Social environment and immunity in male red jungle fowl

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We examined the relationship between social dominance, immune response, and ornamentation in captive red jungle fowl by comparing these variables in males housed individually with a single female to those in the same males after they were placed in flocks with an unfamiliar male and three unfamiliar females. Males with larger combs before being placed in the flocks were more likely to become dominant, and dominant males' combs grew after flock formation, whereas subordinate males' combs shrank. Immune response as reflected in hematocrit, immunoglobulin levels, and wing web swelling (a measure of cell-mediated immunity) was stronger in males that later became dominant, both before and after flock formation, although the difference between dominant and subordinate birds was more pronounced after males were housed in the multi-male groups. Dominant and subordinate males also differed in the relationship between comb length and wing web swelling. Among dominant males, individuals with larger combs had significantly larger swellings after flock formation, whereas within the subordinate males, those with relatively larger combs had worse cell-mediated immunity than those with smaller combs. These results suggest that males of different quality pay different costs to maintain both ornamentation and immune defense. *Key words:* dominance, *Gallus gallus*, immune response, ornamentation, red jungle fowl. *[Behav Ecol 11:146–153 (2000)]*

The social environment has a profound effect on many aspects of an animal's biology. Dominance status, for example, can influence access to food, mating success, and fecundity, all of which in turn may be important determinants of fitness. The social environment can also affect an animal's internal state: physiological variables such as hormone profiles and growth or maturation rates may potentially be modified in response to the presence of conspecifics. Disease resistance has been a recent focus of the effect of social interactions on individuals; the ability to fend off parasites and pathogens is likely to be important for most organisms at some point in their lives, and the role of parasite resistance in host behavioral ecology has been increasingly recognized over the last decade (Folstad and Karter, 1992; Sheldon and Verhulst, 1996; Zuk, 1994). In some species, social rank may change susceptibility to disease, and parental effort involved in feeding offspring may also limit an animal's ability to fight off pathogens (Barnard et al., 1993; Gustafsson et al., 1994; Richner et al., 1995).

Studies of this interaction between social behavior and disease resistance have generally taken one of two approaches. First, several researchers have examined immune suppression or vulnerability to parasite infection as one of the factors mediating the cost of reproduction (Gustafsson et al., 1994; Nordling et al., 1998; Richner et al., 1995). This life-history approach looks for a trade-off between reproductive effort and immune function, so that animals investing heavily in producing young may become more vulnerable to disease. Experimental manipulation of parental effort or brood size in collared flycatchers (*Ficedula albicollis*), zebra finches (*Taeniopygia guttata*), and great tits (*Parus major*) showed that birds forced to work harder either had a decreased ability to respond to an immune system challenge (flycatchers, Nordling et al., 1998; zebra finches, Deerenberg, 1996) or developed higher levels of avian malaria (tits: Nordling et al., 1998; Richner et al., 1995). This response is consistent with the idea that individuals cannot maximize both immune defense and

parental effort and suggests that at least in the short term animals must pay a price for increasing their offspring production and that such trade-offs are potentially important in evolution. Svensson and Sheldon (1998) point out that placing these life-history studies in a social context, such as that of sexual selection or dominance, is potentially fruitful.

A second approach is more physiologically based and examines the immediate effect and proximate causes of changes in disease resistance when animals are under different social conditions, such as changes in dominance status or stress. A variety of laboratory and field studies have determined that acquiring or maintaining dominance is stressful and that immune competence declines under such stressful conditions (Barnard et al., 1998; Fox et al., 1997; Sapolsky et al., 1997; Tuchscherer et al. 1998). In particular, low-ranking individuals in mammals such as laboratory mice (*Mus musculus*) and baboons (*Papio anubis*) appear to have higher levels of stress-related hormones that may make them more vulnerable to disease (Barnard et al., 1998; Sapolsky and Spencer, 1997). The picture is complex; dominant and subordinate animals may differ not simply in their stress hormone levels but in the modulation of those hormones (Barnard et al., 1998), and the relationship between dominance and stress can be seasonal (Kotrschal et al., 1998). In addition, a few field studies have contradicted the generally inverse relationship between dominance and stress, perhaps because animals measured in the field are under different constraints than those measured in captivity (Creel et al., 1996). In any case, the literature on psychoneuro-immunology in both humans and nonhumans underlines a strong connection between the social experiences of an individual and its ability to resist disease (Ader et al., 1991; Blanchard et al., 1993).

Here we attempt to bring these two approaches together by examining the consequences of changes in social environment for immune defense in male red jungle fowl (*Gallus gallus*) and placing the individual-level results in an evolutionary context. We were particularly interested to see how dominant and subordinate individuals responded to changes in their social situation because, although a trade-off between investment in immune defense and reproductive behavior is expected, it may take place differently in individuals of different quality. High quality may be considered as roughly

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equivalent to high fitness, as reflected in traits such as immunocompetence as well as mating success. Although within a given individual immune defense is decreased when reproductive effort is increased and vice versa, high-quality individuals may perform this trade-off differently because they have more resources overall to allocate to either task. Conversely, low-quality individuals may simply have poorer immune responses and poorer ability to perform reproductive behaviors. Variation in these individual-level responses has important implications for the way in which natural selection acts on life history (Roff, 1992; van Noordwijk and de Jong, 1986).

Previous work on immunity and sexual selection in red jungle fowl has revealed a connection between sexually selected ornaments and immune status (Zuk and Johnsen, 1998; Zuk et al., 1995). During the breeding season, males with large combs, a trait important in both mate choice and male competition, have lower levels of lymphocytes but greater cell-mediated immunity, as indicated by a cutaneous hypersensitivity response (Zuk and Johnsen, 1998). Before the breeding season, however, both cell-mediated immunity and proportion of lymphocytes are positively correlated with comb length. These results suggest that males may not all show the same patterns of allocation to ornamentation and immune defense, displaying an immunocompetence handicap (Folstad and Karter, 1992). Other ornaments used by females in mate choice, such as comb color and eye color, also appear to reflect immune status (Zuk et al., 1995).

In this study we assessed immunity in the context of behavior by examining how immune response changes when males are placed in social groups with another male and three females after having been housed in male–female pairs. In the wild as well as in free-ranging captive populations, jungle fowl live in flocks consisting of a dominant male, one or a few subordinate males, and a number of females (Collias and Collias, 1967; Collias et al., 1966). Dominance interactions are frequent, and social status has important consequences for both sexes (Collias et al., 1994; Zuk et al., 1998). Changing the social group composition from male–female pairs to multimale groups therefore alters the competitive environment in a biologically meaningful way. We were particularly interested in whether males responded differently after their social environment was changed depending on the dominance status they attained. Do dominant males pay more or less of a price to maintain immunocompetence? We also examined the relationships among ornamentation, dominance status, and immune function. The condition of a male's immune system obviously cannot be directly detected by another individual, so any response presumably is mediated by morphology and behavior. Males with longer combs are preferred as mates and are more likely to win in aggressive encounters (Ligon et al., 1990; Zuk et al., 1990), so we wanted to see if changes in immune status were reflected in comb length or other ornamental characters.

We measured immunity in several different ways to obtain a more complete picture of immune competence. First, we measured the hematocrit, the proportion of red blood cells in the whole blood. Hematocrit often reflects general health, and in male red jungle fowl it is higher in birds with greater aerobic capacity, or ability to maintain vigorous exercise (Chappell et al., 1997). Second, we examined the agglutination response of the birds to injection of sheep red blood cells (SRBC), a common immune system stimulant. The hemagglutination results from a reaction between antibodies formed against the SRBC and the SRBC themselves, so a higher agglutination score indicates a higher level of antibody formation. Third, we measured the total amount of immunoglobulin G (IgG) produced in the blood in response to phytohemagglutinin and SRBC injections; immunoglobulins are the molecules that are the source of specific antibodies, and higher levels indicate a more vigorous immune response. Finally, we measured cell-mediated immunity using a cutaneous hypersensitivity test.

MATERIALS AND METHODS

Origin and maintenance of jungle fowl

Our study population is descended from 150 individuals obtained in 1985–1986 from a free-ranging population at the San Diego Zoo, which imported 30 red jungle fowl from Asia in 1942 (Zuk et al., 1995). Chicks were hatched in incubators and kept indoors in brooders for the first 6 weeks of their life. At 6 weeks of age, the chicks were moved outside and kept in large communal cages in flocks of up to 80 birds. When they were approximately 5 months old, the males began to exhibit aggression toward each other, and we separated the birds into male–female pairs and housed them in smaller cages (1 m high, 2 m wide, and 1 m deep). The birds all received water and commercial poultry feed (18–21% protein) ad libitum, supplemented with scratch, a mixture of seeds, after they were moved outside. Individual males could thus see and hear, but not contact, other males, a treatment which equalizes the perceived dominance of each individual (Ligon et al., 1990).

When the birds reached sexual maturity at 9–11 months of age, they were put into 37 mixed-sex flocks each consisting of two males and three females. The flocks were housed in large group cages (2 m high, 3 m wide, and 6 m deep) with 10 permanent perches to allow subordinates the opportunity to escape when chased by dominant birds. Sample sizes are <37 for some measures either because certain individuals were not measured for some variables on a given day or because a member of the flock died.

Timetable of experiment

We obtained six blood samples from each male, with four taken before the males were placed in the flocks and two afterward, as follows:

Day −23 to −25: Injection of SRBC (sensitizing), males housed singly with one female
Day 0: Blood sample "alone 1" taken, injection of PHA (sensitizing)
Day 10: Wing web injection (PHA), wing web swelling measured 6 hr after injection
Day 11: Wing web swelling measured 24 hr after injection, blood sample "alone 2" taken, SRBC injection administered
Day 18: Blood sample "alone 3" taken
Day 19–43: Males allowed to remain with females while antibody titers returned to near-baseline levels
Day 44: Blood sample "alone 4" taken, injection of PHA (sensitizing). Males were placed in the mixed sex flocks.
Day 55: Wing web injection (PHA), wing web swelling measured 6 h after injection
Day 56: Wing web swelling measured 24 h after injection; blood sample "flock 1" taken, SRBC injection administered
Day 63: Blood sample "flock 2" taken

The "alone" samples 1–4 in the figures therefore corresponded to those on days 0, 11, 18, and 44, whereas the "flock" samples 1 and 2 were taken on days 56 and 63.

Behavioral observations

To form the mixed-sex flocks, we first placed three females in each cage. On the next day, we added the two males to each flock and observed them as they fought for dominance. Male pairs were chosen at random, and none of the birds in the flock had interacted before. The dominance relationship between the males in each flock was determined by observing the behavior of the males immediately after they had been placed in a common cage as well as once the flocks had been together for several days. In most cases, the males interacted within seconds of being introduced to the cage. These conflicts ended with one male persistently chasing and pecking the other male, and we determined that the winner was dominant and the loser was subordinate. If the aggression continued until it looked as if the subordinate male could become injured, we separated the males before this occurred and gathered no data from that flock. In flocks where the males did not fight, we observed pecks and displacements between the males, and we determined that the winner in these aggressive encounters was dominant to the loser in the same manner as Zuk et al. (1998).

Morphological measurements

We measured tarsus length and comb length to the nearest 0.01 mm using digital calipers. Mass was recorded to the nearest 0.1g using a digital scale.

We measured colors of the comb and iris using the Munsell system. Although we and others have employed a more quantitative technique for color measurement of feathers using a spectroradiometer (Zuk and Decruyenaere, 1994), the eye and comb are virtually impossible to reliably and safely measure using this device. The Munsell system consists of a series of color chips that give each color a score for hue (e.g., red or yellow), value (darkness, or amount of black), and chroma (brightness, or saturation with pigment). For data analysis, we used the variable that varied most among individuals. This was hue for iris color and chroma for comb color; for the other scores, most males had the same iris chroma and value and the same comb hue and chroma. Iris hue and comb chroma were also the color measures of these traits that were most likely to be important in mate choice in previous studies (Zuk et al., 1990).

Immune system assays

We took blood from the alar vein in the wing by inserting a needle into the vein and drawing blood into a vacutainer containing the anticoagulant lithium heparin. The blood was stored on ice for up to 3 h before it was centrifuged, the plasma separated from the hematocrit, and the plasma stored at $-20°C$. Later the same day, we brought the birds into the laboratory and took a second blood sample. We placed the needle in the vein and collected blood in a capillary tube as it emerged from the needle.

Hematocrit
Blood drawn into a capillary tube was spun at 2000 rpm in a centrifuge for 5 min. We determined the percentage of red blood cells, or hematocrit, in the capillary tube by measuring the length of the column of red blood cells relative to the total column of blood.

Hemagglutination assay
We measured the relative concentration of antibodies to SRBC using a hemagglutination assay (Hudson and Hay, 1989). Briefly, 25 μl of PBS buffer was added to the wells rows 2–11 of a 96-well plate (concave bottom). For each sample, we added 50 μl plasma to the well in the first row. We prepared a gradual dilution of the plasma in PBS buffer by transferring 25 μl from the first well into the second well, mixing the solution in that well before we continued to dilute the plasma until we discarded the last 25 μl taken from row 10. We then added 25 μl of SRBC antibody solution to the wells in the twelfth row. Finally, we added 25 μl of a 10% SRBC solution to all the wells. When enough antibodies were present in solution, a matrix formed between the antibodies and the SRBC, preventing the cells from accumulating at the bottom of the wells. When the concentration of antibodies was low, the blood cells accumulated at the bottom of the well and could be seen as a well-defined spot. We scored the plates by determining the lowest concentration of plasma at which the hemagglutination reaction had not occurred. We then used the $\log_{(2)}$ of the inverse of the concentration in our analysis. This calculation allowed us to score the plates as 0 if no reaction takes place at a plasma concentration of 1, 1 at a concentration of 0.5, 2 at a concentration of 0.25, and so on. This number is the reaction score used in analyses.

Immunoglobulin G
We used a sandwich ELISA assay to measure IgG concentrations in plasma samples taken from the males after the challenges with phytohemagglutinin and SRBC. Briefly, chicken anti-IgG (100 μl, 12.5 ng/ml) was allowed to bind to the walls of Corning 96-well polystyrene flat-bottom plates. Nonbinding anti-IgG was washed off and further binding to the walls was blocked by saturating the wells with bovine serum albumin (BSA). Excess BSA was washed off, and IgG (standard curves and plasma samples) was then added to the wells and allowed to bind with its antibody. After washing, chicken anti-IgG labeled with peroxidase (100 μl, 1:4000 dilution) was added to all wells. The wells were washed to remove unbound anti-IgG×. The addition of 3,3′,5,5′-tetramethylbenzidine (TMB) and peroxidase in buffer produced a color change from clear to blue. The reaction was stopped after 10 min by adding 50 μl 2N sulfuric acid, which also changed the color from blue to yellow, and light absorption was measured at 450 nm.

The standard curve consisted of a dilution series from 0.01 to 0.0008 mg/ml^{-1} of chicken IgG, and a duplicate of the curve was added to rows 2 and 11 of each of the 27 plates in the assay. We plotted the log-linear relationship for the standard curve and calculated the least-squares best fit (r ranged from .75 to .97). Rows 1 and 12 and the remaining outer edge of the plates were filled with buffer, both as blanks and to provide a more constant environment for the inner wells on the plate. Plasma samples were diluted by a factor of 1:1000, 1:5000, and 1:50,000, and 100 μl of the solution was added in duplicate in one of the remaining rows. We plotted a least-squares best fit log-linear curve for the plasma samples and calculated light absorption at dilutions of 1:1000 or 1:5000. For most samples, we used the 1:1000 dilution, and we used the 1:5000 when necessary. We calculated IgG concentrations after comparing absorption readings with the standard curve and accounting for the dilution of the sample. Before beginning the assay, we made a pool of plasma and included a subsample on each plate.

Cell-mediated immunity
We evaluated cell-mediated immunity, a generalized short-term response to grafts, allergens, and wounds, using a delayed cutaneous hypersensitivity response (Benjamini and Leskowitz, 1991; Roitt et al.; 1989; Saino et al., 1997; Sorci et al., 1997). This response is a measure of T-cell reactivity and is assessed by subcutaneously injecting an inert protein and measuring the swelling that occurs within 24 h (Benjamini and
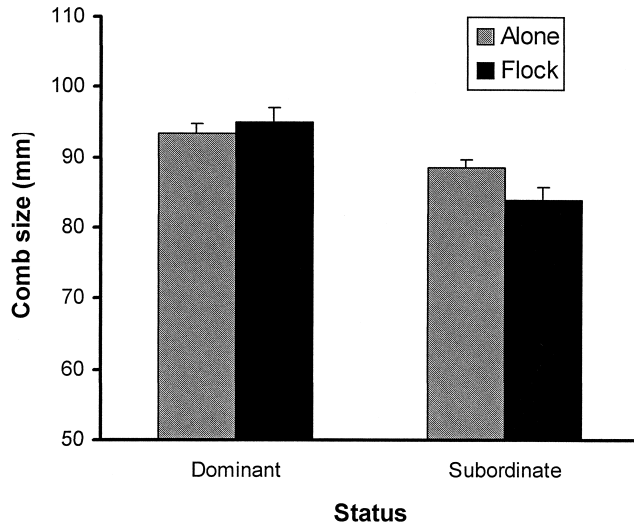
Figure 1
Comb length in dominant and subordinate male red jungle fowl before and after males were placed in flocks of two males and three females.

Leskowitz, 1991). The immune system is thus stimulated without any accompanying pathology. Our procedure is the same as that used in Zuk and Johnsen (1998) and is adapted from Parmentier et al. (1993, 1994). The birds were given a sensitizing injection of 400 µg PHA, suspended in 0.4 ml of PBS, administered subcutaneously into the abdominal region. The thickness of the wing web, a thin layer of skin between the radius and humerus, was measured to the nearest 0.01 mm using digital calipers after placing a metal disc 1 mm thick and 18 mm in diameter on both sides of the extended wing to standardize the measurements. For all measurements, we measured the wing web three times and used the mean in subsequent analyses; as in a study by Sorci et al. (1997), the measures were highly repeatable (Zuk and Johnsen, 1998). A week after the sensitizing injection, the wing web was measured and the birds were injected at the wing web with 0.1 ml of the same solution, and the wing web measurements were repeated 6 and 24 h after this injection. Larger localized swelling indicates a more robust immune response.

Data analysis

All data were analyzed using SAS version 6.12 for personal computer. To compare overall immune response in dominant and subordinate birds, we used canonical discriminant analysis. This multivariate technique uses several characters simultaneously to determine if group membership (i.e., whether a bird is dominant or subordinate) can be successfully predicted (Tabachnik and Fidell, 1983).

RESULTS

Ornaments, body size, and dominance status

At male–female pair formation, before the flocks were formed, males that later became dominant had significantly larger combs than males that became subordinate (Figure 1; Student's $t = 2.764$, $n = 37$ in each group, $p = .007$). The mean difference between birds that eventually were paired was 4.97 mm, with a range of -30.63 to 27.66 mm. At 17 days after flock formation, this difference was even more pronounced (Figure 1; $t = 4.351$, $n = 27$ in each group, $p = .0001$). The mean difference between dominant and subor-
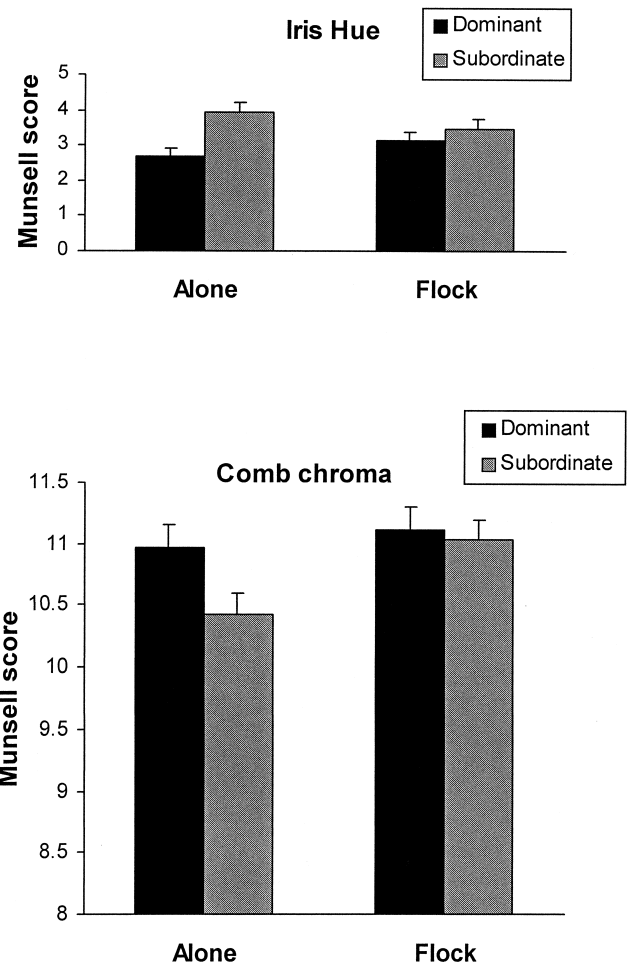

Figure 2
Comb and iris color in dominant and subordinate male red jungle fowl before and after males were placed in flocks of two males and three females. Note that Munsell scores have lower magnitude for redder hues and higher magnitude for higher chroma or saturation.

dinate birds was 5.75 mm; in 24 of 36 pairs, the dominant bird had the larger comb. In addition, comb size increased significantly in dominant males after flock formation (Figure 1; paired t-test, $T = 2.19$, $p = .0375$), whereas the combs of subordinate males became significantly smaller (Figure 1; paired t-test, $T = -2.17$, $p = .0394$).

Iris color and comb color were both duller in subordinate birds before flocks were formed, but not after (Figure 2; iris hue before flocks: Student's $t = -4.351$, $n = 37$ in each group, $p = .001$; iris hue after flocks: $t = -0.669$, $n = 27$ in each group, $p = .50$; comb chroma before flocks: $t = 2.357$, $n = 37$ in each group, $p = .02$; comb chroma after flocks: $t = 0.250$, $n = 27$ in each group, $p = .80$). Note that Munsell scores have lower magnitude for redder hues and higher magnitude for higher chroma or saturation.

Neither tarsus length nor mass differed in dominant versus subordinate males before flock formation (tarsus: Student's $t = 1.321$, $n = 27$ in each group, $p = .19$; mass: $t = 0.927$, $n = 27$ in each group, $p = .36$). Tarsus was not measured again after flock formation, and mass was still not significantly different in the two groups ($t = 0.85$, $n = 27$ in each group, $p = .39$). Both groups, however, lost a significant amount of weight after they were put in flocks (dominant males: paired t-test, $T = -4.53$, $p = .0002$; subordinate males: paired t-test,

Table 1
Results of repeated-measures ANOVA examining the effect of time of measurement and dominance status on immune system measures in male red jungle fowl

Source	df	Type III SS	MS	F	p
Hematocrit					
Status	1	290.29	290.29	4.09	.048
Error	51	3617.93	70.94		
Time	5	159.69	31.94	3.26	.007
Time × status	5	48.57	9.71	0.99	.423
Immunoglobulin G					
Status	1	716.27	716.27	6.23	.016
Error	51	5866.55	115.03		
Time	5	1085.47	217.09	7.10	.001
Time × status	5	67.00	13.40	0.44	.821
Hemagglutination score					
Status	1	1.09	1.09	0.31	.58
Error	43	152.06	3.54		
Time	5	86.44	17.28	39.05	.0001
Time × status	5	4.05	0.81	1.83	.11

SS, sums of squares; MS, mean square.

$T = -4.735$, $p = .0001$). Neither group lost more weight than the other (Student's $t = 1.035$, $n = 20$ in each group, $p = .31$).

Immune response and dominance status

We evaluated immune response in the context of dominance in two ways. First, we compared dominant and subordinate males using a canonical discriminant analysis to see if a combination of all immune system measures could be used to distinguish between the two groups. When IgG levels, hemagglutination scores, wing web swelling, and hematocrit from the blood samples taken just before flock formation were compared in the two classes of males, dominant males had slightly stronger immune responses, but the discrimination was not quite significant (Wilks' λ = 0.878, canonical correlation coefficient = .35, $n = 36$ dominant and 31 subordinate individuals, $p = .08$). At day 17 after flocks were formed, however, the two groups were statistically distinct, with dominant males showing substantially higher scores in each of the measures (Wilks' λ = 0.795, canonical correlation coefficient = .45, $n = 27$ dominant and 27 subordinate individuals, $p = .02$).

We also examined changes in the immune system measures over time by performing a repeated-measures ANOVA on IgG levels, hemagglutination scores, and hematocrit using dominance status as the class variable. Because wing web swelling was only measured once before and once after flock formation, we compared this response in the two groups of males using t tests. The effect of status on both IgG and hematocrit was significant, while dominant and subordinate males did not differ in hemagglutination score (Table 1; Figure 3). The ANOVA also revealed a significant effect of time of measurement, so that all males increased their responses during the experiment, as is expected when their immune system is sensitized by previous exposure to the antigen. No significant time × status interaction effect was seen, however, suggesting that the dominant males' more robust responses were present before the multi-male groups were formed.

Wing web swelling size was similar in dominant and subordinate males before they were put in the multi-male flocks (Figure 4). After flock formation, however, dominant males had significantly larger swellings at 24 h after injection than did subordinate males (Figure 4; Student's $t = 2.276$, $n = 27$ in each group, $p = .027$).

Comb size and immune response

Our previous study showed that males with larger combs also had more robust cell-mediated immunity, as indicated by larger wing web swellings (Zuk and Johnsen, 1998). Here, dominant and subordinate males differed in the relationship between comb length and wing web swelling (Figure 5). Before flock formation, comb length was unrelated to web swelling when all birds were pooled ($F_{1,78} = 2.174$, $r = .09$, $p = .144$). Among dominant males, individuals with larger combs again had significantly larger swellings after flock formation ($r = .48$, $n = 26$, $p = .01$). Subordinate males, however, showed the opposite effect, with a significantly negative relationship ($r = -.47$, $n = 26$, $p = .01$), so that subordinate males with relatively larger combs had worse cell-mediated immunity than those with smaller combs. If the single subordinate individual with the smallest comb is removed from the analysis, the relationship between comb size and swelling is no longer significant for subordinate males but is still different from that apparent in the dominant males.

DISCUSSION

Our results suggest that males of different quality pay different costs to maintain both ornamentation and immune defense. At least in the short term, male red jungle fowl show a "rich get richer" response, so that males with large combs became dominant and even increased their comb size, as well as having more robust immune responses. The disparity between males that achieved dominance and those that became subordinate was only exacerbated by being placed in a presumably more stressful social environment.

This study also provides an example of the car-house paradox, a phenomenon well-described in life-history theory (Roff, 1992; van Noordwijk and de Jong, 1986; Zuk et al., 1996). Although limited funds available for any individual would suggest that funds put into a house would restrict the amount put into a car, the expected negative trade-off does not appear when examining the population as a whole, and people with large houses also tend to drive expensive cars. The paradox occurs because of the differing amounts of resources individuals start out with; people who are wealthy to begin with have more money to spend on everything. Similarly, although allocation of resources to immune defense may
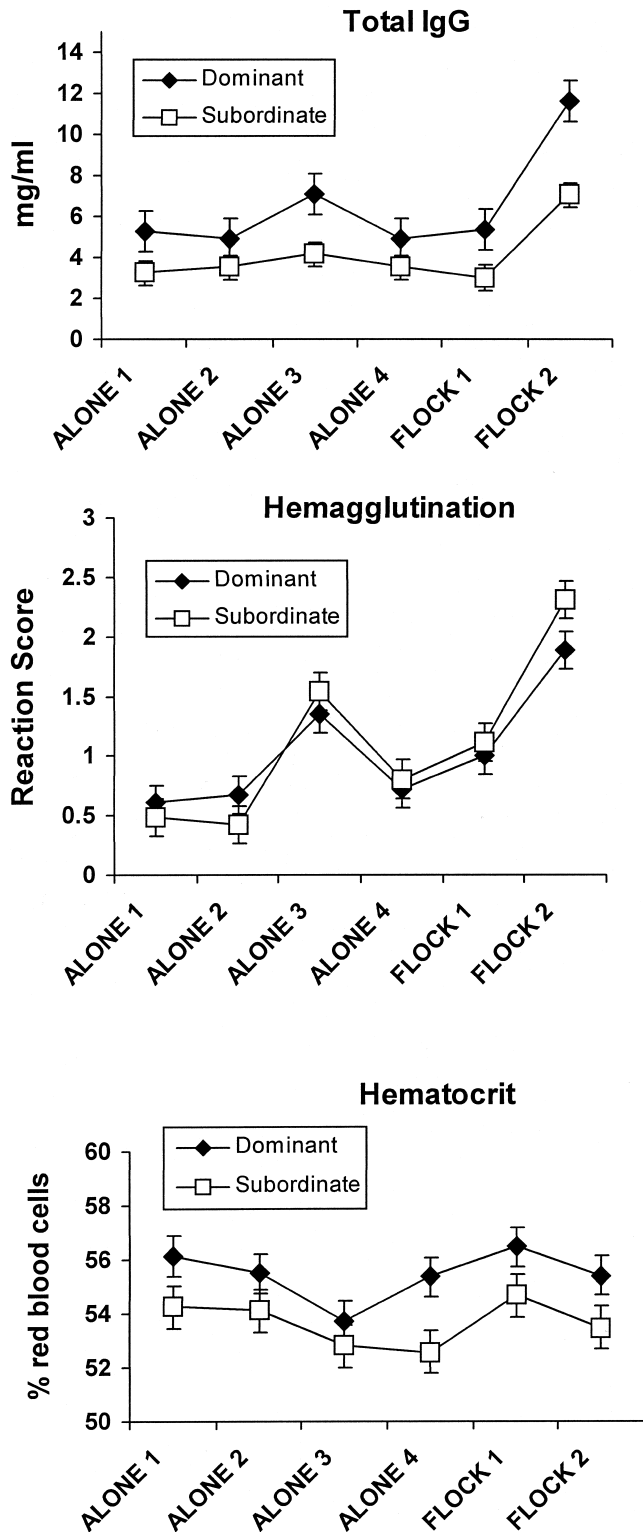
Total IgG

Hemagglutination

Hematocrit

Figure 3
Changes in hematocrit, immunoglobulin G levels, and hemagglutination responses in dominant and subordinate male red jungle fowl before and after males were placed in flocks of two males and three females. See text for details of timing of measurements.
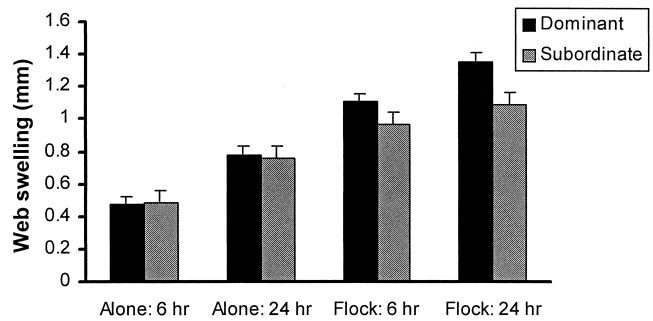

Figure 4
Wing web swelling size in dominant and subordinate male red jungle fowl before and after males were placed in flocks of two males and three females.

come at the expense of investment in ornamentation, this trade-off is not apparent because individuals are of different quality (Qvarnström and Forsgren, 1998).

Support for such a trade-off comes from Verhulst et al.'s (1999) study of artificially selected lines in domestic chickens. Lines selected for high antibody responsiveness showed a correlated decrease in comb length, while lines selected for low responsiveness had large combs (Verhulst et al., 1999). Presumably, the lines are obtained by selecting individuals already at the extremes of the distribution of both comb size and immune response, which allows the trade-off to be exhibited because the selected population is a subgroup of the variation originally present. The males selected for their particularly robust immunity therefore consisted of a subgroup that exhibited the negative correlation between comb length and responsiveness. Our birds were under no such constraints and hence were able to show both dominance and immune competence. This natural spread in variation in quality among individuals may mean that in nature, trade-offs are unlikely to result in selection for either extreme ornamentation or extreme immune competence, but instead may help maintain differences in resources available to individuals within the population.

What caused the initial differences among males? Unlike the situation in field studies, males in our experiment were the same age, had the same rearing environment, and were housed in the same circumstances, making it unlikely that individuals were in very different conditions at the start of the breeding season. It would be interesting to determine if genetic differences were at least partially responsible for both the eventual dominance status and immune response of the jungle fowl. The relatively rapid response of the domestic chickens in Verhulst et al.'s (1999) study suggests that antibody responsiveness, at least, is highly heritable.

It is also possible that the dominant males pay their costs at a different time of year, perhaps after the breeding season is over. Seasonal effects on immune response, social status, and the relationship between ornamentation and both of these measures, are well known (Kotrschal et al, 1998; Nelson and Demas, 1996; Zuk and Johnsen, 1998). Because jungle fowl live in year-round flocks in nature, however, it seems unlikely that dominant birds simply collapse at the end of the breeding season; the birds appear to maintain most aspects of their social system even when chicks are not being produced (Collias and Collias, 1967; Collias et al., 1966).

Although immune defense is frequently presumed to be costly (Deerenberg et al., 1997; Sheldon and Verhulst, 1996; Wedekind and Folstad, 1994), relatively little evidence exists to support this assumption. Demas et al. (1997) found that when laboratory mice were immunized with keyhole limpet
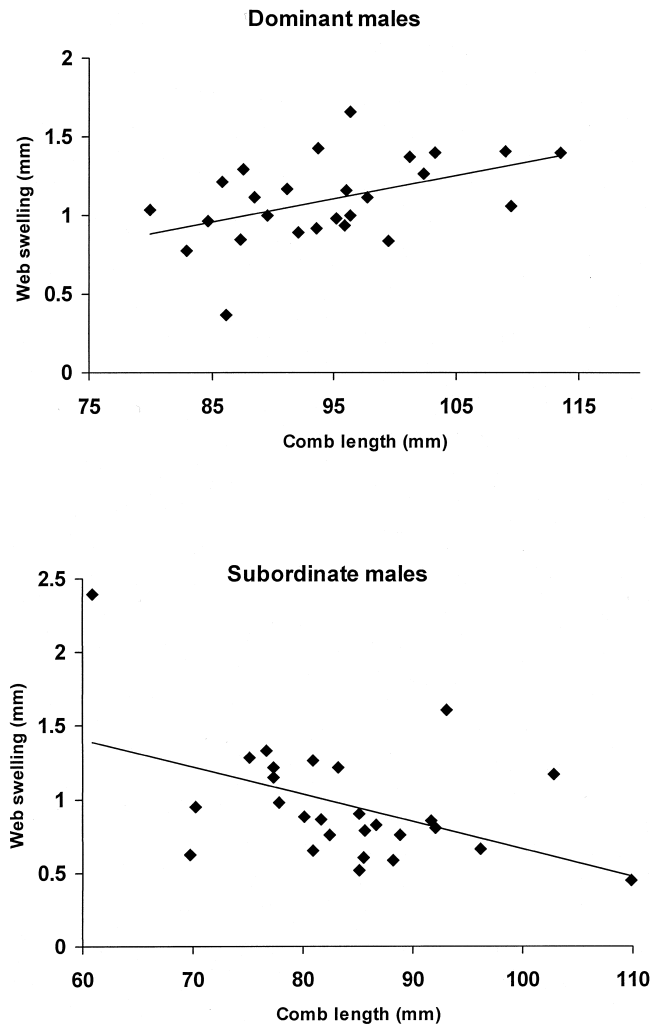
Dominant males

Subordinate males

Figure 5
Relationship between comb length and wing web swelling size in dominant and subordinate male red jungle fowl after males were placed in flocks of two males and three females.

antigen, they had significantly higher metabolic rates, a difference not simply due to a rise in body temperature, and which seems to reflect the actual cost of producing antibodies. Svensson et al. (1998) suggested that any trade-off between immune defense and energetically costly activities may not necessarily be based on energy or nutrient limitation but instead may occur either through the adaptive avoidance of autoimmunity or via the damaging effects of free oxygen radical production. Results such as ours support the idea of costly immune competence, but until the mechanisms are better understood, the exact nature of the trade-off and how it is executed by individuals of different quality remains unclear.

Perhaps somewhat more clear is the cost of maintaining sexual ornaments such as the comb. The finding that dominant males have larger combs than subordinates is consistent with previous studies (Ligon et al., 1990; Zuk et al., 1990). Furthermore, the significant increases in comb size by dominant males and decrease in comb size of subordinate males after flock formation suggests that the comb is a powerful social signal with an accompanying social cost; it seems unlikely that subordinate males gained any physiological, rather than social, benefit by reducing their comb size. The maintenance of a few millimeters more or less of soft tissue is unlikely to significantly alter the energy budget of an individual.

Instead, males with larger combs may be challenged more by other males, increasing the risk of injury during fights (Ligon et al., 1990; Zuk and Johnsen, 1998). Therefore, the shrinking of combs in subordinate individuals could have reduced the likelihood of social interactions with the accompanying risk of wounding.

Additional support for the idea that males of different quality pay different costs for ornaments comes from the finding that only dominant males showed a positive relationship between comb length and wing web swelling; for subordinate males, in contrast, having a large comb was costly in terms of immune defense (Figure 5). The failure of dominant males to maintain their brighter comb and eye colors after the birds were placed in the multi-male flocks suggests that these ornaments are secondary to comb length in importance in sexual selection, a conclusion supported by these traits being less important to females in mate choice experiments (Zuk et al., 1990). Apparently all ornaments cannot be maintained at their peak during social competition.

In our experiment, dominant males had better immune defense than subordinate males, a finding somewhat different from those of Barnard et al. (1993, 1998), who suggested that high-ranking, aggressive laboratory mice modulate their immune responses differently from low rankers, but that the dominant individuals are not uniformly better or worse. Several other studies suggest that subordinate individuals have suppressed immunity, probably mediated through stress-related hormonal changes (Blanchard et al., 1993; Sapolsky et al., 1997; Stefanski and Engler, 1998; Tuchscherer et al., 1998). The timing and stability of a social situation can also influence the degree of stress and stress-related responses (Fox et al., 1997). The generally more robust immune responses of the dominant jungle fowl males even before the flocks were formed and the lack of a significant time × status interaction effect on immune responses in the repeated-measures ANOVA argues against the lower immunity of subordinates being an artifact of a social situation in which they were unable to escape the aggression of dominant individuals. The differences among studies may reflect the degree of variation in initial quality of individuals; where fewer high-quality males in an absolute sense are present, even dominant individuals may not be able to maintain both immune defense and high levels of aggression or ornamentation.

Social dominance has many repercussions. Moore et al. (1997) point out that such interacting phenotypes, or traits that exist exclusively as a product of interactions, have unique genetic effects, both through direct and indirect contributions to the trait itself. They suggest further that the relative rate of evolution in interacting phenotypes may be different from that of more "standard" characters such as morphology (Moore et al., 1997). In the situation described here, one can envision not one but two sets of interacting phenotypes occurring together: the social status that results from interactions with conspecifics and the immune system response that results from interactions with pathogens. Rapid evolution has been proposed for traits expressed in social situations, such as those associated with courtship (West-Eberhard, 1983), as well as for traits associated with immune defense (Ebert, 1998). Whether traits such as comb size in jungle fowl, which are linked to both, show even more exaggerated responses to selection, or whether the need for coevolution with pathogens is antagonistic toward the evolution of ornamentation, remains to be seen.

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