

Interval singing links to phenotypic quality in a songbird

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Darwin was fascinated by melodic performances of insects, fish, birds, mammals, and men. He considered the ability to produce musical notes without direct use the most mysterious endowment of mankind. Bird song is attributed to sexual selection, but it remains unknown how the expected relationship between melodic performance and phenotypic quality arises. Melodies consist of sequences of notes, and both Pythagoras and music theorists in the Middle Ages found that their tonal frequencies form simple ratios that correspond to small-integer proportions derived from the harmonic series. Harmonics are acoustically predictable, and thus form the basis of the natural, just tuning system in music. Here I analyze the songs of the great tit (*Parus major*), a bird with a stereotyped song of typically two notes, and test the prediction that the deviations of the intervals from small-integer frequency ratios based on the harmonic series are related to the quality of the singer. I show that the birds with the smallest deviations from small-integer ratios possess the largest melanin-based black ventral tie, a signal that has been demonstrated to indicate social status and dominance, past exposure to parasites, and reproductive potential. The singing of notes with exact frequency relationships requires high levels of motor control and auditory sensory feedback. The finding provides a missing link between melodic precision and phenotypic quality of individuals, which is key for understanding the evolution of vocal melodic expression in animals, and elucidates pathways for the evolution of melodic expression in music.

bird song | melodies | evolution of music | overtones | Parus major

An essential element of vocal performance in many animals and in most music produced by humans are melodies based on defined intervals between sequential notes. Within given tuning conventions, musicians train for years to sing or play well-tuned intervals. Pythagoras showed that a vibrating string, partitioned at the middle, two thirds, or three quarters of its length, produced more consonant sounds (i.e., a pure octave with a 1:2 frequency ratio of the lower to the upper note, a pure fifth with a 2:3 frequency ratio, and a pure fourth with a 3:4 frequency ratio) than when partitioned in more complex ratios. The inclusion of 4:5 and 5:6 ratios by Zarlino (1) and other music theorists in the Middle Ages led to a natural scale of notes, also known as pure or just tuned scale. The tonal frequencies of the two notes of such intervals are found in the overtones or harmonics of natural sounds. The two notes of an interval with small-integer frequency ratios show greater spectral similarity with the harmonic series of their common fundamental or tonic than notes of intervals with complex frequency ratios (2), and are thus supposed to evoke higher sensory consonance (3). In an experimental study, 6-mo-old children were able to discriminate between intervals based on simple frequency ratios of 2:3 (a pure fifth) and 3:4 (a pure fourth) compared with more complex intervals based on a ratio of 32:45 (one of the several possible tritones); an inherent processing bias for the perception of intervals with simple small-integer frequency ratios has been suggested (3).

Many bird species sing melodies based on sequences of intervals (4) showing similarity to melodic expression in both Western and non-Western music. Among the very few bird species analyzed so far, the melodic songs of the hermit thrush (*Catharus guttatus*) are

composed of flute-like sounds of stable pitch, whose frequency relationships form small-integer ratios based on the harmonic (overtone) series (5). Similarly, musician wrens (*Cyphorhinus arada*) use intervals with small-integer frequency ratios of 1:2 (octave), 2:3 (fifth), and 3:4 (fourth) (6).

In animal vocalization with melodies composed of stable-pitched sounds, the missing link for a Darwinian understanding of the use of intervals based on small-integer frequency ratios is the relationship with life-history traits, and hence the role of such intervals in sexual selection as the main driver for the evolution of birdsong (7). Singing specific intervals is not trivial, as it requires a high level of motor control over the many fine muscles used for producing sounds in the syrinx of birds or the larynx of mammals (8). For fine tuning this control, a highly sophisticated auditory sensory, motor, and neuronal system is required, first for producing a pitched tone, second for the perception and analysis of the produced sound, and third for the feedback to the vocal structures for fine adjustments (8). Furthermore, if the precision in the use of intervals and singing performance has been under selection imposed by receivers, the receiver also requires the sensory and neuronal capacities to judge the precision of the sung intervals. On the basis of the mentioned theoretical and empirical studies (2, 3, 5, 6), not only for the producer but also for the receiver of a signal aiming to deduce information about phenotypic qualities of a singer, the most logical and the only natural reference intervals should thus be based on small-integer frequency ratios derived from the harmonic series. Most important, such a yardstick does not require the use of human-specific concepts of music theory or practice (5), as in the many scales used in Western and non-Western music based on forms of tempered tuning and invented for the needs of performing orchestral music and modulating in all keys.

Here I use the great tit (*Parus major*) as a model species to evaluate interval singing precision, measured as the deviation from

Significance

Many animals—insects, fish, frogs, birds, and mammals—sing sequences of pitched melodic tones to communicate with conspecifics. As noted by Pythagoras (AD 1500) and later music theorists, the tonal frequencies of structurally important notes in melodies form small-integer ratios, based on the harmonic series of natural sounds. Here I show for a songbird with a simple two-note song, the great tit, that males with large black ventral ties, which are a signal of social status and reproductive potential, sing intervals with smaller deviations from the predicted small integers than males with small ties. This study illuminates a pathway for the evolution of melodic singing in animals, and perhaps of music in general, where defined intervals are a key feature.

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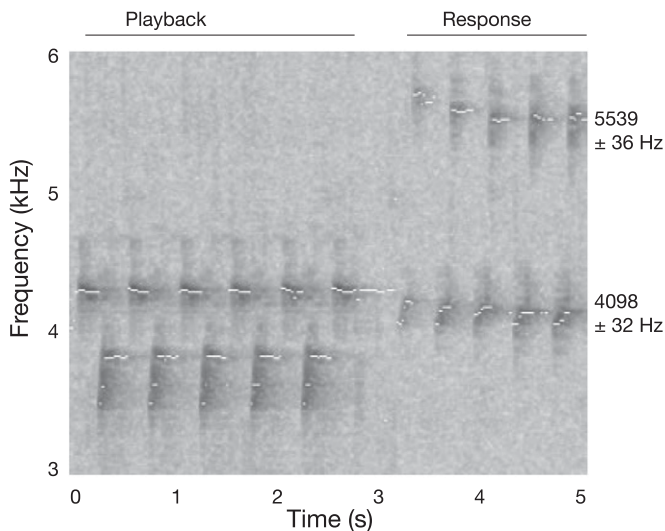


Fig. 1. Typical response of a great tit male to a playback of a computer-generated song. The playback consists of five and a half two-note phrases in a strophe, the response of five two-note phrases in a strophe. Ten such strophes were selected for each song type to calculate the frequencies of the two notes in the response interval. Sound analysis software (Raven Pro-1.4) was used to autogenerate selections and to identify for each selection window the frequency (\pm SD) showing the strongest amplitude (white lines). The response interval shown here has a frequency ratio of 4,098:5,539 Hz = 0.7398, which is 24 cents off the predicted perfect fourth with a frequency ratio of 0.75 (see *Methods* for calculation of deviation in cents).

predicted small-integer frequency ratios, in relation to a phenotypic trait: the black-melanin-based ventral tie, known to signal dominance, social status, and previous parasite exposure. The great tit has a simple and stereotyped song structure, most often a sequence of two-note phrases in which the notes differ in tonal frequency (9). If three notes are sung, then two of them are typically identical in duration and frequency. An individual male can have a repertoire of half a dozen or more different songs (10). It is striking to a human listener that the intervals used are often identifiable as seconds, major or minor thirds, fourths, and fifths, with the major and minor thirds being the most common. It is also evident from listening to great tits that the intervals are not always perfect. Hence, the question arises whether the deviations from a predicted interval carries some information about the quality of the singer and sender of the signal. For assessing the precise tonal frequencies of interval notes used by great tits, I provoked great tit males with a typical but computer-generated great tit song. To this end, I extracted a single note from a great tit song to create an interval of a second and a fourth on the computer, and then repeated it to produce a typical great tit song for playback (Fig. 1). Such a computer-generated song from a single note minimizes the risk that the song may hide other unknown properties of the song related to phenotype that could influence a response. I then played these songs back to male great tits in their breeding territories and recorded their response. Responses were immediate as soon as the challenged male appeared in its territory.

One week, on average, after the playback, the males were captured, biometrically measured, and had a standard ventral photograph taken for assessing the area of their tie, a melanin-based stripe of black feathers that extends from the ventral beak area to the hips. Males have significantly bigger ties than females, and there is large variation in tie size among males (11, 12). Tie size has been shown to be a reliable signal of social dominance (12–14), and also of previous parasite exposure and parasite resistance (15). I then analyzed the frequencies at peak amplitude of both notes in a song sequence, calculated their ratio, and classified

the intervals according to the closest interval, based on small-integer values shown in Fig. 2 (*Methods*). Intervals close to a minor third and a major third were sung most frequently; intervals close to a major second, a perfect fourth, a tritone, and a perfect fifth moderately often; and intervals close to a minor second and a major sixth rarely.

Next I calculated the deviation in cents of each interval (*Methods*) in a song sequence from the closest pure interval, based on small-integer values. The males' precision of singing pure intervals was strongly and significantly related to the area of their tie (Fig. 3) [tie area: $F = 18.39$ ($P = 0.0003$); male age: $F = 0.06$ ($P > 0.81$); tarsus length: $F = 0.66$ ($P > 0.42$); $r^2 = 0.47$; $n = 27$ males], independent of their age or body size. The males with the smallest average deviations from the closest small-integer interval frequency ratios had around 80% bigger ties than the males with strong deviations. The width of the tie was larger for males singing more precise intervals [tie width: $F = 12.44$ ($P = 0.0019$); male age: $F = 0.08$ ($P > 0.77$); tarsus length: $F = 0.15$ ($P > 0.70$); $r^2 = 0.37$; $n = 27$ males]. Using a general linear mixed model that included all 76 songs recorded and male identity as a random effect led to the same results. Furthermore, the males with small mean deviations from such intervals also had larger song repertoires [repertoire size: $F = 5.09$ ($P = 0.009$); male age: $F = 0.03$ ($P > 0.85$); tarsus: $F = 0.85$ ($P > 0.37$); $r^2 = 0.44$; $n = 27$ males], a trait previously demonstrated to correlate with the rate of copulation solicitations by females (16), as well as with lifetime reproductive success (17) of males. The absence of an age effect suggests experience does not play a role.

The great tit is a socially monogamous species with strong defense of a breeding territory, most often in forests but also in gardens and orchards. The rate of extrapair matings of great tit females is relatively high (17), and the proportion of extrapair offspring in broods varies in our populations from 10% to 20%

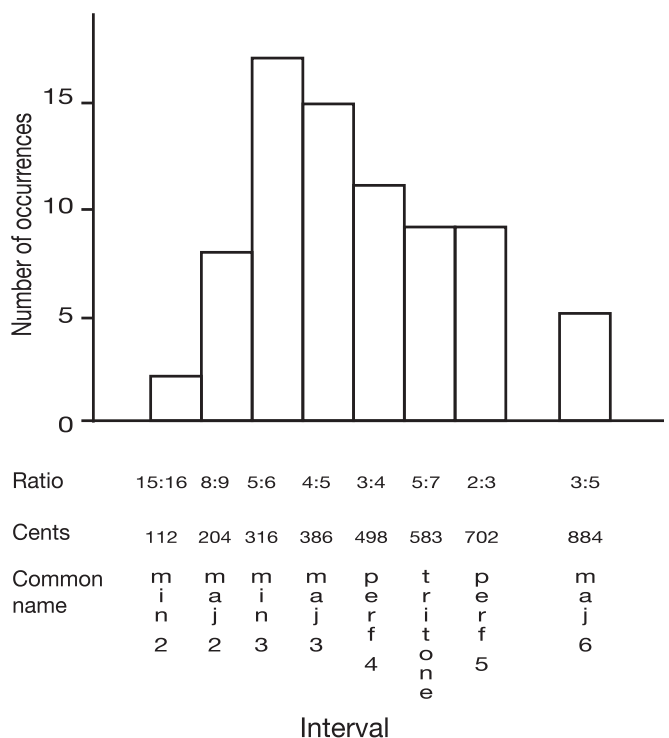


Fig. 2. Intervals used by great tits in response to playbacks. Seventy-six responses of 27 males were classified according to the closest small-integer ratio of the frequencies of the lower to the upper notes drawn from the harmonic series.

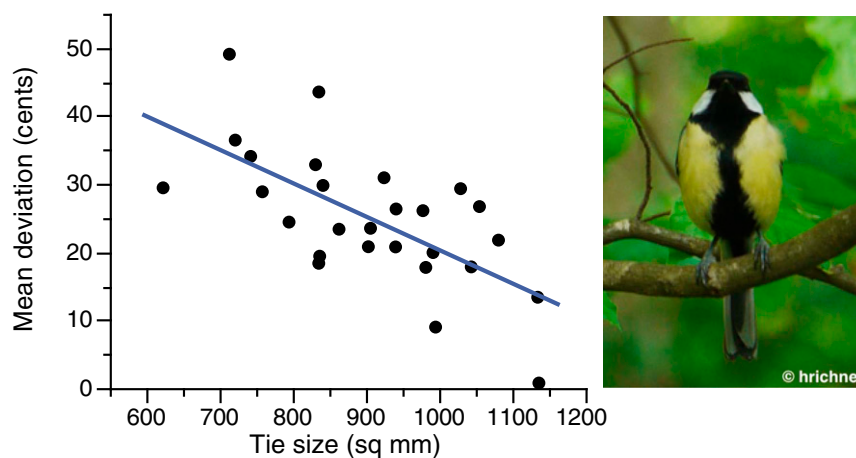


Fig. 3. Interval precision and tie size. Mean deviation ($n = 27$ males) from intervals predicted by small-integer proportion of frequencies of the two notes drawn from the harmonic series in relation to the area of the black melanin-based tie.

among years. Tie size has repeatedly been shown to be a signal of dominance in interactions between males (12, 14), and offspring sired by males with larger ties were found to grow faster, be heavier, and have higher survival (11). Tie size can thus serve as a signal of male quality for both intra- and intersexual selection processes. High-quality females mate with males with larger ties, and males with larger ties have a significantly higher reproductive success (11, 12). Tie size after molt is affected by exposure to ectoparasites during the previous breeding period (15). Thus, the finding that deviations from pure intervals are highly significantly related to the area of the black tie suggests the quality of the interval can be used by males and females for judging singer quality, and hence promote both processes of intra- and intersexual selection. In contrast to a visual signal such as a melanin-based black tie, a song can be heard over long distances, and thus allow for an assessment of an opponent during male conflicts or of a potential mate for an extrapair mating, also at distance in forests and dense vegetation. The suggestion that the males with small deviations are of higher phenotypic quality is supported by the finding that males with larger song repertoires sing intervals with smaller deviations.

Theoretically, a few more intervals with small-integer frequency ratios could be considered for calculating deviations, beyond the ones used here (as listed in Fig. 2); for example, 4:7 (0.57 equals 969 cents), 6:7 (0.857 equals 267 cents), 5:8 (0.625 equals 814 cents), 7:8 (0.875 equals 231 cents), 7:9 (0.778 equals 435 cents), and so on. To test whether the inclusion of additional small-integer ratios would strengthen the relationship between tie size and the deviation from the nearest predicted pure interval, I included all possible small integers up to the 16th harmonic (Table 1).

The inclusion of these further small-integer ratios into the analysis led to several orders weaker or no relationships between tie size and the precision of sung intervals (see P values in Table 1) compared with the correlation using the subset of ratios shown in Fig. 2. This subset is part of the same musical scales that evolved in much of Western and non-Western music. One feature of this subset is the maximization of tonal spacing between semitones, which may reduce perception errors (e.g., with signal degradation) and enhance sensory consonance (2, 3). Semitones are spaced roughly from between 80–120 cents in pure tuning and exactly 100 cents in tempered tunings. A recent study on humans (18) found that sung tones require a mistuning of at least

Table 1. All possible small-integer intervals from the fifth to the 16th harmonic within the interval range of great tit songs, and the significance of the correlation between deviations and tie sizes

Nth harmonic	Small-integer intervals based on the harmonic series	P -value of correlation
5	2:3, 3:4, 3:5, 4:5	0.138
6	2:3, 3:4, 3:5, 4:5, 5:6	0.028
7	2:3, 3:4, 3:5, 4:5, 5:6, 4:7, 5:7, 6:7	0.052
8	2:3, 3:4, 3:5, 4:5, 5:6, 4:7, 5:7, 6:7, 5:8, 7:8	0.065
9	2:3, 3:4, 3:5, 4:5, 5:6, 4:7, 5:7, 6:7, 5:8, 7:8, 7:9, 8:9	0.086
10	2:3, 3:4, 3:5, 4:5, 5:6, 4:7, 5:7, 6:7, 5:8, 7:8, 7:9, 8:9, 7:10, 9:10	0.123
11	2:3, 3:4, 3:5, 4:5, 5:6, 4:7, 5:7, 6:7, 5:8, 7:8, 7:9, 8:9, 7:10, 9:10, 7:11, 8:11, 9:11, 10:11,	0.071
12	2:3, 3:4, 3:5, 4:5, 5:6, 4:7, 5:7, 6:7, 5:8, 7:8, 7:9, 8:9, 7:10, 9:10, 7:11, 8:11, 9:11, 10:11, 7:12, 11:12	0.448
13	2:3, 3:4, 3:5, 4:5, 5:6, 4:7, 5:7, 6:7, 5:8, 7:8, 7:9, 8:9, 7:10, 9:10, 7:11, 8:11, 9:11, 10:11, 7:12, 11:12, 8:13, 9:13, 10:13, 11:13, 12:13,	0.260
14	2:3, 3:4, 3:5, 4:5, 5:6, 4:7, 5:7, 6:7, 5:8, 7:8, 7:9, 8:9, 7:10, 9:10, 7:11, 8:11, 9:11, 10:11, 7:12, 11:12, 8:13, 9:13, 10:13, 11:13, 12:13, 9:14, 11:14, 13:14	0.587
15	2:3, 3:4, 3:5, 4:5, 5:6, 4:7, 5:7, 6:7, 5:8, 7:8, 7:9, 8:9, 7:10, 9:10, 7:11, 8:11, 9:11, 10:11, 7:12, 11:12, 8:13, 9:13, 10:13, 11:13, 12:13, 9:14, 11:14, 13:14, 11:15, 13:15, 14:15	0.534
16	2:3, 3:4, 3:5, 4:5, 5:6, 4:7, 5:7, 6:7, 5:8, 7:8, 7:9, 8:9, 7:10, 9:10, 7:11, 8:11, 9:11, 10:11, 7:12, 11:12, 8:13, 9:13, 10:13, 11:13, 12:13, 9:14, 11:14, 13:14, 11:15, 13:15, 14:15, 11:16, 13:16, 15:16	0.623
Subset as in Fig. 2	2:3, 3:4, 4:5, 5:6, 5:7, 8:9, 15:16	0.0002

60 cents for being reliably identified as out of tune by listeners. Thus, in music a spacing of half-notes of at least 60 cents seems reasonable for reduction of perception errors, but for birds, this threshold is not known. There are other, interlinked reasons for the use of intervals from this specific subset: First, harmonic overlap below the minimum distance for discrimination by human ears, as occurs with more complex frequency ratios, leads to stronger perceived sensory dissonance (ref. 19, but see ref. 20); second, the lower harmonics of a tone are more important in defining virtual pitch (the fundamental) (21); third, intervals based on small-integer frequency ratios elicit more consonant virtual pitches (22); and fourth, the two notes of intervals based on small integers share a higher proportion of harmonics with the set of harmonics of their common fundamental (2); that is, they show higher spectral similarity than is the case for intervals with notes forming more complex frequency ratios, as demonstrated recently (2). The fact that six of the eight intervals recorded here for great tits (Fig. 2) are identical to the six highest-ranked intervals based on spectral similarity of the harmonic series in the mentioned analysis (2), together with the observed relationship between interval precision and a biologically important trait, lend support to the hypothesis of a biological rationale for the choice of specific subsets of notes in melodic vocalization in animals, and thus also to the recent proposition of a biological rationale for the several most widely used five- and seven-note scales in much Western and non-Western music, defined by the overall spectral similarity of intervals among the scale notes to a harmonic series (2).

Very few studies have rigorously investigated the frequency relationships among notes in melodic vocalizations of birds and other animals (5, 6). For more complex melodies than the song of the great tit, the analysis of the frequency relationships requires a rigorous method for estimating the fundamental frequency. So far, only one such approach has been used (5) and applied to the complex song of the hermit thrush. A link with important life-history traits, as was performed in the present study, was not possible because of the absence of such information, although it showed convincingly that the hermit thrush sings melodies with intervals corresponding to small-integer ratios drawn from the harmonic series. The notes from 57 of 71 hermit thrush songs analyzed showed significant relationships with the harmonic series of the estimated fundamental, and it may well be that the remaining songs were from individuals unable to control song properly. Thus, the observed variation may correspond to variation in phenotypic quality among the singers. The singing of intervals is a ubiquitous trait, as Darwin noted in his many descriptions of animal vocalization from fish to frogs to monkeys. Understanding the evolution of singing intervals predicted by the harmonic series requires an understanding of its related selection process; that is, it requires an analysis of the observed variation in relation to life history traits and fitness. In a Darwinian framework, selection can occur if potential receivers can infer phenotypic qualities of a sender from the precision of the sung intervals, as shown here. It also suggests that observed deviations from intervals predicted by small-integer frequency relationships should not be taken as evidence that animals do not use intervals similar to the ones used in pure tuned music (i.e., based on just intonation) but, rather, that one should search for the biological causes of deviations from such intervals.

Great tit song is characterized by sequential singing of interval notes. The notes, as is typical for most songbirds, do not show a strong harmonic spectrum. Hence, it remains an open question of how great tits tune the intervals to correspond to given harmonics. It has been demonstrated (23, 24) that at the source in the syrinx, the sounds comprise the typical harmonics, but then undergo resonance filtering of harmonics, resulting in near pure tone whistles. Thus, the harmonics may be available for use as a reference but would need a sensory pick-up system operating at the source, rather than via the ear. Alternatively, the harmonics may

not be required as a template for singing pure-tuned intervals, and singers may learn these intervals via a preference of the receivers. As suggested (5), a preference may arise via a sensory bias or a facility of memorizing frequencies related by small-integer ratios, and only the males growing up under optimal conditions would have the required sensory and motor control for adjusting the intervals so perfectly as to be able to sing well-tuned intervals.

This study demonstrates a strong relationship between the precision of interval singing and functional traits known to correlate with the social status of males, offspring survival, and reproductive success. Intervals are also the most prominent feature of music in humans, both vocal and instrumental, and thus the current study may have bearing on the evolution of music in our species, which to Darwin remained a mystery, given the absence of a use (25), in his view.

Methods

Study Population. The study was conducted in 2012 in a natural population of great tits breeding in nest boxes in a large wood west of Bern (46°7'N, 7°8'E), Switzerland. The wood is dominated by large beech trees. The average distance between nest boxes corresponds to the diameter of several typical great tit territories in nest box populations. Great tits in this wood have been studied for more than 20 y in the context of host-parasite interactions and maternal effects. As part of a standard procedure, nest boxes were visited regularly from the start of the breeding season at the beginning of March to determine for each breeding pair the onset of egg laying, incubation, and hatching of nestlings. The present study was done within the framework of another study that involved manipulation of reproductive effort, and hence reproductive parameters are not further analyzed in the present study. Two weeks after hatching, both parents were caught, measured, and ringed, if not ringed in previous years, and a photograph of the ventral body side taken using a camera mounted onto a stand at a distance of around 25 cm. The bird was held in a fixed, stretched-out position for a short moment. A ruler was glued to the camera stand and later used as a scale reference during image analysis.

Playbacks. For the construction of playbacks, typical two-note songs of five great tit males were recorded in another large forest close to Bern (46°57'N, 7°24'E), using a condenser shotgun microphone (KMR 82; Georg Neumann) connected to a preamplifier (FP23; SHURE), placed at a distance of 10–15 m, onto a laptop (MacBook Air11 recording with Sound Studio 4.6 Felt Tip, Inc., software). Songs were recorded as mono files in AIFF/WAV format at a sampling rate of 44.1 KHz with 24-bit sample size. A single note with stable nonfrequency modulated pitch was identified and selected, using RavenPro 1.4 software (Cornell Lab of Ornithology, Bioacoustics Research Program), and saved in WAV 24-bit format. This sound file was then used to create a typical two-note great tit phrase, where the pitch of one of the two notes was manipulated to create intervals of approximate (± 20 cents) major second, minor third, major third, and perfect fourth. Intervals were deliberately not fabricated as exactly pure-tuned notes to exclude imitations that could be taken as singing of intervals as predicted. Imitations occurred rarely, and when they occurred, one note only approximately matched a note of the template, and the other note was of a different frequency, resulting therefore in a different interval. The fabricated two-note phrase was then copy-pasted to create a full strophe (Fig. 1), and the strophes were then repeated five times and interspersed with quiet interstrophe periods to create a typical great tit song. A 30-s pause was added at the end of a song. The length of the entire song template was therefore around 60 s.

In the field, I played the looped song back to males in their breeding territory. A speaker (FOXPRO Scorpion x1B; FoxPro, Inc.) placed at the bottom of the nest box tree was remote-controlled (FOXPRO TX-200) from a distance between 20 and 30 m. Males usually responded immediately by singing typical songs and searching for an intruder. The playback was continued for 10 min and was then switched to a new song for another 10 min. Responses of males varied from one to four different song types within the 20 min of recording time per focal male.

Picture Analysis. The surface of the melanin-based black ventral stripe extending from the beak to the hips was measured from photographs, using the free ImageJ software (version 1.46c; National Institutes of Health; <https://imagej.nih.gov/ij/>). See Figs. S1 and S2 for details of the measuring procedure.

Sound Analysis. Sound files were analyzed using the Raven sound analysis software (Raven Pro-1.4 Build 4; Cornell Lab of Ornithology, Bioacoustics

