti* from passage animals that had been infected 10 days earlier with the same batch of the parasite taken from liquid nitrogen. The technique for determining the dose has been detailed by Barnard *et al.* (1993). Following infection, the mice were isolated and housed in individual polypropylene cages (48 × 15 × 13 cm) while the development of the infection was assessed. At the same time 32 tracer mice were also infected with the same dose of *B. microti*. To minimize disturbance to experimental animals, a blood smear was taken daily (from 2 days post-infection) from a superficial caudal vein of each tracer mouse until the parasite had apparently cleared from the fastest responders. The percentage cells infected in smears then provided a guide to the timing of blood samples from experimental mice. On this basis a blood smear was taken when (*a*) the percentage parasitaemia in tracer animals was beginning to rise, (*b*) when the fastest responding tracer mice reached their peak parasitaemia, (*c*) 2 days later, (*d*) when the parasitaemia in the fastest responders had reached a plateau and (*e*) when the fastest responders had apparently cleared the parasite. Tracer mice were sampled between 08.00 and 10.00 h; smears were then stained and the stage of infection assessed so that there was a 1–2 h interval between samples from tracer and experimental mice.

At 21 days post-infection, the mice were killed and weighed and the adrenal glands, kidneys, mesenteric lymph nodes (MLN), heart and spleen dissected out and weighed. Body weight at autopsy was taken into account either in analyses (adrenal weight) or by expressing weights as percent body weight (all other organs). Kidney and adrenal weights were expressed as paired organ weights.

Statistical analyses

Parametric tests were used where data (logarithmically transformed where necessary) met the required assumptions. Where they did not, non-parametric tests were used. Wherever there were *a priori* reasons for expecting trends or differences in a particular direction, probabilities associated with significance tests are indicated as one-tailed.

RESULTS

Group size, dominance rank and aggression

Following the method of Barnard *et al.* (1993), we ranked males within groups on the basis of the ratio of the number of attacks initiated/number received over the period of grouping, with rank 1 having the greatest ratio and mice sharing ratios taking the average of the ranks they would otherwise have been allocated. As in the previous study, the number of attacks initiated over the grouping period was negatively related to rank in all three group sizes ($P < 0{\cdot}0001$ in all group sizes), but discontinuously within group sizes so that certain males (high

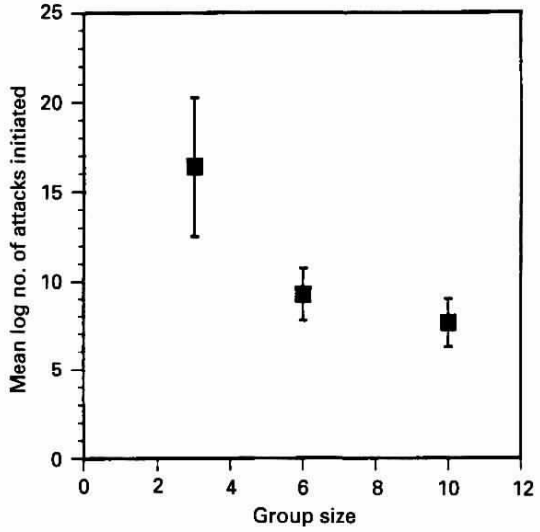

Fig. 1. The relationship between group size and the mean number of attacks initiated/individual during the period of grouping (log transformed for analysis). $F_{2,189} = 4{\cdot}79$, $P < 0{\cdot}01$ (high and low rankers combined), $F_{2,54} = 5{\cdot}20$, $P < 0{\cdot}01$ (high rankers only), $F_{2,134} = 9{\cdot}93$, $P < 0{\cdot}001$ (low rankers only). Bars represent standard errors.

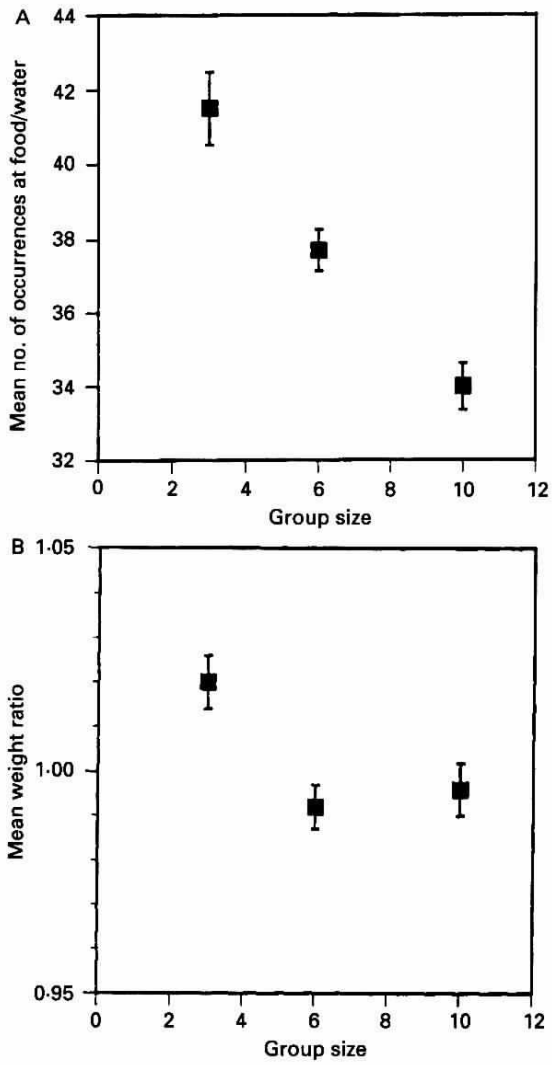

Fig. 2. The relationship between group size and (A) the mean number of times an individual was recorded in the vicinity of food or water dispensers ($F_{2,187} = 21{\cdot}66$, $P < 0{\cdot}0001$ (combined rank categories); $F_{2,50} = 3{\cdot}33$, $P < 0{\cdot}05$ (high rankers only); $F_{2,132} = 19{\cdot}11$, $P < 0{\cdot}0001$ (low rankers only)) and (B) the mean change in body weight among low rankers over the period of grouping ($F_{2,131} = 3{\cdot}18$, $P < 0{\cdot}05$; for high rankers, $F_{2,50} = 1{\cdot}30$, N.S; for rank categories combined, $F_{2,184} = 4{\cdot}12$, $P < 0{\cdot}05$). Bars represent standard errors.

rankers) showed a disproportionately high rate of attack initiation compared with others (low rankers). In groups of 3 and 6, there was one high-ranking male (mean ($\pm$S.E.) number of attack initiations by high rankers in groups of 3 = $35{\cdot}6 \pm 7{\cdot}00$, $N = 10$, by low rankers = $6{\cdot}90 \pm 2{\cdot}13$, $N = 20$; mean number by high rankers in groups of 6 = $28{\cdot}0 \pm 4{\cdot}47$, $N = 10$, by low rankers = $5{\cdot}52 \pm 0{\cdot}91$, $N = 50$), while in groups of 10 there were on average 4 (mean number by high rankers = $18{\cdot}23 \pm 3{\cdot}04$, $N = 35$, by low rankers = $1{\cdot}92 \pm 0{\cdot}62$, $N = 65$). The rate of attack initiation declined significantly with increasing group size (Fig. 1) and the decline was significant for both high and low rankers (see Fig. 1 legend) so that mice showed less overt aggression in larger groups. Because of the discontinuity in attack rate with rank and the lack of equivalence for any given ranking across group sizes, we shall restrict analyses of rank effects to comparisons between high and low rankers as defined above.

In keeping with these differences between rank categories and group size, there was a significant effect of group size on fur score among high rankers ($H = 5{\cdot}23$, one-tailed $P < 0{\cdot}05$), with fur being in poorest condition at the end of the grouping period in the smallest groups, but no significant effect among low rankers. High rankers across all group sizes showed significantly poorer fur condition after the period of grouping than low rankers ($D_{max} = 0{\cdot}42$, $P < 0{\cdot}0001$). While overt aggression decreased with increasing group size, larger groups resulted in any given individual being recorded less often in the vicinity of the food or water dispensers (Fig. 2A), implying that competition for these resources increased with group size. Although the relationship was significant for both high and low rankers, it was more pronounced (the within-group variance was lower) among low rankers (Fig. 2A legend). Weight gain (the ratio of body weight before and after grouping) over the period of grouping decreased significantly with increasing group size (Fig. 2B legend) with mice in groups of 6 and 10 tending to lose weight slightly over the period of grouping. However, the effect was significant only among low rankers when rank categories were analysed separately (Fig. 2B). Although this may suggest a direct effect of time spent near food dispensers, multifactor

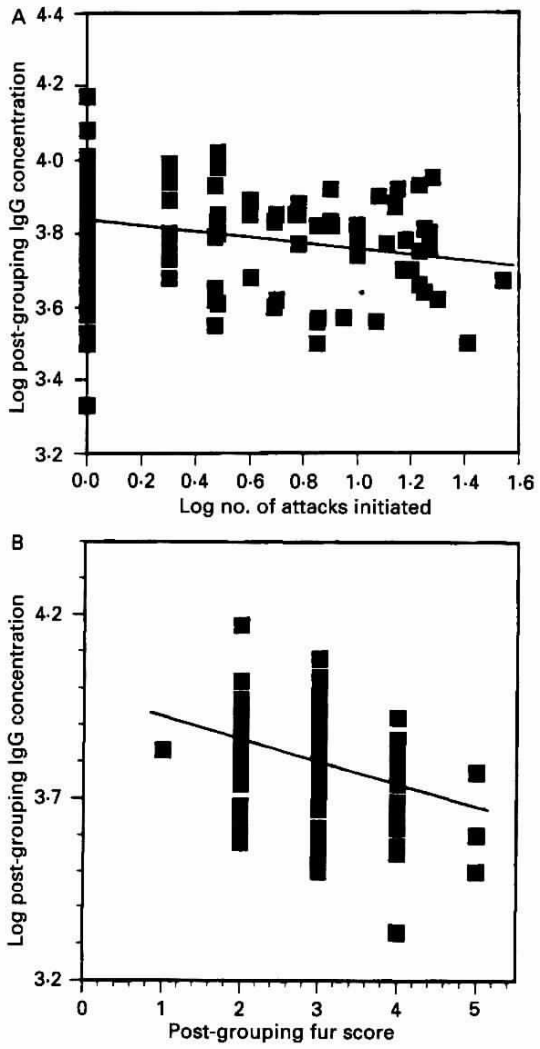

Fig. 3. The relationship among low rankers between total IgG concentration after grouping (mg/l, log transformed for analysis) and (A) the number of attacks initiated ($t = -2{\cdot}05$, D.F. = 131, one-tailed $P < 0{\cdot}05$) and (B) post-grouping fur score ($t = -3{\cdot}49$, D.F. = 131, one-tailed $P < 0{\cdot}001$). Lines fitted by linear regression.

analysis of variance taking frequency of occurrence near food/water into account maintained a significant negative effect of group size ($F_{2,183} = 5{\cdot}12$, $P < 0{\cdot}01$) but no independent effect of occurrence near food/water ($F_{1,183} = 0{\cdot}23$, N.S.).

Hormonal responses, immunocompetence and resistance to infection

From the above, we should predict that high rankers and mice in smaller groups would have shown reduced immunocompetence and resistance to infection with *B. microti*. While there was a significant decline in total IgG concentration during the period of grouping among both high and low rankers (paired t, $P < 0{\cdot}0001$ in both cases), high rankers tended to show a greater decline than low rankers (mean ($\pm$S.E.) IgG after/IgG before grouping for high rankers $= 0{\cdot}87 \pm 0{\cdot}03$, $N = 53$; in low rankers $= 0{\cdot}94 \pm 0{\cdot}03$, $N = 134$). There was no significant difference between rank categories in IgG concentration prior to grouping, though levels were slightly higher among high rankers (cf. Barnard *et al.* 1993). The assumption underlying the prediction regarding rank categories was borne out in that IgG concentration after grouping was significantly negatively related to the number of attacks initiated ($t = -2{\cdot}21$, D.F. = 184, one-tailed $P < 0{\cdot}01$) and both IgG concentration and IgG ratio were significantly negatively related to fur score ($t = -3{\cdot}79$, D.F. = 184, one-tailed $P < 0{\cdot}001$ and $-1{\cdot}69$, D.F. = 184, one-tailed $P < 0{\cdot}05$ respectively) (see also Barnard *et al.* 1993). When high and low rankers were analysed separately, none of the trends was significant among high rankers but the relationships between post-grouping IgG and both attack initiations and fur score remained significant among low rankers (Fig. 3A, B).

Post-grouping IgG and IgG ratio varied with group size in the expected direction in that both were greatest in the largest groups (10 animals). However, the difference was significant only for post-grouping IgG when number of occurrences at food/water dispensers was also taken into account ($F_{2,183} = 3{\cdot}71$, $P < 0{\cdot}05$) and mean IgG concentrations were lowest in groups of 6 rather than 3. Again, the difference in post-grouping IgG across group sizes was significant only among low rankers ($F_{2,130} = 4{\cdot}45$, $P < 0{\cdot}05$) when rank categories were analysed separately. The effect of group size both overall and among low rankers just failed to reach significance ($F_{2,183} = 2{\cdot}93$ and $F_{2,129} = 2{\cdot}88$, $0{\cdot}05 < P < 0{\cdot}1$ in both cases) when number of attacks initiated was included in the analysis ($F_{1,182}$ (number of attacks initiated) $= 5{\cdot}04$, $P < 0{\cdot}05$ overall and $F_{1,129} = 4{\cdot}59$, $P < 0{\cdot}05$ for low rankers), thus confirming group size-related aggression as an important factor in the relationship between group size and IgG concentration.

As expected from the above relationships between rank, aggression and IgG, and from previous results (Barnard *et al.* 1993), high rankers were significantly slower to clear *B. microti* infection than low rankers (mean ($\pm$S.E.) parasitaemia (% cells infected) at fastest clearance by tracer mice for high rankers $= 4{\cdot}26 \pm 1{\cdot}09$, $N = 52$; for low rankers $= 2{\cdot}43 \pm 0{\cdot}41$, $N = 133$, $D_{max} = 0{\cdot}48$, $P < 0{\cdot}0001$). There was also a non-significant tendency for peak parasitaemia to be greater among high rankers (mean ($\pm$S.E.) parasitaemia among high rankers $= 26{\cdot}71 \pm 3{\cdot}45$, $N = 52$; among low rankers $= 21{\cdot}37 \pm 2{\cdot}12$, $N = 133$). While there was no significant independent effect of attack initiation, fur score or group size on any of the measures of infection, stepwise partial regression showed that day of peak parasitaemia occurred earlier as might be expected with increasing ratio of attacks initiated:received, but among high rankers only ($t = -1{\cdot}73$, D.F. = 48, one-tailed $P < 0{\cdot}05$). The

effect of rank rather than attack initiation on the development of infection is consistent with the results of Barnard *et al.* (1993).

Rank-dependent differences in infection were associated with differences in serum corticosterone and testosterone concentrations. As in the study of Barnard *et al.* (1993), high rankers tended to enter their experimental groups with higher corticosterone concentrations (mean ng/ml before grouping in high rankers = $61{\cdot}18 \pm 8{\cdot}18$; in low rankers = $57{\cdot}19 \pm 2{\cdot}67$), but the difference was not significant. A similar non-significant tendency was apparent in testosterone concentration (mean ng/ml before grouping in high rankers = $7{\cdot}76 \pm 1{\cdot}09$; in low rankers = $6{\cdot}26 \pm 0{\cdot}68$). However, inclusion of post-grouping concentrations and post/pre-grouping concentration ratios for corticosterone and testosterone in stepwise partial regression analyses across rank categories revealed a significant association in the expected direction between testosterone ratio and both peak parasitaemia ($t = 1{\cdot}80$, D.F. = 157, one-tailed $P < 0{\cdot}05$) and parasitaemia at fastest clearance ($t = 1{\cdot}81$, D.F. = 157, one-tailed $P < 0{\cdot}05$), both positive, and a negative association between corticosterone ratio and day of peak parasitaemia ($t = -1{\cdot}73$, D.F. = 148, one-tailed $P < 0{\cdot}05$). Analysing rank categories separately showed that these relationships were significant only among high rankers (Fig. 4A–C).

While there was no significant difference in pre-grouping testosterone concentration between rank categories, there was a significant decline in concentration over a period of grouping among high rankers (paired $t = 3{\cdot}11$, D.F. = 50, $P < 0{\cdot}01$) but no significant decline among low rankers. This difference between rank categories is as expected from the greater decline in aggression with duration of grouping among high rankers (Barnard *et al.* 1993). However, there was considerable variation in the change in testosterone level during grouping with some individuals showing pronounced increases related to their aggressive performance (see below). Testosterone ratio differed significantly between group sizes among high rankers ($F_{2,48} = 5{\cdot}72$, $P < 0{\cdot}01$), being lowest in groups of 10 where rates of aggression were also lowest. There was no significant effect of group size on post-grouping testosterone concentration or on either testosterone measure overall or among low rankers. Both corticosterone ratio and post-grouping corticosterone concentration, on the other hand, increased significantly with group size among both rank categories (Fig. 5A, B). Interestingly, when the number of recorded occurrences at food or water dispensers was also taken into account, there was a significant positive association between corticosterone ratio and both group size and occurrences at food/water overall ($F_{2,165}$ (group size) = 16·36, $P < 0{\cdot}0001$; $F_{1,165}$ (occurrences at food/water) = 17·90, $P < 0{\cdot}0001$) and among low rankers ($F_{2,117}$ (group size) = 11·46, $P < 0{\cdot}0001$; $F_{1,117}$ (occurrences at food/water) = 17·35, $P < 0{\cdot}001$); among high rankers, however, there was no significant relationship with occurrences at food/water ($F_{2,44}$ (group size) = 4·25, $P < 0{\cdot}05$; $F_{1,44}$ (occurrences at food/water) = 2·32, N.S.).

Stepwise partial regression analyses of the relationships between hormone measures, aggression and fur score revealed a significant positive relationship between corticosterone ratio and fur score ($t = 2{\cdot}92$, D.F. = 159, $P < 0{\cdot}01$) which was due mainly to a strong association among high rankers ($t = 4{\cdot}20$, D.F. = 44, $P < 0{\cdot}001$), and a significant positive relationship between post-grouping testosterone concentration and ratio of attacks initiated : received among high rankers ($t = 2{\cdot}04$, D.F. = 51, $P < 0{\cdot}05$).

Organ weights

Since organs were weighed after *B. microti* infections had run their course, potential effects of infection on organ weights were taken into account in analyses of relationships with group size, behaviour and physiological measures.

From the positive relationship between group size and corticosterone ratio among both rank categories, a similar positive relationship for adrenal weight might be expected. Taking peak parasitaemia, post-grouping body weight and number of occurrences at food/water into account, there was a significant increase in adrenal weight with group size among both rank categories (F-ratio, $P < 0{\cdot}02$ in both cases), though adrenal weight was greatest in groups of 6 in both cases. There was no significant effect of peak parasitaemia or occurrences at food/water and the effects of group size remained ($P < 0{\cdot}05$ in both rank categories) when attack initiation rate was taken into account.

Kidney weight was also significantly positively associated with group size among high rankers ($F_{2,33} = 4{\cdot}42$, $P < 0{\cdot}02$), but not among low rankers or when ranks were combined. Again, there were no significant effects of peak parasitaemia or occurrences at food/water and the difference with group size remained when attack initiation was taken into account. There were no significant associations for heart, spleen or MLN weights.

Relationships between organ weights and aggressive behaviour were analysed using stepwise partial regression taking into account peak parasitaemia, occurrences at food/water, attack initiation and post-grouping fur score. Further stepwise analyses took into account relationships with post-grouping and ratio measures of testosterone and corticosterone. Relationships in the expected direction emerged in a number of cases. Both spleen and MLN weights decreased significantly with increasing post-grouping fur score overall (Fig. 6A, B) and among low

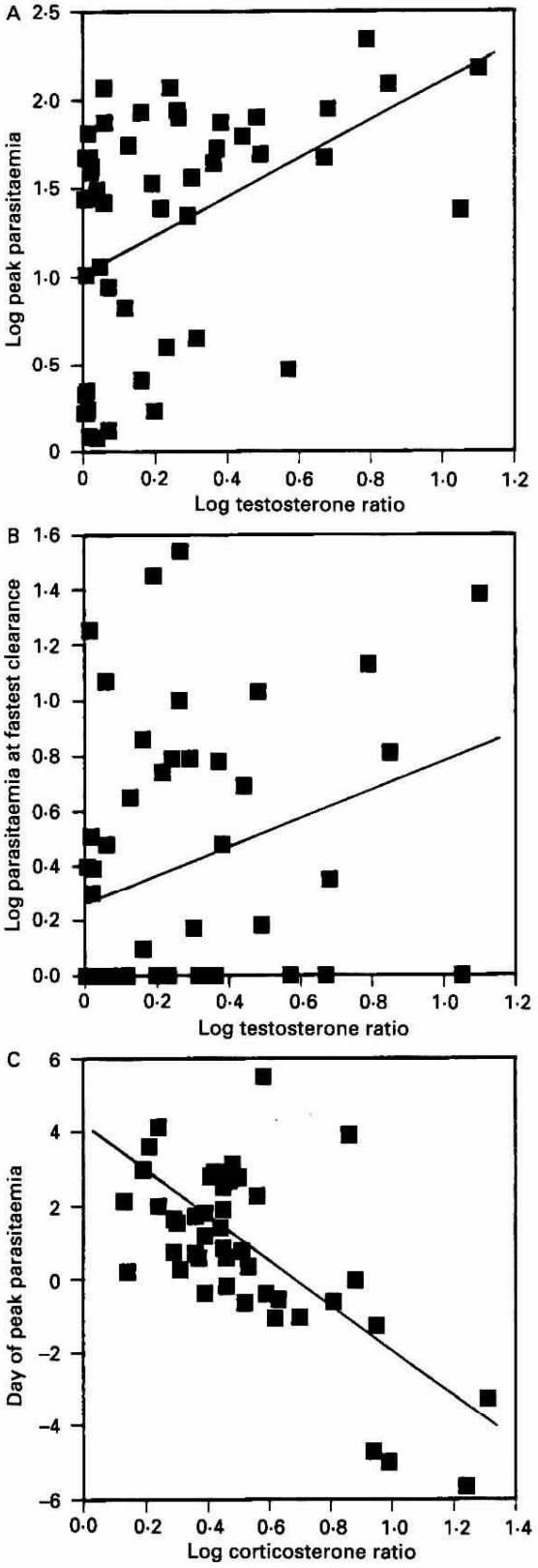

Fig. 4. The partial regression relationship among high rankers between (A) peak parasitaemia (% cells infected) and testosterone ratio (ng/ml), $t = 2{\cdot}13$, one-tailed $P < 0{\cdot}01$; (B) parasitaemia at fastest clearance by tracer mice (% cells infected) and testosterone ratio, $t = 2{\cdot}19$, one-tailed $P < 0{\cdot}01$; (C) day of peak parasitaemia and corticosterone ratio, $t = -2{\cdot}70$, one-tailed $P < 0{\cdot}001$.

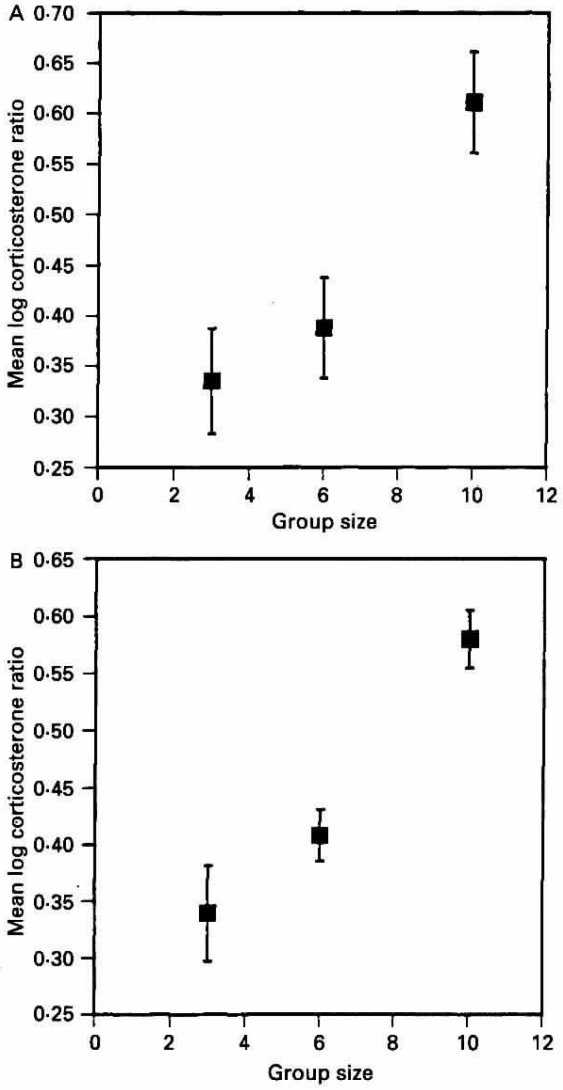

Fig. 5. The relationship between mean corticosterone ratio (ng/l) and group size among (A) high rankers ($F_{2,45} = 5{\cdot}53$, $P < 0{\cdot}01$) and (B) low rankers ($F_{2,118} = 19{\cdot}33$, $P < 0{\cdot}0001$). Bars represent standard errors.

rankers (Fig. 6 legend). Spleen weight also decreased with increased attack initiation among low rankers ($t = -2{\cdot}31$, D.F. = 101, $P < 0{\cdot}05$). Among both rank categories there was a strongly significant relationship between spleen weight and peak parasitaemia ($P < 0{\cdot}0001$ in both cases). MLN weight decreased with increasing corticosterone ratio overall and among both high and low rankers ($t = -2{\cdot}40$, D.F. = 31, $P < 0{\cdot}05$ (high rankers); $t = -2{\cdot}02$, D.F. = 87, $P < 0{\cdot}05$ (low rankers)). There was no significant relationship between any hormone measure and spleen weight.

As expected from the relationship between fur score and corticosterone levels, adrenal weight increased with fur score, but only among high rankers ($t = 1{\cdot}76$, D.F. = 37, one-tailed $P < 0{\cdot}05$). Not surprisingly, there was a positive association between adrenal weight and measures of corticosterone concentration among both high (post-

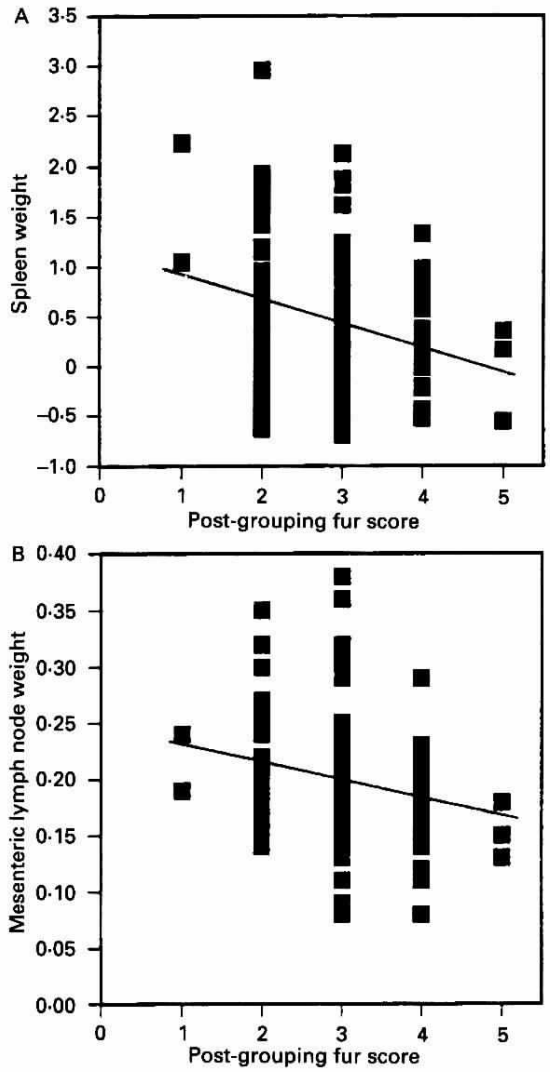

Fig. 6. The partial regression relationship between post-grouping fur score and (A) spleen weight ($t = -2{\cdot}47$, D.F. = 139, $P < 0{\cdot}02$, rank categories combined; $t = -3{\cdot}06$, D.F. = 101, $P < 0{\cdot}01$, low rankers only) and (B) mesenteric lymph node weight ($t = -2{\cdot}53$, D.F. = 139, $P < 0{\cdot}02$, rank categories combined; $t = -2{\cdot}29$, D.F. = 101, $P < 0{\cdot}05$, low rankers only).

grouping concentration $t = 2{\cdot}04$, D.F. = 31, $P < 0{\cdot}05$) and low (corticosterone ratio $t = 2{\cdot}04$, D.F. = 87, $P < 0{\cdot}05$) rankers. Among high rankers, however, there was also a significant positive association with testosterone ratio ($t = 2{\cdot}49$, D.F. = 31, $P < 0{\cdot}02$).

There were no significant associations between attack initiation or fur score and heart or kidney weights, though heart weight increased with post-grouping testosterone concentration overall ($t = 3{\cdot}52$, D.F. = 120, $P < 0{\cdot}001$) and among low rankers ($t = 3{\cdot}67$, D.F. = 87, $P < 0{\cdot}001$).

DISCUSSION

The results for the association between social rank, immunodepression and resistance to *B. microti* infection within single-sex groups of male mice confirm those of Barnard *et al.* (1993) and are consistent with an association between testosterone and rank-dependent resistance. However, as in the previous study, the relationships between rank, behaviour, physiological responses and resistance turn out to be complex, with animals of high and low social rank showing different suites of response.

In the present experiment, high-ranking males again had higher serum corticosterone levels prior to grouping, but only marginally so, and likewise had marginally higher levels of IgG (unlike mice in the earlier experiment) and testosterone. However, high-ranking males showed a more pronounced reduction in serum immunoglobulin concentration over the period of grouping and were slower to clear subsequent infection, implying that their immune system was more depressed than that of low-ranking mice (cf. Vessey, 1964; Jackson & Farmer, 1970; Beden & Brain, 1985). Differences between rank categories in resistance were associated with rank-specific relationships between serum hormone concentrations and measures of infection. In particular, there was a tendency among high rankers for increased serum testosterone over the period of grouping to be associated with (*a*) a greater peak of infection and reduced clearance rates, (*b*) a high ratio of attacks initiated : received and (*c*) smaller groups in which levels of aggression tended to be higher. Since corticosterone measures were taken into account in the analyses, these reflect independent associations with testosterone among high-ranking males. However, independent effects of corticosterone were also implied in that increased corticosterone ratios among high rankers were associated with poorer post-grouping fur condition and an earlier peak of infection.

Previous studies suggest that mice respond to increased group size and/or density with reduced levels of overt aggression (e.g. Poole & Morgan, 1973) but show greater apparent stress as reflected in increased serum corticosterone concentration (Brain & Nowell, 1970), reduced circulating antibodies (Vessey, 1964) and reduced resistance to infection (Brayton & Brain, 1974; Peterson *et al.* 1991). Whilst our data are consistent with the first two, there was no clear relationship between group size and resistance to infection with *B. microti*. As expected from other studies (Brain & Nowell, 1970), serum corticosterone levels rose significantly with increasing group size among both high and low rankers, and levels were highest in the largest groups in which overt aggression was reduced but access to food and water dispensers appeared to be restricted. However, the apparent restricted access was significant only for low rankers and an independent group size effect remained when access was taken into account. Similarly, while some group size effects among low rankers appeared to be attributable to group size-related aggression (reduced IgG), others were in-

dependent of size-related aggression (increased adrenal weight).

There was no association between corticosterone levels and resistance to infection among low rankers, but an immunodepressive effect was suggested by the negative relationship between corticosterone ratio and mesenteric lymph node (MLN) weight. High corticosteroid levels are known to cause a marked, rapid, dose-dependent reduction in the weight of secondary lymphoid organs, notably the spleen and MLN (Dracott & Smith, 1979), evident within 2 days of injection and causing over 80% loss of weight by day 4 following a single dose. The negative relationship between corticosterone ratio and MLN weight is therefore consistent with a dose-dependent loss of immune function. While spleen weight was not significantly associated with corticosterone levels, expected effects of corticosterone on the lymphoid compartments within the spleen may have been masked as a result of enhanced haemopoiesis and erythrophagocytosis (Phillips, 1969; Irvin *et al.* 1981). The negative association among low rankers between IgG concentration and spleen weight (once the strong hypertrophy due to infection was taken into account) and both rate of attack initiation and post-grouping fur score suggest some immunodepressive effect of aggression, though this did not influence the course of infection with *B. microti* as measured here.

Overall, therefore, the results suggest that aggressive social experience has important consequences for immunocompetence and resistance to infection but that relationships with aggressive behaviour are complex. The interesting contrast was between aggressive individuals of high social rank in which reduced resistance to *B. microti* infection was associated with increased serum testosterone and corticosterone levels and reduced serum immunoglobulin concentration, but independently of group size, and much less aggressive individuals of low social rank in which hormonal changes were not associated with resistance to *B. microti* infection but in which corticosterone secretion and immunodepression increased with group size and aggressive behaviour. The complexity of these interactions reflects the possible existence of other components, as yet unidentified, that mediate between social status, stress and resistance to infection. In this context, Wunderlich *et al.* (1991) reported that whereas castration of male mice enhanced resistance to *Plasmodium chabaudi*, depression of serum testosterone levels by treatment with buserelin was not associated with enhanced resistance. Thus other gonadal factors may be involved in down-regulation of protective mechanisms in male mice. The results of this experiment, and that of Barnard *et al.* (1993), also suggest the importance of considering relative as well as absolute changes in the level of physiological factors within individuals in regulating resistance to disease. The rank-specific relationships found here imply different consequences of aggressive social experience for resistance to any particular infection and that resistance and associated physiological responses may be distributed discontinuously across individuals of different competitive ability within social groups. In particular, they suggest that changes in gonadal factors associated with the aggressive maintenance of high social status may impose a cost on dominant individuals in terms of increased susceptibility to disease.

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