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Parasite levels in blue-black grassquits correlate with male displays but not female mate preference

Thais M. Aguilar, Rafael Maia, Eduardo S.A. Santos, and Regina H. Macedo
Laboratório de Comportamento Animal, Departamento de Zoologia—IB, Universidade de Brasília,
70910-900 Brasília, D.F., Brazil

The handicap principle proposes that male sexual ornaments and displays provide honest indicators of quality. Female preference for high-quality males, however, may be driven not only by genetic benefits but also by indirect benefits. We investigated the impact of parasitism on morphological, ornamental, and behavioral characteristics of male and female blue-black grassquits (*Volatinia jacarina*) in captivity. First, we tested whether male displays and morphology were influenced by parasitism. Second, we assessed if females were attentive to variation in male morphology and displays linked to parasitism. Third, we tested whether parasitism in females influenced health and mate preferences. We maintained 2 groups of birds in captivity: nonmedicated birds developed high levels of coccidian parasitism, whereas medicated birds were free of parasitism. Parasitized males developed, relative to nonparasitized males, lower weight/tarsus indices and mass. They also showed relative deficiencies in their displays, with less persistence and lower rates. Despite the negative effects of parasitism on males, females did not prefer nonparasitized males. This held for both parasitized and nonparasitized females. Our data suggest that coccidian parasitism has adverse effects on morphological condition and expression of displays. These effects, however, appear not to be attended to by females; moreover, female mate preferences appear not to be impacted by the threat of parasitism. It thus seems that female mate preferences may not depend only on sexual characters affected by parasitism in this species. *Key words:* blue-black grassquit, mate choice, parasitism, sexual selection, *Volatinia jacarina.* *[Behav Ecol 19:292–301 (2008)]*

For many animals, male ornamentation and behavioral displays play central roles in sexual selection and female choice (Andersson 1982; Torres and Velando 2003; Kraaijeveld et al. 2004). Male secondary sexual characters are thought to provide honest indicators of their genetic and phenotypic quality as potential mates, and females are thought to evolve to select males with the most extravagant ornaments and elaborate displays (Zahavi 1975). The interpretation that such characters serve as reliable indicators of quality has been examined in several contexts. For instance, the "nutritional stress hypothesis" suggests that song complexity and dialect structure indicate male quality because when songbirds are subjected to nutritional stress as juveniles, brain structures essential for song learning do not develop properly (Nowicki et al. 1998; reviewed in Nowicki et al. 2004). Several kinds of stresses, in addition to nutritional deficiencies, could potentially affect the development of ornaments or secondary sexual characters based on learning, including song and other ritualized mating behaviors.

Hamilton and Zuk (1982) proposed that the development of elaborate ornaments could indicate genetic resistance of individuals to disease and parasites and that females might use variation among males in ornament features to choose the most resistant males as mates. Support for this hypothesis requires that several conditions are satisfied (Møller 1990). One condition is that parasites must have a negative impact on the development and expression of secondary characters and that parasite-induced deterioration in general health may negatively affect the expression of behavioral displays. Additionally, females should reject parasite-infected males, thus implying that parasites have a negative effect on the fitness of their male hosts. Numerous studies have examined one or the other of these conditions, but few have asked how parasitism affects both male sexual characters and female mate preferences.

Birds may harbor a large variety and number of ectoparasites, including lice and ticks (Boyd 1951), and among endoparasites, coccidians (especially of the genera *Eimeria* and *Isospora*) are among the most common. They usually invade the gastrointestinal tract (Hadley 1917), liver, and kidneys (Friend and Franson 1999), resulting in death of heavily infected individuals. The negative effects of parasites on male ornamentation have been empirically demonstrated in several studies (Zuk 1991; Brawner et al. 2000; Doucet and Montgomerie 2003; Hill et al. 2005). By contrast, only a handful of studies have demonstrated an effect of parasites on male mating displays and singing. For instance, adult male canaries (*Serinus canaria*) infected with malaria (*Plasmodium relictum*) as juveniles developed simpler songs when compared with adults not infected as juveniles (Spencer et al. 2005). In some studies, the effect of parasites on male ornaments has been shown to influence females, which prefer less parasitized males (Zuk 1988; Polak and Markow 1995; MacDougall-Shackleton et al. 2002; Borgia et al. 2004).

A comparatively overlooked issue in the study of parasites and sexual selection concerns the direct effect of parasites on females' mate choice (Wiehn and Korpimäki 1998; Doucet and Montgomerie 2003) or on their mate-choosing capacity (Poulin 1994; Poulin and Vickery 1996; Lopez 1999; Córdoba-Aguilar et al. 2003; Buchholz 2004). Mate choice is based on sensory and behavioral properties of animals that influence their tendency to mate with certain phenotypes. The capacity to choose may affect the energy and time needed to sample and evaluate potential partners (Jennions and Petrie 1997).

Address correspondence to R.H. Macedo. E-mail: rhfmacedo@unb.br.

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Additionally, a parasitized bird is more likely to have its sensory and neural mechanisms compromised, so that it is unable to make the needed fine-resolution decisions. The possibility that parasitized and nonparasitized females differ in mate choice has not been tested (Poulin and Vickery 1996).

Here, we used an experimental manipulation to investigate 1) whether male morphology and display features are influenced by parasitism; 2) if (nonparasitized) females are attentive, in a mate preference assay, to variation in male morphology and/or displays linked to the presence and absence of parasites; and 3) whether parasitism in females influences their mate preferences. We used 2 groups of individuals in captivity, one of which was treated and kept free of coccidian parasites (treatment group) whereas the other (control group) was allowed to develop high levels of parasite infection. We predicted that through time, males free of parasites would differ from parasite-infected ones in the degree of exuberance of secondary sexual ornaments, including behavioral displays, as well as in body condition. We anticipated that females, too, would suffer adverse effects of parasitism in their body condition. Additionally, we expected that nonparasitized females would be attentive to differences among males, preferring nonparasitized males over parasitized males. Finally, we predicted that parasitized females might not make the same kind of discrimination in mate choice as their nonparasitized counterparts.

METHODS

Subjects

We mist netted 58 males and 48 females on the Universidade de Brasília campus (15°46’S, 47°52’W) in Central Brazil, between November and April from 2003 to 2005. Male, female, and immature blue-black grassquits exhibit an inconspicuous brownish plumage during the nonbreeding season. Prior to and throughout the breeding season, males acquire an iridescent blue-black plumage with white underwing patches. In Central Brazil, they reproduce between November and April, wherein males defend small territories through conspicuous vocal and behavioral displays consisting of repeated leaps from perches, revealing their white underwing patches (Alderton 1963; Almeida and Macedo 2001). A recent study of a population in Central Brazil found that approximately 50% of nestlings in more than 60% of all nests sampled resulted from extrapair fertilizations (Carvalho et al. 2006).

Experimental design

We banded all individuals with permanent metal bands (supplied by the Brazilian bird-banding agency—Centro Nacional de Pesquisa para Conservação das Aves Silvestres) and evaluated them morphometrically and for the degree of parasitism (see below). Forty-five males and 29 females were randomly assigned to 1 of 2 groups, parasitized or nonparasitized, for the 5-month duration of the experiment. We treated individuals in the nonparasitized (hereafter referred to as healthy) treatment group monthly with a coccidiostatic drug (sulphametoxazol–trimethoprim), a vermifuge (mebendazole), and an insecticide talcum (pirethrin). Individuals of the control group did not receive any treatment. Males of both groups were handled for approximately the same amount of time and in equivalent manner; administration of the talcum increased handling time in about 2 min. We housed birds separately by sex and treatment group in an outside aviary (3.0 × 3.0 × 2.0 m). Groups were visually isolated; however, aviaries were within hearing range of each other. Captive birds received equivalent diets of mixed seed ad libitum, sterilized sand, and water with a multivitamin supplement. Maintenance of birds in the aviary was authorized by Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis/Brazil (DIFAS/DIREC permit 237).

Evaluation of parasitism

We documented parasite loads of individuals on capture and then monthly. For ectoparasites, we checked for (1) lice, through visual inspection of the wing and tail feathers held against a lighted background using a hand magnifying glass, and (2) ticks, through examination of feathers over the whole body. We collected and preserved (70% alcohol) some ectoparasites for identification (Kanegae 2003). To document intestinal parasites, we collected fecal samples after 1400 h, when there is maximal excretion of oocysts (Brawner and Hill 1999). Fecal samples were placed in water, homogenized, allowed to settle, and then kept refrigerated until analyses were conducted (Sloss et al. 1999; Foreyt 2005). We used the sedimentation method because flotation does not allow visualization of some trematoid and cestoid eggs (Sloss et al. 1999). Three slides were made from aliquots taken from individual fecal samples and examined under ×400 magnification. Coccidian oocysts were counted and individual degrees of parasitism calculated as the average value obtained from the 3 slides examined.

To quantify hemoparasites, we collected a drop of blood from the medial metatarsal vein and smeared it on a glass slide, which was air-dried and then fixed with methanol and stained with May-Grunwald Giemsa (Sloss et al. 1999; Foreyt 2005). We later scanned 100 visual fields (×1000 magnification) per slide, approximately 10 000 red blood cells, to document the occurrence of hemoparasites, avoiding nonstained areas and slide preparation artifacts.

We calculated 3 measures of parasite infection. The *prevalence* of parasitism, expressed as a percentage value, was calculated by dividing the number of host individuals infected with each type of parasite by total number of host individuals examined. *Individual intensity* of parasitism was calculated as the number of parasites of a particular type found in association with each host individual. The *average intensity* of parasitism was calculated as the total number of parasites of a particular type divided by the total number of host individuals infected, that is, the arithmetic mean of individual intensity (Margolis et al. 1982). Parasite prevalence was calculated only at the moment of capture to document the state of parasitism of wild grassquits in the study area. For the monthly evaluations, we calculated the individual intensity of parasitism.

Body condition, morphology, and male plumage

Males and females in both control and treatment groups were evaluated monthly by the same person to ensure consistency of measurements; treatment group was known when scoring the birds. Standard morphological measurements taken were body mass (±0.1 g), tarsus length, wing length, and beak length (±0.1 mm). For males, we also assessed plumage status by taking 3 measurements. The first measure, percent nuptial plumage coverage, was obtained by visually inspecting the bird’s dorsal and ventral sides, head, tail, and both wings and estimating the proportion of black plumage, ranging from 0% (no black feathers) to 100% (all black feathers). A second measure, percent plumage increment, was used to assess possible differences in the growth of new black feathers through molting in the intervals between evaluations. This was calculated by subtracting the value of the last measure of nuptial plumage coverage from the present value of plumage coverage. This parameter was included because male molting rates could differ during the study period. Finally, we also measured the area of the white underwing patch relative to the area of the wing by lightly pressing the wing against an acetate sheet and tracing the outline of the wing and the white patch (Costa and Macedo 2005). We digitized the outlined drawings and used Autocad (Release 14.0, Autodesk 1997) to obtain the area (square millimeter) of the wing and white patch.

Mate-choice trials: male display and female choice

At the beginning of the experiment, we designated 20 fixed male dyads, each consisting of a parasitized and a healthy male with roughly similar body measurements. The first mate-choice trials occurred after animals were in captivity and under experimental conditions for a full month. We presented dyads randomly to different females that had no prior experience with males they were viewing, and each female was tested with a dyad only once. We conducted 165 mate-choice trials (120 min each), 83 using parasitized females and 82 using healthy females.

Preliminary observations indicated that males display spontaneously in captivity, during the breeding season, and with females in sight (Macedo et al., unpublished data). Approximately 24 h before the beginning of a trial, the male dyad and the female were isolated individually in the experimental room and fed their regular diet under a controlled 12/12 h photoperiod. During the experimental trials (November–April), natural photoperiod in Central Brazil ranged from 11:45 to 12:50 h; thus, the laboratory photoperiod deviated, in all cases, by less than 1 h from the natural photoperiod. It was assumed that exposure of birds to the artificial photoperiod for about 24 h during experiments would not be enough to disrupt their circadian rhythms.

The experimental mate-choice arena (2.54 × 0.60 × 1.25 m) is subdivided into 3 compartments separated by glass and aluminum partitions. At the beginning of each mate-choice trial, we placed the female in the larger, middle compartment (1.38 × 0.60 × 1.25 m) and the 2 males of the dyad in the smaller, lateral ones (0.58 × 0.60 × 1.25 m) and, after an acclimation of 5 min, lifted the aluminum partitions, which allowed visual contact between the female and the males. Males each had a single perch within their compartments, and females had 3 evenly spaced perches, 2 of which were close to each of the lateral compartments. The height of the mate-choice apparatus (1.60 m) and the placement of perches (height of 20 cm) in the lateral compartments allowed males to perform their leaping display at the maximum heights seen under natural conditions (average male leap height = 32.3 ± 13.8 cm: Carvalho et al. 2007). Trials lasted 3 h, with observations during the first and third hours. During the second hour, we switched the positions of the males to control for any side preferences. We recorded the total time females spent close to and observing males, which only occurred when females were on the floor of the arena, at a distance from 0 to 15 cm from the partition between compartments.

For males, we obtained the average height of leaps using a scale on the front panels of the arena through which birds were videotaped. The duration of the leaping display was calculated as the total time in minutes, across each of the two 60-min trials, during which males performed at least one leap display. If, for example, a male leaped at least once during the first, fifth, and ninth minutes of the trial, the time spent in leaping display would be coded as 3 min, independent of number of leaps executed. We also calculated 2 measures of "leap rate" to account for the observation that males often do not display continuously but rather in discrete bouts of activity. Our first measure, "relative leaping display rate," was calculated as the total number of leap displays divided by the duration of leaping display. To refer back to our example, a male that displayed 12 times during the 3 trial minutes in which he gave any display would have a relative leap rate of 4. Our second measure was "absolute leap rate," calculated as the total number of leaps divided by the duration of each of the 2 trials, that is, 60 min. Thus, in our example, absolute leap rate would be 12/60 or 0.2. Finally, we measured "display persistence," which counts not only leaps but also other displays directed toward females including calls, small hops (<20 cm), and dances (opening and closing of wings while wagging the tail) performed in front of the glass partition.

Data analysis

We considered that females had chosen a male during a trial when they associated with a male by remaining <15 cm from the partition to his compartment for at least 3 more minutes (total time) than the time spent near the other male. We videotaped the trials, and analyses are presented pooled for the first and third hours.

We tested for normality using Kolmogorov–Smirnov tests. The only parameter that deviated from normality was beak length, possibly because some birds acquired deformities in their beaks, a common problem in captive birds, usually due to vitamin D deficiency. The different parasite intensity measures also did not present normal distributions. We evaluated the effects of the experimental manipulation for each morphometric and ornamental characteristic (both males and females) through time using univariate repeated-measures analysis of variance (ANOVA). We also report the results of multivariate repeated-measures ANOVA used to evaluate the effects of time and treatment on male and female parameters. This test is considered doubly multivariate because it assesses multiple characters through repeated measures (Tabachnick and Fidell 2007). To avoid multicolinearity issues in this approach, we did not include derived morphometric characteristics (mass/tarsus index for both sexes and plumage increment for males) because they were highly correlated to other parameters (mass and plumage percentage, respectively). When necessary, we replaced missing cells with the individual's mean value for the parameter during the experimental period (Tabachnick and Fidell 2007). We present Wilks's lambda and approximate F values for the multivariate tests.

We used Spearman correlations to evaluate the degree and direction of change in affected characters over time. To assess behavioral differences between the parasitized versus healthy males and to evaluate choice and persistence of choice for females, we used paired Student's t-tests. Unless otherwise indicated, all tests were 2 tailed, and we present means ± standard deviation. We conducted univariate analyses using R statistical software (ver. 2.5.1; R Development Core Team 2007) and the nlme package (ver. 3.1-83; Pinheiro et al. 2007), both available for download at http://cran.r-project.org. For multivariate tests, we used SPSS (ver. 13).

RESULTS

The only ectoparasites found on the birds when captured were feather lice (Mallophaga), which occurred in approximately half of all males and females. However, individual intensity (absolute number) of Mallophaga for males at capture was approximately 5 times higher than for females. This trend was reversed in the evaluation of endoparasites, as females harbored notably more coccidians than did males, in both prevalence and average intensity (intensity: Mann–Whitney $U = 499.0$; $P < 0.001$; Table 1).

Table 1

Initial (preexperimental) parasite prevalence and intensity (see Methods for definitions) during the 2003–2005 breeding seasons

	N		Prevalence (%)		Average intensity ± standard error	
Parasites	Males	Females	Males	Females	Males	Females
Mallophaga (body)	58	46	50	57	39.79 ± 9.99	7.26 ± 3.77
Mallophaga (wing)	58	—	40	—	39.13 ± 8.99	—
Mallophaga (white patch)	58	—	33	—	14.74 ± 5.95	—
Coccidian	53	32	72	88	398.81 ± 27.69	618.00 ± 39.10
Other endoparasites	53	32	4	16	2.80 ± 1.88	0.29 ± 1.20

We assessed the occurrence of parasites on the wing and white patch only for males, as it is a male-specific ornamental trait.

Impact of parasitism: male body condition, morphology, and plumage

After 2 months of captivity, ectoparasites were extremely reduced for both healthy and parasitized groups of males. Coccidian oocysts, by contrast, were higher in the parasitized group and remained so for the duration of the experiment (Figure 1, Table 2). Parasitized individuals after 3 months of the experiment had a mean intensity of 599.33 ± 1107.80 ($N = 19$) oocysts, whereas the healthy group had 174.37 ± 405.01 ($N = 22$). During the months of the experiment, the level of coccidian oocysts remained close to zero for the healthy group whereas that of the parasitized group climbed steadily and reached a peak mean intensity of 739.45 ± 903.09 ($N = 18$) in the fifth month (Figure 1). Thus, our experiment was successful in manipulating the intensity of coccidian parasitism in the grassquits, allowing us to consider how coccidian parasitism might affect individual phenotypes and behavior.

The multivariate tests showed significant effects of treatment and time on male morphometry but no effect for time × treatment interaction (Table 2). Univariate repeated-measures ANOVAs showed that the 2 groups of males differed in 3 parameters: mass, mass/tarsus index, and tail (Table 2). The repeated-measures ANOVA showed that there was a significant effect of time on all traits except for beak and tarsus.

Both healthy and parasitized males showed positive and significant correlations between time and the parameters mass/tarsus index, percent plumage coverage, and percent plumage increment (Figure 2C–E). This suggests that both groups became healthier in captivity and that this possibly had a positive impact on ornamental nuptial plumage; however, there was no treatment effect on these plumage characteristics. There also was no treatment effect on the patch/wing area, but it was affected by the time factor, showing a decreasing pattern for both groups of males (Figure 2F).

Average wing length in the parasitized group was generally smaller than in the healthy group during the experiment; it increased with time in the healthy group but remained unchanged in the parasitized group (Figure 2A). Tail length decreased significantly through time in both treatment groups, probably due to the attrition of the tail feathers against the aviary mesh partitions (Figure 2B). Although both groups showed a similar trend, the healthy group maintained shorter tail values overall. This probably reflects the increased wear of tail feathers of the more active individuals of the healthy group in comparison with the parasitized group.

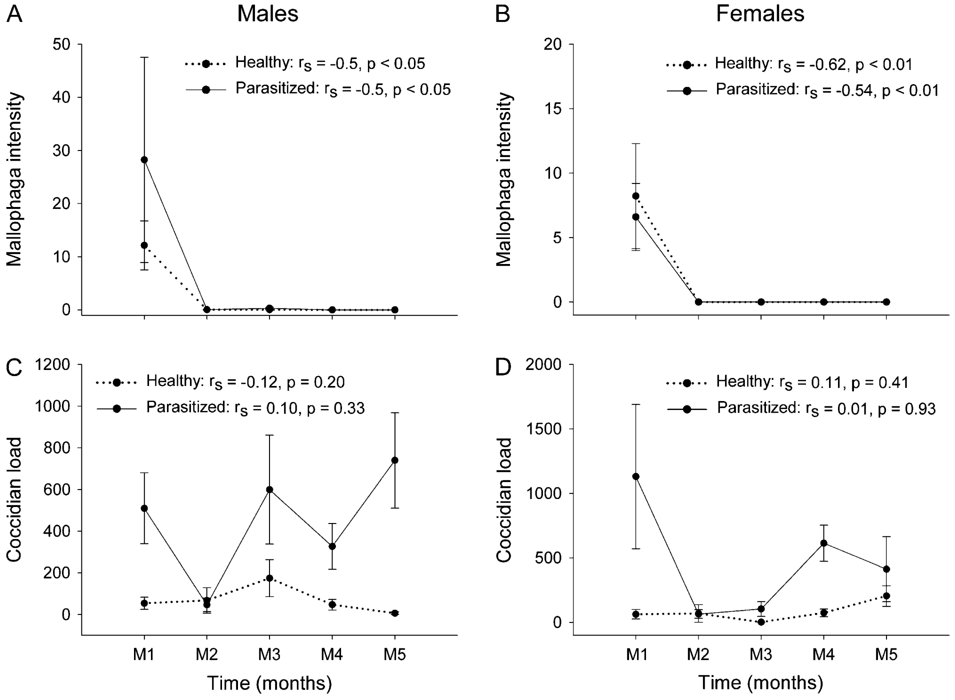

Figure 1

Variation (mean ± standard error) between the treatment groups (parasitized = solid line; healthy = dashed line) through time in Mallophaga infestation and coccidian load for males and females. A significant treatment effect was found for the level of coccidian infection for both males and females. The 5 months of experimental procedures are indicated as M1 to M5, and M1 refers to measures taken 1 month after experimental procedures were initiated.

Table 2

Multivariate repeated-measures ANOVA (MANOVA) and univariate repeated-measures ANOVA for effects of time and treatment on blue-black grassquit male parameters

	Time		Treatment		Time × treatment		Mean ± standard deviation	
	F	*P*	*F*	*P*	*F*	*P*	Parasitized	Healthy
Parasites								
Wilks's λ	0.646		0.752		0.615			
MANOVA	1.92	0.60	5.60	<0.01	2.19	0.03		
Mallophaga	7.90	<0.001	1.29	0.26	2.48	0.05	26.41 ± 102.55	3.27 ± 5.62
Coccidians	3.07	0.01	13.98	<0.001	3.16	0.02	474.84 ± 567.46	70.89 ± 147.84
Other endoparasites	0.58	0.68	1.21	0.28	0.76	0.56	0.69 ± 2.04	0.23 ± 1.20
Morphometry								
Wilks's λ	0.224		0.71		0.39			
MANOVA	3.22	<0.01	2.70	0.02	1.47	0.17		
Mass (g)	9.19	<0.001	10.05	<0.01	1.40	0.24	9.69 ± 0.80	10.52 ± 1.02
Wing (mm)	4.00	<0.01	2.73	0.11	3.39	0.01	49.02 ± 1.34	49.48 ± 0.96
Beak (mm)	1.20	0.31	0.95	0.34	0.48	0.75	6.77 ± 0.31	6.87 ± 0.36
Tarsus (mm)	1.86	0.12	0.00	0.99	1.14	0.34	16.04 ± 0.61	16.04 ± 0.63
Tail (mm)	22.44	<0.001	5.21	0.03	0.43	0.79	46.14 ± 1.64	45.08 ± 2.50
Mass/tarsus[a]	7.00	<0.001	12.01	0.001	1.47	0.22	0.60 ± 0.05	0.66 ± 0.05
Percent plumage	61.88	<0.001	0.10	0.75	2.25	0.07	63.47 ± 23.80	63.19 ± 21.56
Plumage increment[a]	68.12	<0.001	2.00	0.16	1.78	0.14	12.00 ± 13.86	18.70 ± 14.83
Patch/wing	4.18	<0.01	0.00	0.98	0.74	0.57	0.28 ± 0.06	0.28 ± 0.06

Parasite values are for intensity measures. Mean ± standard deviation refers to sample sizes of $N = 28$ and $N = 27$ for parasitized and healthy groups, respectively. Figures 1 and 2 show the direction of the effects of the manipulation.

[a] Variables not included in multivariate analysis (see text).

Impact of parasitism: male behavior

The impact of parasitism on male behavior was assessed during 330 h of observation of males during the mate-choice trials. We found that 4 of the 5 behavioral parameters had significantly higher values for healthy males when compared with parasitized individuals (Figure 3): persistence, duration of leaping, leap height, and relative leap rate. Only absolute leap rate showed no significant difference between treatment groups.

We conducted analyses with the male dyads ($N = 20$) to assess how distinct pairs of males differed through time. In all but one dyad, males differed significantly (all $|t| > 2.4$, all $P \leq 0.05$, degrees of freedom = 38) in at least one morphometric and behavioral characteristic evaluated. Nine dyads (45%) differed in 1 characteristic, 4 dyads (20%) in 2 characteristics, 3 dyads (15%) in 3 characteristics, and another 3 dyads (15%) in 4 characteristics. The morphometric and ornamental characteristics were consistently less robust for the parasitized males (Figures 2 and 3), and these differences could be verified at the dyad level when comparing each dyad through the period of manipulation.

Impact of parasitism on females

The multivariate test, which took into consideration both ecto and endoparasites, showed a nearly significant effect of treatment ($P = 0.078$) and was significant for the time factor (Table 3). The univariate repeated-measures ANOVA suggests that the manipulation of coccidian parasitism was successful for females, as the level of coccidian infection was significantly different between treatment groups. Time had a significant effect on female coccidian parasitism, and the time × treatment interaction term was nearly significant (Table 3). Despite these results, the maintenance of coccidian infection for parasitized females was not nearly as consistent as for parasitized males. Additionally, in sharp contrast to males, there were no significant differences between treatments concerning the effects of the manipulation on morphology in either univariate or multivariate tests. The analysis of individual parameters showed that all characters, except for wing and tarsus, varied significantly through time within each treatment group (Table 3). General condition, as inferred through the mass/tarsus index, improved through time for both parasitized and healthy females (Figure 4). Beak length decreased through time for both groups.

Mate choice and influence of female health

Healthy females did not spend more time with either parasitized or healthy males ($t = 0.001$, $P = 1.00$). We also found no difference in the mean time parasitized females spent with males of either treatment ($t = 0.05$, $P = 0.96$). Thus, the condition of females, whether healthy or parasitized, did not influence their choice for a category of male nor were healthy males favored over parasitized ones overall. Females (pooled: healthy and parasitized) chose healthy males in 74 trials, preferred parasitized males in 76 trials, and expressed no choice in the remaining 15 trials. As could be expected, when females of the 2 treatment groups were pooled, average time spent with a male did not differ between parasitized and unparasitized male treatments ($t = -0.09$, $P = 0.92$). Despite the differences in morphometry and behavior between males of each dyad (see above), females (pooled) preferred healthy males in only 3 dyads and preferred parasitized males in 3 other dyads.

DISCUSSION

We predicted that male blue-black grassquits kept free of parasites would differ, through time, from parasite-infected ones in the exuberance of secondary sexual ornaments, including behavioral displays, as well as in general morphology. Our results partially corroborate the hypothesis of Hamilton and Zuk (1982), as we found that infection with coccidian parasites clearly generated a difference between the 2 treatment

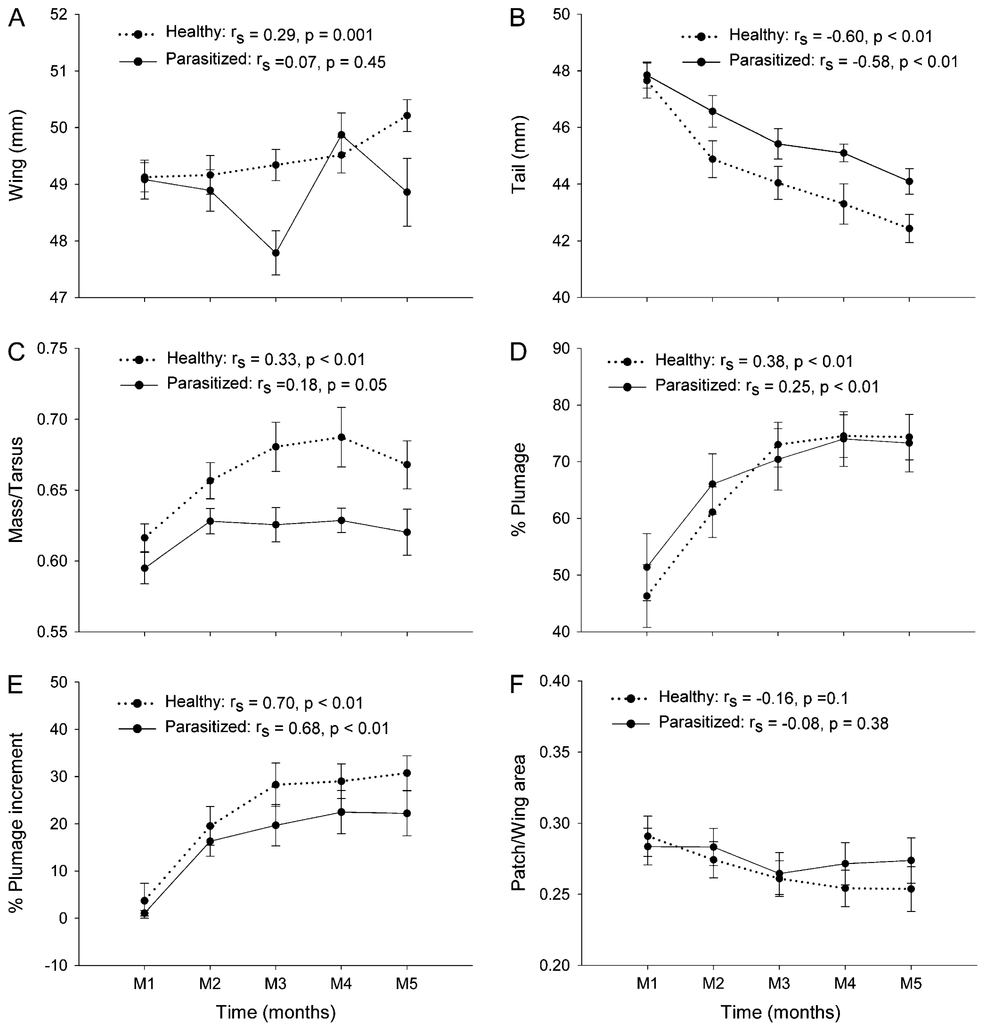

Figure 2
Effect of parasite manipulation on male morphological and ornamental characters (mean ± standard error; parasitized = solid line; healthy = dashed line). Most characters show increasing values through time for both groups, and a positive treatment effect was found for mass/tarsus and mass (not shown) for the healthy group. The 5 months of experimental procedures are indicated as M1–M5, and M1 refers to measures taken 1 month after experimental procedures were initiated.

groups of males relative to their morphology (mass, tail, and mass/tarsus index) and behavioral courtship displays.

Coccidians produce intestinal lesions and decrease nutrient absorption (Hadley 1917; Friend and Franson 1999), which should increase susceptibility to other diseases and also bring about a deterioration in health that will be reflected phenotypically and behaviorally. The fact that males and females from both treatment groups showed a positive increase in most traits during the experiment suggests that under these favorable conditions (i.e., ad libitum food, little energy expenditure) all individuals gained a net health benefit. However, even in this optimal cost/benefit setting, parasitized males exhibited more deteriorated conditions when compared with healthy males in mass, mass/tarsus index, and behavioral displays.

Several studies suggest that coccidian infections may lessen the expression of carotenoid-based ornaments (Hill and Brawner 1998; Brawner et al. 2000; McGraw and Hill 2000; Hõrak et al. 2004; Hill et al. 2005). Additionally, exuberance, color intensity, and size of carotenoid-based ornaments have been shown to affect female choice (Hill 1990; Norris 1990; Mateos and Carranza 1995; Collins and Cate 1996; Hill et al. 1999; Hill 2006). There is conflicting and scanty evidence to support the idea that structural or melanin-based black plumage may function as a secondary sexual ornament, serving as an honest signal of male condition in inter and intrasexual interactions (Sætre et al. 1994; McGraw et al. 2002; Shawkey et al. 2003; Liu et al. 2006). Carotenoid coloration may serve primarily as an indication of health and foraging ability of males, whereas structural plumage ornaments may reflect a delayed response to environmental conditions, including parasites (Fitze and Richner 2002; Hill 2006).

Additionally, few data exist to assess how endoparasites may directly affect the expression of structural plumage. One study found a negative relation between structural coloration and abundance of hematozoa in satin bowerbirds *Ptilonorhynchus violaceus* (Doucet and Montgomerie 2003). Another study found that coccidian-infected male turkeys *Meleagris gallopavo* grew feathers that were more opaque and with decreased UV chroma compared with uninfected males (Hill et al. 2005). However, in house finch *C. mexicanus*, the structural color of males was not affected by coccidian infection, despite the negative impact on the carotenoid coloration (Hill and Brawner 1998).

The structurally based, blue-black plumage of male grassquits is acquired just prior to reproduction, thus generating an expectation concerning its sexual role. The male grassquits initially captured, when divided into the 2 treatment groups, did not differ in the relative percentage of blue-black plumage coverage. This percentage increased steadily through time in the 2 treatment groups, as did the percentage of blue-black plumage increment. In the final 3 months of the experiment, the latter parameter was consistently lower for parasitized males, although statistical tests did not differentiate between the 2 groups. Possibly, a longer experimental period would

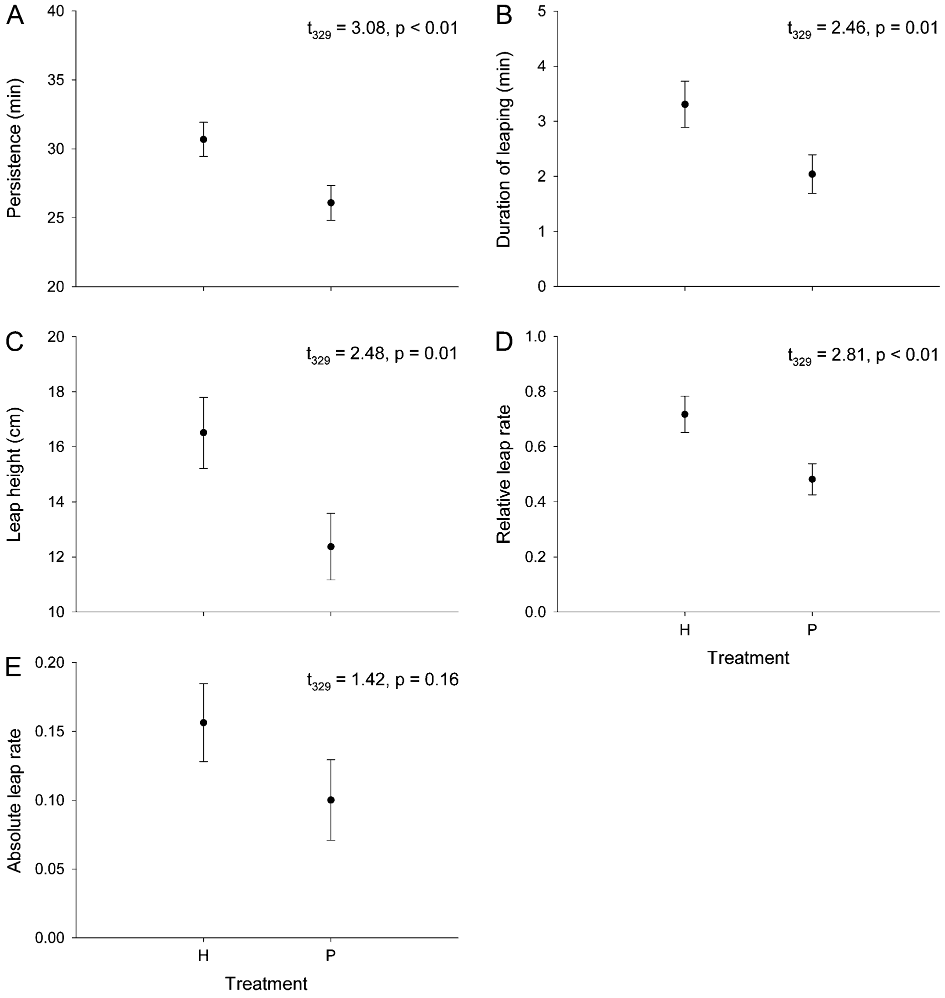

Figure 3
Comparison (mean ± standard error) of healthy (H) and parasitized (P) males for behavioral display parameters during mate-choice trials showing that healthy males exhibited higher values in 4 of 5 parameters examined. Parameters tested included (A) persistence, (B) duration of leaping, (C) leap height, (D) relative leap rate, and (E) absolute leap rate.

have demonstrated an impact of coccidian parasitism on plumage coverage. It remains unclear, however, whether the pattern found occurred through a lowered capacity to molt and produce plumage with structural coloration. This is because both groups improved their increment of black plumage through time, a possible result of better overall health, and this may have counteracted the deleterious influence of parasitism. One intriguing result concerns the effect of time on patch/wing area, which decreased for both groups. There is some evidence that white plumage ornaments are costly to produce and maintain (Gustafsson et al. 1995) and that their production may be condition dependent and rely heavily on protein in the diet (Török et al. 2003; McGlothlin et al. 2007).

Generally, data are scarce concerning the mechanism of feather growth for white plumage and its dependence on environmental and social factors. For the grassquits, it appears that parasites had no deleterious effect on this parameter because there was no difference between treatment groups. However, some condition of captivity is implicated in the simultaneous increase of black plumage and decrease of the white patch through time.

In any case, our results suggest that research into the effects of parasitism on plumage should consider events prior to the reproductive season and molting because past parasite load and condition may be more relevant to present plumage condition than current parasite load or condition.

Our third objective in this study considered one component of the hypothesis of Hamilton and Zuk (1982), which implies that females should interpret the exuberance of male secondary sexual ornaments as reliable indicators of male resistance to parasitism, resulting in a choice for healthy males. We predicted that in mate-choice trials, healthy females would prefer healthy males but females infected with parasites possibly would not express any choice. Differently from males, where the impacts of parasitism can be primarily morphological and/or physiological, it is possible that the effects on females would be limited to behavior, leading to errors in mate choice. For instance, parasite infections in female fish may hinder their discriminatory abilities during mate choice (Lopez 1999; Poulin 1994).

However, we did not find that female blue-black grassquits, whether healthy or parasitized, preferred healthy males, despite the fact that the latter clearly exhibited inferior morphological and behavioral traits. These results were unexpected, as many studies have not only verified the negative impact of parasites on characteristics similar to the ones we used (Fitze and Richner 2002; Doucet and Montgomerie 2003; Hõrak et al. 2004; Hill et al. 2005) but also found evidence that females favor nonparasitized males (Polak and Markow 1995; MacDougall-Shackleton et al. 2002; Borgia et al. 2004). The mate-choice design we used has been applied in many studies, and our sample size and number of trials are many times larger than what is frequently reported in the literature. Additionally, from what we were able to observe, males were actively conducting their displays and females were visually inspecting the males during trials. Thus, we are

Table 3

Multivariate repeated-measures ANOVA (MANOVA) and univariate repeated-measures ANOVA for effects of time and treatment on blue-black grassquit female parameters

	Time		Treatment		Time × Treatment		Mean ± standard deviation	
	F	*P*	*F*	*P*	*F*	*P*	Parasitized	Healthy
Parasites								
Wilks's λ	0.470		0.830		0.631			
MANOVA	2.54	0.03	2.46	0.08	1.31	0.27		
Mallophaga	7.45	<0.001	0.02	0.89	0.01	0.10	2.04 ± 3.46	2.23 ± 5.39
Coccidians	2.71	0.04	8.06	0.01	2.42	0.06	526.54 ± 804.50	147.32 ± 74.17
Other endoparasites	0.89	0.47	1.15	0.29	1.24	0.30	0.27 ± 0.74	1.55 ± 5.13
Morphometry								
Wilks's λ	0.233		0.950		0.478			
MANOVA	3.13	<0.01	0.36	0.87	1.04	0.47		
Mass (g)	14.58	<0.001	0.29	0.59	0.04	0.10	10.17 ± 0.95	10.31 ± 0.92
Wing (mm)	1.20	0.32	0.87	0.36	1.05	0.39	47.65 ± 1.54	47.83 ± 1.66
Beak (mm)	5.34	<0.001	0.10	0.76	0.87	0.49	6.62 ± 0.28	6.65 ± 0.34
Tarsus (mm)	0.73	0.58	0.23	0.64	0.59	0.67	16.27 ± 0.63	16.36 ± 1.45
Tail (mm)	4.57	<0.01	1.15	0.29	0.96	0.43	45.68 ± 2.02	44.94 ± 2.61
Mass/tarsus[a]	8.40	<0.001	0.81	0.37	0.44	0.78	0.63 ± 0.05	0.65 ± 0.05

Parasite values are for intensity measures. Mean ± standard deviation refers to sample sizes of $N = 20$ for both parasitized and healthy groups. Figures 1 and 4 show the direction of the effects of the manipulation.

[a] Variable not included in multivariate analysis (see text).

convinced that our results indicate a lack of choice of female blue-black grassquits for the attributes affected by parasitism.

Possibly, females can identify phenotypic differences among males but do not actively make choices. There are several theoretical models suggesting that females may reduce their threshold acceptance levels for male attraction depending on environmental or social conditions and the urgency of mating (Real 1991; Jennions and Petrie 1997). An empirical study of mate choice in the túngara frog *Physalaemus pustulosus* showed that, although still retaining their discriminatory capabilities, females exhibited higher levels of receptivity when faced with little time to lay eggs (Lynch et al. 2005). The blue-black grassquit is socially monogamous, but approximately 50% of chicks result from extrapair fertilizations (Almeida and Macedo 2001; Carvalho et al. 2006). One possible explanation for the lack of discrimination by females is that they may have fast access to territories and paternal care by accepting social pairing with less attractive males and then seeking extrapair copulations with more attractive/healthy males.

A second explanation for the random choice exhibited by the females is that the full range of ornamental, behavioral, and morphological traits typically used by females was not in evidence during the experiment. Possibly other traits, or combinations of those, are more important for choice in this

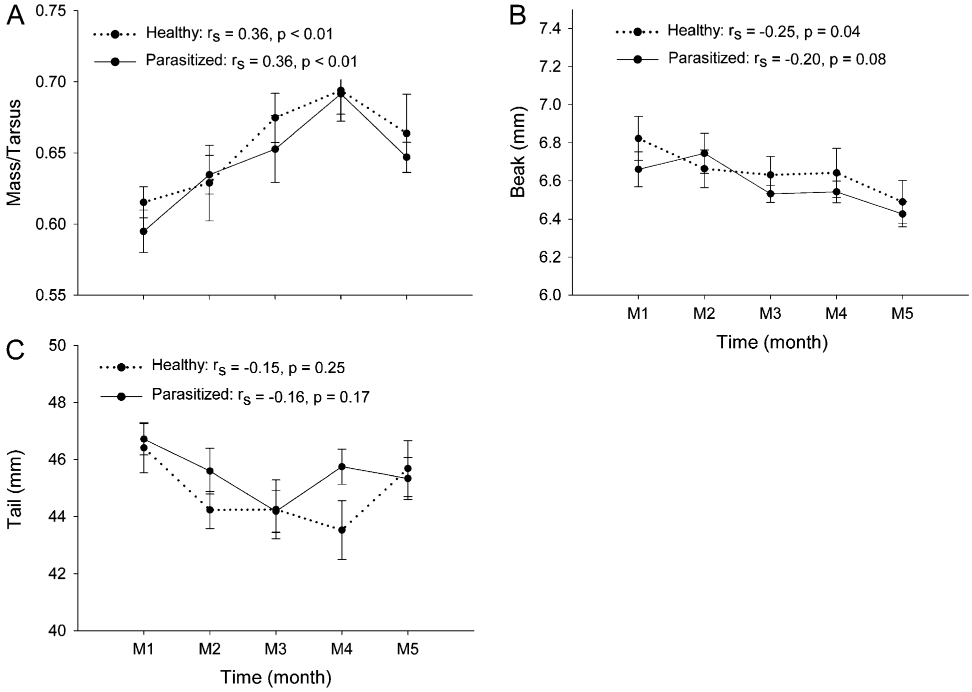

Figure 4
Effect of coccidian manipulation on female grassquit mass/tarsus, beak, and tail parameters (mean ± standard error; parasitized = solid line; healthy = dashed line), indicating that there was no effect of the treatment on female morphology. The 5 months of experimental procedures are indicated as M1–M5, and M1 refers to measures taken 1 month after experimental procedures were initiated.

species and were not affected by the parasite infection. One observation made during the experiments is that males did not vocalize during their display leaps, which is quite different from what occurs in natural conditions, where the display leap is usually coupled with a vocalization. The song component of the display may be important for female choice, and possibly, they may rely more on auditory than on visual cues, at least when both cues are presented together, which is usually the case with blue-black grassquits. Another ornamental trait not evaluated is the reflectance of the structural plumage of males. Both physical condition of male grassquits (Doucet 2002) as well as degree of endoparasitism (Costa and Macedo 2005) may affect the quality of reflectance of the plumage.

A final explanation for the lack of choice favoring healthy males is that the evaluation by females of both ornaments and physical condition of males may not be enough to guarantee greater fitness for females. In some species, the assessment of parenting abilities and/or nest sites by females may be essential (Soler et al. 1998; Svensson and Kvarnemo 2003). Obviously, the experiment in captive conditions did not address this possibility. Blue-black grassquit males invest heavily in parental care and defend territories that vary in size and occur in areas with specific vegetational structure (Barnard 1956; Alderton 1963; Almeida and Macedo 2001; Carvalho et al. 2006).

To summarize, the results of our study, based on a longitudinal sampling of 2 treatment groups of male blue-black grassquits, one healthy and the other infected with coccidian parasites, clearly support one aspect of the hypothesis of Hamilton and Zuk (1982). We found that parasites have a negative effect on male body condition. But more importantly, we demonstrated the adverse effects of parasites on male mating displays. Most studied effects of parasitism on sexually selected characters have focused on ornaments, and much less is known about negative impact on behavior. Our study emphasizes the need for research that examines the link between parasitism and behavior. We also found support for the concept that females are less susceptible than males to deleterious effects of parasitism (Folstad and Karter 1992). It would be enlightening to further examine female response to parasitism, as most studies focus on males. We found no support, however, for the prediction that females (healthy or otherwise) would choose males with more robust morphological and ornamental traits.

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