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

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SUMMARY

Unrelated and initially unfamiliar male CFLP mice, maintained for different periods in groups of 6, differed in both their rate of clearance of Babesia microti and the time taken to reach peak parasitaemia in relation to their aggressive behaviour within groups prior to infection. Males maintained in groups for shorter periods and showing more aggression within their group were slower to clear infection and males showing more marked external evidence of aggressive interaction reached a peak of parasitaemia sooner. Serum IgG and corticosterone analyses were consistent with increased aggression causing stress-induced immunodepression but relationships with aggression and social status were not simple. Males showing more aggression tended to enter their groups with higher levels of corticosterone and, to a lesser extent, reduced levels of IgG compared with other mice. The results thus suggest that increased susceptibility to disease may be a cost to males aggressively maintaining high social status.

Key words: house mouse, Mus musculus, Babesia microti, social status, aggression, stress, immunity, corticosterone.

INTRODUCTION

The relationship between social and sexual behaviour, susceptibility to disease and parasite burdens in host organisms is currently of considerable interest among evolutionary biologists (see Edwards, 1988; Read, 1980; Moore & Gotelli, 1990). Parasite burdens may have complex effects on several aspects of social and sexual behaviour including social tolerance (e.g. Edwards, 1988), maintenance of social status (Rau, 1983), acquisition of breeding sites (Borgia, 1986) and mating preferences (Hamilton & Zuk, 1981; Edwards & Barnard, 1987; Milinski & Bakker, 1990). While such effects can be observed, establishing the cause-and-effect relationship between parasite burden, pathological consequences to the host and host behaviour is often impeded by confounding factors that are difficult to overcome (see Edwards & Barnard, 1987; Read, 1990).

One widely recognized possibility among social species is that the social environment itself may affect susceptibility to infection as a result of stress or sex-hormone mediated immunodepression (Davis & Read, 1958; Christian, 1961; Vessey, 1964; Bronson & Eleftheriou, 1965; Edwards et al., 1980; Rabin et al. 1987). Several studies have suggested that the stressful and potentially immunodepressive effects of the social environment correlate with the social rank or experience of the individuals concerned (Vessey, 1964; Henry & Stephens, 1977; von Holst et al. 1983; Sapolsky, 1983; Schur, 1987; Peng et al. 1989, Mormede et al. 1990). However, it is difficult to draw clear conclusions about the relationship with social rank from existing studies for a number of reasons. First, stress responses, immunodepression and/or parasite burdens are greatest among subordinate individuals in some studies (Vessey, 1964; Sassenrath, 1970; Henry, Stephens & Ely, 1986; Peng et al. 1989) but greatest among dominants in others (Hausfater & Watson; 1976, Halvorsen, 1986). Second, increased levels of social interaction may lead to increased rather than decreased resistance to infection (Hall, Gross & Turner, 1979). Third, infection itself may result in immunodepression (Behnke, 1987) thus obscuring the effects of pre-existing stress. Fourth, the relationship between social status and susceptibility to infection has been investigated directly in the laboratory, animals have been infected experimentally prior to interacting (Davis & Read; 1958; Jackson & Farmer, 1970) leading to potentially confounding effects of infection-induced behavioural modification (Rau, 1983; Edwards, 1988). Fifth, several studies inferring a relationship between social status and stress (Christian, 1961; Adams & Finn, 1972) used wild or wild-derived animals in which individual parasite burdens were initially unknown. A priori predictions about relationships with social status are also not easy to make. Dominance relationships are often dynamic and can change rapidly (Southwick, 1955;
Poole & Morgan, 1973; Berry & Jakobson, 1974) so that status-related stress is likely to vary with the social and physical environment and temporally (Gaudernack et al. 1984; Hurst, 1987). Furthermore, while subordinates within social systems are generally expected to experience the greatest stress, it is often dominant individuals that experience most aggression and the greatest risk of injury (Christian, 1961; Lahr, 1966; Rohwer & Ewald, 1981).

Establishing the cause-and-effect relationship between social behaviour, stress and pathogen burden has important implications for both the dynamics of parasite communities within host populations and the evolution of social and sexual behaviour among host organisms. In this paper, we report an experiment with laboratory mice in which we investigated the consequences of aggressive social experience for measures of stress, immunocompetence and susceptibility to a subsequent infection. The experiment tested the idea that an individual's social experience influenced its susceptibility to later infection and took into account both pre-existing individual variation in measures of stress and immunocompetence and the effects of differences in social experience with time. The effects of social experience were tested by allowing groups of mice to remain established for different periods of time and thus experience different degrees of social stability and levels of aggression (Poole & Morgan, 1973, 1975) and by examining the differential physiological responses of dominant and subordinate individuals as reflected in circulating levels of corticosterone and IgG. The primary adrenal corticosteroid response of mice to stress and other challenges (e.g. ACTH) is enhanced serum corticosterone levels (Brain & Nowell, 1970). While this contrasts with the response of some other mammals, e.g. primates, in which it is levels of cortisol that are enhanced, there is evidence that elevated corticosterone levels have a broad range of effects on cellular and inflammatory responses during infection similar to that associated with cortisol (Riley, 1981; Landi et al. 1982; Stewart et al. 1988). It is also well-established that corticosterone levels correlate with measures of aggressive behaviour in both cause and effect relationships (Henry & Stephens, 1977; Leshner & Politch, 1979; Koolhaas, Schuurmann & Fokkema, 1983; Sachser, 1989). Total serum IgG levels were chosen as a convenient by-stander measure of immunocompetence reflecting overall immune potential rather than a specific indication of protection against the infection used in the experiment (but see Materials and Methods section).

**Materials and Methods**

**Mice and husbandry**

The mice used in the experiment were males of the randomly-bred CFLP strain which has been used extensively in immunological and behavioural studies (Behnke, Waken & Wilson, 1978; Williams & Behnke, 1983; Barnard, Hurst & Aldhous, 1991). 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 90 days. Twenty-four hours before grouping, males were separated and housed individually in smaller polypropylene cages (48 × 15 × 13 cm) to standardize their social experience immediately prior to group establishment. A separation period of 24 h was chosen because pilot observations showed that it was short enough to avoid the escalated and frequently injurious aggression commonly reported among unfamiliar adult male mice grouped after isolation (Cairns, Hood & Midlairm, 1985).

**The parasite**

The parasite selected for this study was Babesia microti because infections with this species are relatively mild (Cox & Young, 1969), thereby reducing the potential for confounding pathological complications. Furthermore, peak parasitaemia can be expected within 1–2 weeks of inoculation and is known to be controlled by an immune response which is well documented (Clark & Howell, 1978). The rapid development of an immune crisis was considered desirable for this experiment since the objective was to assess susceptibility and resistance as near to the point at which animals were removed from their experimental social environment as possible. The King's 67 strain of B. microti was used throughout. The parasite was obtained originally in 1991 from Dr S. Randolph (Oxford University) and passaged 20 times through CFLP mice (both sexes) until parasitaemia was peaking consistently in excess of 60%. Several mice were then infected to provide a larger uniform stock of infectious material. Mice were bled on day 6 of infection, during rising parasitaemia (˃60%) and heparinized pooled blood, diluted 50–50 in glycerol/sorbitol/saline (38/2-9/0/63 g%) solution was aliquoted in 1 ml quantities and frozen in liquid nitrogen. Prior to the infection of each experimental group of mice, an aliquot was rapidly thawed in warm water, diluted 50–50 with 17.5% sorbitol solution and injected i.p. into 6–10 mice which were monitored regularly until a rising parasitaemia was evident. The mice were then killed and exsanguinated. The number of parasitized erythrocytes was calculated from total erythrocyte count/ml blood and percentage parasitaemia. Experimental mice were injected i.p. with a
Volume (0.1-0.25 ml) corresponding to $4 \times 10^8$ parasitized erythrocytes. Parasitaemia was determined from smears of tail blood, fixed in methanol and stained with Wright's stain using standard haematological techniques.

**Collection of serum samples**

Two blood samples were taken from each mouse in the study, one 2 weeks before the establishment of experimental groups and the second immediately after the period of grouping and prior to infection with *B. microti*. To minimize disturbance, mice were anaesthetized in Trilene (BDH) in which they lost consciousness rapidly but without the panic characteristic of other inhaled anaesthetics. To minimize disturbance to mice during the procedure, cages were covered with black polythene when moved to the sampling bench and sampling was carried out in the dark phase under dim red light. Heparinized capillary tubes of 50 µl vol. were used to take a sample of retro-orbital blood, in almost all cases within 1 min of removal of the mouse from its home or experimental group cage. Blood samples were centrifuged for 5 min in a haematrit centrifuge and the serum was stored at $-20^\circ C$ prior to analysis. Approximately 20 µl of serum were available for analysis which was sufficient to allow the estimation of serum corticosterone levels and a measure of immune capacity.

**Measurement of the concentration of serum corticosterone**

Corticosterone levels were measured using a kit (Gamma-B 12°I-corticosterone—Immunodiagnostic Systems Ltd) based on double antibody radioimmunoassay (RIA), as advised by the manufacturer, using 6 µl samples of undiluted serum. Serum corticosterone levels were calculated by reference to standards provided with the kit.

**Measurement of total serum IgG**

Total serum IgG was measured because a strong parasite-specific IgG response is elicited by *B. microti* in mice (Purvis, 1977) and IgG is involved in acquired resistance to various other infections including resistance to helmint infections (Wahid & Behnke, manuscript submitted). Total serum IgG (mg/l) 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 concentration 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 and/or IgG from a particular sample. As a result, sample sizes in some subsequent analyses vary (see below).

**Concurrent pinworm infections**

All mice used in the experiment were autopsied for pinworm (*Syphacia obvelata and Aspiculuris tetrapera*) infections after the *B. microti* infection had run its course (day 27 post-infection). Pinworms are generally regarded as innocuous in mice, individuals appearing to tolerate worm burdens of several hundred without showing clinical distress. However, there is some evidence that the rat pinworm *S. muris* causes mild immunodepression in its host (Pearson & Taylor, 1975), though none that the murine species do. Antagonistic interactions have been recorded between the two murine species and between mouse pinworms and other nematodes (Keeling, 1961) but there is no evidence that pinworm burdens affect the course of *Babesia* infection (Christensen et al. 1987). Nevertheless, it was considered prudent to assay worm burdens in this study (a) to control for potential effects of any pinworm infection on experimental measures and (b) to provide another measure of susceptibility to parasitic infection. The entire caecum and colon of each mouse were thus removed, opened and incubated for 4 h at $37^\circ C$ in a gauze suspended in a 50 ml beaker containing Hanks's saline. The contents of each beaker were emptied into a Petri dish and, with the aid of a binocular dissecting microscope, the worms were identified and counted as they were removed with a Pasteur pipette.

**Experimental procedure**

Males were arbitrarily assigned to one of 16 experimental groups of 6 unrelated individuals (each group contained a maximum of 1 male from any given natal litter) housed in a plywood and glass observation cage (30 x 60 x 30 cm) fitted with a 60 W red light bulb and 2 food and water dispensers that were replenished *ad libitum*. 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 (no bald, damaged or dishevelled patches, fur well-groomed) to 5 reflecting increasing incidence of damage to, or deterioration in the apparent condition of, the fur was recorded. The fur score for all mice at group establishment was considered prudent to assay worm burdens in this study (a) to control for potential effects of any pinworm infection on experimental measures and (b) to provide another measure of susceptibility to parasitic infection. The entire caecum and colon of each mouse were thus removed, opened and incubated for 4 h at $37^\circ C$ in a gauze suspended in a 50 ml beaker containing Hanks's saline. The contents of each beaker were emptied into a Petri dish and, with the aid of a binocular dissecting microscope, the worms were identified and counted as they were removed with a Pasteur pipette.

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The groups of 6 mice were allocated arbitrarily to one of four categories of 4 groups each based on the length of time groups were to remain established. Four groups remained established for 3 h, four for 3 days, four for 8 days and four for 14 days. 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 observation were made during 15 min periods under dim red light in the dark phase. Each 3 h group was observed for $3 \times 15$ min periods at arbitrary intervals through the 3 h period of grouping before each mouse was blood-sampled, infected and rehoused (see below) at the end of the period of grouping. Each group in the other three time categories was observed for $2 \times 15$ min periods on each day of group establishment with all periods except the first (taking place within the first 3 h of grouping) being allocated at arbitrary times through the dark phase to control for any time effects. During observation, the number of social investigatory (sniffing the face, anogenital and other body areas of another individual), aggressive (chase, bite, tail-rattle, allogroom, aggressive upright and aggressive sideways postures) and defensive (flee, defensive upright and defensive sideways postures) behaviours initiated and received by each individual was recorded on audio tape for later analysis (behaviour categories following those of Mackintosh (1981) see for example Kareem & Barnard (1982) and Edwards (1988)). In addition, the individuals in the vicinity (within 5 cm) of the food and water dispensers were noted on an opportunistic spot-check basis during the observation periods at each cage. All behavioural observations were made by the same observer.

After the designated duration of grouping, each mouse was weighed and its fur score recorded. The second sample of blood was taken and mice were infected with $B. \ microti$ as described above. Following infection, mice were separated and housed in individual polypropylene cages while the development of the infection was assessed. At the same time, 28 tracer mice were also infected with the same dose of $B. \ microti$. To minimize disturbance to experimental animals, a blood smear was taken regularly (first on day 2 post-infection then daily until the parasite had apparently cleared from the fastest responders) from a superficial tail vein of each tracer animal to assess the percentage cells infected and provide a guide to the timing of blood samples from experimental mice. On this basis, a blood smear was taken from experimental mice when $(a)$ the percentage parasitaemia in tracer animals was beginning to rise, $(b)$ when the fastest responding tracer mouse reached its 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.

**Statistical analyses**

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; in other cases probabilities are two-tailed. Where necessary, data were logarithmically transformed to normalize distributions for regression analyses.

**RESULTS**

**Aggressive behaviour and susceptibility to $B. \ microti$**

As expected from previous work (Poole & Morgan 1973, 1975), the overall frequency of aggressive interaction per observation period over the period of grouping (thus taking all observation periods into account) decreased significantly the longer groups remained established (Fig. 1). Individuals within groups were ranked according to the ratio of the number of aggressive interactions initiated and the number received (attack ratio) over the period for which they remained in groups. Top-ranking males (rank = 1) thus had the highest attack ratio and the most subordinate males (rank = 6) the lowest. In the few cases where mice had equal attack ratios, individuals were allocated the mean values of the ranks they would otherwise have occupied. Although there was no significant tendency for high-rankers to spend more time near food and water dispensers during observation periods, the use of attack ratio as a measure of dominance was supported by the fact that the high-rankers showed a significantly greater weight gain over the period of grouping than low-rankers ($t = -2.08, \text{D.F.} = 93, P = 0.04$) (there was no significant relationship between eventual rank and weight before grouping). Again as expected, there was a significant decline, when all group
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Fig. 2. The relationship between dominance rank (based on ratio of attacks initiated:received) and the mean number of attacks initiated per observation period. \( t = -4.94; \) D.F. = 95; \( P < 0.00001 \). Bars represent standard errors.

Fig. 3. The number of attacks initiated per observation period by high-ranking (ranks 1 and 2, \( \bullet \)) and low-ranking (ranks 3–6 \( \triangle \)) males with duration of group maintenance. \( t = -5.75; \) D.F. = 30; \( P < 0.00001 \) and \( t = -3.88; \) D.F. = 64; \( P = 0.00025 \) respectively.

durations were taken into account, in the number of aggressive interactions initiated with decreasing dominance rank, though with a tendency for the two top-ranking males to have a disproportionately high mean incidence (Fig. 2). Analysis of the relationship between the time groups remained established and the frequency of initiating aggressive interactions by ranks 1 and 2 and remaining ranks separately showed that the decline with duration of establishment, while significant for both rank categories, was more pronounced in the top two ranks (Fig. 3). The frequency of initiation of attacks was thus much higher in the top two ranks in the early stages of group establishment but declined to levels comparable with those of subordinate ranks in longer-established groups. As a result of their greater involvement in attacks, males with a greater frequency of initiation emerged with a higher average fur score at the end of their period of grouping \( (t = 2.40, \) D.F. = 93, \( P = 0.019) \), though there was no significant relationship between fur score and rank.

If involvement in aggressive interactions has an immunodepressive effect, we should expect susceptibility to experimental infection to have been greater among mice grouped for shorter periods when rates of attack were high. While there were no significant trends for peak parasitaemia or time to reach peak parasitaemia, there was a significant trend in the expected direction for time to clear infection. Fig. 4 shows that, relative to the fastest clearance among tracer mice, there was a significant negative relationship between duration of grouping and remaining parasitaemia. Mice grouped for short periods thus appeared to be slowest to clear infection. That this was due to the relationship between grouping and frequency of aggression and not some other consequence of short-term grouping was supported by stepwise partial regression analysis taking into account both duration of grouping and the number of attacks initiated per observation period which yielded attacks initiated as a significant predictor of parasitaemia at fastest clearance \( (t = 2.05, \) D.F. = 88, \( P = 0.044) \) but no independent effect of duration of grouping. For the reasons discussed in the Introduction section, it is more difficult to predict \textit{a priori} the relationship between susceptibility to infection and dominance rank. However, since the frequency of initiating attacks increased with rank and parasite clearance rate was negatively related to attack initiation, it is not surprising that parasitaemia at fastest clearance increased with increasing rank (one-tailed \( t = -2.12, \) D.F. = 89, \( P < 0.05 \)). Interestingly, stepwise partial regression
analysis taking into account both rank and frequency of attack initiation maintained the significant relationship between clearance rate and rank (one-tailed $t = -2.12$, d.f. = 89, $P < 0.05$) but did not yield a significant independent effect of frequency of attack initiation. Nevertheless, an effect of frequency of attack initiation was implied by significant relationships between fur score and both parasitaemia at fastest clearance (Fig. 5A) and time to reach peak parasitaemia (Fig. 5B); males with poorer fur condition at the end of their period of grouping both reached a peak of infection sooner and maintained a high parasitaemia longer. In contrast to the partial regression analysis of attack initiation and duration of grouping, both rank and duration of grouping yielded significant effects (one-tailed $t = -2.18$, d.f. = 88, $P < 0.05$ and $t = -1.81$, d.f. = 88, $P < 0.05$ respectively) when taken into account simultaneously. A significant effect of rank was also maintained (one-tailed $t = -2.00$, d.f. = 89, $P < 0.05$) when eventual pinworm burden was taken into account, with pinworm burden showing a significant positive association with *B. microti* parasitaemia at fastest clearance ($t = 2.12$, d.f. = 89, $P = 0.037$). Males having the highest parasitaemia at fastest clearance thus also had the highest pinworm burdens. However, there was no significant association between rank or frequency of attack initiation and pinworm burden. The expectation arising from these trends, therefore, is that higher-ranking/more aggressive males will have shown more evidence of stress and immunodepression as a result of their greater involvement in aggressive interactions.

### Stress and immune responses

Analysis of the relationships between rank and frequency of attack initiation on the one hand and measures of serum IgG levels on the other showed a significant trend in the expected direction for the association between frequency of attack initiation and ratio of IgG levels before and after grouping (Fig. 6); males showing higher rates of attack initiation experienced reduced IgG levels relative to pre-grouping measures. There were also non-significant tendencies for IgG ratio to decline with increasing dominance ranking and for higher ranked and males initiating more attacks to have lower absolute levels of IgG before grouping and after grouping prior to infection with *B. microti*. These outcomes were maintained when both rank and frequency of attack initiation were taken into account simultaneously using stepwise partial regression. Inclusion of eventual worm burden in partial regression analysis revealed no significant independent effect on IgG measures and no effect on the significance of the analyses above. Despite its relationship with frequency of attack initiation, there were no significant effects of duration of grouping on measures of IgG level.

While the significant trends in IgG levels were as expected from the analyses of aggressive behaviour and *B. microti* infection, analysis of corticosterone levels revealed more complex relationships. On average, corticosterone levels rose as a result of grouping (mean± s.e. ng/ml corticosterone before grouping = 88.3±11.87, mean after grouping = 175.4±15.76; $t = 6.21$, d.f. = 184, $P < 0.0001$). However, the ratio of increase (ng/ml after: before) within individuals showed a significant tendency to increase with decreasing social rank (Fig. 7) so that lower-ranking males experienced a greater increase in corticosterone levels relative to levels before grouping. There was also a non-significant trend towards males showing higher frequencies of attack initiation having higher corticosterone levels prior to grouping. However, these trends conceal a potential ceiling effect among higher-ranking/more aggressive males. Analysis of absolute levels of corticosterone before and after grouping showed that the males that
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Fig. 6. The number of attacks initiated per observation period and the ratio of serum IgG levels before and after grouping. \( t = -1.91; \) d.f. = 67; \( P < 0.05 \).

Fig. 7. Dominance rank and mean serum corticosterone levels before and after grouping. \( t = 2.45; \) d.f. = 88; \( P < 0.01 \). Bars represent standard errors.

Fig. 8. The number of attacks initiated per observation period and serum corticosterone level before grouping. \( t = 4.06; \) d.f. = 92; \( P < 0.01 \). (- - - ) Relationship with serum corticosterone levels after grouping (data points omitted, see text).

were aggressive in their experimental groups had significantly higher corticosterone levels than less aggressive males before entering the group (Fig. 8). There was a similar, but non-significant, trend with rank. Since there was no significant relationship between the frequency of attack initiation (or rank) and corticosterone levels after grouping (see dashed line in Fig. 8), the relationships with corticosterone ratio appear to be due partly to high initial levels of corticosterone among higher-ranking/more aggressive males and partly to a large relative increase in corticosterone among low-ranking/less aggressive males to levels comparable with those of high rankers. As with IgG levels, there was no significant effect of period of grouping on any measure of corticosterone.

**Discussion**

The results of the experiment support the idea that social experience can influence susceptibility to infection and that this is mediated by effects of social experience on the immune system.

Initially unfamiliar male CFLP mice, maintained for different periods in groups of six, differed in their rate of clearance of an infection of *B. microti* and the time taken to reach peak parasitaemia in relation to their aggressive behaviour within groups. Males that initiated more aggressive interactions and/or had higher social status in terms of their ratio of attacks initiated: received were slower to clear infections and tended to reach a peak of infection sooner. Moreover, mice that were allowed to remain in groups for only short periods, when rates of attack initiation tended to be higher, were also slower to clear infection. The fact that a trend occurred in rates of clearance of parasitaemia is consistent with what is known about the immunological control of *B. microti* infections in mice. Primary infections are curtailed by processes dependent on T lymphocytes since nude mice fail to control primary parasitaemia which eventually stabilizes at approximately 50% and may persist for several months (Clark & Allison, 1974). The onset of a declining parasitaemia is characterized by the appearance of crisis forms (degenerating intra-erythrocytic stages of the parasite (Clark et al. 1977)), possibly as a consequence of exposure of infected erythrocytes to the cytokines TNF and INF secreted T lymphocytes and macrophages (Clark & Howell, 1991). However, all the parasites are not eliminated and there follows a chronic plateau which may last for 4–5 weeks or even longer in some mouse strains (Eugui & Allison, 1980). Parasite-specific antibody appears in the serum in detectable amounts at about the time of rising parasitaemia, reaching a plateau almost concurrently with peak parasitaemia (Purvis, 1977). Immunodepression does not alter the point at which parasitaemia begins to increase (Clark & Allison, 1974). Severe immunodepression, as in
nude mice, prevents parasitaemia from falling after the peak has been attained but milder treatment would be expected to affect the intensity of peak parasitaemia (Irvin et al. 1981), to slow the declining phase and to prolong the survival of the low-intensity post-crisis plateau. However, while an effect on the intensity of peak parasitaemia was not detected here, the time to reach a peak was shorter in aggressive and maintenance of high rank causing stress-induced immunodepression was relatively mild. The inocula used were relatively high and the stimulus for activating the cell-mediated response would have been sufficiently intense to be indistinguishable across mice. However, subsequent events involved in clearing the persisting parasites from erythrocytes took place over a longer period and here the subtle influence of moderate levels of immunodepression are likely to have been more influential in determining the duration of infection.

A significant positive association between clearance rate for Babesia and pinworm burden suggests that increased susceptibility may be general, though there was no independent relationship between pinworm burden and measures of aggression or social rank. Coupled with the lack of any relationships between pinworm burden and measures of immune competence, it is unlikely that the apparent effects of aggression on immunity were influenced by differences in individual pinworm burden during the period of grouping.

The results of serum IgG and corticosterone analysis were consistent with increased aggression and maintenance of high rank causing stress-induced immunodepression, but relationships with aggression and rank were not simple. At first sight, it appeared that more aggressive, higher-ranking males experienced a greater reduction in IgG levels over their period of grouping but a smaller increase in corticosterone compared with less aggressive/lower-ranking males. However, it is important to note that aggressive males tended to enter their groups with higher corticosterone and, to a non-significant extent, reduced IgG levels. Their greater reduction in IgG levels over the grouping period thus occurred from already relatively low levels whereas their corticosterone did not increase much above its pre-existing level. This suggests that aggressive males were predisposed towards greater susceptibility before being grouped with other unfamiliar individuals (either inherently or as a result of social experience in their natal litters). Such apparent predisposition has been found with other physiological correlates of social response. Fokkema et al. (1988) found that rats that turned out to be most competitive in experimental social encounters tended to be those with high pre-existing levels of noradrenaline and that prior noradrenaline levels predicted not only social but also associated catecholaminergic (adrenaline: noradrenaline ratio) responses. The appreciable decline in their immune capability, but only small increase in corticosterone levels, suggests there may have been causes of immunodepression other than a stress response among higher-ranking males, perhaps mediated by testosterone secretion (Grossman, 1985; Folstad & Karter, 1982). This is supported by the fact that lower-ranking males showed a large increase in corticosterone secretion but little evidence of reduction in IgG levels.

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REFERENCE

ADAMS, L. & FINN, J. A. (1972). Behavioral indices of adrenal gland weigh in the California ground squirrel. Ecology 53, 173-6.

BARNARD, C. J., HURST, J. L. & ALDOUS, P. (1991). Of mice and kin: the functional significance of kin bias in social behaviour. Biological Reviews 66, 379-430.

BEHNKE, J. M. (1987). Evasion of immunity by nematode parasites causing chronic infections. Advances in Parasitology 26, 1-71.

BEHNKE, J. M., WAKELIN, D. & WILSON, M. M. (1978). Trychomielia spiralis: delayed rejection in mice concurrently infected with Nematopredis diduis. Experimental Parasitology 46, 121-30.

BERRY, R. J. & JAKOBSON, M. E. (1974). Vigilance in an island population of the house mouse. Journal of Zoology 173, 341-54.

BORGIA, G. (1986). Satin bowerbird parasites: a test of the bright male hypothesis. Behavioural Ecology and Sociobiology 19, 355-8.

BRAIN, P. F. & NOWELL, N. W. (1970). The effects of differential grouping on endocrine function of mature male albino mice. Physiology and Behaviour 5, 907-10.

BRONSON, F. H. & ELEFHERIOU, B. E. (1965). Adrenal response to fighting in mice: separation of physical and psychological causes. Science 147, 627-8.

CAIRNS, R. B., HOOD, K. E. & MIDLARNS, J. (1985). On fighting in mice: is there a sensitive period for isolation? Animal Behaviour 33, 166-80.

CHRISTENSEN, N. O., NANSSEN, P., FABEGEMI, B. O. & MONRAD, J. (1987). Heterologous antagonistic and synergistic interactions between helminths and between helminths and protozoans in concurrent experimental infection of mammalian hosts. Parasitology Research 73, 387-410.

CHRISTIAN, J. J. (1961). Phenomena associated with population density. Proceedings of the National Academy of Sciences, USA 47, 428-49.

CLARK, I. A. & ALLISON, A. C. (1974). Babesia microti and Plasmodium berghei yoelii infections in nude mice. Nature, London 252, 328-9.

CLARK, I. A. & HOWELL, M. J. (1978). Protozoan parasites of erythrocytes and macrophages. In Parasites,
Immunity and Pathology: the Consequences of Parasitic Infection in Mammals (ed. Behnke, J. M.), pp. 146–167. London: Taylor and Francis.

Clark, I. A., Richmond, J. E., Wills, E. J. & Allison, A. C. (1977). Intraerythrocytic death of a parasite in mice recovering from infection with Babesia microti. Parasitology 75, 189–96.

Cox, F. E. G. & Young, A. S. (1969). Acquired immunity to Babesia microti and Babesia rodhaini in mice. Parasitology 59, 257–68.

Davis, D. E. & Read, C. P. (1958). Effect of behavior on the status of host and risk of parasitic infection. Journal of Parasitology 44, 675–80.

Eugui, E. M. & Alison, A.C. (1980). Differences in susceptibility of various mouse strains to haemoprotezoan infections: possible correlation with natural killer activity. Parasite Immunology 2, 277–92.

Edwards, J. C. & Barnard, C. J. (1987). The effects of Trichinella spiralis infection on social interactions in mixed groups of infected and uninfected male mice. Animal Behaviour 36, 529–40.

Edwards, J. C. & Barnard, C. J. (1987). The effects of Trichinella infection on intershare interactions between mice. Animal Behaviour 35, 533–40.

Eugui, E. M. & Alison, A. C. (1980). Differences in susceptibility of various mouse strains to haemoprotezoan infections: possible correlation with natural killer activity. Parasite Immunology 2, 277–92.

Fokkema, D. S., Smits, K., Gugten, J. Van Der & Kooh, J. (1988). A coherent pattern among social behavior, blood pressure, corticosterone and catecholamine measures in individual male rats. Physiology and Behavior 42, 485–9.

Holst, D., von, Fuchs, E. & Stohr, W. (1983). Physiological changes in male Tupai a belangeri under different types of social stress. In Biobehavioural Bases of Coronary Heart Diseases (ed. Dembrowski, T. M., Schmidt, T. H. & Blumenthan, G.), pp. 382–390. Basel: Karger.

Hurst, J. L. (1987). The functions of urine marking in a free-living population of house mice (Mus domesticus Rutty). Animal Behaviour 36, 1433–42.

Irvin, A. D., Young, E. R., Osborn, G. D. & Francis, L. M. A. (1981). A comparison of Babesia infections in intact, surgically splenectomized and congenitally asplenic (Dh/+ ) mice. International Journal for Parasitology 11, 251–5.

Jackson, L. A. & Farmer, J. N. (1970). Effects of host fighting behaviour on the course of infection of Trypanosoma duttoni in mice. Ecology 51, 672–9.

Kareen, A. M. & Barnard, C. J. (1982). The importance of kinship and familiarity in social interactions between mice. Animal Behaviour 30, 594–601.

Keeling, J. E. D. (1961). Experimental trichuriasis. I. Antagonism between Trichuris muris and Aspicularis tetragona in the albino mouse. Journal of Parasitology 47, 641–6.

Koolhaas, J. M., Schuurmann, T. & Fokkema, D. S. (1983). Social behaviour of rats as a model for the psychophysiology of hypertension. In Biobehavioural Bases of Coronary Heart Disease, (ed. Dembroski, T. M., Schmidt, T. H. & Blumenthan, G.) pp. 391–400. Basel: Karger.

Lamb, M., Rediger, I. W., Lang, M. & Bullock, L. P. (1982). Effects of shipping on the immune function in mice. American Journal of Veterinary Research 43, 1654–7.

Lesher, A. I. & Polich, J. A. (1979). Hormonal control of submissiveness in mice: irrelevance of the androgens and relevance of the pituitary–adrenal horrones. Physiology and Behaviour 22, 531–4.

Leuthold, W. (1966). Variations in territorial behaviour of the Uganda kob Adenota kob thomasi (Neumann 1896). Behaviour 27, 215–58.

Mackintosh, J. H. (1981). Behaviour of the house mouse. Symposium of the Zoological Society of London 47, 337–65.

Mancini, G., Carbonara, A. O. & Heremans, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2, 235–54.

Milinski, M. & Bakker, T. C. M. (1990). Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. Nature, London 344, 330–2.

Moore, J. & Gotelli, N. J. (1990). Phylogenetic perspective on the evolution of altered host behaviours: a critical look at the manipulation hypothesis. In Parasitism and Host Behaviour (ed. Barnard, C. J. & Behnke, J. M.), pp. 193–233. London: Taylor and Francis.

Mormede, P., Lemaire, V., Castanon, N., Dulluc, J., Laval, M. M. & Le Moal, M. (1990). Multiple
neuroendocrine responses to chronic social stress: interaction between individual and situational factors. *Physiology and Behaviour* 47, 1099–105.

**Pearson, D. J. & Taylor, G.** (1975). The influence of the nematode *Syphacia obvelata* on adjuvant arthritis in the rat. *Immunology* 29, 391–6.

**Peng, X., Lang, C. M., Drozdowicz, C. K. & Ohlsson-Wilhelm, B. K.** (1989). Effect of cage population density on plasma corticosterone and peripheral lymphocyte populations of laboratory mice. *Laboratory Animals* 23, 302–6.

**Poole, T. B. & Morgan, H. D. R.** (1973). Differences in aggressive behaviour between male mice (*Mus musculus* L.) in colonies of different sizes. *Animal Behaviour* 21, 788–95.

**Poole, T. B. & Morgan, H. D. R.** (1975). Aggressive behaviour of male mice (*Mus musculus*) towards familiar and unfamiliar opponents. *Animal Behaviour* 23, 470–9.

**Purvis, A. C.** (1977). Immunodepression in *Babesia microti* infections. *Parasitology* 57, 197–205.

**Rabin, B. S., Lyte, M., Epstein, L. H. & Caggiula, A. R.** (1987). Alteration of immune competency by number of mice housed per cage. *Annals of the New York Academy of Sciences* 496, 492–500.

**Rau, M. E.** (1983). Establishment and maintenance of behavioural dominance in male mice infected with *Trichinella spiralis*. *Parasitology* 86, 311–18.

**Read, A. F.** (1990). Parasites and the evolution of host sexual behaviour. In *Parasitism and Host Behaviour* (ed. Barnard, C. J. & Behnke, J. M.), pp. 117–157. London: Taylor and Francis.

**Riley, V.** (1981). Psychoneuroendocrine influences on immunocompetence and neoplasia. *Science* 212, 1100–10.

**Rohwer, S. & Ewald, P. W.** (1981). The cost of dominance and advantage of subordination in a badge signalling system. *Evolution* 35, 441–54.

**Sachser, N.** (1989). Short-term response of plasma norepinephrine, epinephrine, glucocorticoid and testosterone titers to social and non-social stressors in male guinea-pigs of different social status. *Physiology and Behaviour* 39, 11–20.

**Sapolsky, R. M.** (1983). Individual differences in cortisol secretory patterns in the wild baboon: role of negative feedback sensitivity. *Endocrinology* 113, 2262–7.

**Sassenrath, E. N.** (1970). Increased adrenal responsiveness related to social stress in rhesus monkeys. *Hormones and Behavior* 1, 283–98.

**Schur, B.** (1987). Social structure and plasma corticosterone level in female albino mice. *Physiology and Behaviour* 40, 689–93.

**Southwick, C. H.** (1955). Regulatory mechanisms of house mouse populations: social behaviour affecting litter survival. *Ecology* 36, 627–34.

**Stewart, G. L., Mann, M. A., Ubelaker, J. E., McCarthy, J. L. & Wood, B. G.** (1988). A role for elevated plasma corticosterone in modulation of host response during infection with *Trichinella pseudospiralis*. *Parasite Immunology* 10, 139–50.

**Vessey, S. H.** (1964). Effects of grouping on levels of circulating antibodies in mice. *Proceedings of the Society for Experimental Biology and Medicine* 115, 252–5.

**Williams, D. J. & Behnke, J. M.** (1983). Host-protective antibodies and serum immunoglobulin isotypes in mice chronically infected or repeatedly immunized with the nematode *Nematospioideus dubius*. *Immunology* 48, 37–47.