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Song in the cold is 'hot': memory of and preference for sexual signals perceived under thermal challenge

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The environmental conditions under which signals are perceived can affect receiver responses. Many songbird populations produce a song chorus at dawn, when, in cold habitats, they would experience thermal challenge. We recorded temperature and the song activity of Lincoln's sparrows (*Melospiza lincolnii*) on a high-elevation meadow, and determined that song behaviour is concentrated around the coldest time of the day, at dawn. We hypothesized that this is because male song in the cold is more attractive to females than song in the warm. To test this, we exposed laboratory-housed Lincoln's sparrow females to songs at 1°C and 16°C, which they naturally experience in the wild. Females spent 40 per cent more time close to the speaker during playback at 1°C than at 16°C. When tested at 16°C 1–2 days later, females biased their movement towards the speaker playing songs previously heard at 1°C over 16°C. Thus, female Lincoln's sparrows remembered and affiliated with songs they heard under thermal challenge, indicating that the thermal environment can affect the attractiveness of a sexual signal.

Keywords: dawn chorus; temperature; *Melospiza lincolnii*; mate choice; songbird

1. INTRODUCTION

Variation in sexual signals can convey information about the quality of signallers [1]. By contrast, invariant signals may also convey such information depending on environmental conditions, as producing a signal under challenging conditions suggests the ability to tolerate this challenge. For instance, male songbirds singing at dawn may advertise their ability to tolerate cold. Indeed, song produced in the cold can be thermally challenging as metabolic rates escalate with temperatures plunging below the thermal neutral zone [2,3].

Lincoln's sparrows (*Melospiza lincolnii*) breed in habitats in which the temperature during their dawn song chorus (mean = 7.8°C, see §2*b*) is well below their lower critical temperature (estimated as 23°C from the closely related and syntopic white-crowned sparrow (*Zonotrichia leucophrys*) [4]) and would thus pose a thermal challenge for the singers. This raises the hypothesis that song heard in the cold is more attractive to female Lincoln's sparrows than the same song heard in warmer conditions. We also hypothesized that females could form a memory of the thermal context in which they hear male songs, and show preferences for songs previously heard in the cold over those previously heard in moderate temperatures.

2. MATERIAL AND METHODS

(a) *Field study*

During the breeding season of Lincoln's sparrows on our field-site (Molas Pass, CO, USA; 37.74° N, 107.69° W; 3250 m [5]), we recorded every 5 days (from 20 June to 10 July 2011) from 14.00 to 13.55: (i) temperature every 5 min with a centrally placed, shaded data logger (HOBO U12, Onset Computer Corp., Bourne, MA, USA), and (ii) Lincoln's sparrow song using an omnidirectional microphone (Sennheiser ME-66/K6, Wedemark, Germany) connected to a digital audio recorder (Marantz PMD 660, Mahwah, NJ, USA).

(b) *Laboratory experiment*

In summer 2010, we collected fledglings from our field site and transported them to the University of North Carolina at Chapel Hill, USA. We exposed them daily to recorded tutoring songs until age 60 days. Zoogen Incorporated (Davis, CA, USA) determined their sex molecularly from blood samples.

On 25 February 2011, we transferred females to indoor cages on an 8 L : 16 D photoperiod, and on 13 April 2011, we changed the photoperiod to 14 L : 10 D. On 26 May 2011, we subcutaneously implanted them with a tube (Silastic Laboratory Tubing, Dow Corning Corp., Midland, MI, USA; 1.47 (i.d.) × 17.00 mm; sealed with silicone at both ends) packed with crystalline 17β-oestradiol (Steraloids Inc., Newport, RI, USA) to elevate sensitivity to song [6].

We exposed individuals to one set of songs in cold temperatures (1°C) on one morning and another set of songs in moderate temperatures (16°C) on another morning before exposing them on a third morning to both song sets in a phonotaxis paradigm in moderate temperatures (see the electronic supplementary material, figure S1). We used two 'temperature chambers' for the temperature manipulations on the first 2 days (75 × 75 × 150 cm internal W × D × H; Percival Scientific, Inc., Perry, IA, USA), and two 'phonotaxis chambers' for the phonotaxis trial (58 × 41 × 36 cm; Industrial Acoustics Company, New York, NY, USA) on the third day. We equipped each chamber with two perches, water and food all within a cage; a video camera (B/W CCD Camera, Super Circuits, Austin, TX, USA) controlled by a computer running MULTICAM SURVEILLANCE software (Ingram Technologies, Price, UT, USA); and one (temperature chambers) or two (phonotaxis chambers) speakers (Pioneer TS-G1041R, Tokyo, Japan). We connected each speaker to an amplifier (Audiosource Amp 5.1A, Portland, OR, USA) attached to an audio interface (M-Audio Delta 1010, Irwindale, CA, USA) and a computer running iTunes 7 (Apple Inc., Cupertino, CA, USA). Lights turned on at 08.00 and off at 22.00. In one temperature chamber, we exposed the female to a cold morning followed by a moderate morning, whereas in the other, we exposed another female to a moderate morning followed by a cold morning. During the first morning, we played songs of male 1, and during the second morning we played songs of male 2. In the evening of the second day, we transferred each female to its phonotaxis chamber constantly held at a moderate temperature. The next morning we antiphonally played songs of male 1 from the left speaker, and songs of male 2 from the right speaker. Song playbacks (rate: 1 song per 10 s) occurred from 08.00 to 08.30 in temperature chambers, and from 08.35 to 09.05 in phonotaxis chambers. We repeated the procedure with seven female pairs.

We used songs from two free-living banded males recorded in our study site, and for which we had three variants (based on the number of syllables within phrases) of two different song types (based on spectral properties and the order in which phrases were arranged, electronic supplementary material, figure S2). Using RAVENPRO v. 1.4 (Cornell Laboratory of Ornithology, Ithaca, NY, USA), we standardized each song according to its peak power.

In temperature chambers, temperature declined progressively from 16°C at 18.00 to 1°C at 08.00 and then increased back to 16°C at 18.00. We chose these temperatures based on data from a United States National Oceanic and Atmospheric Administration weather station located on our field site (minimum, mean and maximum temperatures: −4.9, 7.8 (s.d. 4.5), and 19.8°C, respectively, between 05.00 and 07.00, for the period 1 June to 15 July 1999 to 2010).

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(**c**) ***Analysis***
To estimate the diel singing pattern in the field, we counted songs for each 3 min period beginning at the top of every half-hour over each of the five 24 h recordings.

From video recordings, we calculated the time females spent in the upper part of the third of the cage close to the speakers, either on the perch nearest the speaker or on the cage wire (affiliation time, see the electronic supplementary material, figure S1). In the phonotaxis chambers, we measured both their immediate response and their response over the 30 min of playback. For the immediate response, to correct for the side where females were immediately before the playback, we subtracted the affiliation time for each speaker during the 20 s before playback began from the affiliation time of the first 20 s of playback (one song from each temperature regimen). To analyse affiliation time, we used mixed-effects linear regressions with the temperature treatment as a fixed factor and the individual as a random intercept and random coefficient on temperature treatment with unstructured covariance.

3. RESULTS

(**a**) ***Diel temperature and song activity***
At night, temperatures decreased until 06.35 and increased afterwards. Singing activity started by 05.00, showed a large peak at 05.30, and rapidly decreased thereafter (figure 1).

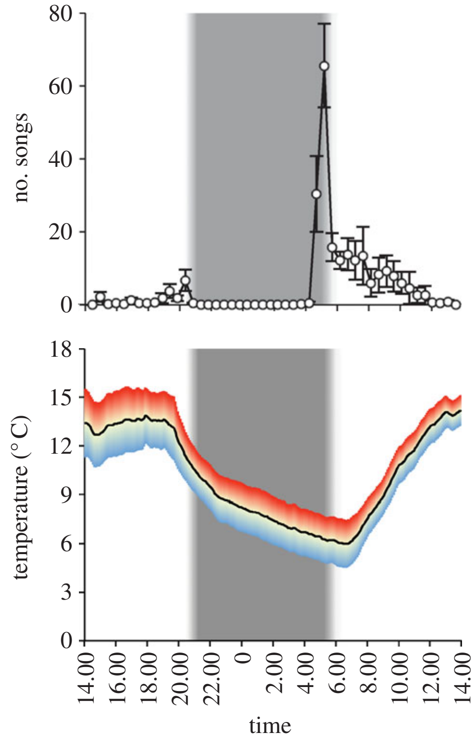

Figure 1. Diel variation in song production and ambient temperature (mean ± s.e.) during the breeding season of Lincoln's sparrows at Molas Pass, CO, USA. The grey area represents night-time estimated from NOAA Solar Calculator.

(**b**) ***Temperature-dependent song preference***
Over the 30 min of playback in temperature chambers, females showed 40 per cent more affiliation time at 1°C than at 16°C ($z = -2.03$, $p = 0.042$; figure 2*a*).

(**c**) ***Memory of and preference for song from the cold***
Females increased their immediate affiliation time on the side of the phonotaxis chamber playing the song from 1°C and decreased their affiliation time on the side of the phonotaxis chamber playing the song from 16°C ($z = -2.69$, $p = 0.007$; figure 2*b*). This bias was also observed over the entire playback (figure 2*c*) but was not statistically significant ($z = -1.15$, $p > 0.2$).

4. DISCUSSION

Male Lincoln's sparrows sing primarily around dawn, when low temperatures are physiologically challenging. Females show behavioural biases towards songs heard in the cold and remember them, at least for a day or two, consistent with the hypothesis that the dawn chorus in this species reflects the collective effort of males advertising their quality, perhaps in the form of tolerating a challenging thermal environment.

For sparrows, dawn represents an energetic challenge owing, in part, to a cold-induced increase in energy expenditure [7]. Dawn thus seems an appropriate time for females to assess the quality of prospective mates. Biasing responses towards song heard in the cold may therefore result in the females' choosing males that meet some minimum requirements in terms of energetic condition. Male songbirds may depart breeding grounds when climatic conditions present challenges [8] and sing later when conditions have improved. However, females would not know they had been present when conditions were difficult, and their song upon their return in less challenging conditions would, according to our results, be less attractive.

Females remembered the thermal characteristics associated with the song of each male, which should allow them to compare males previously heard at different times of the day, in different thermal contexts. This memory would also allow them to remain in their roosts or forage while listening to males, and later respond to their song when conditions have improved. At that time, signals other than song may also be important, perhaps explaining why the bias towards songs previously heard in the cold had declined by 30 min of song exposure. This decline may be owing to the absence of a reinforcing response or additional signals from males (e.g. visual signals) [9].

As dawn choruses may also occur when temperatures are not necessarily challenging [10], our findings are unlikely to suggest a basis for the dawn chorus broadly. Moreover, low temperatures are not the only challenge dawn presents. For example, diurnal songbirds endure a long fast at night that negatively affects their energetic reserves available for singing at dawn [11]. Females may prefer songs they hear at dawn because of its association with the end of this fast [12,13], which, once daylight arrives, might pressure males in poor condition to focus on foraging rather than singing. However, males with sufficient energy reserves may opt to sing instead of foraging, and therefore advertise their capacity to endure the challenge of a fast, which would only be compounded by cold temperatures.

Although we have interpreted our results in the adaptive framework that males may be advertising their cold hardiness with their dawn song, our results do not rule out the possibility that temperature-induced change in song acoustics (e.g. wavelength) or signal processing (e.g. change in metabolic rate, attention or memory

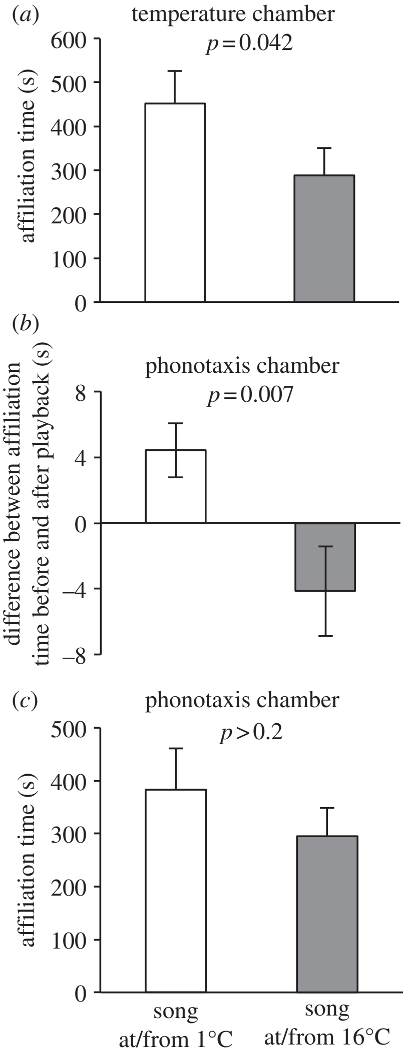

Figure 2. (*a*) Mean (±s.e.) affiliation time of female Lincoln's sparrows during 30 min exposure to male songs at 1°C and 16°C. (*b*) Mean (±s.e.) difference in affiliation time between the 20 s preceding playback and the first 20 s of playback of song previously heard at 1°C and 16°C. (*c*) Mean affiliation time (±s.e.) during 30 min of playback of song previously heard at 1°C and 16°C.

consolidation) drive female responses. Regardless, our results raise questions about how climate change might affect male signalling and female choice in songbirds breeding in cold environments. Unless Lincoln's sparrows move up in latitude or elevation, climate change may impact the persistence and thus the function of song in the cold.

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