

**Tradeoffs Between Mounting an Immune Response and Territorial Singing Behavior in
Mountain White-crowned Sparrows**

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ABSTRACT

Sexually selected traits are often plastic and can thus provide information on infection status and parasite load. Mounting an immune response has been shown to require the diversion of energy and resources away from other life history functions. Previous studies have demonstrated that bird song may be influenced by an immune challenge. We investigated how mounting an immune response by mountain white-crowned sparrows *Zonotrichia leucophrys oriantha* affects specific aspects of territorial song and behavior. We used song playback to simulate a territorial intrusion and elicit baseline song and behavioral defense responses. Individuals were then either injected with a lipopolysaccharide (LPS) solution to induce an acute immune response, or a saline control. Post-treatment territorial song and behaviors in response to playback were recorded 1 day and 7 days later. The immune challenge significantly reduced the number of birds that sang compared to control individuals on Day 1. Alarm behaviors increased, and the few birds that did sing on Day 1 sang abnormal songs at significantly slower rates and with fewer terminal notes. By Day 7, the number of LPS-treated males that sang equaled the number of singing control birds, and song structure and rates returned to baseline levels. However, on Day 7, LPS-treated birds had elevated rates of alarm behaviors. Responses were variable and, with the exception of whether birds sang or not, comparisons that simply compared individuals within a time period across treatment and control were not as sensitive as those that compared the difference between baseline and post-treatment responses across treatment groups. This suggests that for the most sensitive assessment of immune status, prior experience of an individual's behavior is necessary. This study also reveals that simply mounting an immune response to infection, as opposed to the actual infection per se is sufficient to impact avian song production.

KEYWORDS

Zonotrichia leucophrys oriantha, sexual selection, bird song, immune challenge

INTRODUCTION

Song is an important aspect of avian life history because of its role in mate selection and territory defense (Catchpole & Salter 1995). According to Zahavi's handicap hypothesis (1975), song traits that are inter- or intra- sexually selected are hypothesized to be honest signals of an individual's quality if they impose a cost to the bearer. Specific costs of song identified in past studies include maintenance of specialized regions of the brain necessary for song learning and production, increased predation risk during singing bouts, and time and energy invested (reviewed by Gil and Gahr, 2002). Song used in territory defense may signal to potential intruders the resident's social status, in which case the singer may bear an additional cost of increased intraspecific aggression or attacks (Rohwer & Ewald 1981; Molles & Vehrencamp 2001).

As with many sexually selected traits, bird song has been shown to be a relatively plastic behavior and associated with multiple tradeoffs. Many birds increase singing rate in concurrence with female fertility, possibly indicating a greater investment in singing at a time of the year when females freely mate with multiple males (Møller 1988; Hutchinson et al. 1993). Evidence also points to a tradeoff between singing and mate guarding, with low singing rates during periods of mate guarding and increasing again during egg laying and incubation (Gil et al. 1999).

Despite an increasing number of studies assessing the impact of parasites and pathogens on the dynamics of free-living vertebrate populations (see Hudson et al. 2001, Combes 200x, Wobeser 2007 for reviews), as well as on the evolution vertebrate life history (Poulin et al. 200x, Hamilton & Zuk 1982; Gilman et al. 2007), many of the aspects of host-parasite interactions in

nature are not well understood (but see Ilmonen et al. 2000; Garamszegi et al. 2004; and Owen-Ashley et al. 2006). For example, the costs associated with infection are not clear and may either be the result of energy and nutrients being diverted away by the pathogen directly, or the outcome of resources committed to mount an effective immune response. While infection *per se* can have dramatic effects on host physiology, recent laboratory investigations have also revealed the surprising cost of actual immune response either in terms of the committed resources or because of autoimmune damage to the host's own tissue.

Since mounting an immune response requires diverting energy and resources away from other functions, one would expect repercussions on multiple aspects of life history including reproduction and investment in soma (Martin 2005, reviewed by Sheldon & Verhulst 1996; and by Lochmiller & Deernberg 2000).

Bacterial infections are probably the most common challenge on a vertebrate's immune system (CITE). Lipopolysaccharide (LPS) is the primary component of the outer membrane of Gram-negative bacteria and largely responsible for activating the acute phase response (Rietschel et al. 1994). In house sparrows (*Passer domesticus*), injection with LPS, which mimics a systemic bacterial infection and elicits a generalized sickness response, resulted in reductions of body mass, depressed parental feeding rates and a decrease in reproductive success (Bonneaud et al. 2003), thus providing support for a tradeoff between immune investment and reproductive activity (Ilmonen et al. 2000).

Given the patent energetic costs of avian song and demonstrated plasticity in song production (Møller 1988; Hutchinson et al. 1993; Gil et al. 1999), one might expect a tradeoff between mounting an immune response and investment in territorial song. Indeed, Gambell's white-crowned sparrows (*Zonotrichia l. gambelii*) injected with LPS experienced elevated

corticosterone levels and exhibited short-term hypothermia, depressed feeding and other physical activities, attenuated territorial aggression behavior and decreased singing during the parental period of the breeding season (Owen-Ashley et al. 2006).

We aimed to further understand the basis of behavioral adaptation to disease by exploring tradeoffs between disease and territorial song and behavior. Because LPS elicits a generalized immune response and associated sickness behaviors without the damage caused by an actual pathogen infection, it constitutes an ideal tool for evaluating the relative contributions of these factors to total cost of disease. Mountain white-crowned sparrows (*Z. l. oriantha*) are an ideal subject because their vocalizations are easy to record, quantify and analyze (Morton 2002). Building upon the study by Owen-Ashley et al. (2006), spectrograms of territorial song, song rate and territorial behavior were analyzed and compared before LPS exposure, during the acute phase response and 7 days following LPS exposure in order to ascertain any long-term effects of mounting an acute phase response.

If energy and resources to generate an immune response are acquired at the expense of territory-defense behaviors, then we expected that LPS-exposed individuals would reduce behaviors that are energetically costly. Since song rate and body reserves are positively related (Thomas 2002), the singing rate of LPS-exposed individuals was expected to decrease.

The number and quality of terminal notes in avian song is known to positively correlate to female preference (Vallet & Kreutzer 1995, Riebel & Slater 1998) and the degree of male-male response (Leitao & Riebel 2003, Nagle & Courox 2000), suggesting that terminal notes may be especially condition dependent. Soha and Whaling (2002) proposed that the terminal notes in the song of white-crowned sparrows (*Z. l. nuttalli*) carried information about the singer's motivation and were responsible for eliciting a territorial response in conspecifics, suggesting

that presence and number of terminal notes may be particularly condition dependent. If this hypothesis holds for mountain white-crowned sparrows, then the number of terminal notes sung by LPS-treated individuals should be expected to decrease. If song, the primary tool in territorial behavior (Catchpole & Slater 1995), was greatly compromised then a territorial intruder may intimidate an immune-compromised individual, resulting in an increase in non-energy intensive alarm behaviors and a decrease in aggressive behaviors.

MATERIALS AND METHODS

Study Sites and Animals

The study was conducted in two previously established study plots (anonymous, unpublished data) in mountain white-crowned sparrow habitat north of the Rocky Mountain Biological Laboratory, Gothic, CO (38°95'N, 106°98'W, 3150m asl.). The two study plots measured approximately 200 m by 400 m and were 200-300 m apart. The two study plots have similar vegetation cover which consists of a patchwork of alpine meadows and willow (*Salix* sp.) thickets. This study was performed during the breeding season (June and July 2009), when male territory defense was high (Morton 2002). Due to the invasive nature of the study and the strong effects of LPS reported previously (Owen-Ashley et al. 2006), we aimed to use a small, target sample size of 6 LPS-treated and 6 control individuals per study site, and we made an *a priori* decision to set our $\alpha = 0.10$ so as not to miss potentially weaker effects. All mountain white-crowned sparrow captured on the study plots since 1999 has been banded with a sequentially numbered, metal US Fish and Wildlife Service band (Bird Banding Laboratory, Patuxent, MD, USA) and a unique combination of three colored bands for visual identification in the field.

Playback Procedure and Song Recordings

Territories were identified based on locations where individuals had been heard singing. Within two weeks of identifying territories, a territory invasion was simulated in order to obtain baseline territorial song and behavior. Between the hours of 06:00 and 12:00 hours, an iHome iH80 directional speaker (SDI Technologies, Rahway, NJ, USA) was placed 1m off the ground and within 10m from the perch on which a male had been observed singing. One of four white-crowned sparrow songs, normalized to a peak amplitude of 80 dB was randomly chosen and played back from an iPod (Apple Corporation, Cupertino, CA, USA) at a rate of 15 songs every 2 min and was stopped once the resident entered the experimenter's view, at which time behavior was recorded for 5 minutes.

Song recordings were made from a distance of 5-10 m, depending on cover available to avoid alarming the individual. Only individuals that sang during the baseline playback procedure were included in the study. An individual's singing bout was recorded for a minimum of 2 min to obtain 10 songs with minimum background noise. Recordings were made with an Audix OM-3xb microphone (Audix Microphones, Wilsonville, OR, USA) and Tascam DA-P1 digital audio tape recorder (Tascam, Montebello, CA, USA). This playback procedure was repeated for each individual on day 1 and day 7 following treatment.

Song and Behavioral Measurements

The first 10 songs from each singing bout before treatment, and on day 1 and day 7 following treatment were analyzed using Raven Pro v.1.3 (Cornell Laboratory of Ornithology, Ithaca, NY, USA). We counted the number of terminal notes per song from spectrograms and calculated song rates from the first 2 min of each recording of a singing bout.

Territory-defense behavior was assessed by measuring rates over the 5-min period of behavior recording of: (1) attack on the speaker; and (2) flights directly above the speaker; (3) alarm calling [defined as ‘chink’ or ‘pink’ calls (see Morton 2002)]; (4) changes in perch positions less than 1 m away; and (5) flights greater than 1 m in distance. Since approaches to speaker decoys have been interpreted as aggressive behavior (Slater & Catchpole 1990; Leita0 & Riebel 2003), behaviors (1) and (2) were interpreted as direct acts of aggression, while behaviors (3), (4) and (5) were interpreted as lower-energy alarm responses. These behaviors were voice-recorded as point measurements in real time and subsequently analyzed using Jwatcher v. 1.0 (Blumstein and Daniel 2007).

Trapping and LPS Administration

Millet-baited potter traps were used between 06:00 - 12:00 h to capture recorded individuals. As individuals were added to the study, we administered to each either an immune challenge or control treatment on an alternating basis. Individuals undergoing immune challenge treatment were administered an approx. 0.2 ml subcutaneous injection of lipopolysaccharide (LPS; final concentration of 0.8mg/kg tissue) derived from *E. coli* (serotype 055:B5, Sigma product code: L4005) dissolved in saline and emulsified at a 1:1 v/v ratio in Freund’s incomplete adjuvant (Sigma Product Code: F5506). Injected LPS constitutes an effective target for the immune system and its injection mimics a bacterial infection precipitating a brief but generalized sickness syndrome (Adler et al. 2001). Addition of adjuvant to LPS has been shown to extend the acute phase response of white-crowned sparrows in captivity for up to 48 h following treatment (Owen-Ashley et al. 2006). Control individuals received a subcutaneous injection of a saline solution of equal volume (0.2 ml).

Statistical Analysis

We used Fisher's exact tests on the count data of the number of birds that sang on day 1 and day 7 after treatment to determine if LPS had a significant effect on whether or not treated birds sang during a territorial intrusion compared to control individuals. We calculated contingency coefficients C , as indices of the effect size for each comparison. We also used Student's t -tests on the difference between each individual's respective baseline and treatment behavioral responses on day 1 and day 7 to identify any significant behavioral effects of the LPS or control treatments. Effect sizes for t -tests were calculated using Cohen's d using the pooled standard deviation. In order to ascertain the importance of looking at individual differences in singing behavior, data were also analyzed using Student's t -tests as if repeated measures were not made for each individual by comparing treatment and control values for each playback experiment.

RESULTS

We obtained quality baseline song recordings from 43 out of the 53 males identified on the study plots. Twenty-one of these 43 males were successfully trapped; 11 males were administered LPS and 10 males were administered saline. During the day-1 playback experiment, from those individuals administered LPS, 9 approached the speaker, but only three of those sang. In contrast, all 10 control-treated individuals returned and sang during the day-1 playback experiment. Nine LPS and 10 control individuals responded to the speaker during the day-7 playback experiment. During the 7-d playback experiment, 8 LPS individuals sang with two individuals not returning to their territories, and all control-treated individuals returned to

their territories with 1 individual not singing. The number of individuals that returned and sang during all playback experiments is summarized in Table 1.

We found significant effects of LPS administration when we compared the difference from baseline values across control and treatment (Table 2). No significant effects of LPS treatment were found for analyses that simply compared treatments and controls at either the baseline period, day 1, or day 7 (Table 2).

On day 1 (Fig. 1), LPS birds were less likely to sing ($p = 0.001$, $C = 0.815$), sang at a slower rate ($p = 0.057$, $d = 1.518$), decreased the number of terminal notes per song ($p < 0.001$, $d = 3.083$), and had increased flight rates ($p = 0.047$, $d = -0.978$). Those LPS-treated individuals that sang on day 1 exhibited abnormal songs with respect to baseline spectrograms (Fig. 2).

On day 7, the LPS treatment had no effect on whether or not birds sang ($p = 0.587$, $C = 0.220$), and the song rates of LPS-treated individuals recovered to baseline levels ($p = 0.675$, $d = 0.221$). Though not highly significant, LPS had a large effect on the number of terminal notes in songs sung on day 7 ($p = 0.075$, $d = 0.991$). LPS injection had no significant effect on non-song related behaviors on day 7, however, LPS injected individuals increased their rates of alarm calling ($p = 0.100$, $d = -0.819$) and flight ($p = 0.102$, $d = -0.813$) (Figure 2).

DISCUSSION

The effects of the immunological challenge on territory defense were clearly evident on our first assessment following treatment (i.e. day 1). As expected from the plastic nature of sexually selected traits, song, a trait selected for by females and used in male-male interactions (Catchpole & Slater 1995), was the behavior most strongly impacted 1 day following an immune

challenge. By day 7 most behaviors returned to baseline levels, though lingering effects of mounting an immune response were still detected in behaviors unrelated to singing.

The number of birds that sang significantly decreased 1 day following the immune challenge. The few immune-challenged individuals that did sing on day 1, sang abnormal songs at a significantly slower rate and with fewer terminal notes with respect to their baselines compared to control birds. Interestingly, 8 of the 11 immune-challenged birds that returned to their territories during the playback experiment on day 1 while not singing did exhibit typical alarm behaviors. The fact that flight rates increased with respect to baseline levels on day 1 may suggest that a male unable to defend his territory through singing is generally more ‘fearful’ and flies around more and alarm calls more.

The number of terminal notes sung by immune-challenged individuals significantly decreased with respect to each individual’s baseline levels. A study by Soha and Whaling (2002) found that white-crowned sparrow *Z. l. nuttalli* songs incorporating terminal notes elicited a greater territorial response in conspecifics compared to songs lacking terminal notes. The fact that immune challenged individuals reduced the number of terminal notes from their baseline more than control birds supports Soha and Whaling’s results (2002) by demonstrating that number of endnotes is indeed an honest and sensitive signal of a singers’ quality. Male white-crowned sparrows may assess another male’s potential threat level based on the number of terminal notes an individual sings, under the assumption that healthier individuals singing more terminal notes pose a greater threat than males of poor condition. While song, as a whole, is hypothesized to be a sexually selected trait and therefore condition dependent, few studies have demonstrated that particular portions of song are more affected by infection than others (but see

Gilman et al. 2007). This experimental study demonstrates that terminal notes of mountain white-crowned sparrows are exceptionally susceptible to immune challenge.

Individuals faced with mounting an immune response that sang on day 1 also suffered depressed singing rates with respect to baseline levels. This result is consistent with previous studies demonstrating that song rate is linked with health condition. For example, the song rate of collared flycatchers (*Ficedula albicollis*) was found to decrease following an immune challenge (Garamszegi et al. 2004), and the singing rate of barn swallows (*Hirundo rusticca*) negatively correlated with lymphocyte and gamma-globulin concentrations, which are known to increase with pathogen infection (Saino et al. 1997). Female pied flycatchers are known to select males with high singing rates (Gottlander 1987).

The ability to sing recovered by day 7, indicated by the result that the number of immune-challenged birds that sang was statistically the same as control individuals. The song rate of LPS-treated individuals returned to baseline levels by day 7, but lingering effects of the experimental immune challenge were observed by large effect sizes of decreased number of terminal notes and elevated flight and alarm-call rates (Fig. 1).

Our analyses demonstrate that song is a very sensitive, honest signal of individual health status. In contrast to past studies (Gervasi et al. 2007) that revealed the impact of infection on song production, here we actually show that mounting an immune response is sufficient to impact song behaviors.

Our analyses also suggest that when assessing an individual's song, prior knowledge of that bird's song and behavior can greatly increase the accuracy of the conclusions. Thus, differences within a population may not be as important as shifts in an individual's behavior over time. This may have important consequences in terms of inter- and intra-sexual selection.

Implications for assessing immune status

This study implies that females seeking a potential mate can use territorial singing behaviors to assess the health condition in a sparrow. In particular, conspecifics can use both qualitative song characteristics (such as truncated songs lacking terminal notes) and the quantity of song produced to evaluate an individual's health status. Our results suggest that such an assessment is particularly effective if the evaluator has information on the baseline song of an individual: thus *changes* in an individual's singing behavior reflect *changes* in an individual's health status. Consequently, females may follow a male over a period of time to determine his suitability as a mate. Furthermore, if the resident male's singing behavior changes, then a nesting female may evaluate tradeoffs between abandoning her nest and renesting with a different mate, as opposed to raising her brood with decreased parental contribution from a sick male.

Results of this study are also relevant for intra-sexual selection. Male American redstarts *Setophaga ruticilla* (Nagle & Couroux 2000) and Nuttall's white-crowned sparrows (*Z. l. nuttalli*) (Soha & Whaling 2002) have been shown to respond more strongly to songs with terminal notes. The terminal notes of Puget Sound white-crowned sparrows (*Z. l. pugetensis*) are known to carry the singer's identity (Baptista 1977, Nelson 2004, Nelson 2007), and male *Z. l. pugetensis* respond more vigorously when a neighbor's terminal notes were substituted with that of a stranger (Nelson 2007). Although the significance of endnotes has not yet been explicitly tested in *Z. l. leucophrys*, the close phylogenetic relationships between the three subspecies, together with close similarities in song structure, suggest that terminal notes carry important information content in our study population, too. The results of the present study indicate that any shifts in the number of terminal notes resulting from an immune challenge will be communicated to conspecific neighbors allowing them to assess health status. Thus, given the

negative repercussions of communicating its impaired health status, a male may instead temporarily refrain from singing. We may have observed this effect in the immune-challenged males that responded to the simulated territory invasion with alarm behaviors but did not sing.

Some of the behavioral changes we observed, such as the collapse of regular song structure, are sufficiently severe or distinctive to be evident even in the absence of prior knowledge on the condition of a male. Other changes however, even though important, are more subtle and are statistically significant only after the baseline song characteristics of an individual are taken into consideration through across time comparisons. In the real world, this suggests that male sparrows may be best able to evaluate the immune status of a neighbor by continuously comparing song characteristics against a baseline of information of this particular individual. The implications of prior knowledge of individuals must be integrated into future models of condition-dependent mate choice.

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Table 1: Number of birds that sang and did not sing during the three playback experiments. The numbers in parentheses indicate the number of birds that did return to their territories during playback.

Table 1

	Control		Treatment	
	sang	did not sing	sang	did not sing
Baseline	10	0	11	0
Day 1	10	0	3	8 (1)
Day 7	9	1	8	3 (2)

Table 2: Student's t-test results of the repeated-measures analysis and analysis without repeat measures taken into consideration. Significant effects were found only for the repeated-measures analysis, indicating that changes in a specific individual's territorial behavior is important for determining the health status of that individual.

		Repeated-measures analysis								Analysis without repeated measures							
		Control mean $\pm$ S.D.	n	Treatment mean $\pm$ S.D.	n	P	d			Control mean $\pm$ S.D.	n	Treatment mean $\pm$ S.D.	n	P	d		
Baseline	no. terminal notes	3.040 $\pm$ 1.620	10	2.418 $\pm$ 2.027	11	0.450	0.354			3.040 $\pm$ 1.620	10	2.418 $\pm$ 2.027	11	0.445	0.354		
	song rate	6.538 $\pm$ 2.167	10	7.648 $\pm$ 2.775	11	0.323	-0.466			6.538 $\pm$ 2.167	10	7.648 $\pm$ 2.775	11	0.318	-0.466		
	rate of alarm calling	6.900 $\pm$ 10.243	10	1.745 $\pm$ 3.492	11	0.159	0.723			6.900 $\pm$ 10.243	10	1.745 $\pm$ 3.492	11	0.159	0.723		
	rate of speaker attack	0.000 $\pm$ 0.000	10	0.046 $\pm$ 0.106	11	0.178	-0.634			0.000 $\pm$ 0.000	10	0.046 $\pm$ 0.106	11	0.178	-0.634		
	flight rate	1.601 $\pm$ 1.669	10	0.599 $\pm$ 0.751	11	0.087	0.829			1.601 $\pm$ 1.669	10	0.599 $\pm$ 0.751	11	0.159	0.829		
	fly-over rate	0.135 $\pm$ 0.330	10	0.235 $\pm$ 0.545	11	0.621	-0.231			0.135 $\pm$ 0.330	10	0.235 $\pm$ 0.545	11	0.614	-0.231		
Day 1	no. terminal notes	0.130 $\pm$ 0.850	10	-2.633 $\pm$ 0.896	3	<0.001	3.499			3.170 $\pm$ 1.451	10	1.800 $\pm$ 2.163	3	0.390	0.928		
	song rate	-0.538 $\pm$ 1.944	10	-3.500 $\pm$ 2.784	11	0.057	1.518			6.000 $\pm$ 1.080	10	4.500 $\pm$ 2.500	3	0.409	1.128		
	rate of alarm calling	-2.120 $\pm$ 11.767	10	1.691 $\pm$ 4.859	11	0.360	-0.454			4.780 $\pm$ 7.894	10	3.436 $\pm$ 2.997	11	0.623	0.241		
	rate of speaker attack	0.000 $\pm$ 0.000	10	-0.046 $\pm$ 0.106	11	0.343	0.634			0.000 $\pm$ 0.000	10	0.000 $\pm$ 0.000	11	-	-		
	flight rate	-0.764 $\pm$ 1.689	10	1.351 $\pm$ 2.693	11	0.047	-0.978			0.837 $\pm$ 0.567	10	1.950 $\pm$ 2.421	11	0.166	-0.650		
	fly-over rate	-0.135 $\pm$ 0.330	10	-0.085 $\pm$ 0.749	11	0.846	-0.091			0.000 $\pm$ 0.000	10	0.151 $\pm$ 0.432	11	0.273	-0.507		
Day 7	no. terminal notes	0.356 $\pm$ 0.000	9	-0.938 $\pm$ 0.063	8	0.075	0.991			3.167 $\pm$ 1.929	9	2.138 $\pm$ 1.756	8	0.268	0.592		
	song rate	-0.669 $\pm$ 2.141	10	-1.109 $\pm$ 2.097	10	0.675	0.221			5.928 $\pm$ 0.812	9	6.766 $\pm$ 1.958	8	0.288	-0.610		
	rate of alarm calling	-2.000 $\pm$ 12.326	10	8.180 $\pm$ 13.849	10	0.100	-0.819			4.900 $\pm$ 5.223	10	8.920 $\pm$ 13.698	10	0.404	-0.409		
	rate of speaker attack	0.000 $\pm$ 0.000	10	-0.020 $\pm$ 0.063	10	0.343	0.471			0.000 $\pm$ 0.000	10	0.031 $\pm$ 0.098	10	0.343	-0.471		
	flight rate	-0.644 $\pm$ 1.888	10	0.624 $\pm$ 1.356	10	0.102	-0.813			0.957 $\pm$ 0.624	10	1.195 $\pm$ 1.047	10	0.547	-0.291		
	fly-over rate	0.002 $\pm$ 0.424	10	-0.239 $\pm$ 0.558	10	0.291	0.513			0.138 $\pm$ 0.310	10	0.020 $\pm$ 0.064	10	0.268	0.553		

Figure Legends

Fig. 1 The effect of LPS (t) and saline (c) injections on white-crowned sparrow territorial behaviors. Mean changes from baseline levels on day 1 (left) and day 7 (right) of: a) singing rate; b) number of terminal notes; c) flight rate; and d) rate of alarm calling. Each dot represents the value for an individual bird, the horizontal line marks the mean, the box ends indicate the 25th and 75th quartiles, and whiskers extend to the most extreme point lying within 1.5 times the interquartile range.

Fig. 2 Representative spectrographs of songs from baseline recordings (left) and 1 day following an immune challenge (right) of each of the three white-crowned sparrow males that sang on day 1. On day 1 the focal male: a) only sang the introductory whistle of his song at the same frequency as his baseline whistle of about 4.5 kHz; b) sang a song similar in structure and frequency to his baseline song but with fewer terminal notes; and c) sang a song with no terminal notes that differed in structure and frequency from his baseline song.

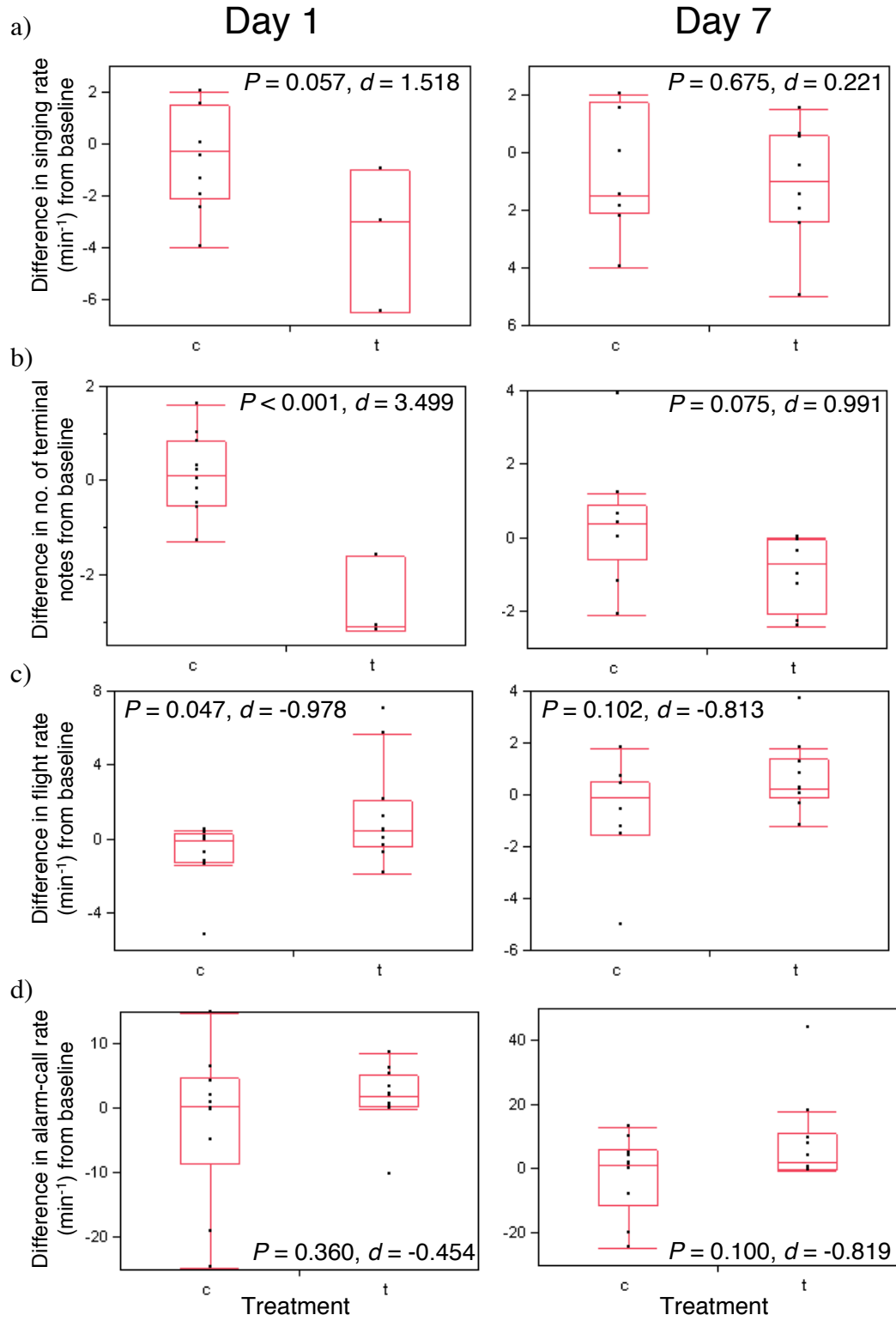
Figure 1

Figure 2