THE ROYAL SOCIETY

Maternal effects on the development of social rank and immunity trade-offs in male laboratory mice (*Mus musculus*)

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Social status in randomly constituted groups of male CFLP mice was predictable from early suckling behaviour and rate of weight gain in natal litters. High-ranking males were those that had suckled on more anterior teats and gained weight more quickly. Rank was not predicted by any measures of sibling interaction or hormone (testosterone, corticosterone) concentration. Aggressiveness in eventual high-rankers was associated negatively with the proportion of males in the litter at birth and the amount of maternal attention received. Aggressive social relationships within natal litters did not predict polarized rank relationships in randomized groups. Nevertheless, while still in their natal litters, and in the absence of aggressive rank relationships, eventual rank categories showed the same difference in modulation of testosterone concentration in relation to current immunocompetence (low-rankers modulating, high-rankers not), as has repeatedly been found in randomized groups by earlier studies. The role of maternal condition in determining rank-related life-history development in male mice is discussed.

Keywords: social rank; maternal condition; mice; immunocompetence; testosterone; modulation

1. INTRODUCTION

Several lines of evidence support the idea that investment in immunocompetence and immune responses to antigenic challenge are subject to trade-offs between fitness components within adaptive life-history strategies (Behnke *et al.* 1992; Folstad & Karter 1992; Sheldon & Verhulst 1996; Barnard & Hurst 1996; Møller 1997). In this view, variation in both components of the immune response itself and associated behavioural and physiological mechanisms (particularly steroid and catecholamine hormones and activities related to 'stress') can reflect adaptive modulation of investment in different components of life history (Barnard & Hurst 1996). While trade-offs may vary more or less continuously within a population, they can frequently be characterized in terms of adaptive suites and thus more discrete categories of life-history strategy (Rohwer & Ewald 1981; Arak 1984; Hutchings & Myers 1994). Social rank classifications are a good example. Rank classifications are usually based on measures of competitive ability, but are often associated with differences in several other important life-history traits, such as growth and body size, disease resistance and reproductive status (Freeland 1981; Schur 1987; Meikle *et al.* 1996; Komers *et al.* 1997).

In a recent series of studies, we have shown that male laboratory mice (*Mus musculus*) can be classified into two discrete rank categories based on the relative amount of aggression initiated and received and the fact that ranks are associated with different strategies of immunity modulation and susceptibility to experimental infections (Barnard *et al.* 1994, 1996*a*,*b*, 1997*a*,*b*, 1998; Smith *et al.* 1996). The differences in immunity modulation hinge on a rank-related tendency to covary behaviour (particularly aggression) and serum hormone (testosterone) concentrations in relation to measures of current immunocompetence (serum total IgG, haemagglutination response) and other potential immunodepressants (corticosterone). High-rankers tend to decouple testosterone secretion from measures of immunocompetence and show a testosterone-dependent reduction in resistance to infection, while the opposite is the case for low-rankers (Barnard *et al.* 1994, 1996*a*, 1998). The difference between ranks is overridden (both categories modulate testosterone), however, when exposed to environments or procedures that depress immunity (Barnard *et al.* 1996*b*, 1997*a*,*b*; Smith *et al.* 1996).

These results have emerged from adult males in randomly established groups of previously unfamiliar individuals, or from singly housed males exposed to odours of unfamiliar individuals (Smith 1996; Smith *et al.* 1996). If they represent strategic life-history trade-offs between short-term competitive ability and longer term survival, however, as suggested by Barnard *et al.* (1997*a*,*b*, 1998) and Barnard & Hurst (1996), we should expect to see predictors of these differences earlier in development. In keeping with this, it is well known in mice and other rodents that the size and sex ratio of litters, and the social relationships of siblings within them, can have marked long-term effects on behaviour, including social competence, parental behaviour, learning ability and aggression (Deitchman & Lavine 1977; Robinson 1976; Gandelman *et*

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al. 1977; Namikas & Wehmer 1978; Mendl & Paul 1991*a*,*b*). While a number of hypotheses, based on differences in exposure to competition or females, degree of maternal care, etc., can account for long-term effects on aggression and competitiveness, studies by Mendl & Paul (1991*a*,*b*) suggest that outcomes are predictable from maternal condition and its expected effects on sex-ratio investment (Trivers & Willard 1973; Clutton-Brock & Iason 1986). Poor-quality mothers give birth to female-biased litters, produce less, or lower quality, milk and thereby inspire aggressive competition among (more demanding) male offspring. Whether competitive differences, and later rank, are in fact determined by postnatal maternal attention or by maternal condition effects *in utero* (through foetal programming: life-history trade-offs in growth and future survival and reproductive potential by the foetus in response to *in utero* nutrition and endocrine events (Barker 1995; Burns *et al*. 1997; Hales 1997)) or by a combination of both, we might expect to see evidence of other differences associated with rank emerging early on if rank categories reflect alternative life-history strategies.

In this paper, we use standardized natal litters of four males to show that high and low rank categories in male CFLP mice are predictable from early postnatal suckling history and differential effects of maternal condition and associated sex ratio at birth on maternal attention and developing aggressiveness among pups. We also show that rank-related immunity trade-offs, which appear to be associated with polarized aggressive relationships in randomly established groups of males (Barnard *et al*. 1994, 1996*a*, 1998) are evident within natal litters prior to the establishment of such relationships.

2. METHODS

Subjects were 128 male mice of the outbred CFLP strain. Mice were derived from 32 litters produced by virgin sires and dams purchased from Bantin & Kingman Ltd, Hull, UK. Each litter was produced by a unique parental pair mated at 50 days of age and maintained under a 12 h:12 h reversed light:dark cycle with lights on at 20.00 h. Litters were maintained with the dam in standard polypropylene laboratory cages (12.5 cm × 45 cm × 14 cm) illuminated by a dim red light (Barnard *et al*. 1993) during the dark phase. At 11 days of age, pups were sexed and each litter reduced to four males. Dams and pups were each weighed, the anogenital distance for each pup was measured, and pups were marked in an individually distinctive pattern with black hair dye (Clairol 'Nice 'n' Easy' Natural Black, Bristol Myers Ltd, UK, see Barnard *et al*. (1993)). Litters were then given three days to settle.

(a) *Pre-weaning procedures (phase 1)*

Pre-weaning behavioural observations began at 14 days of age and continued for 10 d. Observations followed the combined spot check and continuous observation procedures of Barnard *et al*. (1997*a*,*b*, 1998; see also Hurst *et al*. 1996), with 20 instantaneous spot checks per pup and two 5-min continuous observation periods per litter per day (a total of 200 spot checks per pup and 100 min of observation per litter over the ten days). All observations were carried out under dim red light during the dark phase with the order of litters randomized across days. Non-social and social (between siblings) behaviours recorded during spot checks followed the categories of Barnard *et al*. (1997*a*,*b*), and defined in table 1 of those papers, but with the addition of (i) behaviours (any directed movement or contact) directed by dams towards pups or vice versa; (ii) suckling by pups; and (iii) the suckling position of pups (anterior–posterior position of the teat at which a pup was suckling (scored on a scale of 1–3 in 1/3 body lengths from the cranial end)).

(b) *Post-weaning procedures (phase 2)*

At the end of the period of pre-weaning observation, pups were separated from their mothers (weaning) and each mother and pup were weighed for the second time. At this point the first 88-μl blood sample (sample 1) was taken retro-orbitally from each pup following the procedure of Barnard *et al*. (1997*a*). Pups were then maintained in their natal litters for a further 50 days and behaviours recorded as during the pre-weaning phase (but minus interactions with the dam and suckling behaviour) on 19 d spread through the post-weaning period.

(c) *Random groups procedure (phase 3)*

At the end of the post-weaning phase, mice were again weighed and a second 88-μl blood sample (sample 2) was taken from the caudal vein (see Barnard *et al*. 1997*a*). Three days after blood sampling, mice were allocated randomly to new groups of four with the caveat that each group contained only one mouse from any given litter (so individuals were previously unfamiliar). Behavioural observations were continued as for the post-weaning phase for nine days spread over the following 14-day period. At the end of this period, mice were weighed and a further blood sample (sample 3) was taken from the caudal vein. (Two different techniques (retro-orbital and caudal vein sampling) were used to obtain blood during the experiment to reduce the risk of damage from repeated sampling at the same site (see also Barnard *et al*. 1997*a*,*b*, 1998). Comparison of serum hormone and IgG concentrations has shown no consistent differences between the two techniques (Smith 1996; Barnard *et al*. 1997*a*,*b*)).

(d) *Blood assays*

All blood samples were processed and assayed for serum concentrations of testosterone, corticosterone and total IgG using the standard techniques in Barnard *et al*. (1994; 1996*a*,*b*). In a small number of cases, limited serum volumes meant it was not possible to obtain a reliable estimate of all three serum factors from a particular sample. As a result, sample sizes vary slightly between analyses (see, also, Barnard *et al*. 1994, 1997*a*,*b*).

(e) *Statistical analyses*

All analyses were performed using Statgraphics Plus v. 7 (Manugistics Ltd, Maryland, USA). Parametric analyses were used throughout (data were $\log_{10}$ or square root-transformed as necessary and tested for normality using a Kolmogorov–Smirnov one-sample test).

3. RESULTS

(a) *Rank categories*

As in our previous work (e.g. Barnard *et al*. 1997*a*,*b*, 1998), high- and low-rank categories within random groups were defined on the basis of the ratio of attacks (Bite, Chase, Offensive upright, Offensive sideways and/or Circling in table 1 of Barnard *et al*. (1997*a*,*b*)) initiated and received by each male during the period of grouping. As before, high-ranking males were identifiable as having high initiation-to-receipt ratios and initiating significantly and disproportionately more attacks ($F_{1,60}=31.35$, $p<0.0001$). All mice could be classified as high- or low-rankers and all groups contained either one or two high ranking males (see also Barnard *et al.* 1997*a*,*b*, 1998). Analyses relating to social status were based on high- and low-rank categories, and data for each rank category were averaged where there was more than one individual per category within cages to control for potential problems of non-independence (Barnard *et al.* 1996*a*,*b*, 1997*a*,*b*, 1998).

(b) *Predictors of rank category and aggressiveness*

Discriminant function analysis (DFA) showed that rank category in randomized groups (phase 3) was best distinguished by just two variables: position of suckling on the dam and the rate of weight gain over the pre- and post-weaning periods ($\chi^2_2=13.51$, $p=0.005$). DFA incorporating only these two variables classified 68% of future high-rankers and 81% of future low-rankers correctly, with high-rankers having suckled at more anterior teats (mean ± s.e., suckling position = 2.01 ± 0.09) than low-rankers (mean position = 2.36 ± 0.07) and having shown a marginally greater rate of weight gain (33.93 ± 0.55 g versus 32.83 ± 0.30 g). Inclusion of any other pre- or post-weaning variable, physiological, behavioural or physical environmental (litter size, sex ratio, etc.), reduced both the significance and classificatory power of the analysis (but see Collins *et al.* (1997) for effects of postweaning urine marking). Notably, no measure of testosterone, corticosterone or IgG (absolute concentrations ($\log_{10}$ transformed) at weaning and pre-random grouping, change in concentration between these points) predicted future rank. Since other variables, particularly measures of interaction with the dam and siblings, litter size and sex ratio (Mendl & Paul 1991*a*,*b*), were likely to have been instrumental in the effects of body weight and suckling position (through competition for teats), we repeated the DFA with these variables in the absence of weight and suckling position. No significant discrimination of rank category emerged.

(c) *Effects of maternal attention*

The obvious explanation for the weight gain and suckling position effects is that weight increases more rapidly the more a pup suckles, and the more it has access to the most profitable teats. However, these two measures may have opposite relationships with weight gain. Mendl & Paul (1991*b*) found a negative correlation between the amount of suckling and growth rate in male wild house mice (*Mus domesticus*) and attributed this to high suckling rates reflecting milk demand rather than intake. In their case, milk supply was limited by reduced maternal attention in mixed sex compared with all male litters, an effect which Mendl & Paul suggested led to increased aggressiveness as adults. Stepwise partial regression of our data, using a forward inclusion model, concurred with Mendl & Paul's (1991*b*) results, indicating clear differences between future rank categories, but in our case all litters had been standardized at 11 days *post partum* to four males only, thus removing differences in sex ratio for the period during which pre-weaning behaviours were recorded. The results were as follows.

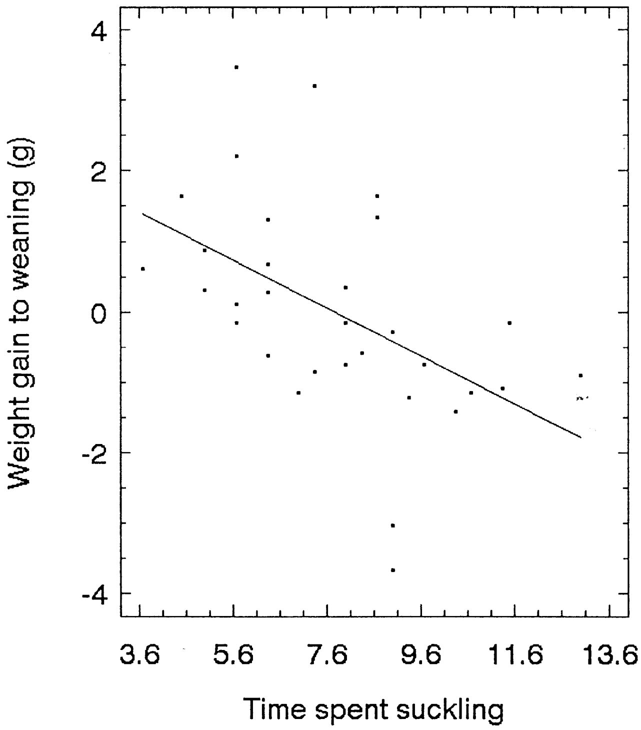

Figure 1. Component effect from stepwise partial regression for the relationship between time spent suckling (number of times recorded) and weight gain from day 1 *post partum* to weaning for low-rankers. Regression equation: $y=18.69-0.34(\text{ts})$, where ts is the no. of times recorded suckling. No other independent variables entered the equation. See text.

First, an analysis of the effects of maternal attention and body weight, time spent suckling, suckling position and initial litter size and sex ratio on weight gain during the pre-weaning phase (phase 1) showed a significant negative relationship between time spent suckling and weight gain across all mice ($t_{60}=-2.79$, $p<0.01$). No other independent variables entered the equation. However, when future rank categories were analysed separately, the relationship was apparent only among low-rankers ($t_{29}=-3.06$, $p<0.01$, figure 1; no independent variable entered the equation for high-rankers).

Second, an analysis of the effect of litter size, number of males in the litter and maternal weight on maternal attention over the pre-weaning phase revealed a significant negative effect of the relative number of males in the initial litter ($t_{60}=-2.86$, $p<0.01$) but a positive effect of maternal weight (at 11 days *post partum*; $t_{60}=2.25$, $p<0.05$). Moreover, the negative effect of the number of males was apparent only among future high-rankers ($t_{29}=-2.33$, $p<0.05$, figure 2) when rank categories were analysed separately. No variables entered the equation for future low-rankers.

Third, analysis of the effects of the same independent variables on aggressiveness (square root number of aggressive acts initiated) in randomly allocated groups (phase 3) showed a marginally non-significant negative relationship between the relative number of males at birth and subsequent aggressiveness ($t_{60}=-1.85$, $p=0.069$), but a significant trend among high-rankers when rank

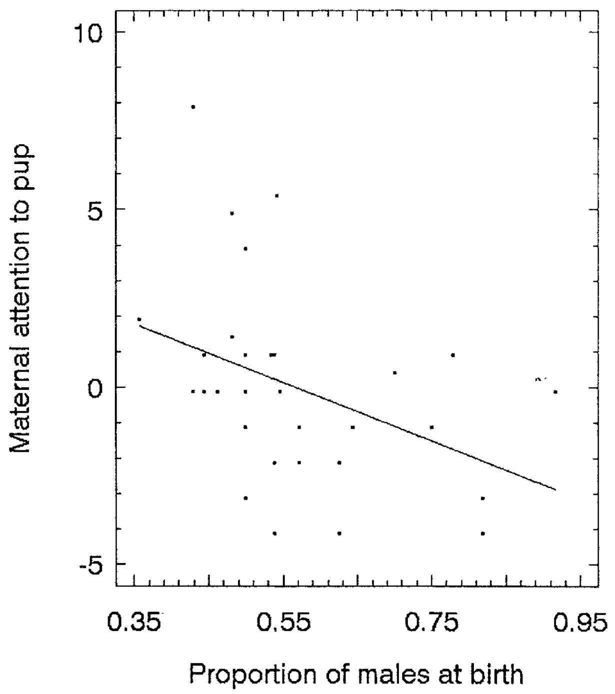

Figure 2. Component effect from stepwise partial regression for the relationship between the proportion of males in the litter at birth and maternal attention to pups (number of attentive acts recorded) in standardized litters of four males. Relationship for high-rankers. Regression equation: $y = -7.45 - 7.93(\text{pm}) + 0.35(\text{mw})$, where pm = proportion of males and mw = weight of mother at 11 days *post partum*. See text.

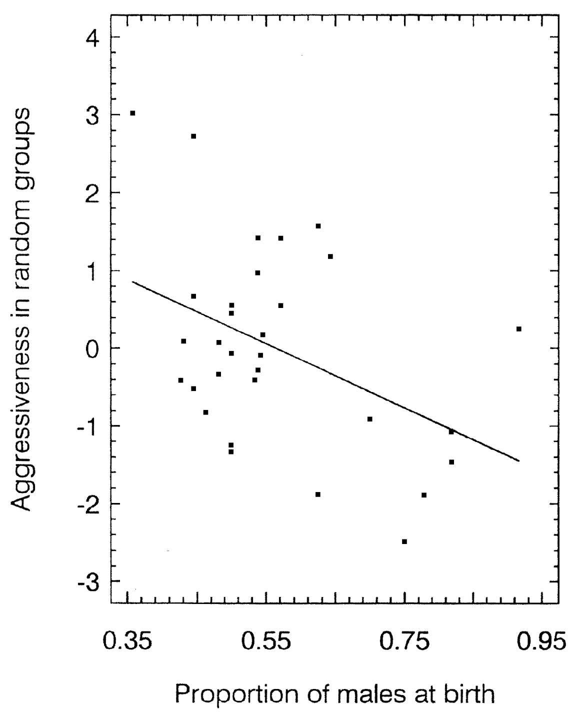

Figure 3. As figure 2, but for the relationship among high-rankers between the proportion of males in the litter at birth and aggressiveness (square root of the number of aggressive acts initiated) in random groups. Regression equation: $y = 8.37 - 0.21(\text{ls}) - 4.12(\text{pm})$, where ls is the litter size (number of pups) at birth and pm is as in figure 2. No other independent variables entered the equation. See text.

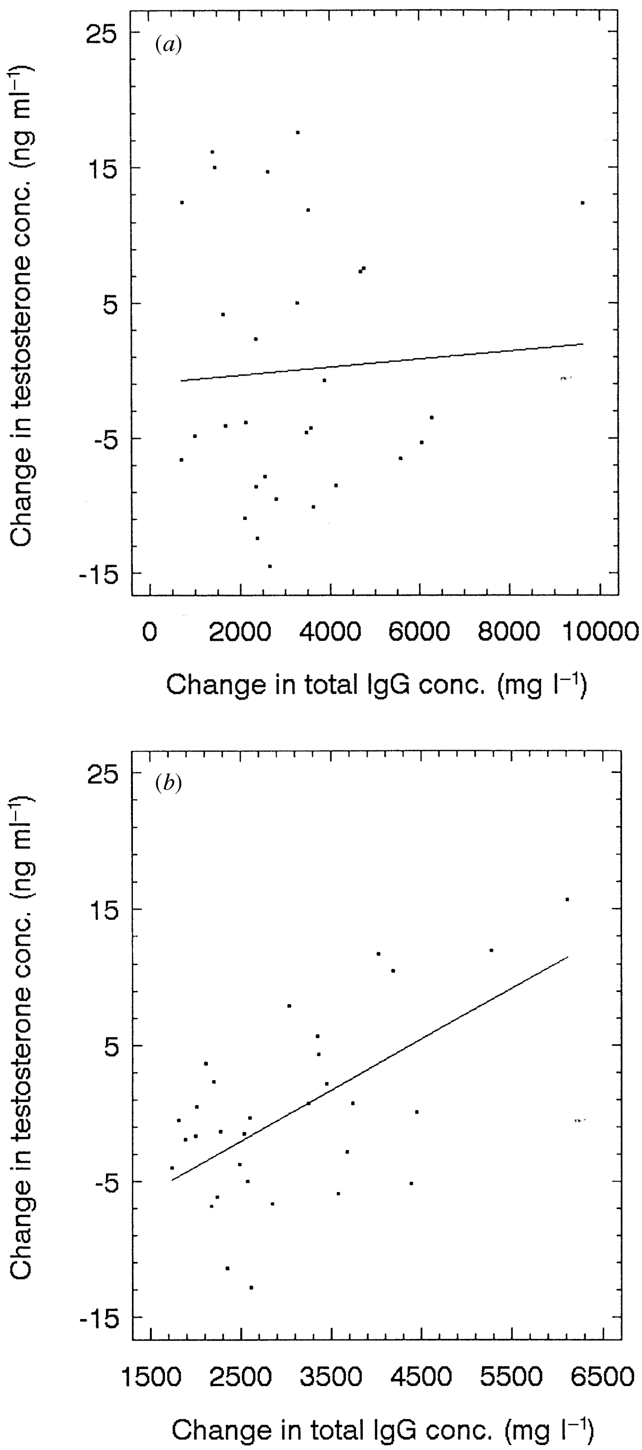

Figure 4. Component effects from partial regression analysis of the relationship between change in total IgG concentration from weaning to immediately prior to random grouping and change in testosterone concentration (*a*) among eventual high-rankers (*b*) among eventual low-rankers. Regression equation for low-rankers: $y = -7.84 + 0.004(\text{igg})$, where igg is the total IgG concentration prior to random grouping minus concentration at weaning. See text.

categories were analysed separately ($t_{29} = -2.46$, $p < 0.03$, figure 3). Aggressiveness among high-rankers also showed a significant decline with increased litter size ($t_{29} = -2.08$, $p < 0.05$). No independent variables entered the equation for low-rankers.

Table 1. *Mean ± s.e. serum concentrations of testosterone* (ng ml^{-1}), *corticosterone* (ng ml^{-1}) *and total IgG* (mg l^{-1}) *at weaning, immediately prior to entering random groups, and at the end of the period of random grouping for high- and low-ranking males in random groups*

(Sample sizes in parentheses.)

		rank category	
		high	low
testosterone	weaning	3.91 ± 0.80(31)	4.43 ± 0.50(31)
	pre-group	8.49 ± 1.56(30)	7.84 ± 1.08(31)
	post-group	9.62 ± 2.23(30)	7.32 ± 1.27(31)
corticosterone	weaning	85.37 ± 19.31(28)	71.73 ± 10.80(31)
	pre-group	66.42 ± 6.77(30)	86.43 ± 11.57(31)
	post-group	62.64 ± 6.64(28)	76.17 ± 9.15(31)
total IgG	weaning	820 ± 43(30)	781 ± 35(31)
	pre-group	4021 ± 327(31)	3818 ± 194(31)
	post-group	4512 ± 406(31)	3997 ± 220(31)

The fact that sex ratio at birth, rather than *post partum*, influenced maternal attention, suckling and weight gain suggests the effects may have been due to dams in poorer condition having fewer male offspring, as predicted by sex allocation theory (Trivers & Willard 1973) and suggested by Mendl & Paul (1991*b*). Some support for this emerged from a significant positive relationship between dam weight at weaning and the number of males in the litter ($r_{60} = 0.30$, $p < 0.02$).

(d) *Effects of physiological measures and body weight*

Several studies have suggested associations between social rank, aggressiveness, body weight and hormone (particularly androgen) concentrations (Drickamer *et al.* 1995; Lucion *et al.* 1996; Girolami *et al.* 1997; Virgin & Sapolsky 1997). Our own work has also indicated a potential regulatory role of immunocompetence in these associations (Barnard *et al.* 1994, 1996*a*,*b*, 1997*a*,*b*, 1998). Although DFA showed no role of body weight or testosterone, corticosterone or total IgG concentration in predicting future rank category, we nevertheless carried out further partial regression analysis to see whether they were associated with aggressiveness before or after random allocation to groups. Independent variables were the change in testosterone, corticosterone and total IgG concentrations between weaning and random group allocation (sample 2 minus sample 1) and change in body weight from 11 days *post partum* to random group allocation. The results showed no effect of any variable on aggressiveness in random groups overall or among high-rankers, but a positive effect of change in body weight among low-rankers ($t_{29} = 2.21$, $p < 0.05$). Analysis of pre-grouping aggressiveness (sum of initiations in pre- and post-weaning natal litters) again failed to reveal any significant effects overall or among future high-rankers, but pre-grouping aggressiveness increased with change in IgG concentration among future low-rankers ($t_{29} = 2.70$, $p < 0.02$). There was no significant correlation between the number of aggressive acts initiated prior to random grouping and the number initiated within random groups in either rank category ($r_{29} = 0.13$, not significant (n.s.) for high-rankers and 0.24, n.s. for low-rankers), neither was there any correlation between the ratios of attacks initiated and received (the basis for defining rank categories (see above), $r_{29} = 0.29$, n.s. for high-rankers and 0.28, n.s. for low-rankers). Indeed, the highly significant difference between rank categories in random groups ($F_{1,60} = 40.1$, $p < 0.0001$) was absent in the same individuals in pre- and post-weaning natal litters ($F_{1,60} = 1.31$, n.s.). Aggressiveness in random groups was thus not simply a continuation of aggressiveness within natal litters (see also Collins *et al.* 1997).

(e) *Modulation of testosterone*

In contrast to our previous experiments (e.g. Barnard *et al.* 1996*a*,*b*, 1997*a*,*b*), there was no significant change in testosterone, corticosterone or total IgG over the period of random grouping in either high- or low-rankers (paired *t*-tests; table 1). This created a floor effect within which neither rank category showed a correlation between change in testosterone concentration and either total IgG or corticosterone concentrations (cf. Barnard *et al.* 1994, 1996*a*,*b*, 1998). However, both testosterone and IgG concentrations showed significant increases in post-weaning natal litters in each future rank category (paired $t_{29} = 2{,}67$, $p < 0.02$ and $t_{28} = 9.20$, $p < 0.0001$, respectively, for high-rankers; $t_{29} = 2.93$, $p < 0.01$ and $t_{29} = 15.88$, $p < 0.00001$, respectively, for low-rankers; table 1). Moreover, partial regression analysis of the relationship between change in testosterone over this period (dependent variable) and change in total IgG and corticosterone (independent variables, with change in body weight controlled as an additional independent variable) showed that future rank categories were already differentiated in their tendency to modulate testosterone concentration with respect to IgG as found previously in random groups by Barnard *et al.* (1994, 1996*a*, 1998). Future high-rankers showed no significant relationship with any independent variable, while future low-rankers showed a strongly significant positive association between change in testosterone and change in IgG ($t_{29} = 4.01$, $p < 0.001$; figure 4*a*,*b*).

4. DISCUSSION

Two important results emerge from the experiment. First, it appears that rank category in randomly constituted, unfamiliar groups of male CFLP mice was predictable from suckling and rate of weight gain within natal litters. Time spent suckling appeared to reflect competition for milk, as suggested by Mendl & Paul (1991*b*), and thus correlated negatively with rate of weight gain among later low-rankers. In this respect it is notable that it was suckling position rather than time spent suckling that predicted rank category. The tendency for later high-rankers to spend more time on anterior teats may reflect their ability to gain access to better nutrition and thus obviate the need to compete for time at the teat. Piglets (*Sus scrofa*) suckling on anterior teats are known to gain weight faster, obtain more colostrum and show reduced mortality compared with those suckling on posterior teats (Fraser & Rushen 1992; Hoy *et al*. 1995).

Second, while this early difference in suckling benefit and development suggests that the potential for competitive high status is established very early on, it is clear that this had little to do with androgen levels. While anogenital distance correlated positively with subsequent testosterone concentration (as expected from numerous previous studies, e.g. Drickamer *et al*. 1995; Palanza *et al*. 1995), neither predicted aggressiveness or eventual rank. What did predict aggressiveness was the number of males in the litter at birth. Moreover, the relationship with aggressiveness was restricted to eventual high-rankers and appeared to reflect reduced maternal attention. Since *post partum* litter composition was standardized experimentally for the period of observation, the relationship between the number of males at birth, maternal attention and aggression among high-rankers supports Mendl & Paul's (1991*b*) inference of a twin effect of maternal condition on sex ratio at birth and pre-weaning care (although Mendl & Paul did not analyse social ranks separately).

These maternal care predictors of social rank were combined with relationships between testosterone, aggressiveness and immunocompetence that reflected rank differences in apparent immunocompetence trade-offs repeatedly found in randomized groups in our previous work (Barnard *et al*. 1994, 1996*a*, 1998). Aggressiveness and testosterone concentration varied independently of a bystander measure of immune responsiveness (total IgG) among future high rankers, but correlated positively with IgG among future low rankers. The fact that these differences preceded the emergence of clear rank categories in random groups suggests deeper differences in life-history strategies between individuals than responses to current aggression. These may have their origin in the effects of maternal condition on early suckling and weight gain, or in its effects on the prenatal environment (Barker 1995). Extensive work in humans and rodents (Barker 1995; Rao 1996; Phillips 1996) has identified a crucial role of nutritional constraints and other mother/foetus conflicts (Haig 1993) *in utero* in determining a suite of life-history attributes in resultant offspring, including patterns of growth and organ development, immune function, menopause and longevity (via susceptibility to adult disease such as cardiovascular disease, hypertension and adult-onset diabetes) (Barker 1995, 1996; Hales 1997; Cresswell *et al*. 1997; Langley-Evans 1997). These differences are underpinned by various endocrine changes involving many different hormones, but particularly glucocorticoids, insulin and growth hormone (Barker 1995; Phillips 1996; Clark *et al*. 1996; Rohner-Jeanrenaud & Jeanrenaud 1997). Such fundamental shifts in development and metabolism, mediated by maternal condition, could account for the early differences in life-history trade-offs in our rank categories prior to the aggressive social environment in which rank is normally expressed.

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