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PHYSIOLOGICAL TRADE-OFFS BETWEEN IMMUNITY AND REPRODUCTION IN THE NORTHERN CRICKET FROG (*ACRIS CREPITANS*)

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Abstract: Investigations of natural history trade-offs between reproduction and immunity are common throughout the literature. Most previous studies of such trade-offs have focused on how resources can be drawn from immune response to fuel reproduction. Our results demonstrate that resources also can be shifted from reproduction to immunity. Immunologically-challenged male northern cricket frogs (*Acris crepitans*) expressed reduced investment in reproduction. Spermatic cyst diameter, germinal epithelium depth, and gonadosomatic index were smaller in antigen-injected males relative to those injected with a sham (saline injected) and noninjected control animals. Although body size increased in all groups during this study, linear growth and body mass did not appear to be significantly different among these three treatment groups. These results demonstrate indirectly that in *A. crepitans* immune response may increase metabolic demand for resources and fuel that need from the stores normally used to support male reproduction. We speculate that anything eliciting an immune response in this species may reduce male fertility, so pathogens and toxins at levels that are currently believed to be relatively harmless may impact populations in ways we could not previously predict.

Key words: *Acris crepitans*; Immunology; Reproduction; Spermatogenesis; Trade-offs

Trade-off theory (Maynard Smith, 1974) predicts that each individual has a limited resource base to fuel all necessary physiological processes (Zera and Harshman, 2001). As demands are placed on one physiological system, the other should experience a compromised supply of resources (Zera and Harshman, 2001). Thus, available resources are distributed so that the organism's evolutionary payoff (Maynard Smith, 1974; Maynard Smith and Price, 1973) is maximized (Zera and Harshman, 2001). Numerous investigations have examined potential trade-offs between reproduction and the immune function indirectly by assessing parasite loads or disease status and relating them to mating success (Hamilton and Zuk, 1989; Hausfater et al., 1990; Liljedal et al., 1999; Zuk et al., 1990). Pathogen or parasite loads may be confounded by ecological or physiological conditions exclusive of an individual's immunological capacity (Beasley et al., in press; Carey et al., 1996). Consequently, recent studies have begun directly examining the relationship between immune response and reproduction (McCallum and McCallum, 2006; Norris and Evans, 2000).

Compton and Derting (2002) conducted one of the few studies of trade-offs between reproduction and immune responses of males. They found that both wet and dry mass of the intestines and of the testes were reduced in antigen-exposed mice. Several studies of birds also have found evidence of such trade-offs. Immune response is suppressed during the breeding season in red jungle fowl (*Gallus gallus*, Zuk and Johnsen, 1998). Black-headed gull (*Larus ridibundus*) chicks from eggs with high yolk androgen levels have suppressed immune function (Groothuis et al., 2005). The breeding success of female pied flycathcers (*Ficedula hypoleuca*) declines in response to increased immune activity (Ilmonen et al., 2000). Conversely, the male mealworm beetle (*Tenebrio molitor*) increases its terminal reproductive effort by enhancing the attractiveness of its pheromone signal in response to immune insult (Sadd et al., 2006).

Norris and Evans (2000) proposed that the following three pieces of evidence are required to demonstrate trade-offs between immunocompetence and reproduction: (1) immunocompetence must compete with life-history components for access to limiting

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resources, (2) increasing investment in a particular life-history component must reduce immunocompetence, and (3) a reduction in immunocompetence must cause a reduction in fitness. That latter requirement is the key feature of the Hamilton-Zuk hypothesis (Hamilton and Zuk, 1982), which states that parasites and pathogens challenge an organism's immune system, and the host's ability to resist those stressors is honestly relayed via their investment in reproduction (e.g., Andersson, 1994; Folstad and Karter, 1992; Sheldon and Verhulst, 1996).

Although many studies have approached questions regarding how reproduction draws resources from immune function, little attention has been paid to the reciprocal relationship (Sadd et al., 2006). If immune response draws resources from reproduction, antigen exposure might lead to reductions in various measurements of male reproductive potential, including the length, diameter, and mass of the testes, spermatic cyst diameters, depth of germinal epithelium, and quantities of sperm present (van Tienhoven, 1983; Norris and Jones, 1987). In addition to reproduction, growth might be stunted as resources are shifted to fuel immunity.

The northern cricket frog (*Acris crepitans*) is a small anuran that is common throughout the eastern two thirds of the United States. Their lifespan is typically one year (Burkett, 1969, 1984). Males of this species tend to maintain spermatogenesis continuously throughout the year after reaching the age of about 1–3 mo (McCallum, 2003). Because males invest in reproduction continuously and are not likely to experience more than one breeding season, they provide a suitable model for testing the influence of the immune response on reproductive activity without complex interference from effects associated with age and experience.

This study addresses the hypothesis that creating conditions that would mobilize an immunological response leads to a reciprocal decrease in spermatogenesis. If this hypothesis is true, then we might expect smaller spermatic cyst diameters, germinal epithelium depths, and testis lengths and widths in antigen-exposed males than in either the saline-injected or not injected groups.

Materials and Methods

Male *Acris crepitans* were collected at the peak of their breeding season (May–June; McCallum, 2003) from a pond located in Craighead County, Arkansas. Thirty frogs were used to verify that a dilute solution of whole sheep blood would elicit an IgM response. An additional 36 adult male *A. crepitans* were collected on 1 June 2002 from the same location for use in hypothesis testing.

All frogs were housed in a modification of Courtland's (2002) recirculating aquarium system to imitate the habitat where these frogs commonly breed (McCallum, 2003). Aquaria were constructed from 60 × 90 × 15 cm plastic containers. The lids were clamped to the aquaria and a hole was cut in each lid and sealed with metal window screen to allow airflow but prevent escapes. Water flow was regulated at about 80 L/min. Containers were tilted approximately 20° to provide both aquatic and terrestrial habitats. A 10 cm × 30 cm plastic lid was floated in the water to provide additional terrestrial habitat. The light cycle was 12/12 h light/dark. Air temperature was maintained at 31–32 C during light periods with a 100 W heat lamp and was allowed to fall to 25 C during the dark cycle. Water temperature was held at 21 C at all times. Frogs were fed approximately two crickets each, three times per week.

Washed whole sheep blood is known to elicit immune responses in amphibia (Manning and Turner, 1976) and was used as a novel antigen (Garvey et al., 1977). Use of a novel antigen circumvents the accumulation of toxins and eliminates the possibility of secondary immune responses associated with the use of known pathogens (Garvey et al., 1977). Whole sheep blood was rinsed three times in 0.85% saline. This blood solution was processed and then diluted to 100 ml with 0.85% saline to form a 1% solution of sheep blood cells (approximately 2×10^8 cells/ml) according to the methods outlined in Campbell et al. (1970). Volumetric dilution is considered sufficiently accurate for immunization of animals (Campbell et al., 1970).

To verify that sheep blood cells would elicit a detectible IgM response, a sample of 30 *A. crepitans* were divided into two treatment

groups. Fifteen frogs were each given a single 0.1 ml injection of sheep blood. Three frogs were sacrificed at 6, 12, 24, 36, and 48 h after an injection. An additional 15 frogs were injected with saline and sacrificed in the same manner. Serum was collected via cardiac puncture and pooled for each group. A simple qualitative agglutination reaction (Campbell et al., 1970; Garvey et al., 1977) was run on the pooled samples to determine if an immune response occurred.

To test whether immune response could drain resources from reproduction, each of 36 *A. crepitans* was randomly assigned to one of three treatment groups containing 12 frogs and allowed to acclimate to captivity until 7 June 2002. Each frog was individually weighed to the nearest 0.01 g, and its snout–vent length (SVL) was measured to the nearest 1 mm to detect changes in growth throughout captivity.

Experimental frogs (EF) were injected with 0.1 ml sheep blood solution, sham frogs (SF) were injected with 0.1 ml 0.85% saline, and control frogs (CF) were not injected. Injections were made into the dorsal femoral lymph sac. Initial injections began 7 June 2002; EF and SF were injected again on 1-week intervals through 26 July 2002 to total eight injections. The interval length was chosen to maintain a sustained immune response; the primary immune response in this species under these conditions subsides in about 6 days (M. L. McCallum, unpublished data). We presumed that a smaller number of injections might not be sufficient to induce a reproductive response, whereas a longer series of injections might confound the results due to expected senescence of the reproductive organs typically associated with the termination of the reproductive season (McCallum, 2003). At the end of the treatment period the frogs were anesthetized in a dilute solution of chloretone and the testes were removed.

Routine histological techniques were used to prepare the testes for light microscopy following standard methods (Presnell and Schreibman, 1997). Testes were dehydrated in a graded series of ethanol, cleared with xylene, infiltrated and embedded in paraffin, sectioned into serial ribbons (8 μm in thickness), affixed to microscope slides using Haupt's adhesive, stained with Harris hematoxylin followed by eosin counterstaining (H & E), and mounted with coverslips. Maximum germinal epithelium depth and average maximum spermatic cyst diameter were determined with an ocular micrometer using the largest spermatic cyst on each of 15 histosections from each frog. The maximum germinal epithelium depth was the maximum depth within the largest spermatic cyst of each section.

The traditional method of calculating Gonadosomatic index (GSI) was not used because of the small size of the testis in this species and the desire not to damage the testicular tissue so that it could be used for histological examinations. Gonadosomatic index was calculated using two different methods. The first technique (GSI-L) was to divide the testis length by the SVL. The second method (GSI-W) was calculated by dividing the testis width by the SVL.

In all data sets, the Anderson-Darling Normality Test was used to choose parametric or nonparametric alternatives. Differences in SVL between treatments were statistically assessed using the Kruskal-Wallis Test. Body mass (BM) at the end of the study was compared among groups using a one-way ANOVA. Where no significant difference occurred, a linear regression was used to determine whether manipulations affected growth. Differences in spermatic cyst diameters were analyzed using a general linear model with individual frogs set as a covariate. A gonadosomatic index based on testis length and testis width was compared between treatment groups using ANOVA followed by Tukey multiple comparisons tests. Germinal Epithelium Depth was analyzed using a Tukey Test. Decisions theory was applied to the data using. $\acute{\alpha} < 0.05$ as significant and $\acute{\alpha} > 0.1$ as not significant. In the single incidence where $0.5 < \acute{\alpha} > 0.1$, a Tukey Test was applied to verify whether differences existed.

Results

No agglutination could be detected in the pooled samples of experimental frogs at 6 h; however, agglutination was detected in all other experimental samples. Agglutination was not detected in the pooled blood samples

Table 1.—A comparison of how selected natural history characteristics respond to exposure to a novel antigen.

	Experimental	Sham	Control
Snout–vent length (beginning and ending)	21 mm (SD = 0.38)	23 mm (SD = 0.34)	22 mm (SD = 0.26)
Body mass (beginning)	1.22 g (SD = 0.17)	1.20 g (SD = 0.15)	1.29 g (SD = 0.18)
Body mass (ending)	1.13 g (SD = 0.19)	1.18 g (SD = 0.13)	1.29 g (SD = 0.15)
Change in body mass	−0.09 g (SD = 0.18)	−0.02 g (SD = 0.10)	0.00 g (SD = 0.05)
Mean maximum spermatic cyst diameter	0.082 µm (SD = 0.014)	0.097 µm (SD = 0.014)	0.099 µm (SD = 0.005)
Mean maximum germinal epithelium depth	0.008 µm (SD = 0.004)	0.012 µm (SD = 0.005)	0.013 µm (SD = 0.003)
Gonadosomatic index based on testis width	0.041 µm (SD = 0.014)	0.055 µm (SD = 0.001)	0.057 µm (SD = 0.013)

from control frogs. Three frogs died in the control group so no 48-h titer was determined.

Responses of the various natural history characteristics expressed in each treatment group are provided in Table 1. Snout–vent length was not normally distributed (Anderson-Darling: $A^2 = 2.142$, $P < 0.001$). Mean SVL in all treatment groups remained unchanged throughout the duration of the experiment. No differences were found in ending SVL among treatments (Kruskal-Wallis: $H = 0.29$, df = 2, $P = 0.864$). It does not appear that linear growth was inhibited by antigen exposure (Fig. 1A). The effect of antigen exposure on body mass (BM) also was not statistically significant among groups (ANOVA: $F = 1.67$, df = 2, $P = 0.211$); however, regression analysis identified a trend ($r^2 = 0.185$, $P = 0.011$) of a decrease in the log of the body mass as stress due to handling and then antigen exposure increased (no handling vs. saline-injected vs. antigen-injected; Fig 1B). Change in body mass between the beginning and the end of the study was not significantly different among groups (ANOVA: $F = 1.35$, df = 2, $P = 0.281$), and no statistically significant trend was evident as stress-level of manipulation increased ($r^2 = 0.100$, $P = 0.132$; Fig 1C).

Maximum spermatic cyst diameter differed among treatments (GLM: $F = 9.01$, df = 2, 21, $P = 0.001$). Tukey Tests indicated that cyst diameter was smaller in EF than in either SF or CF (Fig. 1D). Maximum germinal epithelium depth was somewhat smaller in EF than in either SF or CF (ANOVA: $F = 3.01$, df = 2, 21, $P = 0.072$; Fig. 1E), but this difference was not significant. A larger sample size might be required to elucidate effects on maximum germinal epithelium depth.

Gonadosomatic index based on testis length showed no treatment effect (ANOVA: $F = 0.55$, df = 2, $P = 0.585$); however, when testis width was used to calculate gonadosomatic index, differences were detected (ANOVA: $F = 6.84$, df = 2, $P = 0.005$). A smaller width-based gonadosomatic index was present in EF than in either SF or CF (Fig 1F). There was no difference in width-based gonadosomatic index between SF and CF.

Discussion

Our observations of reduced average maximum spermatic cyst diameter and average germinal epithelium depth suggest that investment in reproduction was reduced during increased immune activity. McCallum (2003) showed that reproductively active spermatic cysts had larger average maximum diameters (0.096 µm, SD = 0.024) than those that were reproductively inactive (0.072 µm, SD = 0.023). The saline injected and noninjected groups had spermatic cyst diameters similar to spermatically active individuals in that study. Spermatic cysts from EF had diameters approaching those found to be reproductively inactive (McCallum, 2003). This result indicates that spermatogenesis had shut down and that these frogs could not reproduce. The combination of increased resource demands and reduced reproductive effort in immunochallenged frogs suggests that these animals shifted resources from reproduction for use in mounting an immune response.

The difference in results based on width-based and length-based gonadosomatic index

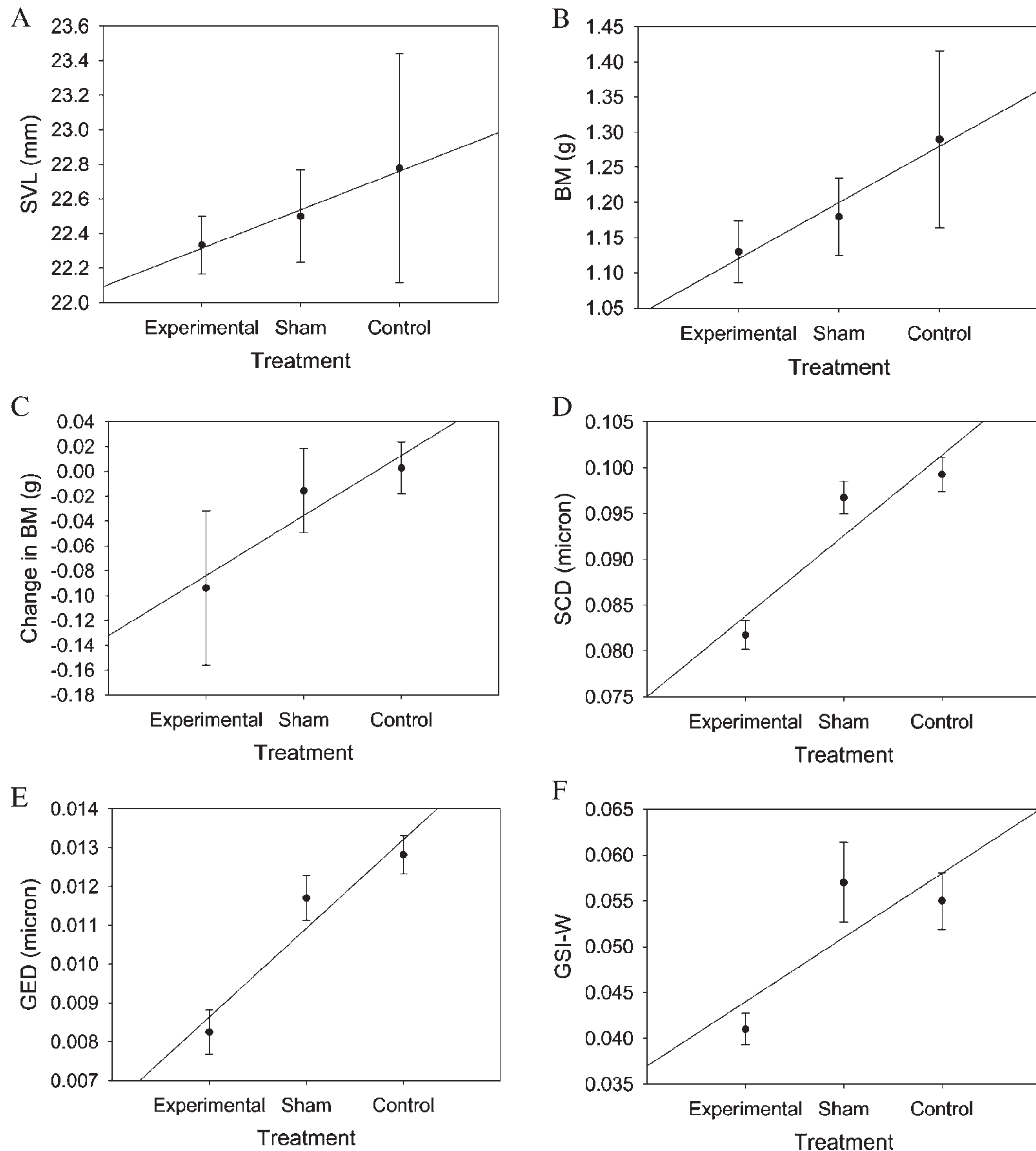

Fig. 1.—Response of selected natural-history characteristics to exposure to a novel antigen. Key: SVL = Snout–vent length, BM = Body mass, SCD = Spermatic cyst diameter, GED = Germinal epithelium depth, GSI-W = Gonadosomatic index.

may reflect the anatomical connection between the testis and the kidney via their longitudinal axis. The connection's length varies but can be limited to the anterior one-third or can extend the entire length of the testis. This connection may allow the width to contract while holding linear shrinkage to a minimum.

The frogs in our study were collected at the peak of the breeding chorus and seasonal reproductive activity (McCallum, 2003) suggesting that the demand of reproduction for resources was at a maximum. We suggest that our data indicate that production of sperm may decrease when under the stress of

immunochallenge, potentially reducing overall fecundity of antigen-exposed individuals.

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Literature Cited

Andersson, M. 1994. Sexual Selection. Princeton University Press, New Jersey, U.S.A.

Beasley, V. R., S. A. Faeh, B. Wikoff, J. Eisold, D. Nichols, R. Cole, A. M. Schotthoefer, C. Staehle, M. Greenwell, and L. E. Brown. in press. Risk factors and the decline of the northern cricket frog, *Acris crepitans*: Evidence for involvement of herbicides, parasitism, and habitat modifications. *In* M. Lannoo (Ed.) The Status and Conservation of United States Amphibians. University of California Press, Berkeley, California, U.S.A.

Burkett, R. D. 1969. An ecological study of the cricket frog, *Acris crepitans*, in northeastern Kansas. Ph.D. Dissertation. Lawrence, Kansas, U.S.A.

———. 1984. An ecological study of the cricket frog, *Acris crepitans*, in northeastern Kansas. Pp. 89–103. *In* R. A. Seigel, L. E. Hunt, J. L. KCGht, L. Malaret, and N. L. Zuschiag (Eds.) Vertebrate Ecology and Systematics – A tribute to Henry S. Fitch. Museum of Natural History, University of Kansas, Lawrence, Kansas, U.S.A.

Campbell, D. H., J. S. Garvey, N. E. Cremer, and D. H. Sussdorf. 1970. Methods in Immunology: A Laboratory Text for Instruction and Research, 2nd ed. W.A. Benjamin, Inc., New York, New York, U.S.A.

Carey, C., G. D. Maniero, and J. F. Stinn. 1996. Effect of cold on immune function and susceptibility to bacterial infection in toads (*Bufo marinus*). Pp. 123–129. *In* F. Geiser, A. J. Hulbert, and S. C. Nicol (Eds.) Tenth International Hibernation Symposium. University of New England Press, Armidale, Australia.

Compton, S., and T. Derting. 2002. Examining the energetic cost of immunocompetence in *Peromyscus leucopus*. *Peromyscus* Newsletter 33:13.

Courtland, S. 2002. Recirculating systems for zebrafish. Lab Animal 31:53–56.

Folstad, I., and A. J. Karter. 1992. Parasites, bright males and the immunocompetence handicap. American Naturalist 139:603–622.

Garvey, J. S., N. E. Cremer, and D. H. Sussdorf. 1977. Methods in Immunology, 2rd ed. Benjamin Cummings, Inc., Reading, Massachusetts, U.S.A.

Groothuis, G. G., C. M. Eising, C. Dijkstra, and W. Muller. 2005. Balancing between costs and benefits of maternal hormone deposition in avian eggs. Biology Letters 1:78–81.

Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: A role for parasites. Science 218:384–387.

Hamilton, W. D., and M. Zuk. 1989. Parasites and sexual selection: Reply. Nature 341:289–290.

Hausfater, G., H. C. Gerhardt, and G. M. Klump. 1990. Parasites and mate choice in gray treefrogs, *Hyla versicolor*. American Zoologist 98:299–311.

Ilmonen, P., T. Taarna, and D. Hasselquist. 2000. Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. Proceedings of the Royal Society of London B 267:665–670.

Liljedal, S., I. Folstad, and F. Skarstein. 1999. Secondary sex traits, parasites, immunity and ejaculate quality in the Arctic charr. Proceedings of the Royal Society of London 266:1893–1898.

Manning, M. J., and R. J. Turner. 1976. *Comparative Immunobiology*. Halstead Press, New York, New York, U.S.A.

Maynard Smith, J. 1974. The theory of games in the evolution of animal conflicts. Journal of Theoretical Biology 47:209–221.

Maynard Smith, J., and J. R. Price. 1973. The logic of animal conflict. Nature 246:15–18.

McCallum, M. L. 2003. Reproductive Ecology and Taxonomic Status of *Acris crepitans blanchardi* with additional investigations on the Hamilton and Zuk Hypothesis. Ph.D. Dissertation. Arkansas State University, Arkansas, U.S.A.

McCallum, M. L., and J. L. McCallum. 2006. Publication trends of natural history and field studies in herpetology. Herpetological Conservation and Biology 1(1):63–68.

Norris, K., and M. R. Evans. 2000. Ecological immunology: Life history trade-offs and immune-defense in birds. Behavioral Ecology 11(1):19–26.

Norris, D. O., and R. E. Jones. 1987. Hormones and Reproduction in Fishes, *Amphibians, and Reptiles*. Plenum Press, New York, New York, U.S.A.

Presnell, J. K., and M. P. Schreibman. 1997. Animal Tissue Techniques, 5th ed. Johns Hopkins University Press, Baltimore, Maryland, U.S.A.

Sadd, B., L. Holman, H. Armitage, F. Lock, R. Marland, and M. T. Siva-Jothy. 2006. Modulation of sexual signaling by immune challenged male mealworm beetles (*Tenebrio molitor*, L.): Evidence for terminal investment and dishonesty. Journal Compilation 19:321–325.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology and Evolution 11:317–321.

van Tienhoven, A. 1983. Reproductive Physiology of the Vertebrates, 2nd ed. Cornell University Press, New York, New York, U.S.A.

Zera, A. J., and L. G. Harshman. 2001. The physiology of life history trade-offs in animals. Annual Review of Ecology and Systematics 32:95–126.

Zuk, M., R. Thornhill, and J. D. Ligon. 1990. Parasites and mate choice in red jungle fowl. American Zoologist 30:235–244.

Zuk, M., and T. S. Johnsen. 1998. Seasonal changes in the relationship between ornamentation and immune response in red jungle fowl. Proceedings of the Royal Society of London 265:1631–1635.

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