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Song rates of dark-eyed juncos do not increase when females are fertile

Abstract Frequency of singing by birds may vary with reproductive stage in ways that reflect variation in the functions of song in intersexual and intrasexual communication. In dark-eyed juncos (*Junco hyemalis*) high-amplitude song is produced only by males. To investigate the function of this song, we tested whether fertility of females affected singing by their mates or by neighboring males. Using focal observations, song censuses, and radiotracking data, we determined whether song production varied between and among periods when females were fertile and non-fertile. Our findings show that males do not increase song production when their mates are fertile, nor do they increase song production when neighboring females are fertile. These results suggest that male juncos do not signal their intent to defend territories (or mates) more when females are fertile and that they do not use song to advertise to specific potential participants in extra-pair fertilizations.

Key words Song · Dark-eyed junco · *Junco hyemalis* · Fertility · Reproductive behavior

Introduction

During the stages of the nesting cycle, songbirds sing at different rates and sometimes use different song types (e.g., Nolan 1978; Catchpole 1983; Logan 1983; Møller 1991). This variation suggests that the costs and benefits of song, as well as song function, vary with stage of the cycle (Brown 1969; Møller 1988). Studies of this variation are limited by the overlapping functions that song

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may possess. Identifying the correlates of differential song use over the nesting cycle provides one way to tease apart the functions of song, including its role in territoriality and mate attraction.

It has long been suggested that song is used in territorial establishment and defense (reviewed in Armstrong 1973; Catchpole 1982; Smith 1991). Experimental demonstrations in a few species have shown that song repels intruders from territories (i.e., song appears to be an announcement of the ability of a bird to defend an area, Catchpole 1982). An important benefit of territory ownership is access to receptive mates (Searcy and Yasukawa 1983). Even after a territory and mate have been acquired, the value of these may be lost if other territory owners expand their own boundaries (McDonald 1989) or obtain extra-pair fertilizations (Stutchbury et al. 1994). If the need to repel intruders is continuous and unchanging, song rates should also remain constant throughout the breeding season. Alternately, variation in song use could reflect differential timing of intrusion pressures. This in turn may be related to time of territorial establishment or stage of the nesting cycle (e.g., if neighbors intrude more often during fertile periods of females).

Møller (1988, 1991) hypothesized that song itself functions as an important means of guarding females from extra-pair copulations and, therefore, that song rates should be high during females' fertile periods. If song indicates a male's ability to defend a territory, high-quality males should sing most because they are most able to defend territories when challenges escalate. Specifically, the "fertility-announcement hypothesis" (Møller 1991) states that in species in which males guard mates, males should announce their mate's fertility – and advertise their own ability to guard her – by increasing the frequency of singing. Two important predictions of this hypothesis are (1) that song rates will peak during the female's fertile period (the period when copulations may result in fertilization of eggs) and (2) that increased song rates will decrease the probability of intrusions by neighboring males.

Song may also function as an announcement by males of their availability as mates to secondary females (Temrin 1986) or extra-pair copulatory partners (Sorjonen 1986; Møller 1988, 1991). In the latter case, males should increase song production in response to the presence of fertile females on nearby territories.

Dark-eyed juncos (*Junco hyemalis*) are territorial and socially monogamous passerines, and males guard females much of the time during their fertile periods (Chandler et al., in press). Despite these apparent efforts to protect paternity, extra-pair fertilizations account for 23–26% of young (bled at age 6 days) (Ketterson et al. 1996, 1997; S. Raouf, unpublished work). Song is most frequent during the breeding season and is thought to function in territorial defense and mate attraction (Hostetter 1961; personal observations).

The principal objectives of this paper are to test (1) whether song rates are higher when males' social mates are fertile, and (2) whether song rates are higher when neighboring females are fertile. We quantified singing behavior of male juncos at different stages of the nesting cycle and assessed whether song peaked during the fertile periods of their mates and of neighboring females.

Methods

We studied juncos during the 1993 and 1994 breeding seasons (April–July) at the University of Virginia's Mountain Lake Biological Station, Giles County, Virginia, USA (37°22′N, 80°32′W). The population at this site has been color-banded and studied intensively since 1983 (for details see Wolf et al. 1990; Ketterson and Nolan 1992; Ketterson et al. 1992, 1996; for a description of the study area see Chandler et al. 1994). Here we describe only methods relevant to this study. All birds in this study were uniquely color-banded for individual recognition.

We used three methods to quantify singing behavior. First, we conducted 15-min focal watches, determining the proportion of time that males spent singing. Every 30 s we recorded whether or not the focal male was engaged in a song bout. Because most songs within a bout are separated by ≤ 8 s (94%; R. Titus, unpublished work), we considered males to be engaged in a bout if they sang at least once within the 4 s prior to or following each 30-s point. During approximately half of all observations, song rate (number of songs per min) was also recorded. Males were followed for an average of three separate focal watches (for an average of 45 min/male). Observations were later assigned to one of two non-overlapping male groups. The first group consisted of males whose social mates were presumed fertile (i.e., during the period from 7 days before laying egg 1 through laying of the 3rd (usually the penultimate) egg (following Birkhead and Møller 1992) (*n* = 12 males). The second group was composed of males whose mates were presumed not to be fertile (i.e., because females were either incubating eggs or tending nestlings; *n* = 10 males). Neither date nor time of day differed between observations of the two groups (median date of observation was 21 May for those whose mates were presumed to be fertile versus 13 May for those whose mates were presumed not to be fertile, Mann-Whitney *U*-test, *U* = 50.5, *P* = 0.13; median time of day 1130 versus 1212 hours EST for the respective groups, Mann-Whitney *U*-test, *U* = 37, *P* = 0.85).

In our second method, we conducted 241 variable-radius point song counts from fixed locations within territories of 24 nesting pairs of color-marked juncos whose stage of reproduction was known (Reynolds et al. 1980). All pairs had complete clutches and were randomly assigned to one of two groups, which we termed residents and neighbors and treated as follows. After conducting counts of songs by a resident and his neighbor for 3 days, we removed all eggs from the nests of the 12 resident pairs. The removal of eggs caused resident females to become fertile and to build nests and complete replacement clutches within 8 days. On adjacent territories, the 12 neighbors retained their clutches. In most cases additional adjacent males were present; these varied in nesting stage. Before and after egg removal, we counted songs during 15-min periods and assigned them to resident, neighbor, or adjacent male categories based on acoustical characteristics (in this population most song types are unique to one male; R. Titus, unpublished work), location of singer, or visual identification. Counts prior to egg removal were considered to be within the non-fertile period. Those beginning on the day after removal through the day females laid their third egg were considered to be within the fertile period. Most counts were performed between 0600 and 1100 hours EST and on any given territory were conducted within 30 min of the same time throughout the trial period.

Our third method of quantifying singing used radiotelemetry to follow 13 male juncos during each of three periods: when their mates were fertile, when their mates were incubating (not fertile), and when the pair was feeding nestlings (not fertile). For this part of the study, fertile-period observations were restricted to the 3 days preceding laying of the first egg and the first 3 days of egg laying (a subset of the fertile period as previously defined). Males had been captured and equipped before monitoring with a small transmitter (*c*. 1.0 g, approximately 4.7% of body mass; Wildlife Materials, Inc.). They were then released and, beginning the next day, located every 0.5 h for approximately 8 h daily using a hand-held Yagi antenna. Each time a male was found, his behavior (e.g., singing, foraging) and the location of his mate, if known, were recorded. Estimates of song rates thus obtained are probably minimums, as some males stopped singing, apparently in response to our approach. Details of the radiotracking procedure, as well as data on male movements and behavior at other stages of the nesting cycle, are described by Chandler et al. (1994, in press). The male juncos in this sample were also part of a study of the effects of testosterone on male behavior; all were controls that carried empty subcutaneous implants, and we assume that their movements and songs were similar to those of unmanipulated males not equipped with transmitters. Ketterson et al. (1991) and Ketterson and Nolan (1992) provide details.

We wished to determine the probability of making type II errors for comparisons that showed no significant differences between groups. Since many comparisons required the use of non-parametric (Mann-Whitney *U*- and Wilcoxon signed-rank) tests and we are not aware of power tables for these statistics, we used the tables for *t*-tests in Cohen (1988; type I error set at 0.05, two-tailed). The power of the analysis of variance test (for radiotracking data) was obtained from Cohen's table for *F*-statistics. Because singing behavior was quite variable within groups (i.e., standard deviations were often as large as means), we expect only large effect sizes to be biologically significant and therefore estimated the power of detecting differences of 50% and 100% between means of the treatment groups. At these levels, probability of detecting a 50% difference ranged from 10 to 99% and that of detecting a 100% difference from 26 to 99% for all comparisons.

Results

Focal observations

The proportion of time focal males (see first method) spent singing did not differ between males whose mates were and were not fertile (*n* = 12, 10; Mann-Whitney *U*-test, *U* = 48, *P* = 0.41; Fig. 1A). Song rates also did not differ between these two groups of males (*n* = 5, 6; Mann-Whitney *U*-test, *U* = 11, *P* = 0.47; Fig. 1B).
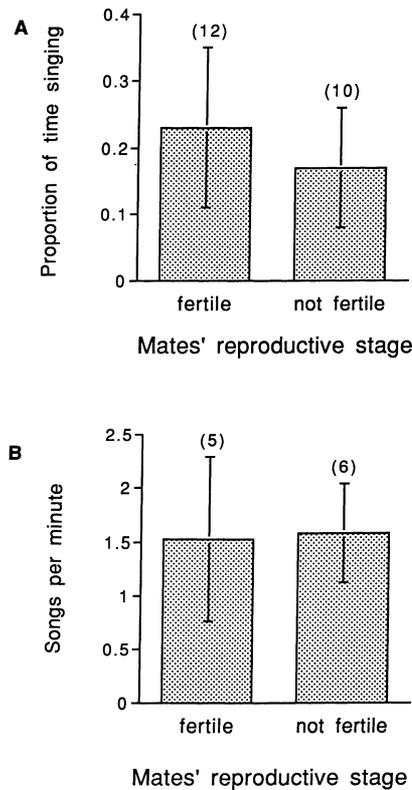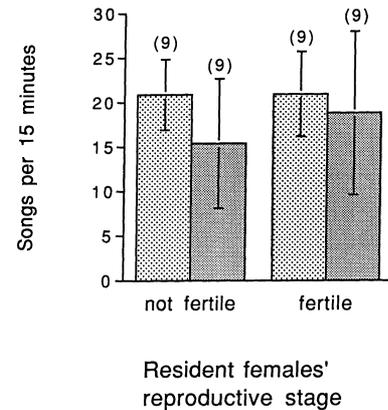
Fig. 1 A,B Singing behavior of male dark-eyed juncos when their mates were fertile and when mates were not fertile, as recorded during focal observations. **A** Proportion of time males were involved in song bouts. **B** Number of songs per 15 min. Sample sizes are shown in parentheses. Mean values are presented ± SE

Fig. 2 Song rates of male dark-eyed juncos before and after induced fertility of females. *Stippled bars* are rates while mates were incubating (not fertile) and then following removal of all eggs from nests (mates fertile). *Solid bars* are song rates of neighbors, whose nests were unmanipulated (thus their mates were not fertile). Sample sizes are shown in parentheses. Mean values are presented ± SE

Song point counts

In 3 of 12 cases (second method), neighboring males' clutches were lost to predation before the residents' mates had laid the third egg in their replacement (i.e., induced) clutches. Including counts only on days prior to loss of the neighbors' nests to predation, overall song production by resident males did not differ significantly with fertility status of females ($n = 12$; 15.8 ± 2.8 SE songs per 15 min during non-fertile period, 21.0 ± 5.1 during fertile period; Wilcoxon matched-pairs signed-ranks test, $z = -0.706$, $P = 0.48$). When neighbors had complete clutches (i.e., suffered no predation) throughout the entire trial, the number of songs resident males produced also did not change with their mates' reproductive stage ($n = 9$, Wilcoxon matched-pairs signed-ranks test, $z = -0.178$, $P = 0.86$; Fig. 2).

Comparisons with song rates of neighbors

Song production by neighbors (second method) did not differ significantly from that of residents with incubating mates (recall that residents and neighbors were in the same reproductive stage; $n = 9$; Mann-Whitney U-test, $U = 22$, $P = 0.10$; Fig. 2). Neither did it differ when

residents had fertile mates ($n = 9$; Mann-Whitney U-test, $U = 41$, $P = 0.97$; Fig. 2). Neighbors did not increase their song rates when fertile females were present on residents' territories ($n = 9$; Wilcoxon matched-pairs signed-ranks test, $z = 0.530$, $P = 0.59$). The number of neighbors and other adjacent males that sang during counts was not affected by the fertility status of the residents' mates ($n = 9$; 0.57 ± 0.12 SE during non-fertile period, 0.61 ± 0.21 during fertile period, Wilcoxon matched-pairs signed-ranks test, $z = 0.070$, $P = 0.94$).

Radiotracking data

Song rates of males in the radiotelemetry experiment (third method) did not differ with reproductive stage. The proportion of observations during which males sang was 0.08 ± 0.01 SE during fertile periods, 0.08 ± 0.01 during incubation, and 0.04 ± 0.02 during nestling stages ($F = 1.16$, $df = 2,27$, $P = 0.33$).

Discussion

Using three methods to quantify song production by male juncos, we found that song rates did not differ significantly between periods when males' mates were presumed to be fertile and when they were presumed not to be fertile. Our results do not support the hypothesis that male juncos increase song rates to announce their ability to defend (1) fertile mates or (2) territories during the period when mates are fertile (Møller 1991). Similarly, recent non-experimental studies (Hanski 1992; Hanski and Laurila 1993) found that male common chaffinches (*Fringilla coelebs*), another socially monogamous, territorial species, do not appear to announce

fertility of mates through changes in song rate. Other studies have found reduced (e.g., indigo bunting, *Passerina cyanea*, Thompson 1972; prairie warbler, *Dendroica discolor*, Nolan 1978) or increased (e.g., yellowhammer, *Emberiza citrinella*, Møller 1988) song rates during fertile periods, indicating that the relationship between song and reproductive stage varies among species.

Males on neighboring territories did not increase their song production when resident females became fertile. Thus, it seems likely that they did not vary their advertisement of availability for copulation according to reproductive stage of potential mating partners. Other possibilities are (1) that neighbors did not detect changes in the fertility status of resident females, or (2) that they did detect the change and modified other, unmonitored aspects of singing behavior (e.g., use of song types or changes in structure within types) to indicate availability. The possibility that neighbors are unable to assess fertility status of nearby females is worth consideration, since juncos occupy large territories in dense habitat (see below). Several species do use different song types over stages of the reproductive cycle (Catchpole 1982), but our data do not allow us to determine whether juncos do so. We did not quantify structural variation within song types in this study, although we did not detect any such variation and evidence from other populations of juncos (Konishi 1964) indicates that such variation most often is minimal. The majority of songbird species produce structurally stereotyped, invariant songs within any one breeding season (Kroodsma 1982). For example, no audible differences were detected in chaffinch songs produced during different reproductive stages (Marler 1956; Hanski and Laurila 1993). A limited number of studies have found that qualities such as frequency range and timing of successive syllables can be modified to announce different motivational states of males (Weary et al. 1990; Smith 1991).

Because of its unvarying production, high-amplitude song in juncos is most likely a signal of territory ownership, a broadcast advertisement for secondary mates, or both. Although this was not tested in this study, previous studies have supported a territorial function of song (Krebs et al. 1978; Yasukawa 1981). The use of song in seeking to attract additional, secondary mates, however, is difficult to address. Increased song rates when mates are incubating are sometimes taken as evidence that males invest more in advertisement for secondary mates when their social mates are preoccupied (Dickinson and Falls 1989). Alternatively, this increase may be the result of increased time available to them to sing. For example, Carolina wrens (*Thryothorus ludovicianus*) provided with supplemental food increased their song rates (Strain and Mumme 1988).

The finding that male juncos do not appear to increase song production when their mates or the mates of their neighbors are fertile suggests that other factors may override any advantage of such announcement. Maintaining constant levels of high-amplitude, long-range signals, regardless of fertility of mates, might allow males to conceal the fact that mates are receptive or at least to make this information difficult for neighbors to obtain. In dense habitats, the observable visual cues produced during mate guarding and nest building may be effectively hidden from conspecifics. Given the dense mixed woodland typical of many junco territories and the relatively large size of junco territories (*c*. 2 ha, Chandler et al. 1994, in press), it seems likely that information potentially available to neighbors during fertile periods would degrade before being detected by them or would be unlikely to be observed. Similar studies of other species in which a female's status is likely to be either easy or difficult to detect seem warranted.

References

Armstrong EA (1973) A study of bird song. Dover, New York

Birkhead TR, Møller AP (1992) Sperm competition in birds. Evolutionary causes and consequences. Academic Press, London

Brown J (1969) Territorial behavior and population regulation in birds: a review and re-evaluation. Wilson Bull 81:293–329

Catchpole CK (1982) The evolution of bird sounds in relation to mating and spacing behavior. In: Kroodsma DE, Miller EH (eds) Acoustic communication in birds, vol 1. Academic Press, New York, pp 297–319

Catchpole CK (1983) Variation in the song of the great reed warbler *Acrocephalus arundinaceus* in relation to mate attraction and territorial defense. Anim Behav 31:1217–1225

Chandler CR, Ketterson ED, Nolan V Jr, Ziegenfus C (1994) Effects of testosterone on spatial activity in free-ranging male dark-eyed juncos. Anim Behav 47:1445–1455

Chandler CR, Ketterson ED, Nolan V Jr (in press) Effects of testosterone on use of space by male dark-eyed juncos, *Junco hyemalis*, when their mates are fertile. Anim Behav

Cohen J (1988) Statistical power analysis for the behavioral sciences. Lawrence Erlbaum, Hillsdale, New Jersey

Dickinson TEJ, Falls B (1989) How western meadowlarks respond to simulated intrusions by unmated females. Behav Ecol Sociobiol 25:217–225

Hanski I (1992) Territorial behaviour and mixed reproductive strategy in the chaffinch. Ornis Scand 23:475–492

Hanski I, Laurila A (1993) Variation in song rate during the breeding cycle of the chaffinch, *Fringilla coelebs*. Ethology 93:161–169

Hostetter DR (1961) Life history of the Carolina junco *Junco hyemalis carolinensis Brewster*. Raven 32:97–145

Ketterson ED, Nolan V Jr (1992) Hormones and behavior: an integrative approach. Am Nat 140:S33–S62

Ketterson ED, Nolan V Jr, Wolf L, Ziegenfus C, Dufty AM Jr, Ball GF, Johnsen TS (1991) Testosterone and avian life histories: the effect of experimentally elevated testosterone on corticosterone and body mass in dark-eyed juncos. Horm Behav 25:489–503

Ketterson ED, Nolan V Jr, Wolf L, Ziegenfus C (1992) Testosterone and avian life histories: effects of experimentally elevated testosterone levels on behavior and correlates of fitness in the dark-eyed junco (*Junco hyemalis*). Am Nat 140:980–999

Ketterson ED, Nolan V Jr, Cawthorn MJ, Parker PP, Ziegenfus C (1996) Phenotypic engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. Ibis 138:70–86

Ketterson ED, Parker PG, Raouf SA, Nolan V Jr, Ziegenfus C, Chandler CR (1997) The relative impact of extra-pair fertilizations on variation in male and female reproductive success in dark-eyed juncos (*Junco hyemalis*). Ornithol Monogr, in press

Konishi M (1964) Song variation in a population of Oregon juncos. Condor 66:423–436

Krebs Jr, Ashcroft R, Webber M (1978) Song repertoires and territory defence in the great tit. Nature 271:539–542

Kroodsma DE (1982) Song repertoires: problems in their definition and use. In: Kroodsma DE, Miller EH (eds) Acoustic communication in birds, vol 2. Academic Press, New York, pp 125–146

Logan C (1983) Reproductively dependent song cyclicity in mated male mockingbirds (*Mimus polyglottus*). Auk 100:404–413

Marler P (1956) Behaviour of the chaffinch. Behaviour 5:S1–184

McDonald MV (1989) Function of song in Scott's seaside sparrow *Ammodramus maritimus peninsulae*. Anim Behav 38:468–485

Møller AP (1988) Spatial and temporal distribution of song in the yellowhammer *Emberiza citrinella*. Ethology 78:321–331

Møller AP (1991) Why mated songbirds sing so much: mate guarding and male announcement of mate fertility status. Am Nat 138:994–1014

Nolan V Jr (1978) Ecology and behavior of the prairie warbler *Dendroica discolor*. Ornithol Monogr 26:1–595

Reynolds R, Scott J, Nussbaum R (1980) A variable circular-plot method for estimating bird numbers. Condor 82:309–313

Searcy W, Yasukawa K (1983) Sexual selection and red-winged blackbirds. Am Scientist 71:166–174

Sorjonen J (1986) Song structure and singing strategies in the genus *Luscinia* in different habitats and geographical areas. Behaviour 91:274–285

Smith WJ (1991) Singing is based on two markedly different kinds of signaling. J Theor Biol 152:241–253

Strain JG, Mumme RL (1988) Effects of food supplementation, song playback, and temperature on vocal territorial behavior of Carolina wrens. Auk 105:11–16

Stutchbury BE, Rhymer JM, Morton ES (1994) Extrapair paternity in hooded warblers. Behav Ecol 5:384–392

Temrin H (1986) Singing behaviour in relation to polyterritorial polygyny in the wood warbler (*Phylloscopus sibilatrix*). Anim Behav 34:146–152

Thompson WL (1972) Singing behavior of the indigo bunting, *Passerina cyanea*. Z Tierpsychol 31:39–59

Weary D, Norris DK, Falls J (1990) Song features birds use to identify individuals. Auk 107:623–625

Wolf L, Ketterson ED, Nolan V Jr (1990) Behavioural responses of female dark-eyed juncos to the experimental removal of their mates: implications for the evolution of male parental care. Anim Behav 39:125–134

Yasukawa K (1981) Song and territory defense in the red-winged blackbird. Auk 98:185–187

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