

The intensity of male-male interactions declines in highland songbird populations

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Abstract

Elevation gradients are associated with sharp environmental clines that play a crucial role in the phenotypic diversification of animal populations. In a variety of organisms, the reproductive output of females declines with elevation in parallel to the drop in environmental productivity and shortening of the breeding season. Little evidence is available on male traits associated with reproductive activities, such as territorial defence and signalling, which may decline because of the low economic defendability of resources and the selective advantage of investing in parental rather than mating (e.g. signalling, chasing intruders) effort in such conditions. Along a broad elevational gradient, we investigated variation in the intensity of territorial defence and sexual signalling in males of the water pipit *Anthus spinoletta* exposed to song playbacks simulating the territorial intrusion of a conspecific. We found that birds from the lower limits of the species distribution approached song stimuli more closely than those from the upper limits. Moreover, physically challenging songs (broad frequency bandwidths and fast trills) elicited a closer approach, and low elevation birds uttered songs ending with the broadest bandwidths. Other responses to the intrusion, such as the number of songs uttered or the latency to approach, exhibited seasonal or spatial variation irrespective of elevation. This study illustrates the decline of some trait associated with aggressive territorial behaviours during male-male conflicts along elevation, and points to the allocation in sexual signalling and motor constraints to signal production, as potential mechanisms underlying it.

Key words: birdsong, elevational gradient, sexual selection, territoriality

Introduction

The sharp variation in environmental conditions along elevational gradients is an important agent of phenotypic diversification in wild populations (Körner 2003; McCormack and Smith 2008). The decline in temperature, solar energy, productivity and the increase in seasonality of resources, are the most striking constraints faced by mountain-dwelling organisms, which have evolved a series of characteristics that permit breeding and persistence in these conditions (Lomolino 2001; Hille and Cooper 2015). Besides direct anatomical or physiological adaptations to cold temperatures, life-history differentiation is the most commonly documented output of shifting selection pressures along elevation. Mainly because of the drop in resources and short breeding or growing seasons, reduced reproductive output and increased investment in self-maintenance (survival) or parental care have been observed both within and among species (Wied and Galen 1998; Wilhelm and Schindler 2000; Badyaev and Ghalambor 2001). Trade-offs among traits come to light along these gradients, and apart from the above mentioned survival-reproduction trade-off, a compromise between offspring quality and quantity has been documented. As an example, females of high-elevation populations lay fewer but larger eggs in many ectotherm species (Berven, 1982; Hancock et al. 1998; Wilhelm and Schindler 2000), and endotherm females may reduce offspring number but augment parental care to enhance juvenile survival (Krementz and Handford 1984; Festa-Bianchet et al. 1994; Badyaev 1997a; Bears et al. 2009).

In species with bi-parental care, such as many songbirds, females and offspring in highlands may depend more strongly on male parental assistance to cope with poor or scattered resources (Badyaev and Ghalambor 2001). In a short breeding season, this forces males to shift rapidly from sexual to parental behaviours. This, in turn, requires testosterone to be maintained at low levels or to rapidly decline to avoid testosterone-driven aggressiveness, which may undermine reproductive success (Ketterson and Nolan 1994; Wingfield and Hunt 2002; Apfelbeck and Goymann 2011; Villavicencio et al. 2014). The balance between

parental and mating effort may further tilt towards the former if high elevation populations present low densities, which reduce opportunities for additional mating and extra-pair fertilizations and thus for aggression involved in the establishment of territories and mate-guarding behaviour (Westneat et al. 1990; Møller and Birkhead 1994). This has important implications for the expression of costly sexual traits: Badyaev (1997b) and Snell-Rood and Badyaev (2008) found less pronounced plumage dimorphism and shorter, less elaborate songs in high elevation Cardueline finches. The density and distribution in time and space of resources also largely change along elevations and may play a role in the spatial variation of behaviours displayed during male-male conflicts (Laiolo 2013; Laiolo et al. 2015).

Apart from their underlying evolutionary importance, sexual selection studies along climate gradients can also provide cues on the responses of species to global warming, and individual behaviour may become a crucial ultimate driver or indicator of a population's response to climate change (Chamailé-Jammes et al. 2006; Candolin and Heuschele 2008). In this study, we analyse elevation clines of male-male interference and signalling in the water pipit *Anthus spinoletta*, a monogamous passerine that shows intense song activity and territorial behaviour during the short alpine breeding season (Rehsteiner et al. 1998). We quantified male responses to the playbacks of conspecific songs, which served to simulate territorial intrusion, and analysed the differences in male songs along elevation. For the hypothesis of declining male-male competition with elevation to be supported, (1) males breeding at higher elevations should show weaker territorial behaviours (i.e., longer distance of approach to stimuli, delayed approach, less intense song activity) than males from lower elevations; and (2) territorial signals (songs) of high and low elevation males should differ in the responses they elicit. In particular, we expect that features of songs associated with male fighting ability will influence male receiver responses, and for a trait to reveal these characteristics, it must be costly to produce (Searcy and Nowicki 2005). We predict that males will respond more strongly to stimuli indicating individual condition or size (songs

with low minimum frequencies), the ability to sing physically challenging songs (fast trills with wide frequency bandwidths), or the investment in singing (long-lasting songs) (Gil and Gahr 2002; Laiolo et al. 2004; Illes et al. 2006; Cardoso et al. 2007; Cardoso and Hu 2011).

Methods

This study was performed in Picos de Europa National Park, eastern Cantabrian Mountains (NW Spain), in June-July 2012 (Fig. S1, Table S1). Due to historical forest clearance, the study area is covered by grasslands and pastures with interspersed limestone and low shrubs from 800-1300 m a.s.l. to the upper alpine belt (Laiolo et al. 2015). This broad elevational range supports high densities of the water pipit *Anthus spinoletta*, a ground-nesting, insectivorous bird that is territorial during breeding but partially erratic outside of reproduction (Rehsteiner et al. 1998). In the study area, water pipit breeding density peaks at 1250 m a.s.l., but remains relatively high from 1000 to 1900 m, dropping outside this range because of warmer temperatures and forest cover (lower limits) and rock cover (upper limits) (Melendez and Laiolo 2014). The winter re-sighting of a few ringed individuals in the Atlantic coast, ≈ 20 km from their mountain breeding grounds, suggests that local water pipits may perform short displacement in winter (GB et al. unpublished data). In spring, as the snow cover melt, birds reach their breeding grounds, and males tend to occupy the breeding sites of previous years: we captured over 450 birds and we detected movements > 3000 m in one individual only (a female, breeding 450 m downhill with respect to the previous season; GB et al. unpublished data).

To attract a female, establish and defend territories, males perform conspicuous parabolic song flights (Rehsteiner et al. 1998). Females incubate and males fed females and chicks during the incubation and nestling phases; second broods are rare but re-nesting after early nest loss can occur (Rauter and Reyer 2000; Bollmann and Reyer 2001). Two trilled strophes, formed by repetitive broadband syllables, constitute the water pipit song in the study

area and are also commonly uttered by birds from the Alps (strophe “A” and “C” of Rehsteiner et al. (1998)) (Fig. S2). We used the song of individuals recorded in the study area as a stimulus to simulate territorial intrusion, a technique that has been successfully used with pipits to study territorial and sexual behaviours (Petruskova et al. 2014) and to lure birds for capture-mark purposes (Melendez et al. 2014). We performed playback tests at four sites, separated by 3.5 – 20 km: two sites were at the lower limits of the species’ distribution (one at 1091 – 1193 m and the other at 1050 - 1225 m) and two sites at the upper limits (one at 1853 - 2152 m and the other at 1763 - 2193 m) (Fig S1, Table S1). These sites were the object of an extensive study since 2009, hosted similar, and high, densities of the water pipit, and had similar cover of grasslands, the preferred habitat of the species (Table S1; see also Melendez and Laiolo 2014; Melendez et al. 2014). Thus, they represented an ideal system to test *ceteris paribus* the effect of elevation.

The study was performed at the peak of the breeding season to minimize variation in another potential factor conditioning behaviour, breeding stage (see also below). In mid-June, we recorded the songs of 25 males in the study sites and their surroundings, and in early July, when snow disappeared from breeding grounds and territory boundaries were more stable, we performed playback tests with the songs previously recorded (Table S1). Tests were performed with 63 subjects, 15-16 per site. Water pipit territories can be localized easily because males display with a manifest behaviour, by repeatedly performing a parabolic song flight that begins and ends at an exposed rock or tussock (Bollmann et al. 1997; Melendez et al. 2014). We are confident that songs were recorded from different males, and that males were tested only once, because of the extensive mapping of territories and the relative stability of borders at the time of the experiment (seven test birds were colour-ringed). In our sample, the minimum distance between a test subject and a recorded male was $330 \text{ m} \pm 15.45 \text{ S.E.}$ ($n=4$ sites), while that between tested males was comprised between 270 and 3430 m, and in just four instances it was lower (108-149 m). In the latter cases, in which we tested territory-

neighbours, two observers controlled individual location to track the movements of both individuals throughout the duration of the experiment. All territories have been the object of a second test in the days following the first experiment, to prove that males were still there and that the recorded response corresponded to a peak phase of territorial defence. Indeed, all territories were still defended, although the response was weaker in at least one behaviour (i.e. fewer songs, longer distance or delayed approach, see below) in 33.3% of second tests in lowland territories, and 36.4% second tests in highland territories. Differences between lowland and highland populations were not significant ($\chi^2_1 = 0.001$; $p = 0.99$), suggesting that territorial behaviour may have declined in parallel in the study males. In the interest of clarity, only the first test provided the dataset for this study.

Individuals were recorded during song flights by means of a Marantz PMD661 recorder and a Sennheiser ME67 microphone during a single visit and in good weather conditions at a distance < 20 m. During recordings, we also noted male geographic coordinates. Playback stimuli were created using Avisoft-SasLab Pro (Version 3.91) Software by Raimund Specht (Berlin, Germany) by selecting one high quality song per individual, discarding recordings in which several males sang. Each song was composed by the two typical strophes. Songs were filtered with a high pass filter at 0.8 kHz and sound levels were standardized to an amplitude of 80% of a volt since birds may use the amplitude of songs as a cue of intruder distance (Beeman 1998; Naguib and Wiley 2001; Laiolo 2013). Vocalization bouts were copied and appended several times to create a 4-minute broadcast consisting of repetitions of ten seconds of song and ten seconds of silence, totalling 12 songs imitating a typical water pipit song rendition.

Each individual song was used as a playback stimulus in 1-4 territories. The 63 subjects for the experiment were located by extensively mapping individual territories, and after surveying the focal male movements for 30 min, we placed the playback equipment (Philips SBA1500/37 speakers and Marantz PMD661) at the most frequently used rock or tussock

post. Each experiment lasted 10 minutes, consisting of three minutes of pre-observation silence, four minutes of playback and three minutes of post-playback silence (Laiolo 2012, 2013). All movements and vocal activities were surveyed from a spot that reduced interference with the behaviour of the focal bird, but that permitted the observations of all movements (20-30 m). We measured variables indicating bird direct interference and vocal responses: the closest approach made to the speaker (hereafter, minimum distance of approach), the time of the closest approach to the speaker (latency to approach), and the total number of songs emitted (number of songs) (Laiolo 2013; Mortega et al. 2014). Observations were made with 10×40 binoculars, distances were established with a laser rangefinder and times were measured with a stopwatch. After each test, we recorded the distance to the nearest neighbour male, as a proxy of the local density of competitors, the time of the day, to account for daily variation, and the distance between the individual territory and the individual song, to control for geographic influences (Laiolo 2013; Hoffmann et al. 2015). Since the start and duration of male territorial and sexual behaviours tend to be tightly associated with photoperiodic stimulation (Ball and Ketterson 2008; Rosvall et al. 2012), we considered the Julian date (1 = 1st July) as a covariate in analyses. In doing this, we accounted for a potential endocrine mismatch among males during the course of the experiment. Ideally, we should have controlled for bird age as well, since in some passerine the song or aggressive behaviour may vary from first breeding to older birds (e.g. Vehrencamp et al. 2013). Our information is however limited to seven ringed birds, only one of which was a male born the year before. Inspecting the capture data in the study sites in 2012 (Melendez et al. 2014), we found that half of the 30 adult males we captured were born in the preceding year, but no difference emerged between lowland and highland populations ($\chi^2_1 = 0.01$, $p = 0.92$). If these proportions were maintained in the test subjects, and if age had a relevant effect, this factor would have probably biased equally observations at all elevations.

We used factorial models to analyse elevation-driven variation in the three variables indicating the territorial responses of males. The elevation of the territorial male and the elevation of the playback individual were entered as fixed factors (each had two levels: low and high elevation territory, and low and high elevation song) together with their interaction. We ran three types of factorial models that differentially control for replication (songs from the same individuals) and pseudo-replication (territorial subjects from the same sites). First, we ran mixed-effects models with playback replication, in which the elevation of the territory and the elevation of the song playback were fixed factors, and the site and playback individual were entered as random factors ($n = 63$). Second, we ran mixed-effects models without playback replication, with the same fixed effects but in which we considered only one test (the first) per song stimulus, and the site alone was entered as a random factor ($n = 25$). Third, we ran nested ANOVAs with three crossed factors: the elevation of the territory, elevation of the playback individual, and the site nested in the elevation of the territory. This test, in our experimental settings, limits type 1 error (false positive) but also reduces the number of degrees of freedom available for hypothesis testing, thus increases type 2 error rate (see Table 1).

To analyse acoustic variation with elevation, and test whether it conditioned the response to the playback stimuli, we centred on eight acoustic variables measured from the 25 song stimuli by means of Avisoft SASLab Pro 3.91 (Fast Fourier Transform FFT length: 512; time resolution: 17.9 ms; frequency resolution: 43 Hz; Window Function: Bartlett). These variables were the overall duration and the minimum frequency of the song, as well as the frequency bandwidth (maximum – minimum strophe frequency), the trill rate (syllable number per strophe/strophe duration) and minimum frequency of each of the two strophes (Fig. S2). Durations were measured in oscillograms, and frequencies in mean spectra. Songs were characterised by a significant trade-off between trill rate and frequency bandwidth in the second song strophe ($r_p = -0.43$, $n = 25$, $p < 0.05$), suggesting that males could not cover a

broad frequency bandwidth while simultaneously singing fast trills. To define the putative performance boundary, we regressed maximum frequency bandwidth from 1 Hz trill rate bins onto trill rate (Podos 1997). The upper bound regression line permitted the identification of male singing at performance limits (see also results). We used one-way ANOVAs to test for differences between low- and high-elevation songs, and general linear mixed models to test whether acoustic variables together with elevation of the territory, explained variation in the behavioural responses to playback, entering the site as a random factor.

Residuals of all models were normally distributed (Kolmogorov-Smirnov tests, all $p > 0.40$). Degrees of freedom may vary depending on missing data in some of the analyses. Analyses were performed in R 3.1 with packages *lmer4*, *lmerTest*, *MuMIn* (R Development Core Team, 2015).

Results

To avoid over-parameterization with a limited dataset, we entered covariates in models only when they significantly influenced the response variables: the hour of the day for the minimum distance ($r_s = -0.41$, $n = 63$, $p < 0.05$) and the day of the season for the number of songs ($r_s = -0.27$, $n = 63$, $p < 0.05$) in the replicated data set. Neither local densities (the distance to the nearest neighbour) nor song geographic origin (distance between the territory and the song source) conditioned the behaviours under study (local density: all $p > 0.24$; song origin: all $p > 0.11$).

All models indicated that lowland and highland males differed in the minimum distance of approach to the simulated intruder, and that this was the sole behaviour affected by elevation (Table 1, Table S2). Males from lowlands approached the stimulus more closely than highland males, and lowland songs appeared to elicit a closer approach by highland males (almost-significant and significant effects of the interaction factor in two out of three models; Fig. 1; Table 1). Between-site and between-(playback) individual variability did not

affect the minimum distance of approach (the variance of random factors in mixed-models was almost null; Table 1), but was substantial in the case of the other two behavioural response variables, explaining up to 29% of their variation (Table S2).

After applying Bonferroni correction for repeated tests with eight acoustic variables, the frequency bandwidth of the last song strophe was the sole acoustic variable that differed among high and low elevation songs (high elevation songs: 2116 ± 390 S.E. Hz, low elevation songs: 3668 ± 369 Hz, $F_{1,23} = 9.1$, $p < 0.05$). Without correction, the minimum frequency of the last strophe also displayed variation, being 760 Hz ± 140 S.E. lower in lowland songs, but this variation did not trigger any significant behavioural response (Table S3). On the contrary, the frequency bandwidth of the last strophe, together with the elevation of the territory, explained 27% of variation in the minimum distance of approach: the broader the bandwidth the closer the approach (Table 2, Table S3). This variable also positively influenced the number of songs emitted by the test subject (Estimate: 0.002 ± 0.0006 , $F_{1,20.3} = 5.2$, $p = 0.03$, $r^2 = 0.24$). Six out of eight males with high song performances (positioned above the triangularly distributed biplot of trill rate by frequency bandwidth of the last strophe) were from lowlands (Fig. 2). Stimuli closer to this performance limit (fast trills and broad bandwidths) elicited a closer approach (Table 2).

Discussion

We found a significant variation in the territorial behaviour of water pipits along elevation with respect to the minimum distance of approach, which was longer in high elevation males with respect to their lowland counterparts. The structure of the song also changed, and songs ending with broader frequency bandwidth, characterising lowland males, stimulated a stronger response especially when associated with fast syllable production. The results for these variables are in line with our predictions of less intense territorial behaviours and poorer song performance in the environmental conditions of the highlands. The other two

behavioural responses to playback did not display similar elevation cline and followed the influence of individual, local or seasonal factors.

Sexual or environmental drivers associated with opportunities for mating and defendability of resources, but also the physiological context at the individual level related to social status and body condition, may underlie the observed patterns in the distance of approach and song performance. Studies performed in the Alps have shown that when food conditions are favourable water pipit females anticipate breeding and produce more young but do not increase their quality (e.g. size; Brodmann et al. 1997), although early-settling females show a longer time lag between settlement and clutch initiation than late ones (Bollmann et al. 1997). In our study area, grassland productivity markedly declines with elevation (Laiolo et al. 2013, 2015), and the cover of habitat elements that reduce the quality of habitat for pipits, such as rocks and snow patches, increases with it (Brodmann et al. 1997; Bollmann et al. 1997; Rauter and Reyer 1997; Rauter et al. 2002; Laiolo and Melendez 2014). The breeding season is also inevitably delayed and more synchronous, following patterns of snow melting (Melendez and Laiolo 2014). In this scenario, the selective advantage for male mating effort, in terms of sexual signalling, mate guarding, and aggressiveness towards intruders, should decline in parallel with the reduced opportunities for pursuing matings and extra-pair copulations (Westneat et al. 1990). In fact, extra-pair paternity in the species, affecting on average $\approx 12\%$ of nests, drops in conditions of breeding synchrony (Reyer et al. 1997). In parallel, the value of male parental effort should increase, triggered by the low likelihood of cuckoldry and by substantial gains in offspring survival due to male care in the restrictive environmental conditions (Moller and Birkhead 1994; Badyaev and Ghalambor 2001; Hille and Cooper 2015). Since the attack of song stimuli is considered an unambiguous assay of aggression (Hof and Podos 2013), the longer distances of approach displayed by high elevation water pipits may be interpreted as the result of reduced aggressive motivation and, more broadly, weaker strength of intrasexual selection (i.e., fewer fitness benefits of male-

male competition). A similar pattern has been observed in the black redstart *Phoenicurus ochruros*, in which highland males had weaker responses to intruder stimuli than lowland ones (Apfelbeck and Goymann 2011).

The reduction in territory quality and thus defendability, or the settlement of low quality individuals in highlands, may have contributed to the observed differences in male-male interference. Male territorial behaviour may for instance reflect the extra effort that males of higher quality territories make to invest in territorial defence (Manica et al. 2014), although this does not explain the differential responses to low and high elevation song stimuli, or why specific signalling patterns likely associated with male quality predict attack reliably. In several species of birds, repetitive syllable production in the form of trills is associated with aggressive contexts (Leitao and Riebel 2003; Illes et al. 2006; Cardoso and Hu 2011). The hypothesis of motor constraints in the production of trilled songs predicts a negative relationship between frequency bandwidth and trill rate, which depend on the ability to produce rapid breaths and at the same time modulate syrinx and beak musculatures during song production (Podos 1996, 1997). These mechanic vocal constraints are used in female mate choice (e.g. Ballentine et al. 2004) and male-male territorial conflicts (Illes et al. 2006) to assess the quality of singing individuals. In our study system, males approached song stimuli ending with broad frequency bandwidths and fast syllable repetition more closely, possibly because males singing at the performance limits represent a greater threat in terms of likelihood of cuckoldry or territorial takeover (Hof and Podos 2013). Lowland males do not sing faster trills but do produce songs ending in broader bandwidths, and qualitatively tend to display a superior song performance with respect to highland males (Fig. 2). These characteristics may be enhanced by the motivation associated with a challenging context, of competitive neighbours (Laiolo and Obeso 2012; Vehrencamp et al. 2013). All in all, these results indicate that the allocation in signalling (vocal performance) accompanies some

aspects of aggressive behaviour (approach to intruder) along elevation, suggesting that both aspects of male-male competition decline in concert, as we move uphill.

Water pipit males uttered more songs when a signal with broad frequency bandwidth was broadcast, strengthening the idea that such songs are challenging and reinforcing the results obtained with the minimum distance. Besides this finding, however, it is worth saying that the number of songs, together with the latency to approach, did not vary in response of elevation as the minimum distance did. The number of songs declined through the season, possibly following endocrine mechanisms as observed in other passerines (Ball and Ketterson 2008) and both variables were subject to between-individual and -site variability. These results, however, do not weaken the hypothesis of reduced intra-sexual selection with elevation obtained with song analyses and experimentally induced aggression (only if males approach the intruder the attack can take place), but rather highlight the multifaceted nature of aggressive behaviours and their underlying mechanisms.

This study, performed across a pronounced environmental cline, provides insight into the evolution of territorial behaviours and mating strategies, and identifies variation potentially attributable to a specific context of fluctuating and restrictive environmental conditions. Spacing and sexual behaviours, albeit restricted to a local scale, may have consequences for many ecological processes, from population regulation to the distribution and abundance of organisms. Given the potentially great environmental changes induced by global warming, knowledge of how space-use behaviours adjust along climate clines is critical for understanding population dynamics from a more applied point of view.

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Figure captions

Fig. 1 Effects of the elevation of the territory and of the song stimuli on the minimum distance of approach to the stimuli during playback experiments. Mean values \pm SE are shown

Fig. 2 Relationship between trill rate and frequency bandwidth of the last song strophe in water pipit song revealing a performance trade-off in vocal production. The upper bound regression line is also shown, as estimated by regressing the maximum frequency bandwidth from 1 Hz trill rate bins onto trill rate ($r^2 = 0.58$, $p = 0.007$). Letters L and H indicate songs from lowland and highland males, respectively, that sang above the putative performance limits

Table 1 Results of three types of factorial analyses testing for the effect of territory elevation and elevation of the song stimuli on water pipit minimum distance of approach to playback stimuli. In mixed-effects models, the degrees of freedom were estimated by using the Satterthwaite method, the explanatory power of fixed factors was determined by marginal r^2 in keeping with Nakagawa and Schielzeth (2012). The hour of the day was entered as a covariate in the mixed-effects models with replication

<i>Mixed effect ANOVA with replicated stimuli</i>	
<i>(Random factors: Site, Playback individual)</i>	
	<i>Statistics</i>
Elevation of the territory	$F_{1,43} = 7.01; p = 0.011$
Elevation of the song stimuli	$F_{1,43} = 1.14; p = 0.29$
Elevation of the territory × Elevation of the song stimuli	$F_{1,43} = 3.56; p = 0.06$
Time of the day	$F_{1,43} = 1.84; p = 0.18$
Variance explained by fixed factors	0.24
Variance explained by random factors	0.00
 <i>Mixed effect ANOVA without replication</i>	
<i>(Random factor: site)</i>	
	<i>Statistics</i>
Elevation of the territory	$F_{1,3.6} = 4.6; p = 0.10$
Elevation of the song stimuli	$F_{1,17.7} = 5.41; p = 0.03$
Elevation of the territory × Elevation of the song stimuli	$F_{1,17.7} = 4.8; p = 0.04$
Variance explained by fixed factors	0.40
Variance explained by random factors	0.02
 <i>Nested ANOVA</i>	
	<i>Statistics</i>
Elevation of the territory	$F_{1,2} = 29.2; p = 0.03$
Elevation of the song stimuli	$F_{1,2} = 2.95; p = 0.22$
Elevation of the territory × Elevation of the song stimuli	$F_{1,2} = 5.52; p = 0.14$
Site (Elevation)	$F_{2,2} = 0.32; p = 0.75$
Site (Elevation) × Elevation of the song stimuli	$F_{2,40} = 0.65; p = 0.53$
Adjusted- r^2	0.10

Table 2 Results of general linear mixed models explaining variation in the minimum distance of approach of water pipit males exposed to song stimuli of varying frequency bandwidth of the last strophe (a) and of varying frequency bandwidth and trill rate of the last strophe (b).

The site was entered as a random factor and the elevation of the territory as a covariate.

Degrees of freedom were estimated by using the Satterthwaite method. The explanatory power of fixed factors was determined by marginal r^2 in keeping with Nakagawa and

Schielzeth (2012)

(a)	Estimate	S.E.	t_{19}	$F_{1,19}$	p
Intercept	34.741	10.745	3.23		0.004
Frequency bandwidth of the last strophe	-0.007	0.003	-2.55	6.49	0.020
Elevation of the territory	-14.992	8.270	-1.81	3.29	0.086
Variance explained by fixed factors	0.27				
Variance explained by random factors	0.00				

(b)	Estimate	S.E.	t_{17}	$F_{1,17}$	p
Intercept	88.693	13.296	6.671		<0.001
Frequency bandwidth of the last strophe	-0.020	0.004	-5.368	28.82	<0.001
Trill rate of the last strophe	-6.888	1.433	-4.806	23.10	<0.001
Frequency bandwidth x trill rate	0.002	0.000	3.092	9.56	0.007
Elevation of the territory	-9.992	5.750	-1.738	3.02	0.100
Variance explained by fixed factors	0.66				
Variance explained by random factors	0.00				

Fig. 1.

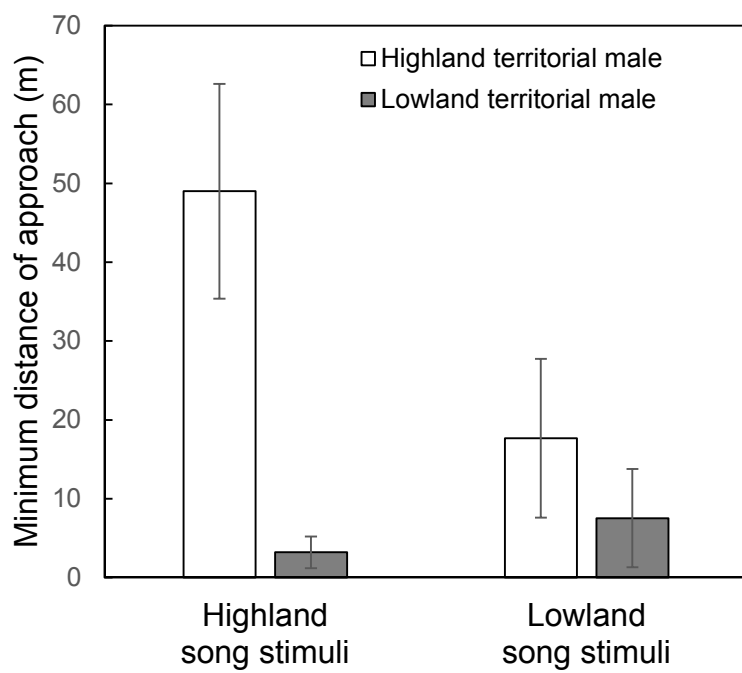


Fig.2.

