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PARASITES, MORPHOLOGY, AND BLOOD CHARACTERS IN MALE RED JUNGLE FOWL DURING DEVELOPMENT[1]

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Abstract. Parasites have significant effects on the fitness of their hosts. We used an intestinal nematode, *Ascaridia galli,* as an experimental treatment of young male Red Jungle Fowl (*Gallus gallus*) to investigate the effect of this parasite on their growth and development through sexual maturity. After treatment, 26% of those fed parasites and 31% of controls became infected. Infected males had lower body mass, lower hematocrit, a higher percentage of lymphocytes among their white blood cells, and smaller combs than uninfected males. Infected and uninfected males did not differ in plasma levels of testosterone (T). The differences between parasitized and unparasitized males persisted from 5 months of age through sexual maturity. The classic example of hormonal action is the effect of testosterone on the comb of male chickens, but the difference in comb length between parasitized and unparasitized male Red Jungle Fowl along with no difference in T suggests that the effect of parasites on male secondary sexual characters does not necessarily involve a change in circulating levels of testosterone.

Key words: Ascaridia galli, *hematocrit, immunocompetence, ornaments, Red Jungle Fowl, testosterone.*

Recently, the importance of parasites in the evolution of sexually selected traits has been recognized. Hamilton and Zuk (1982) proposed that male ornaments signal parasite load, and that females benefit in choosing parasite free males if resistance to prevalent parasites is passed on to their offspring. Folstad and Karter (1992) proposed the immunocompetence handicap hypothesis to explain how male secondary sexual characters are linked to a compromised immune response. In vertebrates, males usually have a higher parasite load and a higher mortality rate than females, and their immune system is weaker (Zuk and McKean 1996). Castrated males have a stronger immune response than intact males, and testosterone treatment of castrates eliminates this difference (Grossman 1984). Folstad and Karter (1992) suggested that testosterone stimulates male ornamental displays and is a handicap because it weakens the immune system. In this hypothesis, high circulating levels of testosterone (T) in males increases their susceptibility to parasites, and males that are not resistant to prevalent parasites pay a disproportionately high cost of compromising their immune system. Females benefit because, by mating with these males, resistance is passed on to their offspring. This hypothesis predicts that males infected with parasites have smaller ornaments than parasite free males, and that the effect of parasites on the male's ornaments results from changes in T.

Red Jungle Fowl (*Gallus gallus*), the wild ancestor of the domestic chicken, are native to southeast Asia, and in nature, live in flocks of one or more males and several females (Collias and Collias 1967). At the San Diego Zoo in California, the social system of free ranging flocks is similar to that of wild Red Jungle Fowl (Collias and Collias 1996). These free ranging flocks have one dominant male that gains most of the copulations with receptive females, but subordinate males also have access to females. Aggressive interactions among males are common, and eventually the dominant male loses his status to a challenge by one of his subordinates. In staged mate choice trials, females prefer to mate with males that have larger redder combs (Zuk et al. 1990). Male comb size depends upon T (Zuk et al 1995), and the size and color of the comb are condition dependent and both change rapidly when males are sick or become infected by parasites (Zuk et al. 1990). *Ascaridia galli* is an intestinal nematode that infects domestic chickens and Red Jungle Fowl. It has a direct life cycle (Ruff 1978). Eggs are present in the feces of infected birds, and the ingestion of eggs or developing larvae can lead to infection. Susceptibility to this parasite is age dependent. Chicks are easily infected by the parasite, whereas at approximately 3 months of age, chickens develop resistance to *A. galli* (Tongson and McCraw 1967, Ruff 1978). In earlier studies, *A. galli* has been shown to affect male morphology, including comb size, and infected males are less attractive to females than males that are free of this parasite (Zuk et al. 1990).

In this study, we investigate changes in male Red Jungle Fowl morphology, hematocrit, lymphocytes, and T between 5 and 8 months of age, and we compare males infected with *A. galli* with males that are free of this parasite. We also test the prediction of the immunocompetence handicap hypothesis that the smaller ornaments in parasitized males is caused by a decrease in testosterone.

METHODS

We worked with a captive population of Red Jungle Fowl that originated from the free-ranging birds at the San Diego Zoo in California (Zuk et al. 1995). Beginning when the chicks were 1 week old, half the males

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and females were fed 50–100 developing *A. galli* in 0.5 ml saline in each of four weekly treatments. Young were hatched in incubators and then kept in commercial brooders for 6 weeks, and while they were in the brooders, we kept parasite treated and control birds separate. At 6 weeks of age, we moved the birds outdoors and kept them in mixed-sex flocks of 10–20 individuals, and at this time, parasite treated and control birds were housed together. When males began fighting with each other at approximately 4 months of age, we separated the birds into male-female pairs and housed them in individual cages. When the males were 15 months old, we killed them and examined their gut for parasites.

We used digital calipers to measure the length of the comb and the tarsus to the nearest 0.1 mm, and we used a digital scale to weigh the birds to the nearest 1.0 g. The males hatched over a period of slightly more than 1 month, and with monthly measurements, we organized our data to obtain three sets of measurements. In our first set of measurements, all males were 5 months old. In the second set, approximately half the males were measured once when they were 6 months old and the other half were measured both when they were 6 and 7 months old (the mean of these two measurements was used in the analysis). In the third set of measurements, half the males were 7 and half were 8 months old. Although infection by *A. galli* was determined up to 7 months after the end of the experiment, the increased resistance to infection that chickens acquire by 3 months of age makes it unlikely that males became infected during or after the completion of the experiment (Ruff 1978).

When the males were 4 months old, we gave half the males in the parasite treatment group and half the controls subcutaneous implants of testosterone. Testosterone treated males were given three 4-cm-long implants of silastic tubing (3.2 mm outer and 1.6 mm inner diameter) packed with testosterone and sealed with silicone adhesive A; sealed empty tubing was used as a control.

We took blood samples by inserting a needle into the alar vein in the wing and drawing blood into a vacutainer containing lithium heparin. We kept the blood on ice for up to 3 hr before processing. We prepared blood smears, fixed and stained them, and counted white blood cells (Zuk et al. 1995). Each slide was read by two people and they identified the percentage of lymphocytes among the first 100 white blood cells; we used the mean of the two counts of lymphocytes in our analysis. We measured hematocrit by drawing blood into a capillary tube, spinning it in a centrifuge at 2,000 rpm for 5 min, and comparing the length of the hematocrit column to the total column of blood. For hormone assays, we separated the plasma from the hematocrit using a centrifuge and stored the plasma at −20°C. We measured T using radioimmunoassays from Diagnostic Products (Zuk et al. 1995). We did four assays, one for samples from each age group of males. The standard curve ranged from 0.2 to 16 ng ml^{-1}, and each assay included a standard tube containing 4.0 ng ml^{-1}. The mean value for all four standard tubes was 3.8 ng ml^{-1}, and the interassay coefficient of variation was 8.2%.

Both hematocrit and lymphocytes were measured as percentages and were square root arcsine transformed for analysis. We used repeated measures ANOVA to analyze changes in male morphology, T, and blood cells for our three sets of measurements during development. At each age, we used discriminant function analysis to compare the males that were found to have *A. galli* in their intestine with uninfected males, using mass, tarsus length, comb length, T, hematocrit, and lymphocytes as the independent variables. In the data set when the males were 6–7 months old, we also used *t*-tests to contrast infected and uninfected males; for six tests the adjusted alpha level was 0.0085. In addition, we used this data set to calculate the correlation coefficient between T and comb length.

RESULTS

Of the 34 males that had been fed *A. galli* as chicks, 26% were infected with the parasite, and 31% of the 35 control males had *A. galli* in their intestine, probably acquired from pecking at contaminated feces. When the males were 5 months old, both parasite and testosterone treatment had an effect on their morphology. Using discriminant analysis, parasite treated and control males differed (Wilks' Lambda = 0.78, $P < 0.05$), and parasite treatment was associated with greater mass ($t_{66} = 2.24$, $P < 0.05$). Similarly, testosterone treated and control males differed (Wilks' Lambda = 0.69, $P < 0.005$), and testosterone implants caused increased T ($t_{67} = 3.04$, $P < 0.005$), increased comb length ($t_{66} = 2.89$, $P < 0.01$), and increased hematocrit ($t_{57} = 3.61$, $P < 0.001$) relative to controls. Neither parasite treatment nor testosterone treatment affected the males after they were 6 months old. The effect of testosterone treatment was transitory and we do not discuss these data further. Greater mass in parasite treated males also was transitory, and the effect does not remain significant when we correct for multiple tests.

During development, males changed significantly in almost all characters (Table 1). Mass increased from a mean of approximately 950 g at 5 months of age to 1,200 g at the beginning of their first breeding season ($F_{2,234} = 30.7$, $P < 0.001$), although tarsus length did not change during this period ($F_{2,234} = 0.1$, $P = 0.88$). Comb length increased in males from a mean of 54 mm to more than 80 mm ($F_{2,234} = 173.1$, $P < 0.001$). T increased from approximately 0.2 ng ml^{-1} (not detectable in our assay) to 1 ng ml^{-1} at the beginning of the breeding season ($F_{2,231} = 36.5$, $P < 0.001$). Hematocrit increased ($F_{2,204} = 90.6$, $P < 0.001$) and the percentage of lymphocytes among white blood cells decreased ($F_{2,231} = 9.5$, $P < 0.001$).

At all three stages of development, the 20 males later found to have *A. galli* in their intestine differed in both morphology and blood characters from the 50 males that were free of the parasite (Table 1). Using six variables (mass, tarsus length, comb length, T, hematocrit, and lymphocytes), we could statistically distinguish between these two populations of males at 5 months, 6–7 months, and 7–8 months of age (Wilks' Lambda = 0.76, $F_{6,58} = 3.1$, $P = 0.01$; Wilks' Lambda = 0.60, $F_{6,59} = 6.66$, $P < 0.001$; Wilks' Lambda = 0.76, $F_{6,58} = 3.1$, $P = 0.01$, respectively). Most, but

TABLE 1. The effect of the presence of *A. galli* in the male's intestine on male morphology and blood characters (mean ± SE) as they grow to sexually mature adults. Parasitized males (P, $n = 20$) had this parasite present in their gut, while parasite free males (C, $n = 50$) did not. * indicates a statistically significant difference in post-hoc tests at all three male ages.

	Parasite status	Male age		
		5 months	6–7 months	7–8 months
Mass (g)*	P	940.0 ± 34.8	1,104.2 ± 39.3	1,143.5 ± 35.8
	C	1,071.5 ± 23.5	1,227.6 ± 22.3	1,274.1 ± 23.7
Tarsus (mm)	P	84.0 ± 1.2	84.4 ± 1.2	84.1 ± 1.2
	C	87.4 ± 0.8	87.6 ± 0.7	87.3 ± 0.7
Comb length (mm)*	P	47.0 ± 1.9	66.9 ± 2.9	80.7 ± 2.1
	C	57.3 ± 1.6	77.2 ± 1.4	88.4 ± 1.2
Testosterone (ng ml^{-1})	P	0.21 ± 0.05	0.79 ± 0.13	1.11 ± 0.15
	C	0.30 ± 0.05	0.76 ± 0.08	1.05 ± 0.11
Lymphocytes (%)*[a]	P	1.15 ± 0.03	1.12 ± 0.02	1.10 ± 0.02
	C	1.06 ± 0.02	1.04 ± 0.01	0.97 ± 0.02
Hematocrit (%)*[a]	P	0.69 ± 0.01	0.75 ± 0.01	0.81 ± 0.01
	C	0.73 ± 0.01	0.79 ± 0.01	0.84 ± 0.01

[a] Square-root arcsine transformed.

not all, of the male traits differed between parasitized and unparasitized males. When the males were 6–7 months old, parasitized males had lower body mass ($t_{68} = 2.86$, $P < 0.005$), smaller combs ($t_{68} = 3.69$, $P < 0.001$), lower hematocrit ($t_{65} = 3.0$, $P = 0.005$), and a higher percentage of lymphocytes ($t_{66} = 3.13$, $P = 0.005$) than unparasitized males, and there was a trend for infected males to have shorter tarsae ($t_{68} = 2.4$, $P < 0.02$, $\alpha = 0.0085$). Parasitized and unparasitized males did not differ in T ($t_{65} = 0.21$, $P > 0.5$). In addition, T and comb size in males were positively correlated ($r = 0.53$, $n = 66$, $P < 0.001$).

DISCUSSION

In Red Jungle Fowl, experimental treatment with *A. galli* causes males to have smaller combs, and at some ages, lower body mass and shorter tarsus (Zuk et al. 1990). Between the ages of 5 and 8 months, male Red Jungle Fowl grow and develop into sexually mature adults. Looking at actual infection with the parasite as opposed to parasite treatment, males with *A. galli* in their intestine differ from those free of this parasite in their morphology and blood characters, but not in T. One interpretation is that the parasite causes male Red Jungle Fowl to have less hematocrit and more lymphocytes in addition to the previously reported effects on male morphology. On the other hand, all our birds may have been exposed to the parasite, and some either did not become infected or were able to shed the parasite. Our results also could be explained if males in poor condition were more likely to become infected when exposed to the parasite. Although we cannot distinguish between these explanations, earlier results showing an experimental effect of *A. galli* treatment on mass and comb length (Zuk et al. 1990), suggest that, at least in part, the presence of the parasite caused males to have lower body mass, a smaller comb, a higher percentage of lymphocytes, and lower hematocrit.

At 6–7 months of age, parasitized males had a higher percentage of lymphocytes among their white blood cells, presumably indicating an immune response to *A. galli*. The changes in the immune system associated with the parasite did not vary over time, suggesting a continued immune response to the presence of the parasites. The alternative, that males with a higher percentage of lymphocytes are more susceptible to infection, is unlikely because the proliferation of lymphocytes in male domestic chickens makes them more resistant to other parasites such as *Eimeria maxima* and *E. tenella* (Bumstead et al. 1995). Most studies of the effects of parasites on the fitness of birds use either nestlings or adults (Lehmann 1993). In Red Jungle Fowl, the differences between infected and uninfected males persists from when they are young until they are sexually mature, and, as in an earlier study (Zuk et al. 1990), the infection with an intestinal nematode in young can have far-reaching effects on the fitness of infected males that survive to become adults.

A smaller comb in males infected with *A. galli* supports predictions of the immunocompetence handicap hypothesis (Folstad and Karter 1992), but this hypothesis also predicts that the effect of parasites on an ornament is directly mediated through testosterone. Parasitized male rats and sheep have lower T than uninfected males (Lin et al. 1990, Mutayoba et al 1994), but we found no difference in T between infected and uninfected male Red Jungle Fowl. In male Red Jungle Fowl, comb size is positively correlated with T, but the parasite effect on comb size is not mediated through changes in circulating levels of testosterone. Our results do not support this prediction of the immunocompetence handicap hypothesis.

The effect of the parasite could be mediated through changes in the metabolism of testosterone in the comb, or comb size could be affected through a separate mechanism. In the comb, dihydrotestosterone is the active hormone and circulating testosterone is metabolized by the enzyme 5α-reductase before it affects the comb (Dube et al. 1975). If the parasite causes a change in enzyme activity or a down-regulation of receptors in the comb, comb size could differ between

parasitized and unparasitized males with no difference in T. On the other hand, increased corticosterone levels are associated with the activation of the immune response (Bateman et al. 1989). If corticosterone affects comb size, we may be able to explain the difference in comb size between parasitized and unparasitized male Red Jungle Fowl.

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LITERATURE CITED

BATEMAN, A., A. SINGH, T. KRAL, AND S. SOLOMON. 1989. The immuno-hypothalamic-pituitary axis. Endocrine Rev. 10:92–112.

BUMSTEAD, J. M., N. BUMSTEAD, L. ROTHWELL, AND F. M. TOMLEY. 1995. Comparison of immune response in inbred lines of chickens to *Eimeria maxima* and *E. tenella*. Parasitology 111:143–151.

COLLIAS, N. E., AND E. C. COLLIAS. 1967. A field study of the Red Jungle Fowl in north-central India. Condor 69:360–386.

COLLIAS, N. E., AND E. C. COLLIAS. 1996. Social organization of a Red Jungle Fowl, *Gallus gallus*, population related to evolutionary theory. Anim. Behav. 51:1337–1354.

DUBE, J. Y., R. R. TREMBLAY, R. LESAGE, AND G. VERRET. 1975. *In vivo* uptake and metabolism of testosterone by the head appendages of the cock. Mol. Cell. Endo. 2:213–220.

FOLSTAD, I., AND A. J. KARTER. 1992. Parasites, bright males, and the immunocompetence handicap. Am. Nat. 139:603–622.

GROSSMAN, C. J. 1984. Regulation of the immune system by sex steroids. Endocrine Rev. 5:435–455.

HAMILTON, W. D., AND M. ZUK. 1982. Heritable true fitness and bright birds: a role for parasites? Science 218:384–387.

LEHMANN, T. 1993. Ectoparasites: direct impact on host fitness. Parasitol. Today 9:8–13.

LIN, Y. C., Y. RIKIHISA, AND Y. GU. 1990. Effects of larval tapeworm (*Taenia taeniaeformis*) infection on reproductive functions in male and female host rats. Exp. Parasitol. 70:344–352.

MUTAYOBA, B. M., P. D. ECKERSALL, I. A. JEFFCOATE, V. CESTNIK, AND P. H. HOLMES. 1994. Effects of *Trypanosoma congolense* infection in rams on the pulsatile secretion of LH and testosterone and responses to injection of GnRH. J. Reprod. Fert. 102:425–431.

RUFF, M. D. 1978. Nematodes and Acanthocephalans, p. 705–736. *In* M. S. Hofstad [ed.], Diseases of poultry. Iowa State Univ. Press, Ames, IA.

TONGSON, M. S., AND B. M. MCCRAW. 1967. Experimental ascaridiasis: influence of chicken age and infective egg dose on structure of *Ascaridia galli* populations. Exp. Parasitol. 21:160–172.

ZUK, M., T. S. JOHNSEN, AND T. MACLARTY. 1995. Endocrine-immune interactions, ornaments and mate choice in Red Jungle Fowl. Proc. R. Soc. Lond. B 260:205–210.

ZUK, M., AND K. A. MCKEAN. 1996. Sex differences in parasite infections: patterns and processes. Int. J. Parasitol. 26:1009–1024.

ZUK, M., R. THORNHILL, J. D. LIGON, AND K. JOHNSON. 1990. Parasites and mate choice in Red Jungle Fowl. Am. Zool. 30:235–244.