# Chapter 1

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Male Tawny Pipit singing during the breeding season in the Gran Sasso and Laga Mountains National Park (Italy), 2020

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ORIGINAL ARTICLE

# Guess who? Evaluating individual acoustic monitoring for males and females of the Tawny Pipit, a migratory passerine bird with a simple song

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## Abstract

Individual acoustic monitoring (IAM), based on the analysis of vocal cues, is particularly suitable for the identification and tracking of birds with temporally stable song or call characteristics. Unlike mark-recapture methods, IAM does not require the physical manipulation of individuals, which can have long-lasting behavioural effects. So far, IAM has usually focused on males, as singing females tend to be overlooked in temperate zones. Here, we evaluated the suitability of IAM for both sexes in an isolated population of the Tawny Pipit (*Anthus campestris*, Motacillidae), a migratory Palearctic species critically endangered in Central Europe, for which female singing has been occasionally documented. We confirmed that songs of all 101 studied individuals, both males and females, were individually distinct. Most individuals used only a single song type in their repertoires, with only three males using two. Of 45 ringed males (that could be unambiguously recognized visually), only two changed their song structure to some extent, either within or between seasons. Multiple individuals often sang structurally similar song types, which nevertheless consistently differed in minor characteristics; such differences were detectable by visual inspection and also affected quantitative analyses of song similarity. Songs sung by females did not have any apparent sex-specific characteristics. Unlike previously suggested, females did not adapt their vocalization to their breeding partner, and we presume their song is also temporally stable. Our findings support IAM as a reliable approach for studying the behaviour and ecology of this passerine species with a small repertoire and simple songs.

**Keywords** *Anthus campestris* · Bird song · Female vocalizations · Individual acoustic monitoring · Vocal signatures

## Zusammenfassung

**Wer singt denn da? Analyse des individuellen akustischen Monitorings von Männchen und Weibchen des Brachpiepers, eines Zugvogels mit einfachem Gesang**.

Individuelles akustisches Monitoring (IAM) auf der Basis von Lautäußerungen ist besonders geeignet für die Identifizierung und Verfolgung von Vögeln mit über längere Zeit hinweg stabilen Gesangs- oder Rufmerkmalen. Im Gegensatz zu

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Wiederfangmethoden erfordert das IAM keine physische Handhabung der Einzeltiere mit möglicherweise daraus folgenden, länger anhaltenden Auswirkungen auf ihr Verhalten. Bisher hat sich das IAM in der Regel auf Männchen konzentriert, da singende Weibchen in den gemäßigten Zonen eher übersehen werden. In dieser Untersuchung bewerteten wir die Eignung von IAM für beide Geschlechter in einer isolierten Population des Brachpiepers (*Anthus campestris*, Motacillidae), einer in Mitteleuropa stark bedrohten paläarktischen Zugvogelart, für die gelegentlich der Gesang auch von Weibchen dokumentiert worden ist. Wir stellten fest, dass die Gesänge aller 101 untersuchten Einzeltiere, sowohl die der Männchen als auch die der Weibchen, individuell unterschiedlich waren. Die meisten Tiere hatten nur einen einzigen Liedtyp in ihrem Repertoire, nur drei Männchen hatten zwei Liedtypen. Von 45 beringten Männchen (die visuell eindeutig erkannt werden konnten) änderten nur zwei ihre Gesangsstruktur in gewissem Umfang, entweder innerhalb oder zwischen den Jahreszeiten. Mehrere Individuen sangen oft Liedtypen, die sich in ihrer Struktur ähnelten, aber durchweg in kleineren Merkmalen unterschieden; solche Unterschiede waren durch visuelle Kontrolle nachweisbar und wirkten sich auch auf die quantitativen Analysen der Gesangsähnlichkeit aus. Die Gesänge der Weibchen wiesen keine offensichtlichen geschlechtsspezifischen Merkmale auf. Anders als bislang angenommen, passten die Weibchen ihren Gesang nicht an den Brutpartner an, und wir vermuten, dass ihr Gesang auch über längere Zeit hinweg stabil ist. Unsere Ergebnisse unterstützen das IAM als einen zuverlässigen Ansatz für die Untersuchung des Verhaltens und der Ökologie dieser Sperlingsart mit einem kleinen Repertoire und einfachen Gesängen.

## Introduction

Bird songs have multiple important roles, especially for intraspecific interactions such as mate attraction and territory defence. In addition, birds often rely on vocalizations to recognize conspecifics from others (Matyjasiak 2005; Hick et al. 2016; Hodgson et al. 2018; Darolová et al. 2020). Recognition might also take place at the individual level if birds show consistent individually distinctive patterns in their acoustic signals (e.g., Terry et al. 2005; Osiejuk et al. 2007; Petrusková et al. 2016). In this way, birds can discriminate between familiar and unfamiliar conspecifics in different social contexts (e.g., neighbour vs. stranger discrimination, parent–offspring recognition, kin recognition; Lambrechts and Dhondt 1995). Not only do vocal signatures play an important role in social interactions, but under some circumstances they can also be effectively used to monitor bird individuals (e.g., Blumstein et al. 2011; Petrusková et al. 2016, 2021; Sebastián-González et al. 2018; Pérez-Granados et al. 2019).

Individual acoustic monitoring (IAM) is a non-invasive monitoring approach based on the analysis of vocal cues of individuals, which is particularly suitable for bird taxa that are highly vocally active (reviewed in Terry et al. 2005). Acoustic monitoring does not require the manipulation of trapped individuals, unlike more traditional mark-recapture methods (e.g., use of mist nets or clap traps for capturing and subsequent ringing). Though these catch-and-ring techniques do not necessarily have a negative effect on bird survival and fitness (e.g., Calvo and Furness 1992; Clewley et al. 2018; Petrusková et al. 2021), playback luring plus capturing and handling may have cumulative effects on the subsequent behaviour of passerines (Linhart et al. 2012; Budka et al. 2019; Oñate-Casado et al. 2021). In contrast, due to its non-invasive approach IAM can be used on wild species with fewer concerns about impacts on the behaviour of target individuals (reviewed in Terry et al. 2005), and might even provide more accurate information about behaviour or population dynamics (e.g., Laiolo et al. 2007; Petrusková et al. 2016). Besides being a good alternative for species that are sensitive to capturing and handling, IAM is also suitable for studying birds that are difficult to spot because they inhabit areas with poor visibility, such as dense vegetation (e.g., Hobson et al. 2008; Celis-Murillo et al. 2009; Petrusková et al. 2016).

Despite the advantages that IAM offers in identifying and tracking birds, it has not been used very frequently. Most of the existing studies have focused on non-passerine species with simple vocalizations, such as Great Bitterns (Gilbert et al. 2002), ducks (Volodin et al. 2005), and owls (Galeotti and Sacchi 2001; Tripp and Otter 2006; Grava et al. 2008; Choi et al. 2019). Studies on songbirds, whose songs develop under the influence of vocal learning and are culturally transmitted, have also mainly concerned species with simple song structures (e.g., Laiolo et al. 2007; Vögeli et al. 2008; Adi et al. 2010). This is because the implementation of IAM for passerines with higher song complexity is time-intensive, especially at the beginning, when the temporal stability and individual uniqueness of target species' song must be evaluated (Petrusková et al. 2016). When vocal signatures are temporally stable, IAM can be effectively used to follow songbirds over time and provide information both within a single season (e.g., population density) and over several years (e.g., survival rates in sedentary species, return rates in migratory species, changes in territorial dynamics and replacements). This has been reported for species with simple vocalizations such as the calls of Dupont's Lark (*Chersophilus duponti*; Laiolo et al. 2007; Vögeli et al. 2008), songs of Mexican Ant-thrushes (*Formicarius moniliger*; Kirschel et al. 2011) and Ortolan Buntings (*Emberiza ortulana*; Adi et al. 2010), as well as in a species with a substantially more complex song structure, the Tree Pipit (*Anthus trivialis*; Petrusková et al. 2016).

Most, if not all existing IAM studies on passerines have only targeted male individuals. In songbirds, songs are traditionally considered to be primarily produced by males (Catchpole and Slater 2008). However, recent reviews have shown that female song is widespread among songbird species (Passeri). Odom and Benedict (2018) reported (based on data from Webb et al. 2016) that sex-specific song information is available for only 27% of all songbird species; of those, however, almost two thirds have females that sing at least occasionally. A review by Garamszegi et al. (2007) indicated that approximately 40% of European songbird species have females that may sing, while among North American passerines, females seem to sing in 43% of duetting and 34% of non-duetting species (Benedict 2007). Nonetheless, there is still a lack of documentation on female songs for most songbirds, especially in temperate areas where studies on female songs are much less prevalent than in the tropics (Langmore 1998; Odom et al. 2014; Hall and Langmore 2017).

Part of this above-described discrepancy may be due to the fact that in temperate regions, songbird females can be relatively quiet and visually cryptic (Price 2019). That leads to the increased detection of songbird males, which usually are more vocally active (Bennett et al. 2019). Additionally, birds in these regions only sing intensively for a short period during the breeding season, and this time window might be even shorter for females (e.g., McDonald and Greenberg 1991). Many species are also monomorphic, which makes it difficult to distinguish between the sexes (Langmore 1998; Hahn et al. 2013; Odom and Benedict 2018), and singing females, even when in fact observed, may be assumed to be males (Sierro et al. 2022). Despite this, however, in some temperate songbird species it has been shown not only that females sing but also that songs can be used to discriminate between males and females (Yamaguchi 1998). In such cases, acoustic monitoring might also provide reliable information about the sex of the vocalizing individuals.

The Tawny Pipit (*Anthus campestris*) is one of the temperate songbird species for which field studies have reported that both sexes sing (Neuschulz 1986; Alström and Mild 2003; Calero-Riestra and García 2019). Although the Tawny Pipit song is simple, males of this species apparently possess individually unique song types (Neuschulz 1986; Osiejuk et al. 2007), and Neuschulz (1986) mentioned that they seem stable between two consecutive seasons. The individuality and stability of their song would make this pipit species suitable for IAM. In the same study, however, Neuschulz (1986) also claimed that females copy the song of the males they are paired with. If true, this would suggest that female Tawny Pipits are able to adapt their vocalization over time. However, there are no publications following the preliminary report by Neuschulz (1986), and all his conclusions were only illustrated by a few spectrograms of male and female songs.

In our study, we performed a detailed analysis of inter-individual and temporal variation of song from an isolated Central European Tawny Pipit population. This way, we assessed the suitability of IAM for tracking both males and females of this territorial migratory songbird. Further, we compared the song structure of males and females, and for the available data on male–female breeding pairs, we also evaluated the claim of Neuschulz (1986) that females copy the song of their partner.

## Methods

### Study species and fieldwork

The Tawny Pipit (*Anthus campestris*) is one of over 40 globally distributed species in the genus *Anthus*. These sub-Saharan migrants are widespread in the Palearctic, and on a whole-European scale their conservation status is categorized as "Least Concern" (IUCN 2022). However, they are undergoing severe population declines, especially in central and western Europe, due to the loss of suitable habitats (Briedis et al. 2016; Tyler and Christie 2016). Because of this, the species is categorized differently in the regional red lists of many European countries (e.g., Switzerland: Endangered, Czechia: Critically Endangered, Netherlands: Regionally Extinct; www.nationalredlist.org).

During the breeding season, Tawny Pipits inhabit dry, sandy steppe-like habitats, and require bare ground with sparse vegetation cover for nesting (Beran et al. 2018; Calero-Riestra and García 2019). They are socially monogamous and cryptically coloured with no sexual dimorphism (Alström and Mild 2003). The song should thus be critical for male success in territorial defence and mate attraction. According to a few published reports (Neuschulz 1986; Alström and Mild 2003; Calero-Riestra and García 2019), females of this species also occasionally sing, mainly in flight while approaching and leaving the nest with nestlings (see the "Results" for more details).

Our study was conducted on the only remaining population of Tawny Pipits in Czechia (Central Europe), located in an active brown coal mine area in the Ústí nad Labem region (50.48–50.56 °N, 13.48–13.58 °E; Briedis et al. 2016). Suitable habitats for the species are mostly found at the edges of open-cast mines, which host up to 200 breeding Tawny Pipit pairs (Briedis et al. 2016; Beran et al. 2018). The population has been intensively monitored for several years (most intensively between 2015 and 2017), and the birds captured during the survey were equipped with a ring with a unique alphanumeric code allowing their visual identification from a distance. Individuals in our study were recorded from two coal mines: ČSA (50.54 °N, 13.53 °E) and Vršany (50.49 °N, 13.54 °E).

Singing individuals were repeatedly recorded during four different breeding seasons (from May to mid-July 2015, 2016, 2017 and 2021) using a Sennheiser ME-67 shotgun directional microphone connected to a Marantz PMD 661 recorder. We noted the date, time, geographic coordinates and bird behaviour for each recording and observation. For those individuals that were ringed at the time of recording, we additionally took note of their ring code, if visible. For both sexes, most of the recordings were obtained in the seasons 2016 and 2017 (Table 1).

We attempted to record at least 3 min of singing bouts from each male; however, substantially shorter recordings of good quality were also considered fully adequate because male Tawny Pipits generally use a single song type (Osiejuk et al. 2007). Since female pipits sing rarely, the number of songs for females was much more limited, and we analysed all songs of sufficient quality that were available.

## Individual identification and song measurements

Individual identification of Tawny Pipit males and females was based on ring observations and/or song recording analyses, following the method of Petrusková et al. (2016). We identified non-ringed birds as males or females based on their behaviour and pairing status with ringed individuals, if known. In total, we recorded 81 males and 20 females (Table 1), 45 (56%) and 8 (40%) of them ringed, respectively. From all the recordings, we obtained the songs of nine breeding pairs formed by nine female and eight male individuals (one ringed male was paired with two different ringed females in different breeding seasons).

To characterize individual repertoires, we first visually inspected all recordings in the program Avisoft-SASLab Lite 5 (Specht 2007) with the following set of parameters: FFT length 256; Frame 100%; and Hamming window. We created standardized spectrograms that were visually inspected and catalogued as distinct song types according to differences in their element structure. Following Lachlan (2011), we defined an "element" as a discrete unit of continuous sound separated from other elements by silence, and a "song" as the sum of all the elements it contains. Minor but highly consistent differences in element shapes between song types (i.e., those consistently retained across different songs and recordings), observable in the spectrograms by an experienced user, were considered sufficient to classify songs as distinct (see Figs. 1, S2, S3).

Additionally, we used the bioacoustics software Luscinia 2.20.03.11.01 (http://github.com/rflachlan/luscinia) to measure and quantitatively compare acoustic characteristics for representative recordings of each song type observed in the population. When needed, we removed the noise and improved the signal by altering the dereverberation (100%), dereverberation range (50 ms), dynamic range (40 dB) and high pass threshold (1000 Hz) settings. We then characterized the parameters for every single element within a song, only considering recordings with songs of sufficient quality (i.e., those in which song elements could be clearly distinguished from the background noise).

## Song analysis and validation of the method

We compared in a pairwise manner the structure of all song types using the "dynamic time-warping algorithm" (DTW) in Luscinia. DTW aligns two acoustic (time-dependent) sequences, which may vary in speed, and calculate dissimilarity scores based on the Euclidean distance between their acoustic features. This is achieved by warping the time and/or frequency axis until an optimal match between those two sequences is found. Weights and parameters of the DTW analyses (for their explanation, see Luscinia documentation) were set as follows: time (5), fundamental frequency (1), fundamental frequency change (1), FF norm (1), compression factor (0.25), minimum element length (2), time SD weighting (1), ArcTan transform weight for frequency slope (0.02), cost for alignment error (0.2), stitch threshold (100 ms), and maximum warp (100%). Fundamental frequency was log-transformed.

**Table 1** Summary of recorded Tawny Pipit individuals in the study population

| Category | No. of recorded individuals | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2015 | 2016 | 2017 | 2021 | Within season | Between seasons | Total |
| Ringed males | 19 | 30 | 19 | 1 | 26 | 20 | 45 |
| Non-ringed males | 5 | 11 | 18 | 4 | 8 | 2 | 36 |
| Ringed females | 1 | 1 | 7 | 0 | 0 | 1 | 8 |
| Non-ringed females | 0 | 6 | 4 | 2 | 0 | 0 | 12 |

The column "within season" includes birds recorded at least twice within the same breeding season (more than a week apart), while the column "between seasons" counts returning birds recorded in at least two years. Altogether, recordings of 101 individuals were included in the study; details about individual birds and each analysed song are provided in Tables S1 and S2, respectively

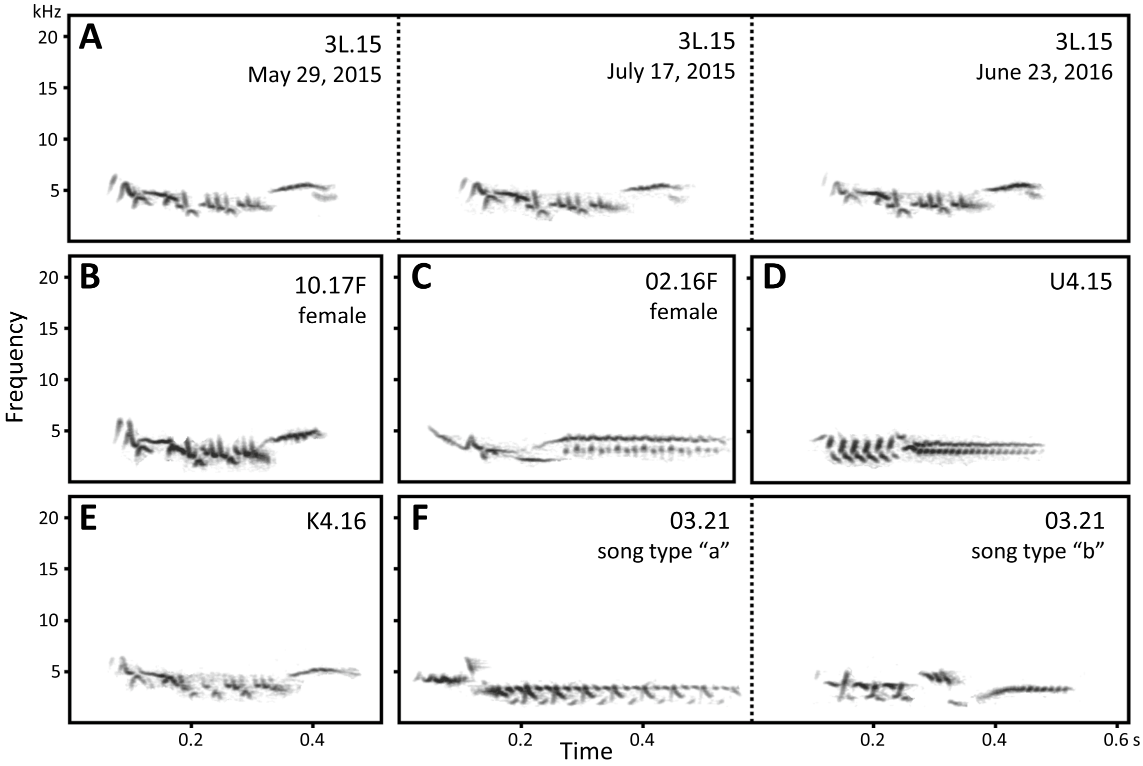

**Fig. 1** Spectrograms illustrating the variation of song types of six Tawny Pipit individuals from the studied population (**A**, **D**–**F**: males, **B**–**C**: females). **A** illustrates the temporal stability of songs sung by the same bird on different dates across two years. **A**, **B** and **E** are distinct but structurally similar song types sung by different individuals (two males and one female), while **B**, **C** and **D** demonstrate structurally different song types sung by different individuals. Two distinct song types sung by the same male are shown in **F**. The position of all song types shown here is indicated in the dendrogram of song similarity in Fig. 3A. More spectrograms are provided in Supplementary Figures S1 (different song types sung by the same males), S2 (song types sung by individuals paired together) and S3 (spectrograms representing the overall variation in the dataset, highlighting "song families", i.e., groups of structurally similar song types)

Through the DTW employed by Luscinia, we obtained a pairwise matrix of song dissimilarities, which was used to visualize and interpret the patterns of acoustic similarity between analysed songs as well as in formal tests of specific hypotheses about song similarity. To visualize the patterns in a dendrogram, we carried out a hierarchical clustering analysis using a unweighted pair group method with arithmetic mean (UPGMA).

Assuming that male Tawny Pipits possess individual acoustic signatures (Osiejuk et al. 2007), different songs from the same bird should be acoustically more similar to each other than songs from different ones, at least for males. Hence, we inspected results from Luscinia to evaluate whether different song types clustered according to the male and female individuals they belonged to. Firstly, we successfully tested that the quantitative approach was able to cluster songs from known birds on a subset representing ringed individuals only, in which songs were unambiguously assigned to a particular male. This subset was formed by three songs from each bird, recorded in the same time period (within one day or two consecutive days).

Then, we evaluated the temporal song stability in known (ringed) males by comparing the acoustic similarity between songs from the same bird at different moments within the same breeding season, as well as between seasons. We included up to three periods for a given year, at least a week apart, and spread as much as possible in time from each other. If available, we tried to cover the beginning (until mid-May), middle (mid-May to mid-June) and end (mid-June to late July) of the season, but we occasionally also included recordings from the same period if taken more than one week apart. Finally, for returning birds, we also analysed songs representing different years (an additional song for each extra season in which the returning bird was recorded).

After confirming the assumptions of song individuality and stability on a subset of recordings from ringed individuals, we also considered recordings from non-ringed males and females for further analyses. As with ringed individuals, we tried to process at least three songs per non-ringed bird, and cover within- and between-season temporal variation. However, in the case of three females and one male, we only included two songs due to the scarcity of available recordings of sufficient quality.

Based on this comprehensive dataset, we generated a single circular UPGMA dendrogram depicting the acoustic similarity of representative songs for all individuals (Figs. 2, 3, S3), which was visualized and annotated in iTOL 6 (Letunic and Bork 2021). On this dendrogram, we highlighted features of interest (e.g., ringed birds, males vs. females, individuals paired together), as well as indicated groups of multiple song types (representing at least four birds) clustering together below a certain arbitrary threshold ("song families"; Figs. 3A, S3). These groups were also highlighted in a multidimensional scaling plot (Fig. 4) that was based on the same pairwise dissimilarity matrix as the dendrogram.

### Statistical analyses

In order to corroborate the results obtained through visual inspection and clustering analyses, we applied Mantel tests (with 10,000 random replicates) to test specific hypotheses about patterns of song (dis)similarity at the individual level (within and between individuals) and sex level (within and between sexes, within and between male–female pairs). We created pairwise binary dissimilarity matrices to represent individuals, sex, and couple membership, using 0 and 1 to denote songs from the same and different individual/sex/couple, respectively. These matrices thus characterized alternative hypotheses of a priori models that were tested by randomization of the dataset.

The selection of songs to be included in the tests depended on the tested hypothesis. When evaluating how consistent the individually unique songs are, each song type was represented by three renditions (except for five above-mentioned individuals with two songs only). For all other comparisons, each song type was represented just once. This way, we performed Monte Carlo tests to investigate possible relationships between group membership and the acoustic dissimilarity values obtained from the DTW algorithm in Luscinia. Significant results of the test can be interpreted as the acoustic similarity being higher between members of the same group than between members of different groups. The Mantel tests were run in R 4.1.3 (R Core Team 2021) using the package *vegan* (Dixon 2003).

## Results

### Male and female singing patterns

Based on our field observations, Tawny Pipit males frequently sing throughout most of the breeding season, usually from higher perches in their territory or during flight, often with many songs in a sequence. Females sing much more rarely than males, and most females were not recorded or heard singing at all. Of those that did sing, we noted their singing activity only occasionally, and these were usually no more than 3–5 flight songs while approaching or leaving the nest. If singing occurred while flying to the nest, females tended to land away from the nest (ca. 10–30 m) and approached it on the ground. We mainly observed females singing when feeding older nestlings (from the age of ca. 5 days), but occasionally also during the incubation period. Accordingly, the period in which the recordings of females were obtained was short (from June to July).

Both male and female Tawny Pipits sing relatively short songs (on average about 0.5 s) composed of several elements forming a simple song type (Fig. 1). Most of the studied individuals sang only one song type, but three males (i.e., 3.3%) produced two different song types (Figs. 2B, S1A) that were both consistently captured in recordings of those individuals, either singing multiple repetitions of the same song type before switching to the other one or alternating between short sequences of 1–3 songs of each type.

### Individuality and the temporal stability of songs

The song types were individually distinct. When analysing songs of ringed birds, we never encountered identical song types sung by different individuals. However, multiple birds often used structurally similar song types, only differing from each other in minor structures (Figs. 1, S2). These resemblances were reflected in lower dissimilarity scores of such song types, which thus formed distinct clusters of "song families" in the UPGMA dendrogram (Figs. 3A, S3). Songs belonging to clusters also formed distinct aggregates when their variation was visualized in the multidimensional scaling plot (Fig. 4).

In most cases, song renditions of the same singing individuals (whether male or female) were alike, regardless of whether these originated from the same recording, from recordings obtained in different parts of the same season, or from different years (Figs. 1, S2). In two out of 45 ringed males (NA.16 and 7H.16), however, we observed within-individual temporal variation (Fig. S1B). Specifically, the terminal part of songs of the male NA.16 varied in the duration and number of repeated elements, both between and within recordings. In late June 2016, this male had a tendency to sing longer variants than in late May, 28 days earlier. Songs of the male 7H.16 differed between two subsequent breeding seasons by an extra terminal element that was consistently absent in 2017 but almost always present in 2016 (except for one single song in one of the 2016 recordings, which also lacked this element).

Accordingly, the clustering in the UPGMA dendrogram (Figs. 2, 3, S3) was mostly arranged by individuals. For 70 out of 81 males and all 20 females, all songs of the same bird clustered together (Figs. 2, S3). The exceptions included the three males singing two distinct song types, as well as the two above-mentioned males that showed temporal variation in song structures (one of the songs of NA.16 was clustered with structurally similar songs of male SX.17, and songs of 7H.16 from consecutive years were separated in two clusters representing "song families" differing in the terminal elements; Figs. 3C, S3). All remaining cases where one of the songs of a particular individual was not clustering with the others in the dendrogram (star symbols in Fig. 2) were apparently caused by variation of the overall

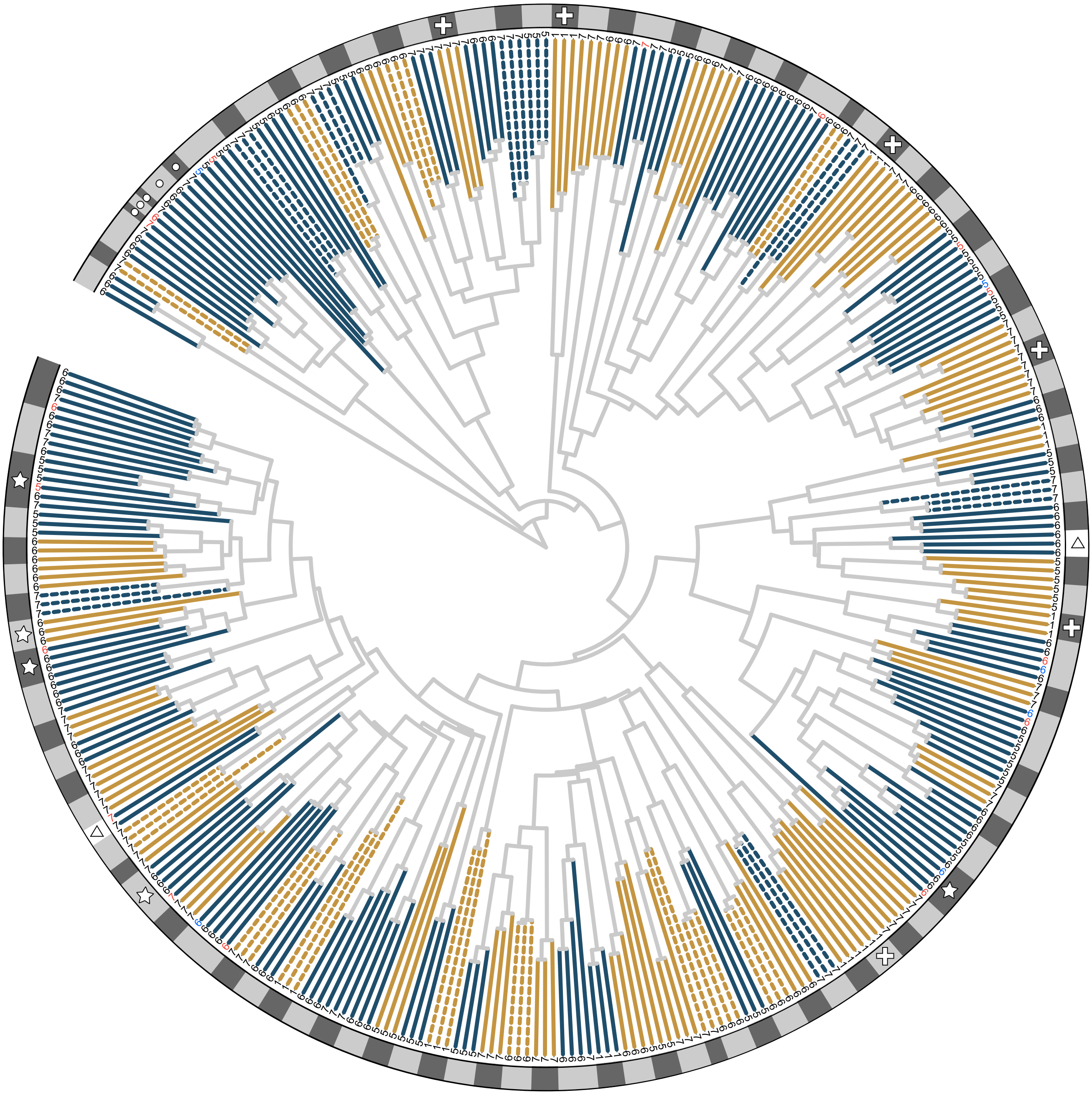

**Fig. 2** UPGMA dendrogram of song similarity based on the output of the dynamic time warp algorithm implemented in Luscinia software. Ringed birds are denoted by blue and non-ringed by brown branches; males by solid and females by dashed branches. The year of recording (2015–17, 2021) is abbreviated by its last digit, numbers in different colours mean different parts of the respective season. Segments in the outer ring indicate songs sung by the same individuals (whose codes are provided in Fig. S3). Specific symbols in the ring mark those individuals for which not all songs formed a single cluster in the dendrogram: males singing two song types (denoted in colour in Fig. 3C) are marked by a plus sign, the male 7H.16 (dark grey in Fig. 3C) by a triangle, interspersed songs of males NA.16 (black in Fig. 3C) and SX.17 by dots. Stars indicate adjacent songs of the same individual that do not cluster together due to variation in recording quality

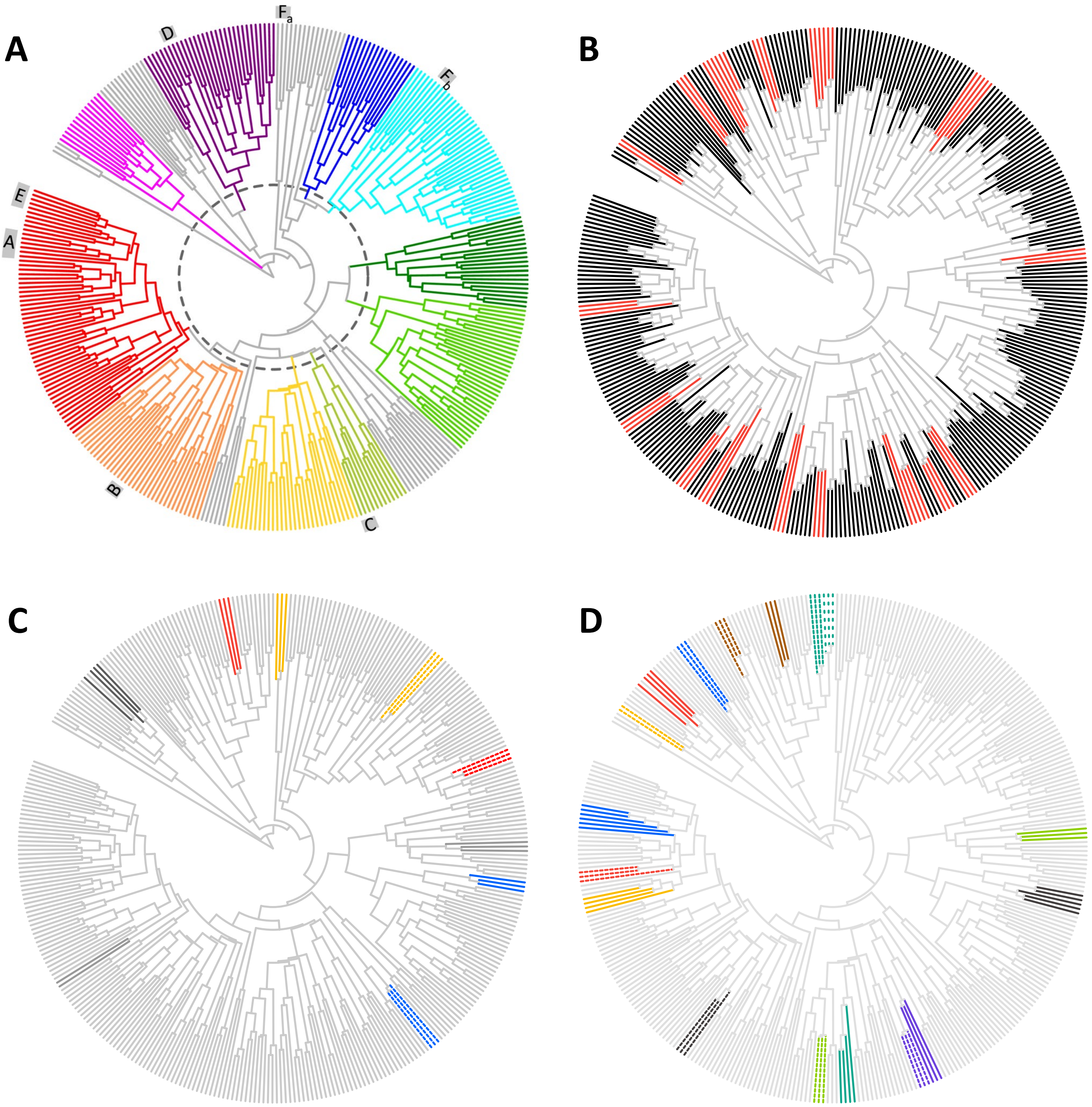

**Fig. 3** UPGMA dendrograms (same topology as in Fig. 2) with highlighted features of interest. "Song families", i.e., structurally similar songs of at least four individuals that cluster together under an arbitrarily chosen threshold (marked by a dashed ring), are marked by colour in **A**; letters along the dendrogram edge indicate the position of song types shown in Fig. 1. Representative spectrograms across these "song families" are shown in Fig. S3. Male songs (black) and female songs (red) are highlighted in **B**, males with different or variable song types in **C**, and paired individuals (males by solid and females by dashed lines in corresponding colour) in **D**. Spectrograms of song types highlighted in **C** and **D** are provided in Figs. S1 and S2, respectively

recording quality, not structural differences among the songs themselves. The prevailing within-individual consistency of songs was reflected in the highly significant results of the test that evaluated the differences between songs sung by the same individual and the others ($n_{songs} = 242$, $n_{birds} = 81$, $p < 0.001$).

For all three males singing two different song types, both types were individually distinct but not similar to each other (Figs. 3C, S1A). Correspondingly, the dissimilarity scores of

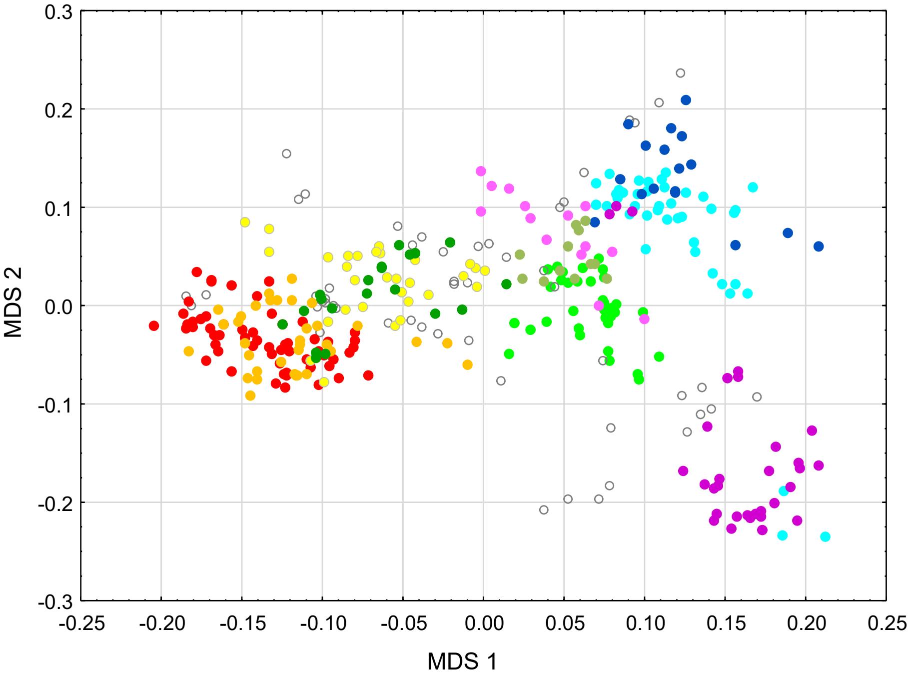

**Fig. 4** A multidimensional scaling plot as an alternative visualization of the variation in song similarity among studied Tawny Pipit individuals, based on the dissimilarity matrix provided by the dynamic time warp algorithm implemented in Luscinia software. Each dot indicates one song; colours match those of the "song families" in Figs. 3A, S3. Grey open dots are songs not assigned to any family, which are scattered across the dendrogram (Fig. 3A)

song types sung by the same male were comparable to those picked at random from all males ($n_{songs} = 251$, $n_{birds} = 81$, $p = 0.99$).

## Female songs

As was found for the males, females also had individually unique song types. Some of these song types were highly distinct, others closely similar to either that of one of the males or of another female (Figs. 2, 3B). Only one female (T3.16F) was recorded in two different seasons (during which it was paired with the same male, 3L.15), and its song did not vary (Fig. S2). There was no song feature that could be considered sex-specific; male and female song types were interspersed in the dendrogram. However, the pattern was not entirely random due to the occasional clustering of two females directly next to each other or within the same "song family" (Figs. 2, 3B, S3). This was reflected in the results of the test evaluating whether the acoustic similarity of songs of the same sex is higher than between sexes ($n_{male\ birds} = 81$, $n_{female\ birds} = 20$, $p = 0.06$).

When nine confirmed breeding pairs were considered, songs of the male and the respective female were usually not located close to each other in the dendrogram (Figs. 3D, S2, S3). Only for one of the pairs (female 02.16F and male 74.15) were the partners' songs more similar to each other than to songs of other birds (Figs. 3D, S2). However, this was not the case for the remaining pairs; other birds with no social bond often sang songs similar to those of a given male or female (Fig. S2). Correspondingly, the Mantel test indicated that the acoustic similarity of a female's songs was not higher to songs of her partner than to songs of a randomly chosen male in our recorded study population ($n_{couples} = 9$, $n_{songs} = 90$, $n_{female\ birds} = 9$, $n_{male\ birds} = 81$, $p = 0.59$). Interestingly, the songs of two ringed females (NK.16F and YT.17F) that were paired with the same male (HT.16) in different years (2015 and 2016–17, respectively) were very similar, but they clearly differed from this male's song (Figs. 2D, S2).

## Discussion

Our findings confirm that the songs of male and female Tawny Pipits are individually distinct, although consistent differences between similar songs are often minor, and require careful assessment of the respective spectrograms. Identification based on song recordings was consistent with the observation of ring codes, indicating that when analysing the spectrograms with care, we could distinguish individuals of both sexes with a considerably high level of certainty. In the case of males, these results support the claims of Neuschulz (1986) and Osiejuk et al. (2007). As the male songs were confirmed to be temporally stable both within and between seasons, we may conclude that the Tawny Pipit, as a migratory passerine species with a small repertoire and low song complexity, seems suitable for IAM. This methodological approach may be thus used for studying the behaviour and ecology of the species (see Oñate-Casado et al. 2021).

Traditional capture-mark methods involve physical manipulation of the individuals, which might have subsequent negative effects on the health (Marco et al. 2006;

Mulcahy et al. 2011; Spotswood et al. 2012) or the behaviour of the studied animals (Linhart et al. 2012; Budka et al. 2019; Oñate-Casado et al. 2021). In a previous study on the same population (Oñate-Casado et al. 2021), we found that male Tawny Pipits associate playback stimulation with capturing events and remember such an experience over a period comparable to their life span in the wild. This implies that long-term negative associations might affect the results of studies that use similar methods for re-capturing individuals (e.g., for retrieving data loggers, estimating survival and return rates, etc.). IAM offers the great advantage of being non-intrusive, and thus can be used on Tawny Pipits with fewer concerns about impacts on their behaviour. As reported in a congeneric species, the Tree Pipit (Petrusková et al. 2016), IAM seems more reliable than ring observations for determining the density of singing individuals as well as estimating their return rates and within-season spatial territorial dynamics. It may also reveal complementary or more detailed information about species behaviour than classical methods (Laiolo et al. 2007; Kirschel et al. 2011; Petrusková et al. 2016).

Our approach was very time-efficient in the field. We initially intended to spend a maximum of 5 min to obtain a recording of sufficient quality for a spontaneous singing male. However, we usually just needed one minute once the male started singing to record enough songs for reliable identification. This took longer (ca. 10–30 min) for singing females, since they sing much less frequently than males, have only short song sequences, and apparently only sing in a specific context (see also Neuschulz 1986; Alström and Mild 2003; Calero-Riestra and García 2019). However, even this amount of time was substantially lower than usually required to unambiguously read ring codes, in particular for females whose behaviour is very secretive. Our method was also not overly time-consuming from a technical perspective: a dataset of three recorded songs per individual was sufficient to characterize an individual's acoustic signature, and to assess with certainty whether an individual was already known or not. Once the individual's song structure had been characterized, even a single well-recorded song was sufficient for its identification.

Our visual observations of differences in song mostly corresponded to the dissimilarity scores obtained from the DTW algorithm implemented in Luscinia. As a result, songs clustered correctly according to individuals for most Tawny Pipits, both males and females. However, for about 10% of individuals, the analysed songs were not arranged in a single cluster (usually, one song was slightly separated from the others). After visually examining the respective spectrograms, we concluded that such songs did not differ from the others sung by the same bird in structure but rather in recording quality, which affected the precision of designating individual song elements in the software and thus the corresponding dissimilarity scores. This highlights that in the case of very similar songs, the DTW algorithm as applied by us seems sensitive to even small differences in fine element structure. While the clustering based on dissimilarity scores alone was surprisingly efficient and may be applicable on large-scale datasets, repertoire-based IAM still requires the assessment and/or correction of results by experienced users. This is particularly true for highly similar song types that only differ in tiny but consistent features. In our study population, we recorded several such "song families" that required careful inspection to reveal individually specific characteristics (Fig. S3).

The songs of two males (7H.16 and NA.16) did not all cluster together in the dendrogram due to clear structural differences rather than recording quality. These differences might be related to the song learning process. Tawny Pipits seem to be age-limited learners (also known as closed-ended learners; Brainard and Doupe 2002). In such bird species, the song is learned for a limited period during the early stages of life, presumably during their first and sometimes the second year of life. During that time, birds memorize a conspecific song model from one or more tutors, and they then start producing a generic, variable and quiet vocalization called a subsong (Brenowitz et al. 1997). They then go through a period in which they produce, repeat, reorder, add and drop vocal elements until they eventually produce a stereotyped or "crystallized" song that resembles the one memorized from tutors (Immelmann 1969; Marler 1991; Williams 2004).

All but one song of male 7H.16 recorded in 2016 contained an extra element that completely disappeared in all songs recorded in 2017. Similarly, the prevailing song structure of male "NA" differed between late May and late June 2016. Since birds tend to produce more sounds during the song learning phase, with several plastic changes occurring over many weeks and vocal performances before reaching a crystallized song (Mooney et al. 2008), it is possible that both males were young (presumably one year old) in 2016 and thus still undergoing song stabilization.

As in previous studies studying the song of male Tawny Pipits (Neuschulz 1986; Osiejuk et al. 2007), we found that most males in our study population sang a single song type. However, we recorded a second song type in the vocalization of three males, which, to our knowledge, has not yet been reported for this species. Both song types sung by the same male were unique but their structures were not acoustically similar to each other. Osiejuk et al. (2007) suggested that there is no sexual selection towards an increased repertoire size for this species since its repertoire is formed by a single song. The proportion of two-song males in our study (3/81; 3.7%) seems too low to have resulted from selection, so this could simply be a learning issue with no adaptive value. Two-song repertoires are not exclusive to male Tawny

Pipits from our study site since we have also observed this phenomenon in other populations in Spain and Italy, always in a proportion comparable to the Czech population (J. Oñate-Casado, A. Petrusek, T. Petrusková, pers. obs.). A more intensive study of such individuals would be needed to determine whether there are any consistent patterns in the use of the different song types, or other behavioural differences from males that only sing one song type.

Females in our study site always sang under the same conditions (i.e., 3–5 flight songs when arriving to the nest, and rarely when leaving it), supporting what was previously described by Neuschulz (1986), Alström and Mild (2003), and Calero-Riestra and García (2019). Notably, however, our study provides the first evidence of female Tawny Pipits singing individually unique song types. We additionally observed that songs of males and females are neither distinguishable by ear in the field nor structurally different between sexes. Apparently, sexing Tawny Pipits based on the song acoustic structures alone seems impossible, although we cannot dismiss that pipits themselves may recognize the sex of conspecifics based on song. However, females seemingly sing under the same specific circumstances across several European populations, and we never observed ringed males singing while feeding (V. Beran and M. Porteš, pers. obs.). Hence, we believe that it is possible to deduce the sex of singing non-ringed Tawny Pipits by considering the behavioural and seasonal context in which their song is produced.

Female vocalizations when leaving and/or arriving at the nest, usually calls, have only rarely been reported for other temperate passerine species (Yasukawa 1989; McDonald and Greenberg 1991; Grunst et al. 2014), and such female calls have been suggested to prevent mistaken attacks by their mates in habitats with low visibility. The same function for the female song in Tawny Pipits was proposed by Neuschulz (1986); however, they breed in open habitats so males can easily use visual cues to identify their partners. We observed that our female pipits tend to land a certain distance (a few to tens of meters) from the nest, and then approach it by walking, just like Swamp Sparrows and Meadowlarks (McDonald and Greenberg 1991). Considering this, females may also use songs to alert their mate about an unprotected nest, to which they return by sneaking silently through the sparse vegetation in order to counteract the increased risk of nest predation associated with their singing activity.

Although always observed in the same context, and thus likely functional, female singing in Tawny Pipits remains rare. It is possible that the female propensity to sing is influenced by some intrinsic factors, such as age or hormonal shifts. For example, increased testosterone levels promote singing in female European Robins (Kriner and Schwabl 1991; Schwabl 1992). The level of this hormone in females often fluctuates temporally (Ketterson et al. 2005), and may differ substantially among individual females in various monogamous songbird species (e.g., Moreno et al. 2014; Těšický et al. 2022). Unfortunately, we lack any specific information about the status, age or physiological state of the females recorded in our study.

Although Neuschulz (1986) reported that Tawny Pipit females copy the song of the male they are paired with, from our observations it appears that most females have songs with a structure that clearly differs from those of their mates. Indeed, the male and female had very similar songs in only one of our recorded pairs. In contrast, we also found one male paired consecutively with two females, each in different years, and the songs of those two females were very similar to each other but not to that of their male partner. Furthermore, numerous males and females sang structurally similar songs irrespectively of their social relationship. We assume these "song families" result from the learning and introduction of fine individual-specific variation, and thus pairing of mates with similar songs arises by chance. Unfortunately, the results and spectrograms presented by Neuschulz (1986) lack sufficient detail, so it is not possible to check whether there were minor song differences between males and females of the pairs he recorded, or if other individuals in that population also sung similar songs. Although our results indicate that female Tawny Pipits do not adapt their song structure to that of their actual mate, we cannot rule out that they copy the song of their first nesting partner. However, it seems at least as plausible that females learn from other males in the population.

To determine whether female vocalization is influenced by their partners' songs or not, it is necessary to record the songs of paired birds—ideally also including females that switch partners—in consecutive years. By doing so, it could be also assessed with certainty whether female Tawny Pipit songs are temporally stable between seasons. Previous studies on White-crowned Sparrows and Northern Cardinals evaluated sex differences in the timings of the sensitive phase of song learning (Nelson et al. 1997; Yamaguchi 2001). In both studies, females and males were both confirmed to be closed-ended learners, but song acquisition was shorter in females. Assuming the same pattern, we may expect that female Tawny Pipits are also age-limited song learners, as expected for males, and thus also have a temporally stable song. In our dataset, one female was recorded in two different years and its song indeed did not change. However, there is no systematic study addressing whether female and male songbirds differ in the timing of song learning (see Riebel 2016).

To conclude, our study clearly demonstrates that songs of Tawny Pipit males and females are individually unique and suitable for IAM, although numerous individuals within a population may share highly similar song types. Clustering based on song comparison using the DTW approach was surprisingly successful in grouping songs of the same individual, but could not fully replace visual inspection by an experienced observer when songs differed only in fine structures and the recording quality was suboptimal. Neither our observations nor DTW-based clustering distinguished between the sexes. However, we cannot exclude that birds themselves do differentiate between songs of males and females; this hypothesis may be testable by playback experiments. Studies focusing on recording and evaluating songs from a greater number of female Tawny Pipits across years can bring more evidence for their presumed temporal song stability.

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**Author contributions** TP, MP and JO-C conceived the study. MP and VB recorded most of the songs, performed long-term monitoring of the study population, ringed and identified the ringed birds. JO-C performed bioacoustic analyses under the supervision of TP and analysed the data with the support of AP and TP; JO-C and AP prepared figures. JO-C, TP and AP wrote the first draft. All authors approved the interpretation of the results and the final version of the manuscript.

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**Availability of data and materials** The dataset generated and analysed during this study, including raw recordings and cut-outs of analysed songs in the WAV format, is available online (Zenodo data repository: https://doi.org/10.5281/zenodo.7670228). In addition, the recordings of songs used to create spectrograms in Figs. 1, S1 and S2 have been deposited in the Animal Sound Archive at the Museum for Natural History in Berlin and are available under the CC-BY-SA Licence at the following link: https://doi.org/10.7479/0k86-kd69.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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Oñate-Casado *et al.*: Guess who? Evaluating individual acoustic monitoring for males and females of the Tawny Pipit, a migratory passerine bird with a simple song.

## Supplementary Figures

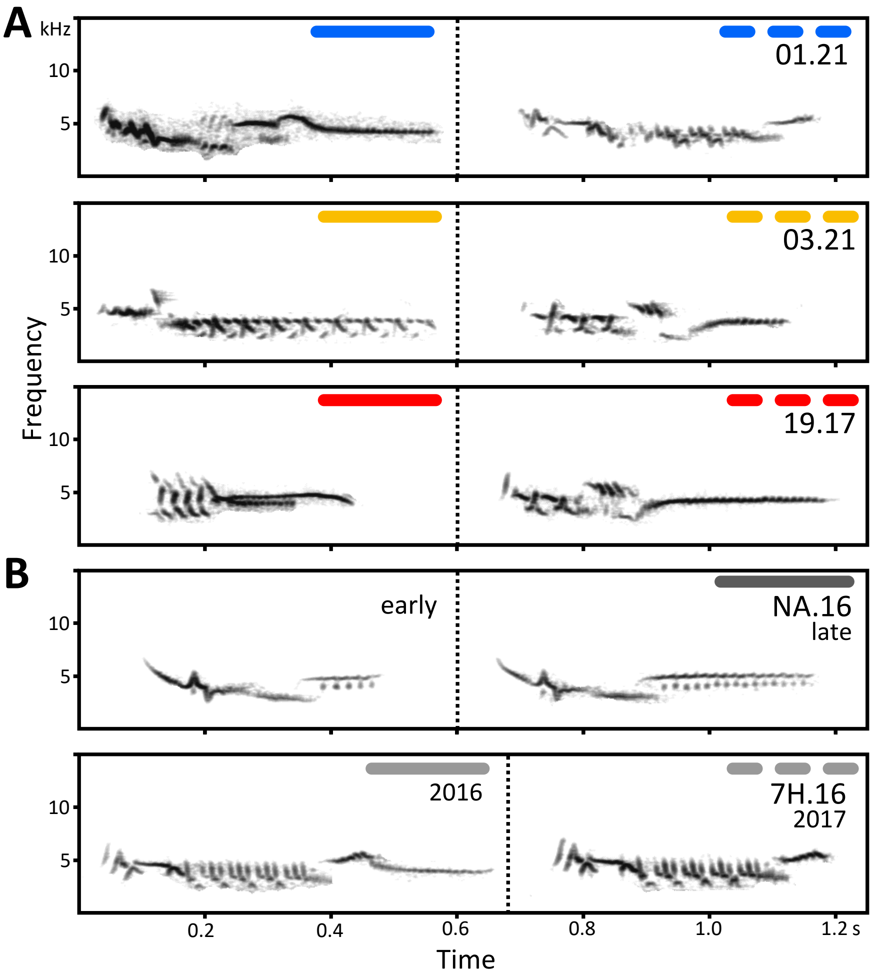

**Fig. S1** Spectrograms of songs of the three Tawny Pipit males that included two song types in their repertoires (**A**), and of the males that changed their song structure over time (**B**), either within a season (NA.16) or between seasons (7H.16). Song types on the left in **A** are marked by the letter "a" in the bird code in Fig. S3, those on the right by the letter "b". Colour bars match highlighted branches on the dendrogram in Fig. 3C. Due to the continuous nature of the variation in the ending part of NA.16's song, we did not visually distinguish "short" and "long" variants in Fig. 3C.

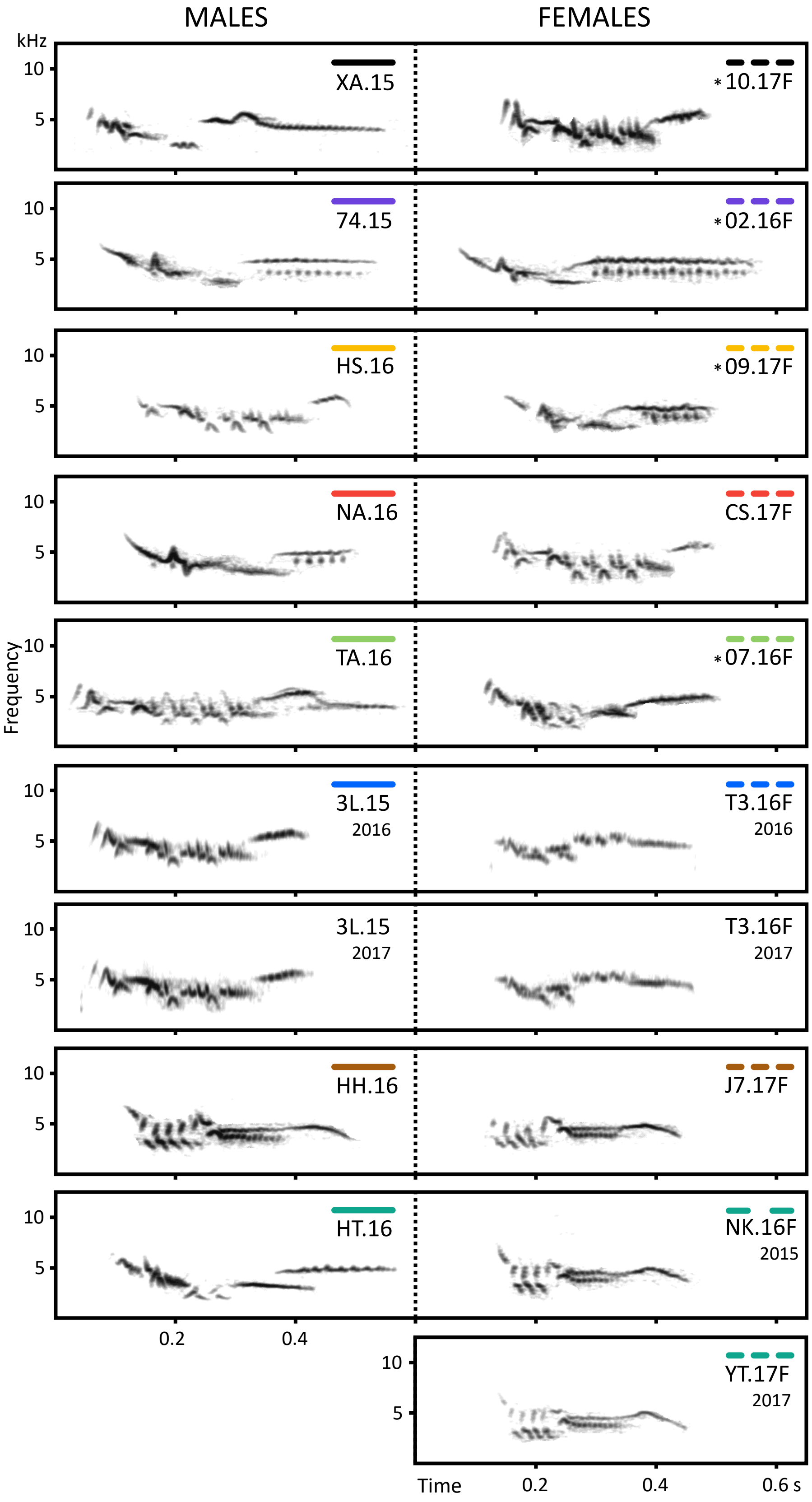
MALES
FEMALES
kHz
10
5
Frequency
XA.15
*10.17F
74.15
*02.16F
HS.16
*09.17F
NA.16
CS.17F
TA.16
*07.16F
3L.15
2016
T3.16F
2016
3L.15
2017
T3.16F
2017
HH.16
J7.17F
HT.16
NK.16F
2015
YT.17F
2017
0.2
0.4
Time
0.6 s

**Fig. S2**

**Fig. S2** Spectrograms of songs of paired males (left) and females (right) from the study population. Colour bars match highlighted branches on the dendrogram in Fig. 3D. Females marked by asterisks in front of their codes were not ringed. Male 3L.15 and female T3.16F were paired in three consecutive years (2015–2017); the male's songs were recorded in all three years, those of the female in 2016 and 2017. Songs from both years are shown to demonstrate their stability. Note the similarity of songs of the male 74.15 and the non-ringed female 02.16F (2nd row), and females YT.17F and NK.16F (paired in different years with the male HT.16) but also female J7.17F and male HH.16 (last three rows, all four ringed individuals clustering in the dendrogram in the same "song family" marked by the purple colour in Fig. S3).

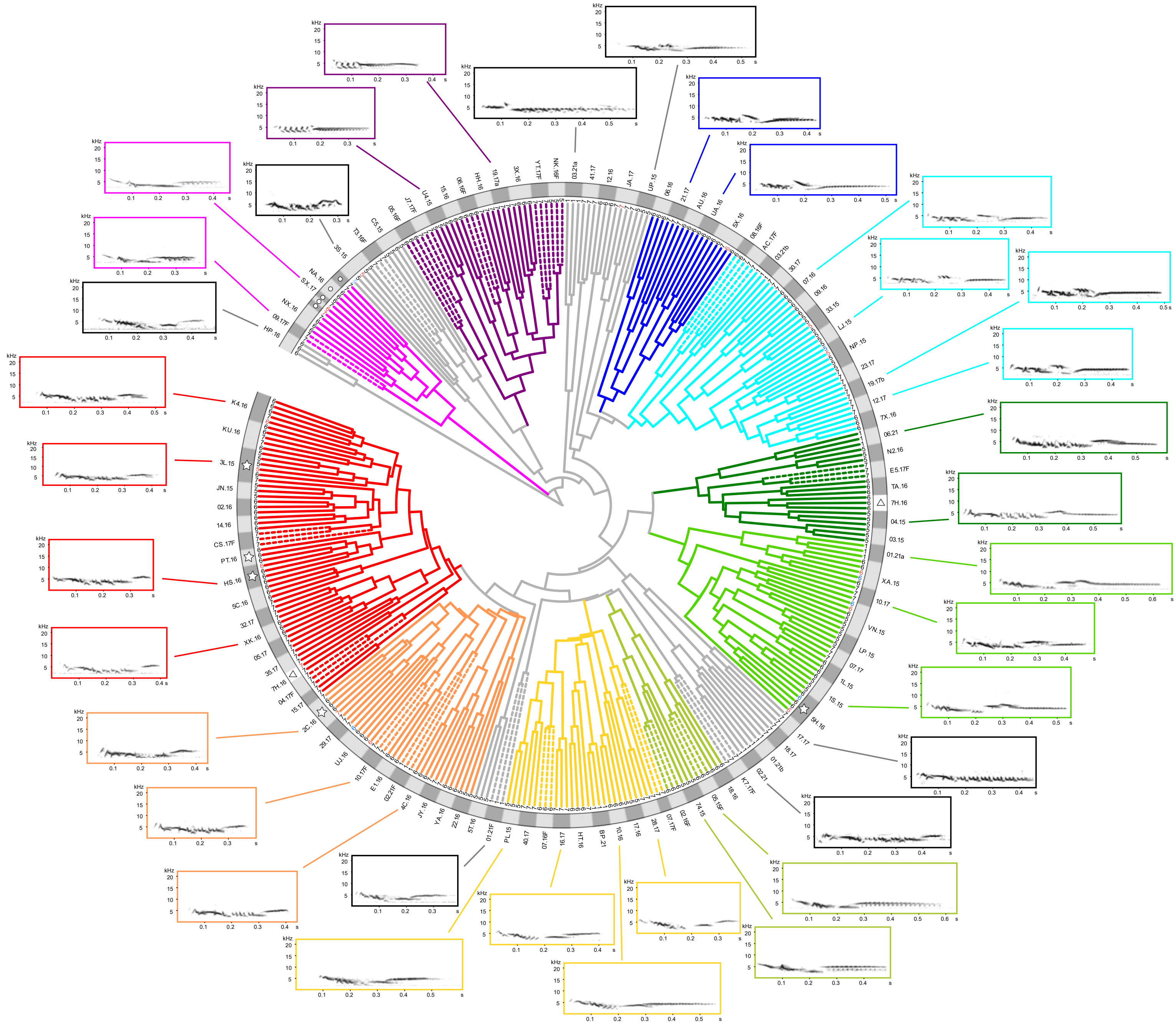

**Fig. S3** Song similarity dendrogram (as in Figs 2, 3) including codes of all individuals ("F" at the end of the code denotes females, letters "a" and "b" differentiate two song types of the same male). Representative spectrograms highlight the overall variation of song types observed in the studied population as well as the similarity of those belonging to the same parts of the dendrogram ("song families", marked by the matching colours).

# SUPPLEMENTARY TABLES

**Table S1**. Summary information about recorded birds, their sex, ringing status, and the number of days in each of the studied seasons their songs were recorded. This table is available as online supplementary material (.xlsx) or from the author upon request.

**Table S2**. Information about analysed recordings (recording date, individual assignment, period of the season, and a file name under which the recording is available in the file repository). This table is available as online supplementary material (.xlsx) or from the author upon request.

# Chapter 2

**Oñate-Casado, J**., Porteš, M., Beran, V., Petrusek, A., Petrusková, T. (2021). An experience to remember: lifelong effects of playback-based trapping on behaviour of a migratory passerine bird. *Animal Behaviour*, 182: 19–29.

A Tawny Pipit individual approaching a loudspeaker during a playback experiment conducted in a population in the coal mines near Most, Czechia.

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# An experience to remember: lifelong effects of playback-based trapping on behaviour of a migratory passerine bird

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Ringing is one of the most commonly used techniques to monitor bird individuals and collect data on various aspects of their life history. However, this method involves practices (capturing and handling) that might affect individuals' behaviour. Wild animals can remember and learn from previous experiences, and, thus, they anticipate risks and modify their response in subsequent similar situations. In this study, we aimed to evaluate the long-term effects of playback-associated mist netting on the behaviour of the tawny pipit, *Anthus campestris*, a medium-sized migratory passerine bird. In playback experiments simulating territorial intrusion of a conspecific male, we examined aggressive responses of individuals attracted in the past by conspecific song playback to a mist net ('experienced' males) and compared them to the responses of individuals without such an experience ('naïve' males). The interval between the capture of experienced males and the playback experiments ranged from several weeks to more than 5 years. Our results revealed prominent differences between the behaviour of naïve tawny pipit males and those experienced with a capturing event. Although all tested males responded to the stimulus, experienced males tended to be substantially less aggressive and more cautious in comparison to naïve individuals; prominent differences were observed, in particular, in the avoidance of close proximity to the loudspeaker by the majority of experienced males. There were no significant differences in responses to playbacks between the experienced males captured and exposed to subsequent experiments within the same or next season and those that underwent playback experiments later, up to 5 years after having been previously trapped. These findings suggest that male tawny pipits may negatively associate playback stimulation with capturing events and remember such experiences over a time period comparable to the expected life span of this species in the wild.

Bird ringing is one of the most commonly used techniques to monitor individuals, as it is an effective method to study several aspects of bird biology such as ecology, physiology, behaviour and population dynamics (Baillie et al., 2007). However, it might be considered an invasive method since practices such as mist netting, biometric and weight measurements, and marking using metal and/or colour rings might carry risks to the manipulated individuals. Several factors, such as the material and size of the mist net, weather conditions, number of birds caught and presence of predators of the trapped bird species (NABC, 2001), as well as experience of the ringer (Spotswood et al., 2012), can determine whether this practice is harmful to the birds' welfare. However, capture and subsequent ringing do not necessarily involve a risk for trapped birds. Clewley et al. (2018) showed with a large sample size of different bird species that mist netting is a relatively safe capture technique. Furthermore, a review paper (Calvo & Furness, 1992) reported that most studies did not find negative effects associated with metal and/or colour rings. Capturing birds for research purposes might also involve invasive methods such as blood sampling, but blood collection is not likely to negatively affect wild birds (reviewed in Sheldon et al., 2008). No negative effect of capture, ringing or blood sampling on territory maintenance or apparent survival of a small passerine in comparison with nonhandled birds was also recently demonstrated with the use of individual acoustic monitoring (Petrusková et al., 2021).

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Some studies nevertheless show that bird-ringing events might be stressful, with consequences at the physiological, physical and behavioural levels. The stress of capture and handling can increase the level of stress hormones (Wingfield et al., 1982), and even lead to capture myopathy. This metabolic disease damages muscle tissues as a result of extreme exertion, struggle or stress (Marco et al., 2006; Mulcahy et al., 2011) and might increase predation risk by affecting mobility and flight reaction from predators for days after release (Ponjoan et al., 2008). Additionally, the handling of captured birds itself can result in physical damage (NABC, 2001; Spotswood et al., 2012).

Apart from stress and injuries, these activities might also alter bird behaviour. Wild animals can remember and learn from previous experiences, and in this way, evaluate the associated risks and make decisions in future similar situations accordingly (Bradbury & Vehrencamp, 2011). In birds, results on, for example, pied flycatchers, *Ficedula hypoleuca* (Camacho et al., 2017), and great tits, *Parus major* (Seress et al., 2017), show that previously captured individuals are more difficult to catch in subsequent attempts using the same technique than individuals never trapped before. Capture avoidance may influence recapture rates, and thus the estimation of population sizes and of individual survival (Pradel, 1993). Furthermore, capture experience can also affect other behaviour beyond recapture rates, as reported in playback studies (Budka et al., 2019; Linhart et al., 2012).

The use of playback recording is common among bird ringers to attract birds into their nets (Falls, 1992). This process consists of playing a birdsong recording to bring birds closer, and it is also commonly used in behavioural studies on bird communication (e.g. Møller, 1992; Oñate-Casado et al., 2020; Petrusková et al., 2014) and social interactions (e.g. Douglas & Mennill, 2010; Grafe & Bitz, 2004; Otter et al., 1999). However, a few studies have assessed the impact of capture events on bird response through playback experiments. For instance, Linhart et al. (2012) observed that the use of playbacks to lure willow warblers, *Phylloscopus trochilus*, into mist nets decreased successful recapture after repeated stimulation, even in tests conducted in the subsequent season. Their results suggest that the process in which bird ringers capture and release individuals might mirror events of a bird being caught by a predator with a subsequent escape. An experimental study conducted under laboratory conditions on common mynas, *Acridotheres tristis*, indicates that capturing of individuals by humans can be successfully used as simulated encounters with predators (Griffin & Boyce, 2009). Besides these studies, Budka et al. (2019) assessed how luring with playbacks of rival songs, capturing and subsequent release affected the behavioural response of male chaffinches, *Fringilla coelebs*. They found that previously captured males, which were subjected within the same season to simulated territorial intrusions again, sang fewer songs than noncaptured males; this was probably because experienced males were more cautious at the time of responding to a stimulus associated with a previous negative experience.

In our study, we focused on the potential long-term effects of playback-based mist netting on the behaviour of the tawny pipit, *Anthus campestris*, a socially monogamous and territorial migratory songbird with a simple song. Based on incidental field experience that suggested the increased difficulty of recapturing once-caught males (e.g. for retrieval of attached geolocators), we hypothesized that mist netting after playback influences males' behaviour in comparable situations later in life. To indirectly test this, we conducted playback experiments in which we examined aggressive responses to a simulated territorial intrusion of individuals previously attracted by playback to a mist net ('experienced males', captured several weeks to several years before the actual experiment) and compared them to the responses of individuals without that experience ('naïve males'). We predicted that at least within the next year after trapping, experienced males would be more cautious than naïve ones, and thus show lower aggressiveness, as a result of previous negative experience with mist netting.

## METHODS

### Study Species and Locality

The tawny pipit (Passeriformes: Motacillidae) is a medium-sized migratory passerine, cryptically coloured with no sexual dimorphism (Cramp, 1988). Despite being widely distributed in the Palaearctic, this species has faced a severe decline in parts of its range during the last few decades (Briedis et al., 2016; Grzybek et al., 2008). Tawny pipits are habitat specialists and require dry, sandy, steppe-like habitats with bare ground and sparse vegetation for breeding. Activities such as forestry, intensification of agriculture and farmland abandonment cause the loss of suitable habitats for this species (Birdlife International, 2020). Thus, tawny pipit populations are very patchy in central and western Europe.

Our study was conducted in the only remaining tawny pipit population in Czechia, intensively monitored in an active surface coal mine area in North Bohemia (50.48–50.56° N, 13.48–13.58 °E; Briedis et al., 2016). During the breeding season (early May until late July) in 2012–2017, most singing males in the population (over ca. 80% in the core area of the mines) were captured and subsequently marked by unique colour rings. In 2015–2017, singing territorial males were repeatedly recorded after territory establishment during the breeding season. Tawny pipit males usually sing from high perches in their territory or during flight. The song is relatively short with an average length of 0.5 s and formed by several syllable types combined in an individually unique song type (Osiejuk et al., 2007). Moreover, song types of individual males are stable both within and between seasons (Oñate-Casado et al., n.d.); therefore, we could use them to verify the identity of unringed males, using a similar approach as described in Petrusková et al. (2016).

### Playback Experiments

We created 3 min playback recordings from songs of different males recorded at the same locality in previous years, each originating from a different individual. We used only recordings of solo singing; that is, in a clearly nonaggressive context, with minimal background noise. The song rate of the playback recordings was on average 15 songs/min, following the natural singing activity of the species. Subsequently, we standardized the amplitude between these preprocessed recordings by equalizing the root-mean-square sound pressure in the individual WAV files, using the script 'rms equalize' by G. J. L. Beckers for the software Praat (Boersma & Weenink, 2013).

The playback experiments were conducted by the same person (M.P.) during the breeding seasons of 2016 and 2017 (late May to July), between 0600 and 1800 hours (CET) in suitable weather conditions, following the species' natural activity. Only males actively singing in the territory were exposed to the playback stimulus. Each male was tested with a unique randomly chosen playback stimulus, and neighbouring males were never tested during the same day, to avoid habituation.

The subject males consisted of two different groups: experienced ($N = 29$) and naïve ($N = 31$). The experienced males had been previously lured by a playback recording (different from those in the experiments) to mist nets, to be captured and ringed (one of these, however, managed to escape before ringing). A blood sample (to be used for another independent project) was taken from a subset of 17 experienced males (most of those captured in 2015 and 2016). Most of the experienced males were captured and handled in one of the seasons preceding the playback experiment (1 year ago: 12 males; 2 years: 2; 3 years: 4; 4 years: 2; 5 years: 3), while six males were captured earlier in the same season as tested (Supplementary Material, Table S1). All these males were adult and actively defending their territories when originally captured (i.e. they were at least in their second year of life; more precise ageing is not possible in this species). Most of the experienced males were only captured by playback once; two of them (KU and XT; Table S1), however, were recaptured using the same technique approximately a year after having been originally caught. All males in the experienced category were therefore already familiar with the playback of conspecific songs and potentially might have associated this with a negative experience.

The naïve males had never been exposed to the playback recordings nor captured by a mist net before, and thus they had no such experience. Apart from three individuals ringed as nestlings in 2015, naïve males were not ringed. To verify their identity, we recorded them while singing in their territory, and then during the playback experiment itself. We then compared the song type in both recordings to ensure that we tested the local territorial male and not an accidentally passing individual. After the playback experiments, time permitting, we lured naïve males using playback to a mist net that was subsequently set up next to the loudspeaker (for ringing purposes).

All playback experiments were performed when the focal male was confirmed to be present in its territory. We placed the wireless, remote-operated, portable loudspeaker (Yuecheng CP-387; the same that had been used while mist netting the experienced birds) and walked approximately 25 m away to conduct the experiment. This distance, from which the tested bird was observed by binoculars, was chosen to avoid the potential effect of human presence on male responses. (Note that birds in this active mining area are familiar with occasional human activities in their territories.)

Prior to playback, we recorded the focal male's spontaneous song using a digital recorder (Marantz PMD661 MKII) connected with a shotgun microphone (Sennheiser ME 67). Then, the 3 min playback stimulus was initiated once the focal male could be either observed within the audible range or heard singing. During the playback and 3 min after its end, the focal male's activity (including singing) was recorded, and behavioural responses (see next section) were noted by voice comments on the recording.

### *Behavioural Assessment and Data Analysis*

The recordings obtained during the playbacks were further assessed in Avisoft SASLab Pro, version 5. After confirming that the tested male was indeed the long-term holder of the territory, we quantified several aspects of the male's aggressive behaviour as in, for example, Petrusková et al. (2007) and Turčoková et al. (2011). During the playback of the stimulus and for 3 min afterwards, we counted the instances of each of the three categories of active territorial defence behaviour with increasing level of aggression: flyovers (male flies directly over the area where the speaker is located); flight attacks (male approaches within 1 m of the speaker, in flight); and physical contacts with the loudspeaker (from briefly hitting the speaker in flight to sitting on it). Further, we determined the latency of reaction (the time when the focal male started to react after the playback started), estimated the minimum distance to which the male approached the loudspeaker, quantified the time spent by the male in the immediate vicinity (within 0.5 m) of the loudspeaker as well as the total time when the individual showed interest in the stimulus (e.g. by moving closer to the loudspeaker, intently watching or exploring the area where it was placed, performing short flights in the vicinity, or expressing some of the stronger aggressive reactions: flyovers, flight attacks or physical contacts).

These variables (except for the total time of interest) were included in a principal component analysis (PCA) to summarize the overall aggressive reaction of males to the playback stimulus (Fig. 1, Table A1). The first principal component (PC1), which accounted for almost 50% of the variation in the data set, correlated negatively with the minimum distance to the loudspeaker and latency to reaction and positively with the other variables summarizing various aspects of the aggressive behaviour (Fig. 1, Table A2). This composite score was thus used as a proxy for the overall male aggressiveness. The values characterizing the responses of experienced and naïve males (both original variables and PC1) were compared by the Mann–Whitney *U* test, due to the non-normal distribution of some variables. We also compared, with the same test, the intensity of aggressive reactions (expressed as PC1) of 17 experienced males from which blood samples were taken with the 11 males that had been also handled and ringed but without blood sampling. (An additional experienced individual that had been captured but escaped before ringing was excluded.)

We split the experienced males into subgroups of those tested relatively shortly after the previous capture event (the same or next breeding season, 18 males) and those tested 2–5 years later (11 males). This allowed us to assess the potential effects of increased delay since capture and/or the males' ages on their behaviour. Owing to the already mentioned difficulty in assessing the age in the study species (the only way to determine it being ringing at the nestling stage), we could not include age as a factor to account for variation in aggressiveness of the tested individuals. However, we assume that the age structure of naïve males and those tested relatively soon after the original capture was comparable, and if male age had an important effect on the aggressive behaviour, the two groups of experienced males should differ in their responses. We thus compared the responses (both original variables and PC1) between all three male groups (naïve and two experienced subgroups). First, we ran Kruskal–Wallis tests (which revealed significant differences between groups in all cases) and then performed post hoc pairwise comparisons between groups using Dunn's test with the Benjamini-Hochberg *P* value adjustment to control for false discovery rates.

To check in more detail whether the aggressiveness of experienced males might have been affected by the time elapsed since their previous experience with playback and capture, we evaluated the relationship between the delay since capture (in days) and the composite aggressiveness score (PC1) of experienced males, using a general linear model (GLM). Furthermore, as the territorial males were tested throughout the breeding season, we assessed whether there was a significant relationship between the timing within the season (day of the year from 1 January) and the aggressive response of the males. We ran general linear models with the aggressiveness scores (PC1) as a continuous response variable, and one continuous variable (day within the season) plus two categorical variables (experimental group: naïve versus experienced males; year when the experiment was conducted) as predictors. Apart from a simple model, we also ran one including interactions between the predictor variables.

For the 26 males tested in 2016 (13 experienced and 13 naïve), we also evaluated the effect of playback on song output. Specifically, we calculated the song rate (songs/min) in 3 min of spontaneous singing recorded before the experiment, and up to a 3 min period after the first vocal response since the playback started. (The evaluated period could be shorter in cases where the male stopped singing and flew away.) We then quantified the difference in song rate as the ratio between song rate after and before stimulation. We used a generalized linear model (GLZ) with a Gaussian family to investigate changes in song rate before and after playback experiments between our experimental groups (experienced and naïve males). For this, we assessed the influence of two predictors, a continuous one (song rate before playback) and a categorical one (experimental group), on one continuous response variable (song rate after playback). As no significant difference was observed between the experienced and naïve groups (see Results), the song rate was not evaluated for 34 males tested in 2017.

Statistical analyses were conducted in Statistica version 13.5 (TIBCO Software, Palo Alto, CA, U.S.A.) and R version 4.0.2 (R Core Team, 2020). The former software was used for PCA and basic nonparametric tests, and the latter for general and generalized linear models and for Kruskal–Wallis tests with post hoc Dunn's tests (included in the package FSA: Fisheries Stock Analysis; Ogle et al., 2021).

### *Ethical Note*

The experimental work was compliant with the relevant legislation of the Czech Republic (Act 246/1992 Coll., on the protection of animals against cruelty). The playback experiments conducted in the study itself did not involve any physical handling of focal individuals. After the experiment, naïve males were captured by a mist net and ringed by an experienced ringer (M.P. or V.B.) with an appropriate licence issued by the relevant national authority (Bird-ringing Station of the National Museum in Prague). There is substantial evidence that appropriately performed standard ringing methods usually do not have long-term negative impacts on passerine birds (for more information and discussion on potential impacts of ringing, and for recent data from a conspecific species, see Petrusková et al., 2021).

## RESULTS

All 60 tested tawny pipit males reacted to the test stimulus, most of them promptly, within seconds of the initiation of the stimulus playback (mean value of latency to reaction was 9 s). Only 10 males (eight of them experienced) reacted later, with latency exceeding 15 s, but all responded within the first minute of the recording playback. There was a small but significant difference in the latency to react between naïve and experienced males, with most experienced males reacting slightly later than most naïve males (Table A2). When the experienced males were split into those tested relatively shortly after the previous capture event (within the same or next year) and those captured 2–5 years later (Fig. 2a), there was no significant difference between them (Dunn's post hoc test: $Z = 1.05$, adjusted $P = 0.29$) but the group of later-tested males did not differ significantly from the naïve ones ($Z = 1.49$, adjusted $P = 0.21$). This indicates that both naïve and experienced males recognized the playback stimulus and considered it relevant.

Most males, both naïve and experienced, kept showing some level of interest in the playback stimulus for the rest of the time that the recording was played, and usually also in most of the subsequent 3 min silent period (median time of interest: experienced males 340 s, naïve males 352 s). A notable outlier was an experienced male that responded to the playback after 10 s, but flew away 30 s later to join the female, in whose close vicinity it spent the rest of the experimental period, presumably mate guarding; all other males expressed interest for at least 210 s. Overall, naïve males showed interest significantly longer than experienced ones (Mann–Whitney $U = 219.5$, $N_1 = 31$, $N_2 = 29$, $P = 0.0007$).

Substantially more pronounced (and highly significant) differences were observed in all variables that characterized the aggressive behaviour of the males (numbers of flyovers, flight attacks and physical contacts, minimum distance to the loudspeaker

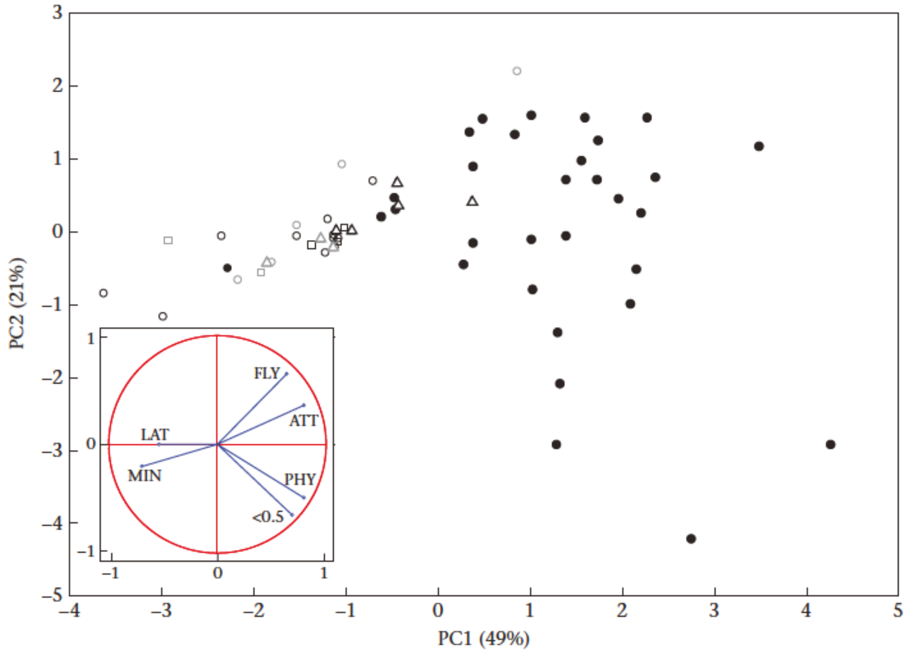

**Figure 1.** Principal component analysis summarizing the aggressive reactions of tested males in the playback experiment simulating a singing male intrusion into the territory. The first principal component (PC1) is a proxy for the overall male aggressiveness in the experiment. Males naïve to the stimulus ($N = 31$) are marked by full black circles, those with previous experience with mist netting accompanied by playback ($N = 29$) by open symbols. Shapes and colours indicate the time between capture and experiment for experienced males (circles: grey: same season, 6 birds; black: 1 year, 12 birds; triangles: grey: 2 years, 2 birds; black: 3 years, 4 birds; squares: grey: 4 years, 2 birds; black: 5 years, 3 birds). The inset chart indicates the correlations between the original variables and the first two principal components: MIN: minimum distance from the loudspeaker; LAT: latency of reaction; <0.5: time spent within 0.5 m from the loudspeaker; FLY: number of flyovers; ATT: number of flight attacks; PHY: number of physical contacts. (PC1 and PC2 values for each variable and each individual male are given in Table A2 and Table S1 in the Supplementary Material, respectively.)

and time spent in its close vicinity; for details, see Fig. 2 and Table A2). Experienced males tended to express much more cautious behaviour: they stayed further from the loudspeaker and showed a much lower tendency for direct attacks. While most males in both groups (30 naïve and 27 experienced, i.e. 97% and 93%) performed at least some flyovers at a distance of more than 1 m from the loudspeaker, only four experienced males (14%), in contrast to 28 naïve ones (90%), also performed flight attacks, and only one experienced male (3%), in contrast to 22 naïve males (71%), attacked the loudspeaker physically.

The most striking differences reflecting the more cautious behaviour of experienced males were observed in the minimum distance to the loudspeaker, and correspondingly, in the time spent within a 0.5 m radius around it (Fig. 2c and d, Table A2). All but three experienced males (i.e. 90%) avoided approaching closer than 1 m to the speaker, and the remaining three did not spend more than 5 s in its vicinity. In contrast, all but three naïve males (90%) approached closer than 1 m, and most of these spent substantially more time within 0.5 m of the speaker (median 19 s, range 4–110 s).

The consistent differences in behaviour between the two male groups were reflected in a significant difference in the composite aggression score (PC1; Figs. 1, 2h and 3) obtained from the PCA (Mann–Whitney $U = 45$, $N_1 = 31$, $N_2 = 29$, $P < 10^{-6}$). There was no significant difference between the PC1 score of experienced handled males from which a blood sample had been taken and of those only captured and ringed (Mann–Whitney $U = 62$, $N_1 = 17$, $N_2 = 11$, $P = 0.14$). Apart from the overall difference in PC1, the scatter in the PCA plot, especially along the second principal component (PC2) tended to be higher in naïve males (Fig. 1), which reflects much higher variation in individual aggressiveness within this group (some males having a stronger tendency for physical contacts and longer stays near the loudspeaker than others). There was, however, no significant difference in PC2 values between naïve and experienced males (Mann–Whitney $U = 367$, $N_1 = 31$, $N_2 = 29$, $P = 0.23$).

When the males were split into three groups, the naïve males and two subgroups of experienced males pooled according to the time elapsed since the negative experience (0–1 years versus 2–5 years later), all the original variables characterizing male behaviour as well as the PC1 score differed significantly between the groups (Fig. 2; Kruskal–Wallis tests, $P < 0.007$ in all tests). However, the difference between the two subgroups of experienced males was never significant (Dunn's post hoc tests, adjusted $P > 0.29$ for all variables). In contrast, both subgroups of experienced males differed strongly from the naïve ones in most variables (Fig. 2), except for the latency of reaction in later-tested experienced males (Fig. 2a), which was not significantly different from that of naïve males (see above), and the time of interest (Fig. 2b), for which the difference between later-tested experienced males and naïve males was only weak, though still significant (Dunn's post hoc test: $Z = -2.17$, adjusted $P = 0.045$).

On a closer look, there was no significant relationship between the intensity of reaction of experienced males (expressed as PC1) and the time (in days) elapsed between the mist netting and the playback experiment (Fig. 3, Table A3). This is also apparent in the scatter of respective males in the PCA plot (Fig. 1). Five individuals with the highest PC1 scores, exhibiting generally stronger or more aggressive reactions than the remaining experienced males, included one male tested in the same season as it was ringed, one ringed in the previous season and three ringed 3 years before.

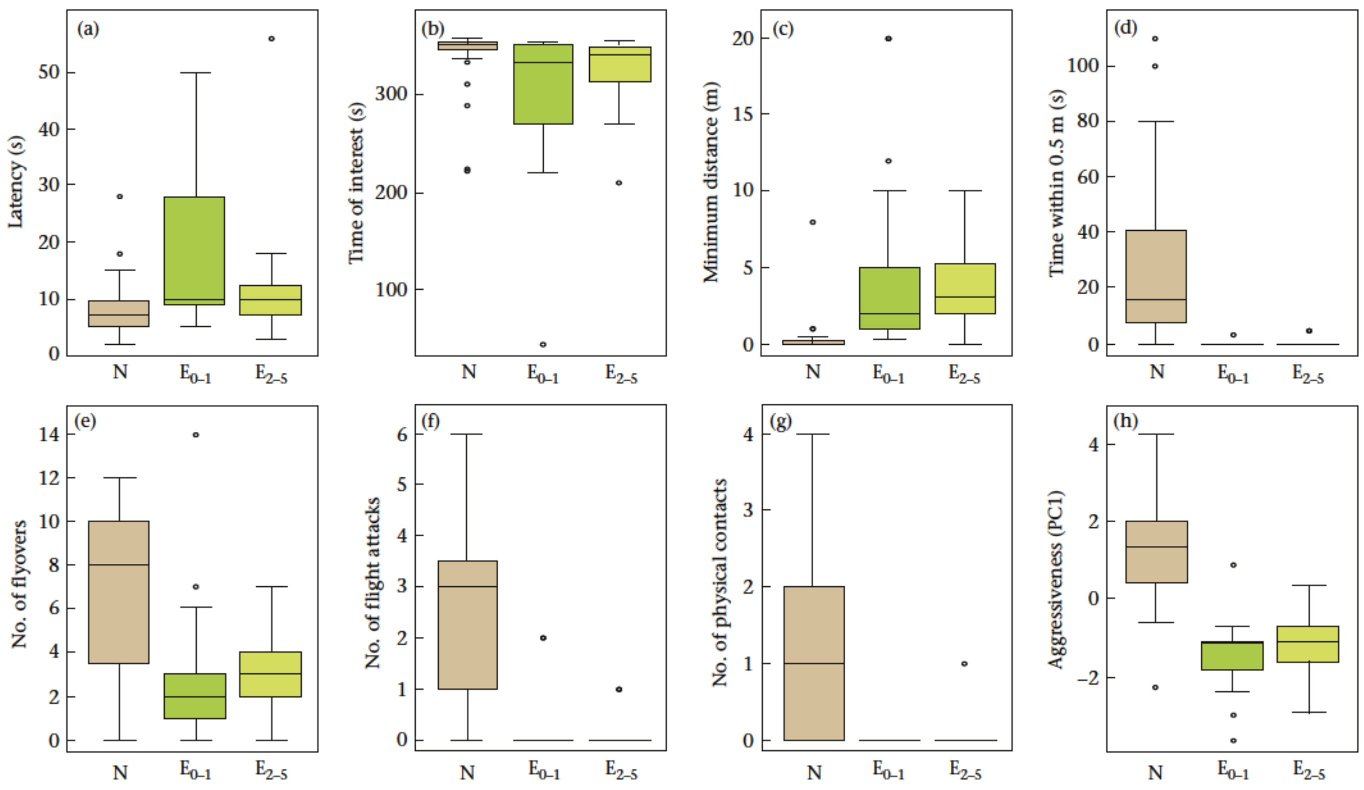

**Figure 2.** Comparison of responses to playback experiments of naïve males (N; $N = 31$), experienced males tested within the same or next breeding season after capture ($E_{0-1}$; $N = 18$) and those tested later, within 2–5 years after capture ($E_{2-5}$; $N = 11$). (a)–(g) Original variables characterizing the male reaction and (h) the aggressiveness score (first principal component from the PCA). The box plots show the median, first and third quartiles and the values within 1.5 times the interquartile range; the circles are outliers.

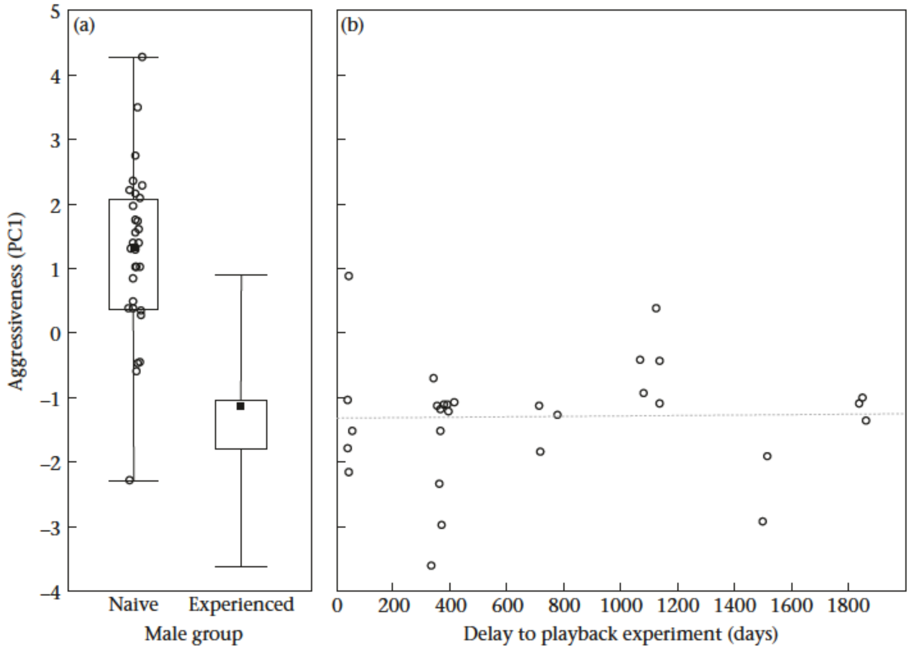

**Figure 3.** Differences in the aggressiveness score (values of the first component from the PCA) between naïve and experienced males. (a) Box plots depict the median, first and third quartiles and range; circles indicate individual data points for naïve males, with jitter introduced to reduce overlap. (b) Scatterplot of the individual values for experienced males, shown against the delay between the negative experience associated with capture and the playback experiment; the trendline is shown as a dashed line.

There was no significant relationship between the intensity of aggressive reaction and the timing of the experiment within the season (Fig. A1, Table A4) or the year in which the experiments were conducted (Table A4); the only significant difference was between the experienced and naïve males, consistent with the other analyses (Table A4). When interactions were included in the GLM, none was significant (results not shown).

The song rate of most males tested in 2016 increased in response to the playback stimulus (Fig. A2). The spontaneous song rate (before playback) ranged from 13 to 19.7 songs/min (median 15). Of 26 tested males, 24 increased their song rate (by 2–66%), which ranged from 16.7 to 24.3 (median 18.5) songs/min after playback stimulation. One of the two remaining males did not change its song rate at all; the last one (an experienced bird) did not sing after playback and responded by calls only. There was no significant difference between experienced and naïve males in their song rates either before or after acoustic stimulation (Table A5), and the male groups did not differ in the magnitude of song rate increase either (Table A5).

## DISCUSSION

In this study, we demonstrated that male tawny pipits that had been lured to mist nests by playback recordings of conspecific songs before exposure to a new playback stimulus reacted less aggressively and more cautiously than naïve males without such an experience. In comparison with naïve males, the previously captured males neither approached the speaker nor attacked it, and the overall aggressiveness in their response to acoustic stimuli in the playback experiment remained low, regardless of whether the experiment was carried out within the same season or after several years. This corresponds to the field observations that birds caught once tend to be more difficult to recapture, a fact well known to many ringers but infrequently reported in peer-reviewed literature (but see e.g. Camacho et al., 2017; de Lima & Roper, 2009).

We assume this behaviour results from the long-term memory of the captured birds, which presumably connected playback stimulation (comparable during the original capture event and in our experiments) with the subsequent negative experience, which included getting entangled in a net and being handled by humans. In our study design, it was impossible to disentangle the contribution of different aspects of this previous experience (acoustic stimulus broadcast from an unusual object, capture and handling by humans). However, the playback itself did not seem to initiate more cautious behaviour, as naïve males tested in playback experiments were usually easy to capture in a net later (M. Porteš, personal observation). Cumulative effects of the playback lure plus capturing and handling on the subsequent behaviour of birds have already been reported (e.g. Budka et al., 2019; Linhart et al., 2012). However, our study is the first one indicating that a short-lived passerine may remember such an experience and modify its future behaviour to avoid it, over a time period equal to or exceeding its expected life span in the wild.

Such avoidance might hinder recapture success and affect the results of long-term studies dependent on individual monitoring (de Lima & Roper, 2009; Linhart et al., 2012; Petrusková et al., 2016). The general persistence of these stressful events on birds' behavioural responses is unclear; both short (within a season; Budka et al., 2019) and longer-term (to subsequent years; Linhart et al., 2012) effects have been reported for passerines. Similar to willow warblers studied by Linhart et al. (2012), experienced male tawny pipits in our study tended to show decreased aggressiveness in comparison with naïve individuals, being prone to express more cautious behaviour. However, we observed that the behavioural response to playback songs did not differ substantially between males captured and exposed to experiments within the same season they were trapped and those tested several years later.

Unfortunately, we could not evaluate the potential effects of the actual age of tested males on their behavioural response. The age of adult tawny pipits cannot be reliably determined even when individuals are captured, preventing us from disentangling the effect of age from the effect of delay since capture. Male age might be a factor influencing the response to the stimuli both in naïve and experienced males and could contribute to some of the observed variation. However, the behaviour of males tested the same or next year after the capturing event, whose age structure is probably close to the naïve males, was comparable to that of older experienced males, and differed strongly from the behaviour of naïve males (Fig. 2). It thus seems likely that the negative experience with the capturing event had a much stronger effect on male behaviour than age.

Other characteristics such as the male's personality may affect the intensity and characteristics of individual response independently of age (e.g. Garamszegi et al., 2009). However, considering that most tawny pipit males within our study population were captured and ringed in the period when the study was conducted (including the naïve males tested in this study, which were usually captured after the playback experiment itself), we are convinced that there was no consistent difference in personality traits between the experienced and naïve groups.

The three males captured as adults and tested in our study ca. 5 years later are apparently among the oldest tawny pipits ever documented from the wild. However, they did not exhibit behaviour substantially different from those tested a year after capture, which were presumably much younger birds. Considering this, we assume that the song playback (possibly associated with a visual stimulus: the presence of a loudspeaker) may act as an informative signal for male tawny pipits to recall the negative context in which they were captured, during their entire lifetime. This suggests that previous negative trapping events may trigger lifelong behavioural changes in this species, and probably in other small passerines as well.

Studies documenting long-term memory associated with negative experiences have rarely been reported in passerines, and those few available were conducted on long-lived corvid species. American crows, *Corvus brachyrhynchos*, which have an average life span of 4–6 years in the wild (Johnson, 1994), can recognize the facial traits of 'dangerous' humans for at least 3 years (Marzluff et al., 2010), and ravens, *Corvus corax*, for at least 4 years (Blum et al., 2020). Rapid learning and long-term memory retention of negative experiences in American crows and ravens may apparently happen after a single and brief stimulus (Blum et al., 2020; Marzluff et al., 2010).

We presume that experienced male tawny pipits recognized the whole playback experiment (conspecific song from a stationary object, no conspecific bird in sight and humans present within tens of metres of the site) as a threatening event, which resulted in more cautious and less aggressive behaviour. However, the reduction in the response to repeated acoustic stimulation might arise also because of habituation to playback (Dong & Clayton, 2009). Such habituation reflected in reduced response towards the playback stimulus repeatedly presented within a single season was reported, for example, in plain-tailed wrens, *Pheugopedius euophrys* (Harris & Haskell, 2013) and chaffinches (Budka et al., 2019). Great tits seem to remember the playback stimulus presented just once, even over 1 year (Rivera-Gutierrez et al., 2015). None the less, it is very unlikely that the differences we have observed in the behaviour of naïve and experienced males in our study resulted from a loss of interest, which is typically associated with habituation (e.g. Latham et al., 2019; Price et al., 2020; Pullen et al., 2012).

In the context of playback experiments, habituation is presumably caused by the gradual perception of a repeated stimulus as a nonthreatening signal. However, our experienced individuals still reacted to playback within the first minute of the trial, on average just a few seconds later than naïve males. Additionally, they kept expressing interest even after the playback recording stopped. In the case of habituation, we would have expected a stronger decrease of interest, especially when the acoustic stimulus was no longer present. Also, we found no differences in the response of males captured and tested within the same year and those that underwent the playback experiment in the successive years. Moreover, both naïve and experienced males did not differ in singing behaviour; both groups significantly increased song rate after playback. The same was documented by Rivera-Gutierrez et al. (2015) in great tits, which seemed to remember a playback stimulus presented just once, and in playback experiments carried out a year after they reduced locomotor behaviour but not vocalization. The authors assumed that such responses could be due to the association with the complete experimental set-up rather than acoustic stimulus only, which is likely in our case as well.

To our knowledge, our study, which revealed behavioural differences in male tawny pipits probably associated with a capture experience up to several years earlier, represents the first experimental field evidence of such long-term memory in a short-lived passerine. Our data are based on a comparison of two groups of males, however. An ultimate proof that the capture alters individual behaviour for years would be a comparison of the responses of the same male tested as a naïve individual, then captured, and later retested with the same experimental design. While we lack such data from repeated experiments, our field experience with attempts to recapture experienced tawny pipit males (e.g. carriers of geolocators) indeed confirms that they behave much more cautiously in response to playback (in particular when a mist net is set up).

Altered behaviour of territorial males during a simulated intrusion of a conspecific male to the territory may raise concerns that the commonly used mist-netting method, in which birds are lured into the net by playback, could also change their behaviour in natural territorial interactions, with a negative impact on their long-term fitness. However, our observations on this and other species suggest that such an impact is unlikely. Captured males did not leave their territories after the handling, and successfully defended them during the whole season (M. Porteš and V. Beran, personal observation). A recently published long-term study on a congeneric species, the tree pipit, *Anthus trivialis*, showed that neither the territory tenure nor apparent survival (within and between seasons) differed between handled males (captured, ringed and blood sampled) and the unhandled ones tracked by individual acoustic monitoring only (Petrusková et al., 2021). Future behavioural studies should nevertheless consider the differences shown here between experienced and naïve males when planning and carrying out experiments in which subject individuals are repeatedly exposed to the same stimuli. Our results also have implications for studies that use similar methods for capturing individuals (e.g. for retrieving data loggers, estimation of survival and return rates, etc.), since their success might be affected by the birds' long-term memory.

## Author Contributions

**Javier Oñate Casado:** Writing – original draft, Writing – review & editing, Formal analysis, Visualization; **Michal Porteš:** Conceptualization, Investigation, Methodology, Writing – original draft; **Václav Beran:** Investigation, Data curation, Funding acquisition, Project administration; **Adam Petrusek:** Formal analysis, Visualization, Writing – review & editing, Funding acquisition; **Tereza Petrusková:** Conceptualization, Supervision, Methodology, Project administration, Writing – review & editing.

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## Supplementary Material

Supplementary material associated with this article can be found online at https://doi.org/10.1016/j.anbehav.2021.09.010.

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## Appendix

**Table A1**
Eigenvalues of correlation matrix and variation explained in the principal component analysis

| | Eigenvalue | Explained variation (%) | Explained variation (cumulative, %) |
|---|---|---|---|
| PC1 | 2.927572 | 48.8 | 48.8 |
| PC2 | 1.257156 | 21.0 | 69.7 |
| PC3 | 0.852611 | 14.2 | 84.0 |
| PC4 | 0.568204 | 9.5 | 93.4 |
| PC5 | 0.245701 | 4.1 | 97.5 |
| PC6 | 0.148756 | 2.5 | 100.0 |

**Table A2**
Correlations between the original variables used in the principal component analysis and the first two principal components (as shown in Fig. 1), descriptive statistics for the groups of naïve and experienced males, and their comparison (by Mann-Whitney *U* test) for each variable separately

| Variable | PC1 | PC2 | Naïve males ($N = 31$) | | | Experienced males ($N = 29$) | | | Mann–Whitney *U* test | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Median | IQR (Q1–Q3) | Range (min–max) | Median | IQR (Q1–Q3) | Range (min–max) | *U* | *P* |
| Latency of reaction | −0.535 | −0.001 | 7 | 5–9.5 | 2–28 | 10 | 9–16 | 3–56 | 248 | 0.00286 |
| No. of flyovers | 0.638 | 0.647 | 8 | 3.5–10 | 0–12 | 2 | 2–4 | 0–14 | 202 | 0.00023 |
| No. of flight attacks | 0.798 | 0.360 | 3 | 1–3.5 | 0–6 | 0 | 0–0 | 0–2 | 73.5 | $<10^{-6}$ |
| No. of physical contacts | 0.797 | −0.492 | 1 | 0–2 | 0–4 | 0 | 0–0 | 0–1 | 141 | $<10^{-6}$ |
| Minimum distance from the loudspeaker | −0.695 | −0.205 | 0 | 0–0.25 | 0–8 | 2 | 1–5 | 0–20 | 57.5 | $<10^{-6}$ |
| Time spent within 0.5 m of the loudspeaker | 0.691 | −0.652 | 16 | 7.5–41 | 0–110 | 0 | 0–0 | 0–5 | 52 | $<10^{-6}$ |

IQR: interquartile range.

**Table A3**
Results of a general linear model evaluating the effect of the delay since capture (in days) on the intensity of the aggressive response for the experienced males

| Predictor variable | β | SE | *P* |
|---|---|---|---|
| (Intercept) | −1.42 | 0.265 | $1.2 \times 10^{-5}$ |
| Delay | 0.0001214 | 0.0002997 | 0.69 |

Values indicate the coefficient estimates (β), standard errors of estimates (SE) and associated *P* values.

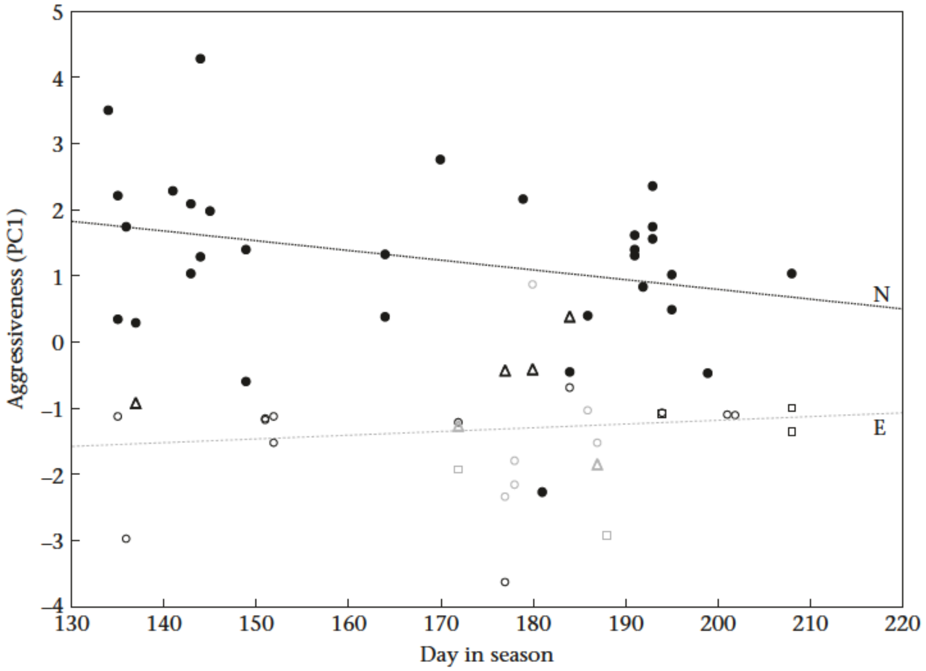

**Figure A1.** Scatterplot depicting the relationship between the timing of the playback experiments within the season (number of days since the beginning of the year, 1 January) and male aggressiveness (expressed as PC1 score). Male groups are marked as in Fig. 1: naïve males by full black circles, experienced males by open symbols differentiated by a delay between capture and experiment (circles: grey: same season; black: 1 year; triangles: grey: 2 years; black: 3 years; squares: grey: 4 years; black: 5 years). Trendlines are provided separately for naïve (N) and experienced (E) males.

**Table A4**
Results of a general linear model evaluating the intensity of the aggressive response of males in relation to experimental group (experienced and naïve), date (days since 1 January) and year

| Predictor variable | β | SE | *P* |
|---|---|---|---|
| (Intercept) | −662.8 | 711 | 0.35 |
| Experimental group | 2.561 | 0.2934 | $5 \times 10^{-12}$ |
| Progress of the season | −0.002751 | 0.007729 | 0.72 |
| Year of experiment | 0.3282 | 0.3522 | 0.35 |

Values indicate the coefficient estimates (β), standard errors of estimates (SE) and associated *P* values.

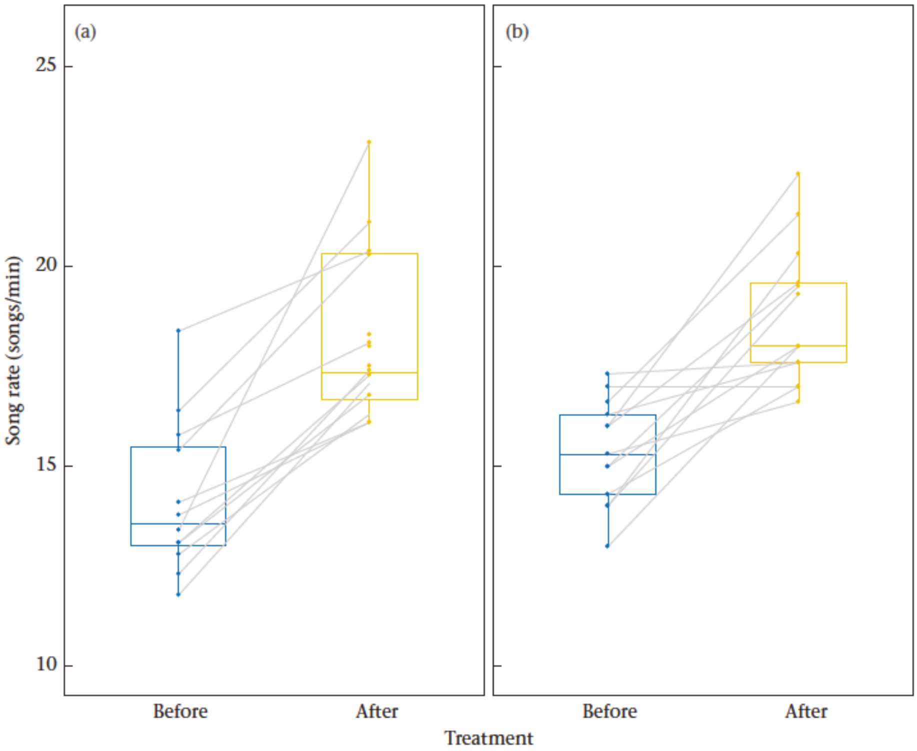

**Figure A2.** Paired box plots illustrating differences in song rate before and after playback experiments for (a) experienced and (b) naïve males tested in 2016. Song rate of each individual is shown by points, grey lines connect pairs of points for each male individual before and after playback to visualize changes in song rates. Box plots depict the median, first and third quartiles and range; circles indicate individual data points.

**Table A5**

Results of a generalized linear model comparing the song rate before and after playback experiments between the two experimental groups (experienced and naïve males)

| Predictor variable | β | SE | *P* |
|---|---|---|---|
| (Intercept) | 13.2322 | 3.923 | 0.003 |
| Song rate before | 0.4097 | 0.252 | 0.12 |
| Experimental group | −0.7521 | 0.7906 | 0.35 |

Values indicate the coefficient estimates (β), standard errors of estimates (SE) and associated *P* values.

# SUPPLEMENTARY TABLE

**Table S1**. Supplementary data to this article is provided in this table. This table is available as online supplementary material (.xlsx) or from the author upon request.

# Chapter 3

**Oñate-Casado, J**., Porteš, M., Beran, V., Pérez-Granados, C., Traba, J., Barrero, A., Sakhalkar, S. P., Petrusek, A., Petrusková, T. (manuscript submitted). When individuality obscures geographic song variation: a comparison of two passerine sister species with different migratory strategies

Berthelot's Pipit perching during sunset, Tenerife (Canary Islands, Spain), 2021

# When individuality obscures geographic song variation: a comparison of two passerine sister species with different migratory strategies

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABSTRACT

Songbirds are a well-established model group to study cultural evolution as their songs are learnt, i.e. culturally transmitted, which may lead to divergence in song characteristics between populations. Song variation may be affected by various factors, including geographic isolation, timing and duration of learning period, and life strategies such as migratory behaviour. In this study, we explored geographic song variation in two congeneric sister species of songbirds with a simple song: a Palaearctic migrant Tawny Pipit (*Anthus campestris*), and a sedentary Berthelot's Pipit (*A. berthelotii*) endemic to Macaronesian archipelagos. We recorded songs of territorial males from six Tawny Pipit populations in the European mainland, and of Berthelot's Pipits in three Canary and two Madeira islands. We confirmed that both species have individually unique repertoires, usually consisting of a single song type per male. The structural characteristics of song types within each species were compared by dynamic time warp analysis, allowing for quantifying song dissimilarity and analysing its geographic patterns. Contrary to our expectations, we did not find any regionally specific song characteristics that would allow assessing the origin of recorded songs recording in either species, not even between the two Berthelot's Pipit subspecies from different Macaronesian archipelagos. In both species, we observed very high within-population variation of song types, possibly reflecting rapid cultural evolution of song driven by the constant introduction of novel, although minor, individually-specific differences. Mean song dissimilarity was higher when song types were compared between different regions (mainland localities or islands) than within them. Only in Berthelot's Pipits, however, we observed significant differences in geographic song variation at the smallest and largest spatial scales, which might reflect Berthelot's Pipits' sedentarity and the isolation of Macaronesian archipelagos, respectively.

KEYWORDS: pipits, *Anthus*, cultural evolution, archipelagos, population fragmentation

## INTRODUCTION

Passerine birds use song to convey important information for interspecific (species recognition) and intraspecific purposes (mate attraction, territory defence). In oscine passerines, song develops under the influence of vocal learning and is culturally transmitted, resulting in great song variation within and between species (Slater & Ince 1979; Podos et al. 2004; Lachlan et al. 2018). By learning from parents and neighbours, cultural transmission may maintain certain song types within populations over generations (Marler & Tamura 1964; Baptista 1977; Baptista & Morton 1988). However, song learning strategies in oscine species vary in copying strategy and timing of learning (Beecher & Brenowitz 2005; Brenowitz & Beecher 2005), which subsequently affects geographic song variation, and leads to a whole range of song sharing patterns among conspecific birds from the same location, from a perfect overlap of neighbours' song types to the complete absence of sharing (Handley & Nelson 2005).

Geographic variation in birdsong is a widely studied phenomenon (Podos & Warren 2007; Singh & Price 2015; Nelson 2017), which can be used to test the relationship among geographic signal variation, reproductive isolation, and speciation (reviewed in Edwards et al. 2005). Being culturally transmitted, songs can diverge faster than genes, and be an early indicator of speciation (Irwin & Price 1999) or contribute to it by promoting prezygotic reproductive isolation. Additionally, acoustic geographic variation can help estimating the effects of population fragmentation on song traits, and thus assess potential associations with local extinction risk (e.g. Laiolo & Tella 2005; Laiolo et al. 2008; Pang-Ching et al. 2018).

Cultural evolution of learned vocalizations is strongly affected by the choice of tutors. In songbirds, it is common that juveniles learn from most males singing around and thus are more likely to pick a common variant of the song than a rare one, a phenomenon known as the conformist bias (e.g. Morgan & Laland 2012). Together with moderately precise learning it leads to stable traditions in bird populations (Lachlan et al. 2018). Imprecise song learning might accelerate song divergence, and potentially contribute to allopatric speciation when populations become geographically isolated by various barriers (Lachlan & Servedio 2004).

Oceanic islands, being isolated geographic entities, provide suitable systems to understand evolutionary diversification processes. Populations on archipelagos tend to be smaller and less connected than on the continents, leading to differences in the effects of evolutionary processes compared to mainland systems (e.g. Frankham 1997; Frankham 1998; Leroy et al. 2021). This extends to cultural evolution, as island isolation allows for the independent evolutionary trajectories of local populations (e.g. Emerson 2002; Parker et al. 2012; Lachlan et al. 2013; Illera et al. 2014). Each colonization event represents an independent evolutionary episode, offering opportunities to estimate how isolated populations diverge over time. Considering this, songbirds with spatially isolated populations are suitable models for studying both cultural (acoustic) and genetic differentiation.

Tawny Pipit (*Anthus campestris*) and Berthelot's Pipit (*A. berthelotii*), sister species in the songbird genus *Anthus* (Passeriformes: Motacillidae), offer a valuable framework for studying and comparing the geographic song variation, given their different natural histories and life-history strategies. Berthelot's Pipit, endemic to Macaronesia, diverged from its sister species broadly distributed across the Palaearctic about 2.5 million years ago by colonizing the Canary Islands from mainland Africa (Voelker 1999). A much more recent colonization (ca. 8000 years ago) from the Canaries to the Madeira and Selvagens archipelagos took place in two independent, northward colonization waves (Illera et al. 2007; Spurgin et al. 2014), resulting in clear genetic and phenotypic variation among archipelagos (Armstrong et al. 2018; Martin et al. 2021). In fact, the populations from the Madeira archipelago are recognised as a distinct subspecies, *A. berthelotii madeirensis* Hartert, 1905 (Martín & Lorenzo 2001).

Although closely related (Voelker 1999), Tawny and Berthelot's Pipits differ in history of population isolation and migration behaviour. Tawny Pipits are trans-Saharan migrants, which have undergone substantial population declines and fragmentation in many parts of their European range in recent decades (Briedis et al. 2016, Tyler & Christie 2020). In contrast, Berthelot's Pipits are a strictly sedentary taxon restricted to the three archipelagos mentioned above (Martín & Lorenzo 2001), with population connectivity clearly constrained by oceanic barriers (Martin et al. 2021). Both species have similar songs with a simple structure (Alström & Mild 2003). Tawny Pipit songs are apparently individually unique (Neuschulz 1986; Osiejuk et al. 2007; Oñate Casado et al. 2023) and temporally stable both within and between breeding seasons (Oñate Casado et al. 2023). The characteristics of Berthelot's Pipit songs have not yet been studied in such detail. However, considering that other pipit species are closed learners (Petrusková et al. 2016; Oñate Casado et al. 2023) and learning abilities appear to be conserved among congeneric songbirds (Catchpole & Slater 2008), temporal stability may also be assumed in that species. Nothing specific is known about the timing and duration of the learning phase in pipits; we may assume that it takes place before the individual's third year of life, as is the case for most closed learners for which such information is available (Brenowitz & Beecher 2005). However, considering the relative rarity of Tawny Pipit males exhibiting temporal variation in their song (Oñate Casado et al. 2023), the learning phase is probably substantially shorter, with songs usually stabilizing during the first breeding season.

In this study, we explored the intraspecific geographic song variation of Tawny and Berthelot's Pipits in representative populations from mainland Europe and Macaronesia, respectively. By collecting and analysing extensive datasets, we seized the opportunity to characterize spatial patterns of song variation at different geographic scales in two closely related species with similar song complexity but different life histories, patterns of geographic distribution, and dynamics of their distribution ranges. We expected substantial differences among geographically distant populations and regions for both species. In particular, we hypothesized that strong divergence will be observable for Berthelot's Pipits from different islands and especially between populations from the Canaries and the Madeira archipelago (i.e., between the two subspecies), reflecting the colonisation trajectory pattern. We also quantitatively compared the song variation of Berthelot's Pipits between males and among different renditions of the same song type by a given male, to evaluate whether this species also possesses individually unique songs.

## METHODS

### Study species

The Tawny and Berthelot's Pipits are two sister species of the motacillid genus *Anthus*, which comprises over 40 species distributed worldwide (Fitzpatrick et al. 2004). Both species require bare ground with sparse vegetation cover for nesting. Tawny Pipits breed in dry open habitats with low vegetation, such as fallow agricultural land, dry steppes, sandy heaths, coastal sand-dunes, and dry mountain slopes (Alström & Mild 2003). This species shows a wide altitudinal range from coastal to alpine areas; in Europe it mostly occurs at relatively low altitudes between 500 and 1000 meters but also in the mountains (Alström and Mild 2003; Calero-Riestra & García 2019; Table 1). Berthelot's Pipits are found in various open habitats of the volcanic landscape of the oceanic Macaronesian islands, such as rocky areas in plains, hillsides with sparse vegetation, clearings in wooded areas and scrubby slopes, from the sea level up to the alpine habitats of over 2500 m above sea level in Tenerife (Illera et al. 2007; Table 1). Both species are territorial and socially monogamous with no sexual dimorphism, and the song, similar between the two species

(Alström & Mild 2003), should therefore be critical for male success in territorial defence and mate attraction.

Both species usually sing while perching or during the flights in their territory and chase away other male intruders during the breeding season (Calero-Riestra & García 2019), readily reacting to playback stimulation by approach and usually also by vocalization (pers. obs.). Tawny Pipits begin to sing in early May, as soon as they arrive to the breeding areas, in order to establish and defend a territory, but the breeding season starts later in populations located at high altitudes (Calero-Riestra et al. 2013; pers. obs.). Berthelot's Pipits sing intensively for several weeks at the beginning of breeding season, typically from late January to late February with some delay in the highest altitudes (Tyler 2020).

## Study areas

We recorded singing male Tawny Pipits in six different populations across mainland Europe (Fig. 1, Table 1), specifically in Czechia, Italy, Spain (two populations), and Greece (two populations). Song recording in Czechia was restricted to the only remaining Tawny Pipit population in the country, located in an active brown coal mine area near Most in northern Bohemia (Briedis et al. 2016). Detailed results on song variation in this populations have been recently published (Oñate-Casado et al. 2023). In Italy, we focused on a dense, high-elevation population within the Gran Sasso National Park in the Apennine Mountains. The first of the two Spanish sites, Altos de Barahona, is a Special Protection Area (SPA) relevant for the conservation of steppe birds characterized by flat areas dominated by short shrubs (Gómez-Catasús et al. 2019), which supports a high-density Tawny Pipit population at two areas (Altos de Barahona and Páramos de Layna; Calero-Riestra & García 2019) approximately 15 km apart. The second Spanish site was in Rincón de Ademuz, where Tawny Pipits occur in relatively low densities at three remaining fragments with suitable habitats (Pérez-Granados et al. 2016). In Greece, we also recorded songs at two separate sites: at Mount Parnassus, a limestone mountain range in Central Greece with Tawny Pipit population breeding in an alpine zone, and in the Chalkidiki peninsula, Northern Greece, where most birds were concentrated in Agios Mamas marsh, a small coastal wetland with standing water, sand-dunes, and reedbeds.

Berthelot's Pipits were recorded on two islands of the Madeira Archipelago (Madeira and Porto Santo) and three Canary Islands (La Palma, Tenerife and Fuerteventura) representing the western, central, and eastern parts of the archipelago, respectively (Fig. 1, Table 1). We actively searched for singing males in suitable habitats across each island (except Porto Santo, with only southwestern half of the island covered), and attempted to record multiple males in each chosen area. As a result, we obtained multiple clusters of recordings, usually separated by several kilometres, from each larger island (see detailed maps of each region in Suppl. Fig. S1).

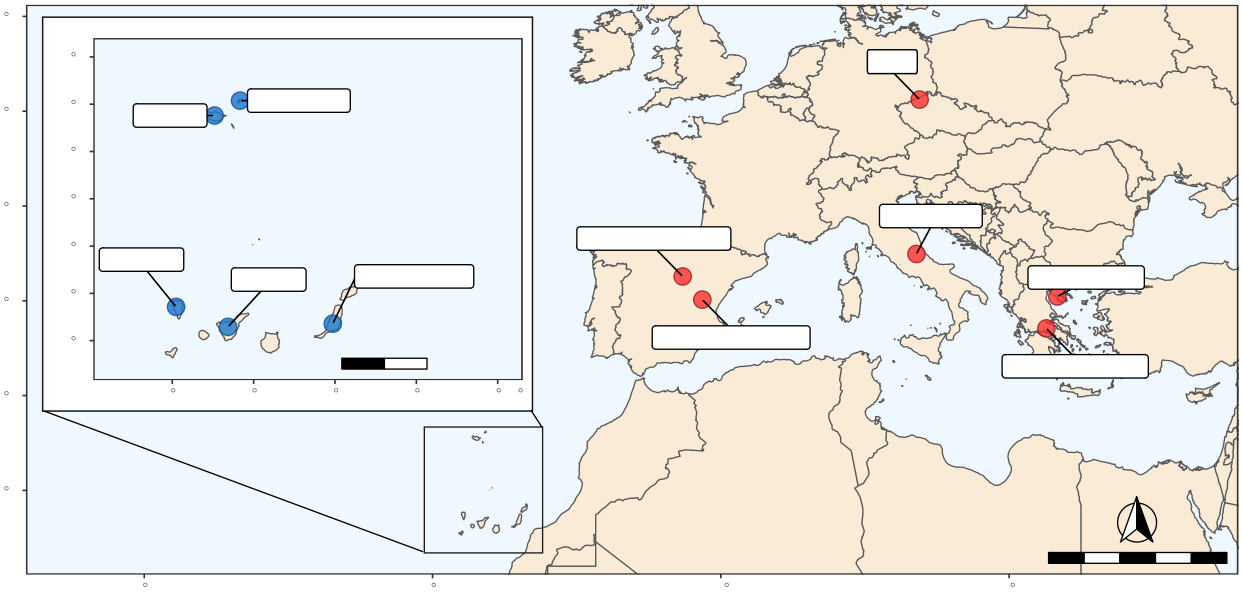

**Fig. 1**. Distribution of our study sites across mainland Europe for Tawny Pipits (red dots) and the Madeira and Canary archipelagos for Berthelot's Pipits (inset map, blue dots). Countries are indicated by 2-letter ISO codes.

## Song recording

Singing males of both species were recorded using Sennheiser ME-67 shotgun directional microphones connected to Marantz PMD 661 recorders. Tawny Pipit individuals were recorded close to the peak of the breeding season at each field site, depending on site altitude; some of the regions were visited in two or more years (Table 1). Berthelot's Pipits were recorded on each island during one breeding season (Table 1), always in mid-to late February.

Territorial males were recorded while singing spontaneously whenever possible; alternatively, we used playback of conspecific songs, as its use usually stimulates singing behaviour of targeted male without changing the structure of the song (Oñate-Casado et al. 2021). We attempted to record at least 3 min of singing bouts from each male; however, substantially shorter recordings of good quality were also considered fully adequate because male Tawny Pipits generally use a single song type (Osiejuk et al. 2007, Oñate-Casado et al. 2023), and the analysis of long high-quality recordings in this study confirmed the same for the Berthelot's Pipits (see Results).

**Table 1**. Characteristics of study areas (with countries indicated by two-letter ISO codes), recording periods, and the number of recorded males (those with two song types in the repertoires are enumerated in parentheses). Median and maximum distances between males in each area were calculated from birds recorded in the same season. For the field sites with Tawny Pipits, months of recording are provided; all recordings of Berthelot's Pipits were obtained in mid- to late February of the respective years.

| Sp. | Field site / Island | Location | Altitudinal range (m) | Median / max. distance (km) | Years recorded | No. males |
|---|---|---|---|---|---|---|
| Tawny Pipit | Most (CZ) | 50.30–50.55°N,<br>13.31–13.64°E | 150–370 | 3.8 / 29.4 | 2015–2017, 2021 (early May to mid-July) | 81 (3) |
| | Gran Sasso (IT) | 42.34–42.45°N<br>13.54–13.75°E | 1400–2210 | 3.9 / 15.7 | 2019, 2020 (early to mid-July) | 63 (1) |
| | Altos de Barahona (ES) | 41.09–41.35°N<br>2.33–2.82°W | 1030–1220 | 19.3 / 45.4 | 2021, 2022 (mid to late May) | 95 (3) |
| | Rincón de Ademuz (ES) | 39.95–40.17°N<br>1.32–1.40°W | 1000–1200 | 3.9 / 25.2 | 2021, 2022 (mid to late May) | 51 (6) |
| | Agios Mamas (GR) | 40.22–40.54°N<br>22.82–23.34°E | 0–580 | 22 / 52.7 | 2023 (mid-May to early June) | 18 (1) |
| | Mount Parnassus (GR) | 38.49–38.57°N<br>22.54–22.61°E | 1160–2120 | 3 / 9.3 | 2023 (mid-May to early June) | 32 (2) |
| Berthelot's Pipit | Madeira (PT) | 32.71–32.82°N<br>16.70–17.26°W | 0–1660 | 16.8 / 53.5 | 2020 | 38 |
| | Porto Santo (PT) | 33.03–33.05°N<br>16.36–16.39°W | 50–140 | 1.1 / 2.7 | 2020 | 25 |
| | Fuerteventura (ES) | 28.05–28.66°N<br>13.83–14.39°W | 0–370 | 21.7 / 84.1 | 2022 | 75 |
| | Tenerife (ES) | 28.01–28.53°N<br>16.15–16.85°W | 0–2310 | 24.9 / 76.9 | 2021 | 139 |
| | La Palma (ES) | 28.46–28.85°N<br>17.77–17.99°W | 0–910 | 16.9 / 44.7 | 2021 | 99 (1) |

## Individual identification, song measurements and song analysis

Individual identification of Tawny and Berthelot's Pipits was primarily based on visual analyses of song recordings and comparison with song type catalogues, following the study of Oñate-Casado et al. (2023). In that study, using 45 ringed and repeatedly recorded Tawny Pipit males, it was demonstrated that this species' song is individually unique and generally temporally stable both within and between seasons. Male identity could also be confirmed thanks to ringing for almost all males recorded at both Spanish field sites in 2021 (26 in Altos de Barahona, 18 in Rincón de Ademuz). Therefore, we considered that recordings with song types of identical structure, obtained within hundreds of meters from each other, represented the same male. When two or more birds sang in the same area (sometimes just a few dozen metres from each other), we carefully tracked the individuals and noted by voice comments in the recordings which birds were singing. In total, we recorded and analysed 340 Tawny Pipit and 376 Berthelot's Pipit males (Table 1).

To characterize individual repertoires, we first visually inspected all recordings in the software Avisoft-SASLab Lite 5 (Specht 2007), and subsequently used Luscinia 2.20.03.11.01 (Lachlan 2020) to measure and quantitatively compare the acoustic characteristics for representative instances of each song type observed in the study populations. (In cases when a male sung two distinct song types, we included each of them separately in the analyses if the recording quality was sufficient). Structures of all song types within each species were compared in a pairwise manner using the dynamic time-warping algorithm (DTW) in Luscinia, which provides a distance matrix with song dissimilarity measures. Weights and parameters of the DTW analyses (for their explanation, see Luscinia documentation at https://github.com/rflachlan/Luscinia/wiki) followed the settings used in Oñate-Casado et al. (2023): time (5), fundamental frequency (1), fundamental frequency change (1), FF norm (1), compression factor (0.25), minimum element length (2), time SD weighting (1), ArcTan transform weight for frequency slope (0.02), cost for alignment error (0.2), stitch threshold (100 ms), and maximum warp (100%). Fundamental frequency was log-transformed.

## Data analyses

Subsequent statistical analyses were mostly performed in R 4.3.1 (R Core Team 2023), using the package 'ggplot2' version 3.5.0 (Wickham 2016) for visualization. The results from DTW were used to visualize and interpret the patterns of acoustic similarity between the analysed songs, as well as to formally test specific hypotheses about song similarity across different spatial scales. For visualization, we created two-dimensional multidimensional scaling (MDS) scatterplots, and dendrograms based on a hierarchical clustering using an unweighted pair group method with arithmetic mean (UPGMA), which were annotated in iTOL 6 (Letunic & Bork 2021).

To evaluate the consistency of song performance of Berthelot's Pipit males, and to ensure that one instance of each song type is appropriate for subsequent analyses, we followed the same approach as in the previous study on individual song variation of Tawny Pipits (Oñate-Casado et al. 2023). We used a subset of 50 birds randomly chosen from two different islands, 25 from Tenerife and 25 from Fuerteventura. This subset consisted of three non-adjacent songs for each bird (usually extracted from different parts of the same, sufficiently long, recording). We then generated a UPGMA dendrogram depicting the acoustic similarity of all processed songs in this analysis.

To corroborate the results obtained through visual inspection and clustering analyses, we formally tested whether songs of the same origin (sung by the same individual, individuals from the same population, from the same region, etc.) are overall more similar to each other than those of different origin. Specifically, for Tawny Pipits we compared songs within and between field sites, and within and between countries. For Berthelot's Pipits, this was examined at both individual level (within and between individuals) and geographic level (within and between islands, within and between archipelagos). When evaluating the consistency of song delivery by Berthelot's Pipit males, each song type was represented by three renditions, otherwise each song type was represented just once. To characterize alternative hypotheses for each test, we created pairwise binary matrices to represent individuals, field sites, countries, islands, and archipelagos, using 0 and 1 to denote songs of the same and different origin, respectively. These matrices were then used along with a pairwise song dissimilarity matrix (output of the Luscinia DTW algorithm) in Mantel tests (with 10,000 random replicates) implemented in the R package 'vegan' (Oksanen et al. 2022).

To assess the similarity of male songs across different geographic scales, we chose five distinct categories of geographic scale for each species. For Tawny Pipits, categories spanned from the local scale (birds within a 3.5 km radius of the focal individual) to broader scales including birds from the same field site but between 3.5 km and 10 km, and over 10 km away, birds from different field sites in the same country (excluding Czechia and Italy with only one site), and birds from different countries. Similarly, for Berthelot's Pipits, the categories ranged from local scale (within 3.5 km) to four more broader scales: same island (3.5 to 10 km, and >10 km away), different islands within the same archipelago, and different archipelagos (Canaries vs. Madeira).

To test for differences in song similarity patterns between these geographic scales, we first calculated for each bird a single value of the mean song dissimilarity from all relevant pairwise comparisons within each distance scale category. In the case of Tawny Pipit localities visited in multiple years, we only considered in small-scale comparisons the birds recorded in the same year. The mean song dissimilarities for each geographical scale category were then compared, separately for each species, through one-way ANOVA followed by post-hoc Tukey tests to identify which categories differ from each other. We also ran the same analyses excluding Porto Santo from small-scale comparisons, as all males there were located within a 3.5 km radius. Furthermore, to check for potential bias caused by lower numbers from Madeira than Canary archipelago, we also compared the between-island and between-archipelago scales on 1000 randomized datasets where both archipelagos were represented by the same number of males (63; i.e., 21 males randomly selected from each of the three Canary islands). A similar analysis was run to assess the mean song dissimilarities within each island and field site, which were compared separately for each species by ANOVA followed by post-hoc Tukey tests.

## Data availability

Key information about recorded males are provided in Supplementary Table S1. WAV files of song types used for Luscinia analysis, geographic coordinates of recorded males in GPX format, song dissimilarity and geographic distance matrices, and R code used to analyse the data are available in the Zenodo repository (doi: *[to be provided after revisions]*).

# RESULTS

## Song structure and individuality

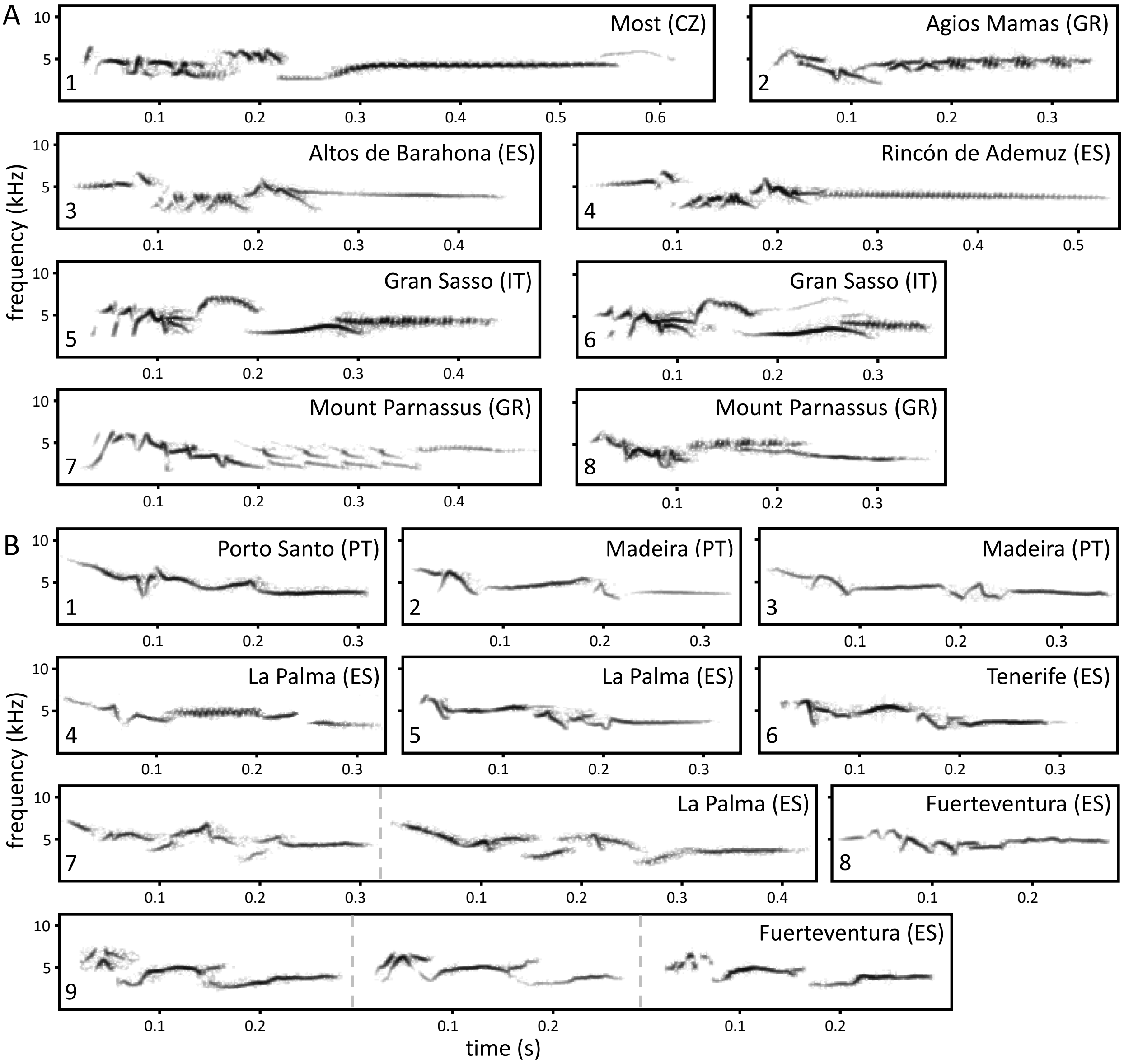

**Fig. 2.** Spectrograms illustrating the variation of song types of Tawny Pipit (A) and Berthelot's Pipit (B), and individuals from the studied populations across Europe and Macaronesia, respectively. Origin of each bird with countries indicated by 2-letter ISO codes is shown. Spectrograms no. 3 & 4 and 5 & 6 in A show structurally similar song types sung by Tawny Pipits from the different and the same field sites, respectively. Spectrograms no. 5 & 6 and 2 & 3 in B illustrate corresponding patterns in Berthelot's Pipits males from the different and the same island, respectively. No. 7 in B shows two distinct song types sung by a Berthelot's Pipit male from La Palma, no. 9 unusually high variation among song renditions by a single male from Fuerteventura. (The latter bird was represented by the leftmost variant in all analyses, except of that of within-individual song stability).

The vast majority of males of both species used a single song type composed of several elements (Fig. 2). The songs were short, usually around 0.4 to 0.5 s for Tawny Pipits and 0.3 to 0.4 s for Berthelot's Pipits, and varied in complexity among birds. Overall, Tawny Pipit song types tend to be more complex, and contain more elements than those of Berthelot’s Pipits (Fig. 2). Visual inspection confirmed that no recordings that could be unambiguously assigned to different birds contained identical songs, although some males within local populations often used structurally similar song types, only differing from each other in minor details. In such cases, these differences could be consistently observed in various song renditions from the recordings of the given individuals.

In all studied populations of Tawny Pipits, we occasionally observed individuals singing two different song types, between which they irregularly but frequently alternated. Usually, the proportion of these ranged between 3 to 6% at a given field site (Table 1) but in Rincón de Ademuz (Spain), they exceeded 10% of the recorded birds. In contrast, only one Berthelot's Pipit male included in this study (from La Palma) was observed singing two different song types (Fig. 2B, no. 7).

In most cases, song renditions of the same Berthelot’s Pipit male were visually identical. Accordingly, the UPGMA dendrogram (Fig. 3) based on dissimilarity values from the Luscinia DTW algorithm consistently clustered together songs by individual birds. The prevailing consistency of songs within individuals was reflected in the highly significant results of the Mantel test ($n_{songs} = 150$, $n_{birds} = 50$, $p < 0.001$) that showed clear differences between songs sung by the same individual and those by others. However, one particular male exhibited more substantial variability among its three song renditions (Fig. 2B, no. 9). This variability exceeded the usual song dissimilarity observed between renditions of the same song type by other birds in our analysis, and even surpassed the differences observed between different birds singing similar song types (Fig. 3). Despite this variation, the different renditions of the song sung by this male were distinct from those of any other male recorded by us in Fuerteventura (or elsewhere).

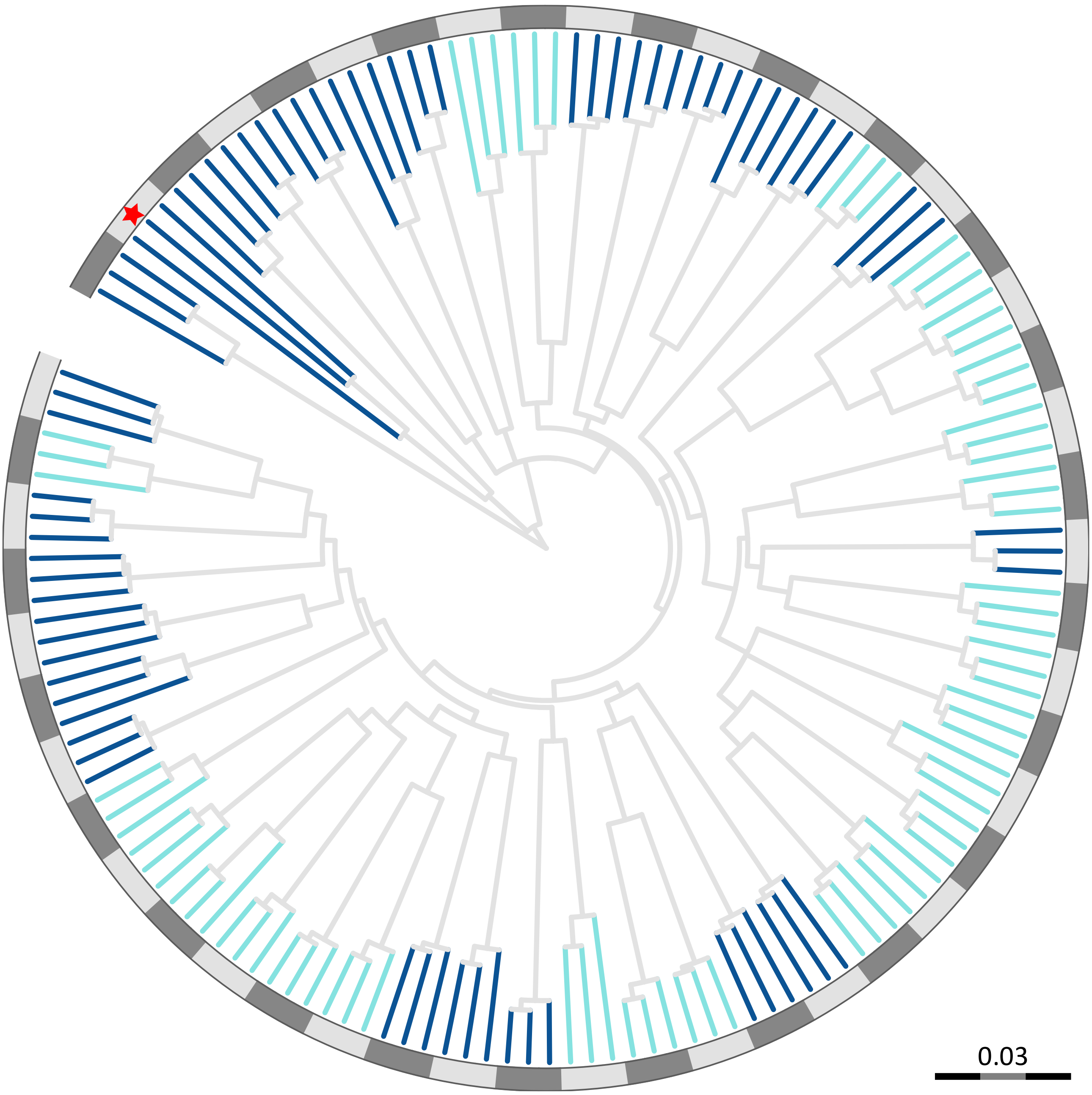

**Fig. 3.** UPGMA dendrogram of song similarity based on the dynamic time warping algorithm implemented in Luscinia software. Birds from Fuerteventura and Tenerife are denoted by dark blue and light blue colours, respectively. Grey segments in the outer ring connect songs sung by the same individual; a red star indicates the individual for which a substantial variability in song renditions was observed (Fig. 2B, no. 9).

## Song variation at different geographic scales

For both pipit species, there were no general song features indicating geographical origin, either field site or country for Tawny Pipits, or island or archipelago for Berthelot's Pipits. Even birds from the same neighbourhood often sang songs that were structurally very different, as revealed by visual inspection of spectrograms as well as reflected in the dissimilarity values from the DTW algorithm. Accordingly, song types from different field sites or islands were interspersed in the multidimensional scaling plots when all birds of each species were considered (Fig. 4). However, the arrangement of song types in the dendrograms (Fig. 5) clearly indicated non-random patterns – songs of several individuals of the same geographical origin (field site or island) were frequently clustered next to each other. These local “song families” (i.e., closely similar song types observed among birds of the same origin; Oñate-Casado et al. 2023) drove highly significant results of all Mantel tests, which consistently indicated higher acoustic similarity of songs from birds of the same (vs. different) origin at various scales. Specifically, Tawny Pipit song similarity was higher among birds from the same field site ($n_{songs} = 355$, $p < 0.001$) and the same country ($n_{songs} = 355$, $p < 0.001$) than elsewhere, a and the same was true for Berthelot’s Pipits from the same island ($n_{songs} = 377$, $p < 0.001$) and the same archipelago ($n_{songs} = 377$, $p < 0.001$).

Despite the high overall intrapopulation variation in songs, the analysis of mean song dissimilarities suggested some differences in patterns of song variation among study sites (Suppl. Fig. S2). Specifically, song variation seems lower in two Tawny Pipit populations, Czech coal mines (thoroughly covered by recording over several years; Oñate-Casado et al. 2023) and Agios Mamas in Greece, than in other continental field sites. This was also reflected in lower scatter in the multidimensional scaling plots (Fig. 4A). However, the seemingly lower variation among songs recorded at the two islands of the Madeira Archipelago (Madeira and Porto Santo) in Fig. 4B was not supported by evaluation of mean within-island song type dissimilarities; these were in fact higher for these Madeira Islands than for La Palma and Tenerife (Suppl. Fig. S2).

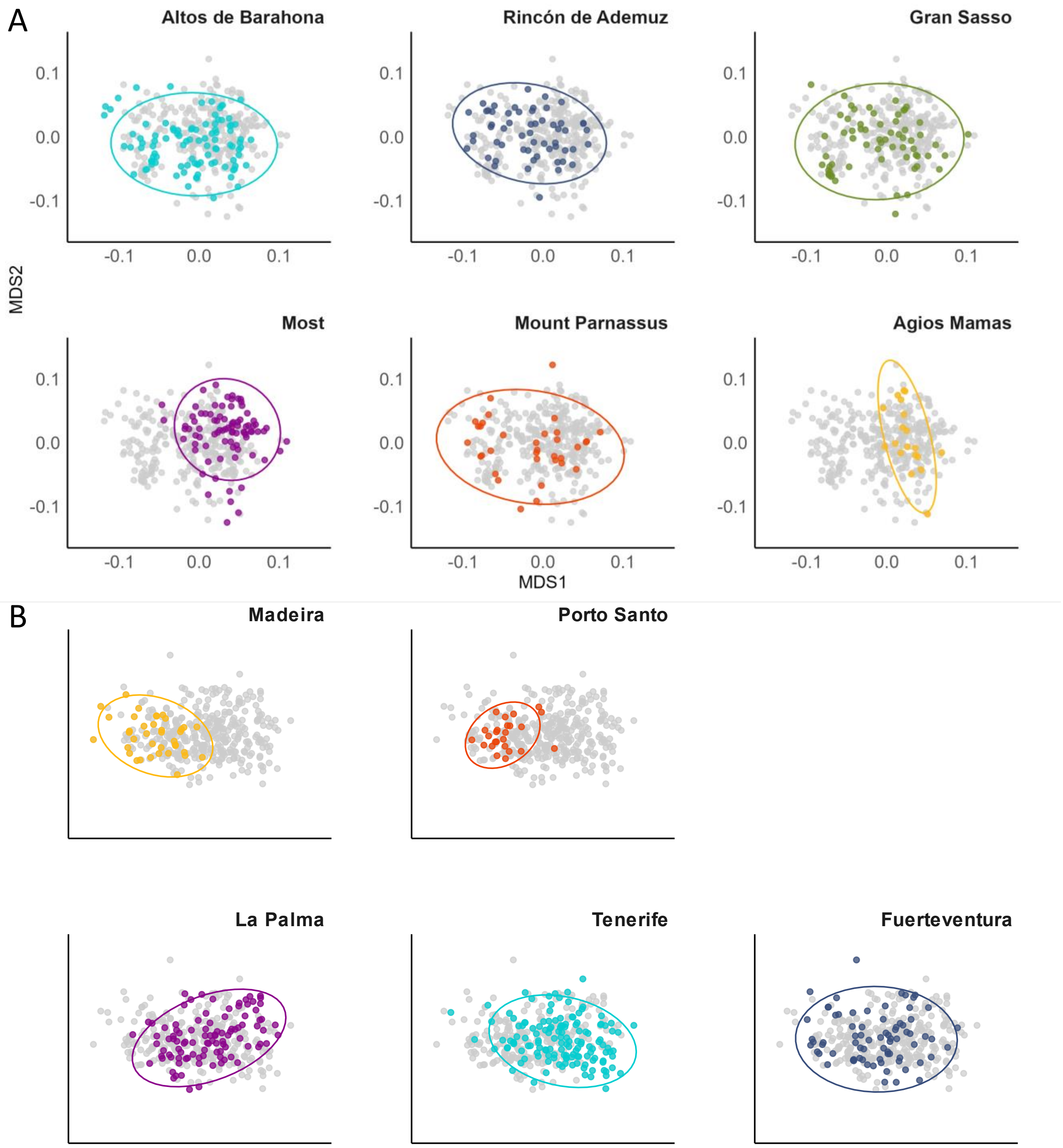

**Fig. 4.** Multidimensional scaling (MDS) plots visualizing variation in song similarity among studied Tawny (A) and Berthelot's Pipit (B) individuals, based on the dynamic time warping algorithm implemented in Luscinia software. MDS analysis was run independently for each species. Each dot represents a single song type, focal field site/island in each plot is highlighted by colour, grey dots represent song types from elsewhere. Ellipses indicate 95% confidence interval from the centroid, based on a multivariate T distribution.

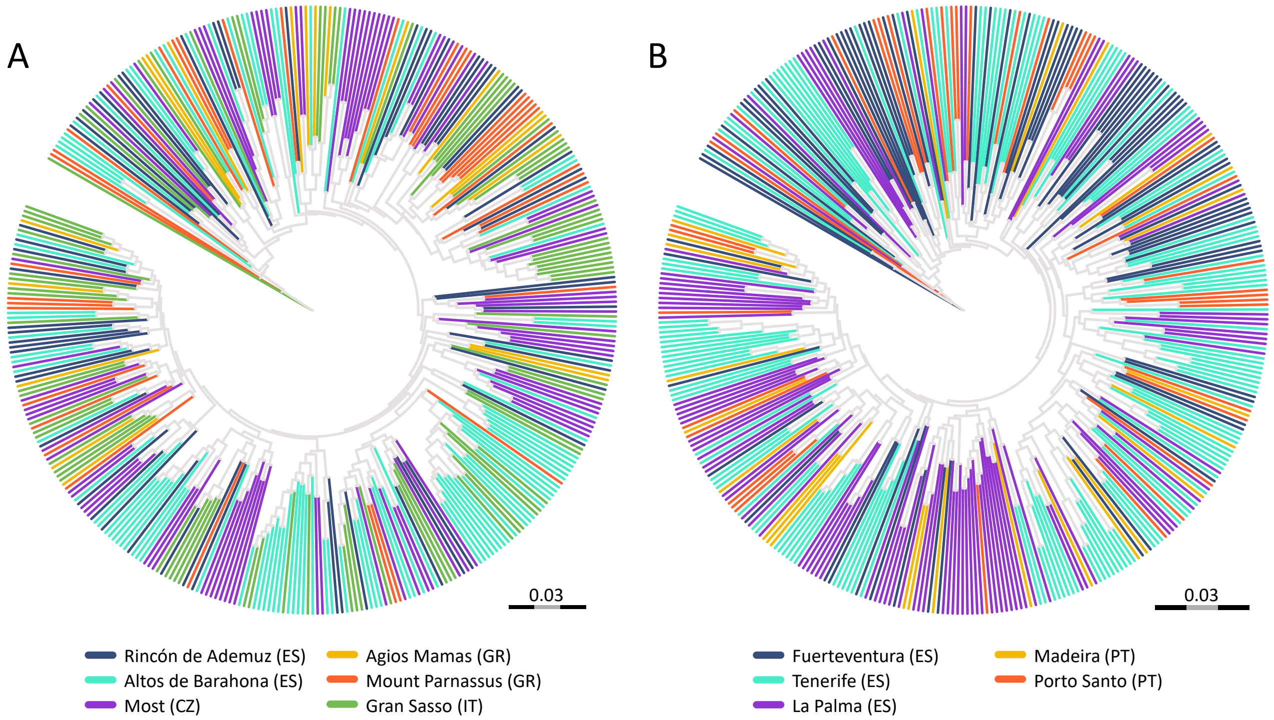

**Fig. 5.** UPGMA dendrograms of song similarity based on the dynamic time warping algorithm implemented in Luscinia software. Each branch represents a single male's song type. These are colour-coded to indicate different field sites and islands for Tawny (A) and Berthelot's Pipits (B), respectively.

As indicated by Mantel tests, mean song similarity between birds from the same region (i.e., field site, island) was higher than between birds from distant populations. This pattern was consistent in both species; however, the spatial scale at which the similarity was most pronounced differed between them (Fig. 6; Suppl. Table S2). In Tawny Pipits, song similarity was consistently higher when comparing any of the three finer geographic scales (local and field-site) to the two broader scales (same-country and different-country). However, there were no significant differences in song similarity between various spatial scales within a field site (different distance categories), or between the two broader scales (same vs. different country; Fig. 6A). For Berthelot's Pipits, the gradient in song dissimilarity was more apparent. Songs were consistently most similar among local birds (within the 3.5 km radius), with significantly lower mean dissimilarity values than at any broader spatial scale (whether within island, between islands, or between archipelagos; Fig. 6B). When birds from different archipelagos (i.e., different subspecies) were compared, song dissimilarities were always significantly higher than in any comparisons within subspecies, regardless of spatial scale (from the local scale to between islands). However, there were no significant differences in song dissimilarity when birds from the same island (but over 3.5 km apart) were compared, and when birds between islands within the same archipelago were compared (Fig. 6B, Suppl. Table S2). The observed patterns were consistent even when Porto Santo was excluded, and when randomized subsets of males from Canary Islands were used to compare within- and between-archipelago scales instead of the full dataset.

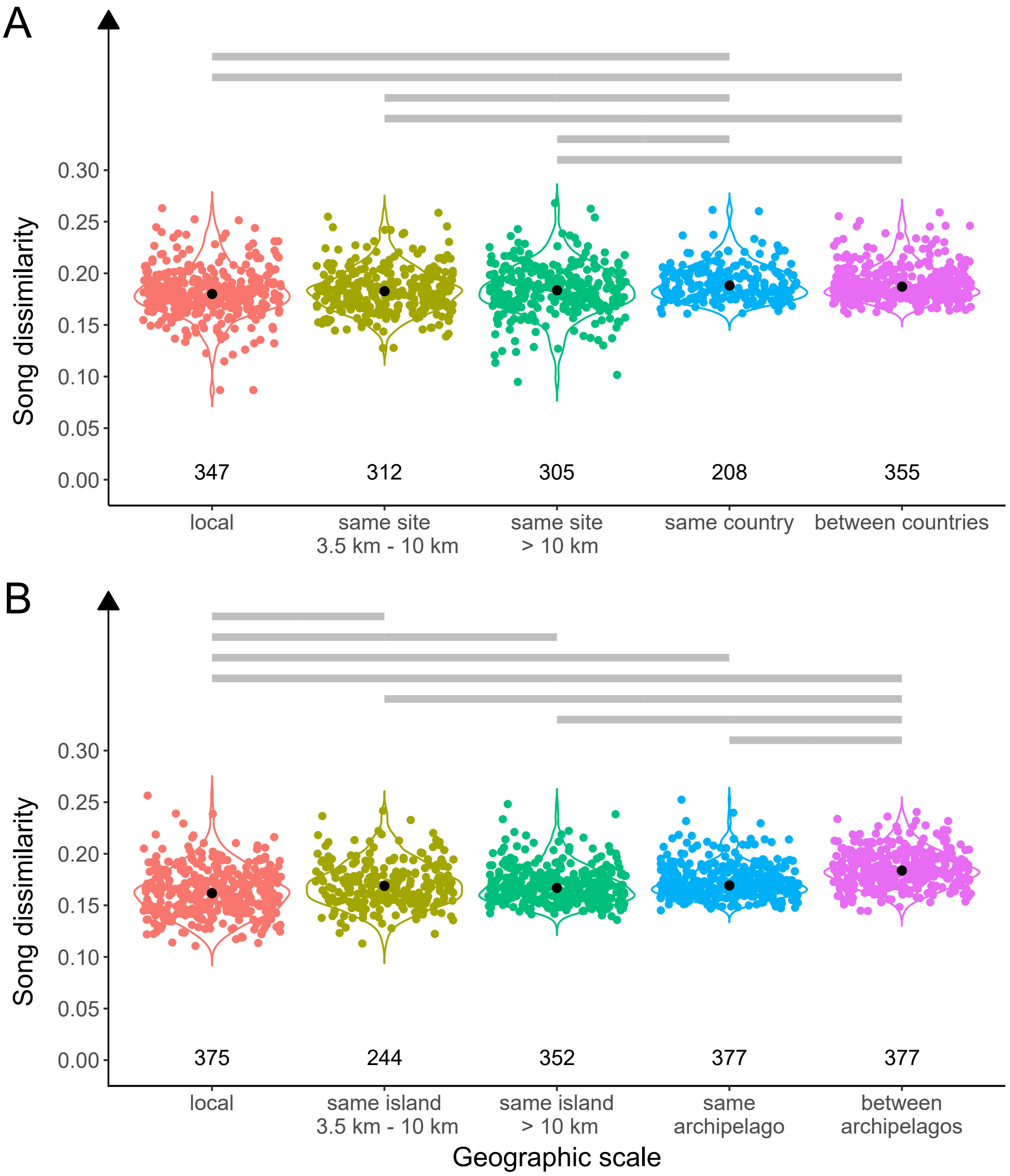

**Fig. 6.** Variation in mean song dissimilarity at various geographic scales for Tawny (A) and Berthelot's Pipits (B). Numbers above Y axis indicate the total number of song types included in the comparison within a given spatial scale. Each dot represents the mean song dissimilarity for each bird from all relevant pairwise comparisons within a given geographic scale; black dots represent median values. Horizontal bars above the plots indicate significant differences ($p < 0.005$) in song similarity between geographic scales, based on the results of one-way ANOVA followed by post-hoc Tukey tests (see Suppl. Table S2 for details).

## DISCUSSION

Our study demonstrates that Tawny and Berthelot's Pipits, congeneric sister species differing in migratory strategy as well as history and present state of fragmentation of their distribution ranges, show similar patterns of geographic song variation at various spatial scales. We confirmed that both species possess individually distinct repertoires, usually comprising a single song type per male. Even at a local scale, both species exhibited very high variation among individuals, with some males singing very similar song types (apparently originating from the same template) while others very differentiated ones. There were no general features that would allow inferring the geographic origin of our pipit species based on their song, not even between Berthelot's Pipit subspecies from the different Macaronesian archipelagos. Over larger geographic distances, mean song dissimilarity increased in both species. This pattern was, however, not primarily driven by further song divergence of dissimilar songs but rather by the lack of closely similar song types. Our hypotheses, however, were partly supported for the Berthelot's Pipits, as we found that mean song dissimilarity between geographically most distant males (from different archipelagos) was significantly higher than between males within the same archipelago.

In Tawny Pipits, the patterns of individual song variation observed in our study agree with those reported previously for several European populations (Neuschulz 1986, Osiejuk et al. 2007, Oñate-Casado et al., 2023). We may therefore conclude that the individual distinctness of male repertoires is indeed generally valid for this species. For the Berthelot's Pipit, this is the first in-depth analysis of song structure and variation, revealing that both species' features are very similar. The quantitative analysis of intra-individual song consistency (Fig. 3) confirmed that most Berthelot's Pipit males sing their unique song type in a very stable manner. In only one individual, we noted structural variability among different renditions of its song (Fig. 2). Such a lack of song consistency was also previously observed in a few Tawny Pipit males (Oñate-Casado et al. 2023) and was attributed to their youth. Birds tend to produce more varied sounds during the song learning phase, with numerous plastic changes in vocal performances before reaching a crystallised song (e.g., Brenowitz & Beecher 2005, Mooney et al. 2008). It is likely that higher variation is observed in vocalization of some male pipits early in their first breeding season, before their song performance becomes consistent. Long-term temporal song stability was demonstrated over two or more years for Tawny Pipit males (Oñate-Casado et al. 2023), so we may presume the same is true also for its sister species.

A vast majority of males of both species in our study used a single song type. However, at all Tawny Pipits' localities, we occasionally observed males singing two song types as previously reported from the Czech site (Oñate-Casado et al. 2023). This phenomenon seems less frequent in Berthelot's Pipits (only one male from La Palma using two song types was included in our dataset) but we recorded a few more males singing two song types in other Canary Islands (JOC, AP, TP, unpublished results). This trait is thus also shared between the two sister species. Female song is another interesting feature occasionally observed in Tawny Pipit vocalization (Neuschulz 1986, Oñate-Casado et al. 2023), typically while feeding the nestlings (Oñate-

Casado et al. 2023). Revealing whether female Berthelot's Pipits also sing (and under which circumstances), however, would require a dedicated study of ringed individuals throughout the whole season.

The individual distinctness of male repertoires, observed for Tawny and Berthelot's Pipits at the level of whole song types, seems to be a general feature within the genus *Anthus*. Individuality and temporal repertoire stability, a prerequisite for individual acoustic monitoring, was demonstrated for the Tree Pipit *A. trivialis* (Petrusková et al. 2016), and the same applies also to other congeners breeding in Central Europe, the Meadow Pipit *A. pratensis* and the Water Pipit *A. spinoletta* (TP & AP, unpubl. data). All those species have more complex songs than Tawny and Berthelot's Pipits, and the differences between individuals are observed at the level of syllable repertoires (as described for Tree Pipits; Petrusková et al. 2016). Together with observations from our study species, however, this suggests that regardless of the level of song complexity, individually distinctive structural features of the song might also be found in other members of this songbird genus.

Contrary to our expectations, both species showed lack of clear, geographically structured song variation, which has been repeatedly documented in many passerines (e.g., Osiejuk et al. 2003, Barišić et al. 2018, Czocherová et al. 2022), including the congeneric Tree Pipit (Petrusková et al. 2010). Although Tawny and Berthelot's Pipits from the same field sites and islands, respectively, had on average more similar songs than individuals from elsewhere, this trend was driven primarily by the presence of very similar song types sung by small groups of local males. The overall song type variation within each studied populations was nevertheless very extensive (following the same trend as observed in Czech Tawny Pipits; Oñate-Casado et al. 2023), and apart from a possible close similarity to a song type of another male, there were no features that would allow inferring the geographic origin of the recorded song types.

We did observe a weakly but significantly higher mean song dissimilarity between Berthelot's Pipit populations representing its two recognized subspecies than at any smaller spatial scales. However, we expected substantially more pronounced song divergence between the subspecies, considering the documented lack of gene flow and clear phenotypic variation between the Canaries and Madeira (Illera et al. 2007; Spurgin et al. 2014; Armstrong et al. 2018; Martin et al. 2021), only very weak gene flow between island populations within archipelagos (Martin et al. 2021), and the species' sedentary behaviour. Despite this, we did not find any structural patterns allowing us to assign the song type to particular archipelago (Figs 2 and 6). The song types from both archipelagos were interspersed, and within-island song variation from Madeira and Porto Santo was comparable with than in the Canary Islands (Suppl. Fig. S2).

The lack of clear differentiation in geographically isolated populations of songbirds, even those from oceanic islands, may not be exceptional. Illera et al. (2014) found neither genetic, morphological nor acoustic differences in Spectacled Warblers (*Sylvia conspicillata*) from three Macaronesian archipelagos (Canaries, Madeira, and Cape Verde). However, their acoustic analysis was based on measurements of spectro-temporal parameters, which may not sufficiently capture variation in song structural characteristics, and the genetic structure of Berthelot's Pipit populations is clearly different. The colonization of the Madeira Archipelago by that species took place ca 8000 years ago (Illera et al. 2007; Spurgin et al. 2014; Armstrong et al. 2018; Martin et al. 2021), and the initial founder effect coupled with the absence of gene flow between archipelagos is the likely reason for the lower genetic diversity of Berthelot's Pipit across that archipelago (Armstrong et al. 2018; Martin et al. 2021). At the early stage of Madeira colonization, introduction bottleneck has likely affected also the song variation, but fast cultural evolution seems to have erased such founder effect since then, similarly as documented for the Silvereyes (*Zosterops lateralis*; Potvin & Clegg 2015).

Indeed, the accumulation of many differences (both small and large) contributing to individual-specific song variation is a likely explanation for the surprising lack of recognizable regionally specific song structures in both Berthelot's and Tawny Pipits. As the individuality in song structure seems prevalent within the genus, some improvisation is probably an inherent feature of the learning process in these songbirds. It might be particularly important in our study species that mostly sing a single but individually unique song type. If each male introduces some change against the template while learning from a tutor, this may result in a strong cultural drift that erases any locally specific features in evolutionary relevant time scales. Even in "recently" colonised islands of the Madeira archipelago, Berthelot's Pipit song has been evolving over several thousand generations (Illera et al. 2007; Spurgin et al. 2014), which must have resulted in emergence of many novel song type structures, obscuring any originally present founder effects. In fact, temporal changes in song characteristics may progress very fast in song-learning passerines. For example, prominent song changes were documented in a population of the house finch (*Haemorhous mexicanus*) at its suspected historical introduction site, where the song types experienced a complete turnover after less than four decades, although remaining structurally similar (Ju et al. 2019). Also, no more than 14 years were sufficient for a complete change of regional song structure of Corn Buntings (*Miliaria calandra*) in Cornwall (Holland et al. 1996). It is thus likely that a fast cultural evolution also takes place in much more recently isolated or newly founded Tawny Pipit populations.

The extent of intrapopulation song variation nevertheless does differ between Tawny Pipits' field sites, in which we observed lower song variation in the Czech coal mines and around the Greek site Agios Mamas (Suppl. Fig. S2). As the song structure at the former locality has been studied intensively (Oñate-Casado et al. 2023), the observed pattern could not be caused by insufficient sampling. Both populations are substantially more isolated than the other studied Tawny Pipit field sites: the closest breeding populations to the Czech site are in similar isolated habitats (open-cast coal mines) in Saxony, Germany, ca 110 km northwest or 130 km northeast (Steffens et al. 2013), and the closest one to Agios Mamas is in the Delta Nestos National Park, about 170 km northeast (D. Papandropoulos, pers. comm.). Both in Saxony and Greece, those populations are also separated from more contiguous areas inhabited by Tawny Pipits. We assume that fragmented and isolated populations of this migratory species may exhibit lower intrapopulation song variation due to smaller population size and limited dispersal, which reduce the number of potential song tutors for juveniles (Laiolo & Tella 2007; Laiolo et al. 2008; Briefer et al. 2010).

However, even a relatively “low” intrapopulation variation means, in case of Tawny Pipits, a presence of numerous highly diverse song types (Oñate-Casado et al. 2023).

The song type variation within a population of a migratory songbird species may also be influenced by its migratory connectivity, i.e., degree to which animals from the same breeding area migrate to the same non-breeding area (Webster et al. 2002). Briedis et al. (2016) reported that Tawny Pipits from the isolated Czech population overwinter together in a small region of the Western Sahel. Birds from a population with such high migratory connectivity are more likely to interact with each other during migration and wintering, potentially increasing song similarity among individuals within that population and limiting introduction of new song types. That way, the migratory connectivity may maintain the isolation of fragmented Tawny Pipit populations throughout the whole year. However, as the species tends to congregate in suitable wintering habitats either in Western or Eastern Sahel (BirdLife International and NatureServe 2011; Cramp 1988), it is plausible that males from different populations get into contact there during winter. Some mixing may also occur during migration, as the primary migration routes for Tawny Pipits breeding in Czechia (through Spain and Morocco) align with those documented for populations in Western Europe (Briedis et al. 2016, Calero-Riestra & García 2019). Whether the migratory behaviour enhances or decreases the cultural isolation of fragmented Tawny Pipits populations thus remains unclear.

Other factors linked to migratory behaviour, which might impact cultural evolution in songbirds, are natal philopatry and adult site fidelity that may both reinforce local song traditions within populations. Remarkable fidelity of adult Tawny Pipits to breeding areas has been observed in various European populations (Calero-Riestra & García 2019; VB, unpubl. data). Also, Czech Tawny Pipits show high natal philopatry (VB & MP, unpubl. data), contrasting with much lower philopatry in one Spanish population (María Calero-Riestra, pers. comm.). Such differences may be due to the patchy distribution of Central European populations resulting from the lack of suitable habitats (Briedis et al. 2016) in comparison with more contiguous distribution in the Iberian Peninsula (Calero-Riestra & García 2019). Lower song variation in the highly isolated Tawny Pipit population could have been influenced by both factors mentioned above.

Although we did not observe any clear differences in patterns of song similarity between Tawny and Berthelot’s Pipits on medium spatial scales (Fig. 6), at the smallest scales there were differences that may be related to their different migratory strategies. Berthelot’s Pipit songs were on average more similar to each other when neighbouring and nearby individuals (up to 3.5 km distance) were considered than at any larger scale. In Tawny Pipits, the song similarity did not differ between such nearby males and those from the same field sites further away. Although the spatial distributions of recorded birds were different in the two species (both median and maximal distances between Berthelot’s Pipits within islands were usually higher than within field sites for Tawny Pipits; Table 1)., we controlled for this by evaluating separately pairs of males below and over 10 km from each other, which revealed that the similarity patterns did not further change with increased distance within island or field site. It is thus plausible that the highest mean song similarity at the smallest scale in Berthelot’s Pipits is a direct consequence of their sedentarity. In oscine passerines, song is learned by juveniles from adult tutors like parents and neighbours (Slater & Ince 1979; Podos et al. 2004), and distribution of such tutors is likely much more stable in sedentary species, thus reinforcing local song traditions among individuals in the same area (e.g., Hansen 1999, Goodale & Podos 2010). A narrow range of potential tutors seems plausible for Berthelot's Pipits since they are territorial year-round (Alström & Mild 2003) and maintain stable territories over time (Juan Carlos Illera, pers. comm.). In contrast, migratory birds may learn songs from a broader range of individuals during breeding, wintering, and migration (Hultsch & Todt 2004; Beecher & Brenowitz 2005), and several studies have shown that song sharing among neighbours is higher in sedentary species than in migratory ones (Ewert & Kroodsma 1994; Kroodsma & James 1994; Kroodsma et al. 1999; Wilson et al. 2000; Nelson et al. 2001; Handley & Nelson 2005). We may assume that differences in song learning opportunities between sedentary and migratory species apply also to Berthelot's and Tawny Pipits and contributed to different patterns of their local-scale song variation.

Despite these results, however, it should be noted that high levels of song similarity, specifically song sharing, may be observed at small geographical scales for bird species regardless of their migratory behaviour. For example, both resident Skylarks (*Alauda arvensis*) and migratory Black Redstarts (*Phoenicurus ochruros*) showed high syllable and song type sharing, respectively, with neighbouring individuals, but very little sharing just 2 km away or less (Briefer et al. 2008; Draganoiu et al. 2014). In case of Skylarks, a species with very rich repertoire, males likely adjust their singing behaviour in interactions with their neighbours (as do for example Great Reed Warblers, *Acrocephalus arundinaceus*; Wegrzyn & Leniovski 2010). The songs in Black Redstarts, however, were stable and represented local microdialects (Draganoiu et al. 2014). Similarly, migratory Savannah Sparrows (*Passerculus sandwichensis*) showed greater acoustic similarity to neighbours within a few hundred metres than to distant individuals within the same island population, possibly due to the high degree of natal site philopatry (Hensel et al. 2022). These findings demonstrate that small-scale patterns of acoustic similarity may be present in both migratory and non-migratory passerine species.

In conclusion, despite the differences between the evolutionary history and migratory strategies between Tawny and Berthelot's Pipits, we observed substantial similarities in the characteristics and spatial patterns of variation of their songs. Overall high within-population variation of song types and lack of further differentiation over large distances may result from relatively fast evolution of song types, driven by individuality in vocalization in both species. The differences in geographic song variation were observed at the smallest and largest spatial scales, which might reflect Berthelot's Pipits sedentarity and isolation of Macaronesian archipelagos, respectively. However, disentangling the effects of migratory and sedentary behaviour from other aspects of natural history and ecology that differ between species remains challenging. To determine whether song variation is truly influenced by migratory behaviour, it would be ideal to compare populations within the same species that differ in this trait (e.g., Nelson et al. 2001, Linossier et al. 2016) from a broad geographical range. Within the genus *Anthus* with its pronounced individuality in songs, suitable taxa for such research might be the Meadow Pipit and the Rock Pipit (*A. petrosus*), with some populations in Europe migratory but other resident all year (Alström & Mild 2003).

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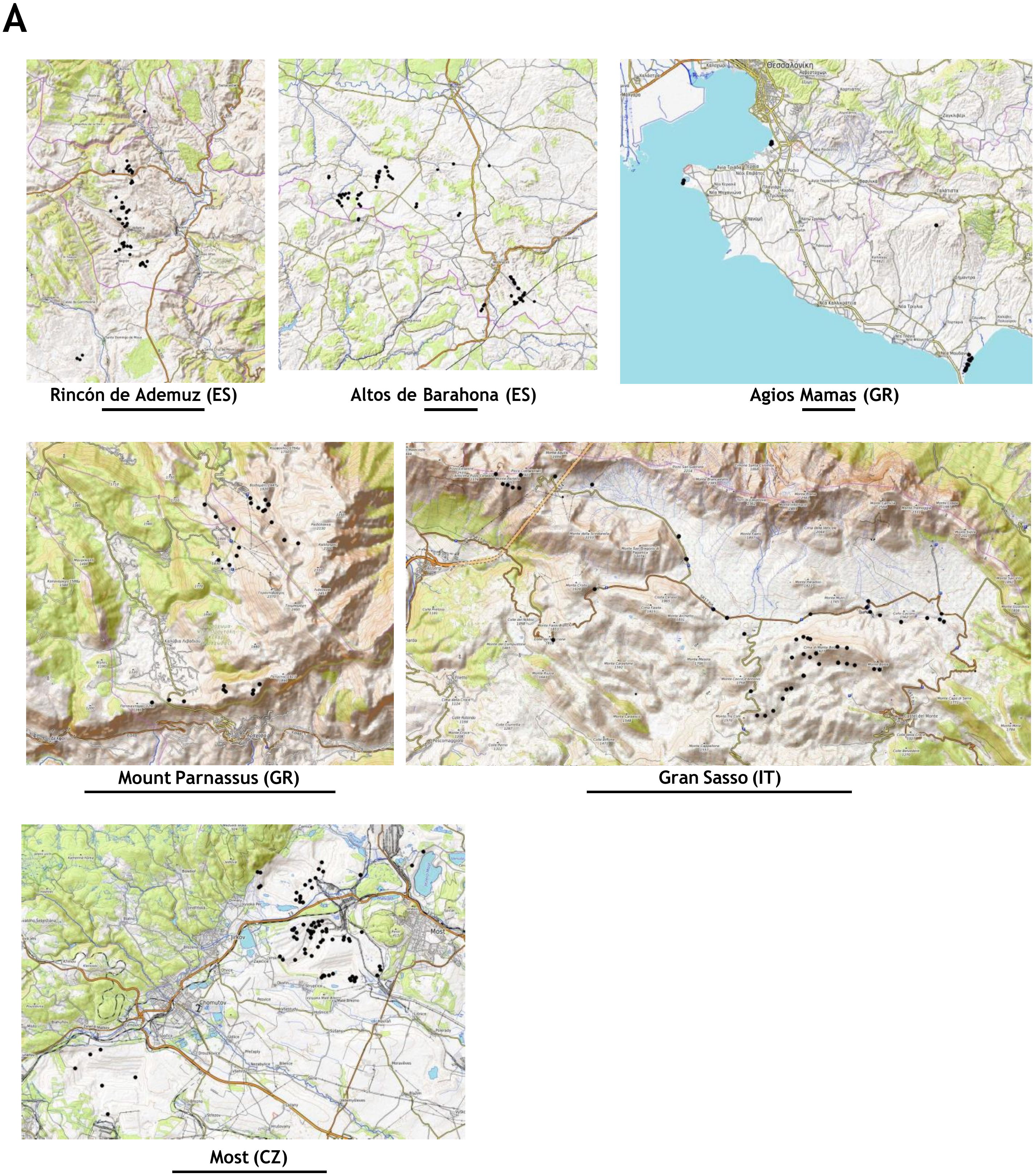


Rincón de Ademuz (ES)

Altos de Barahona (ES)

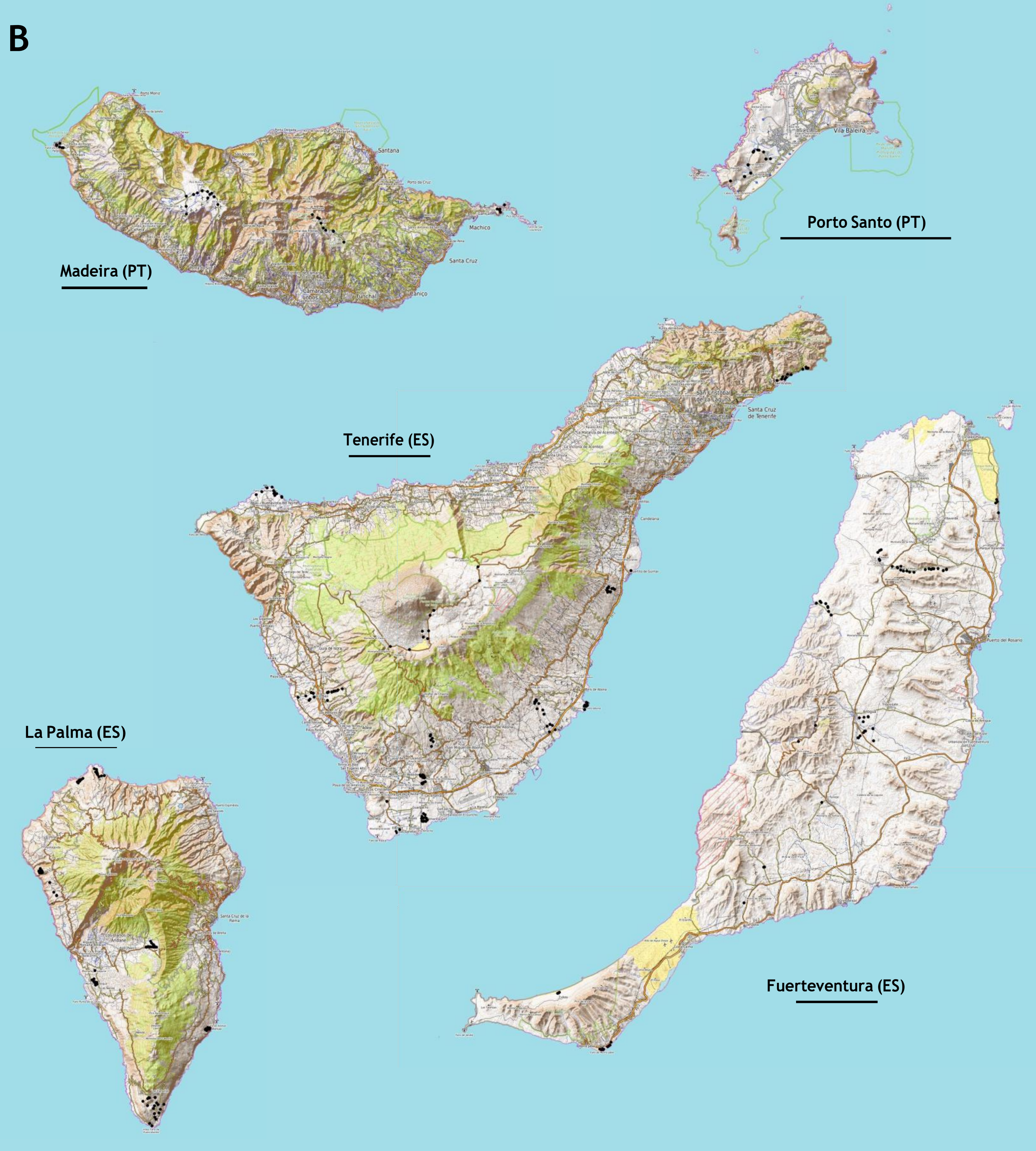
Agios Mamas (GR)

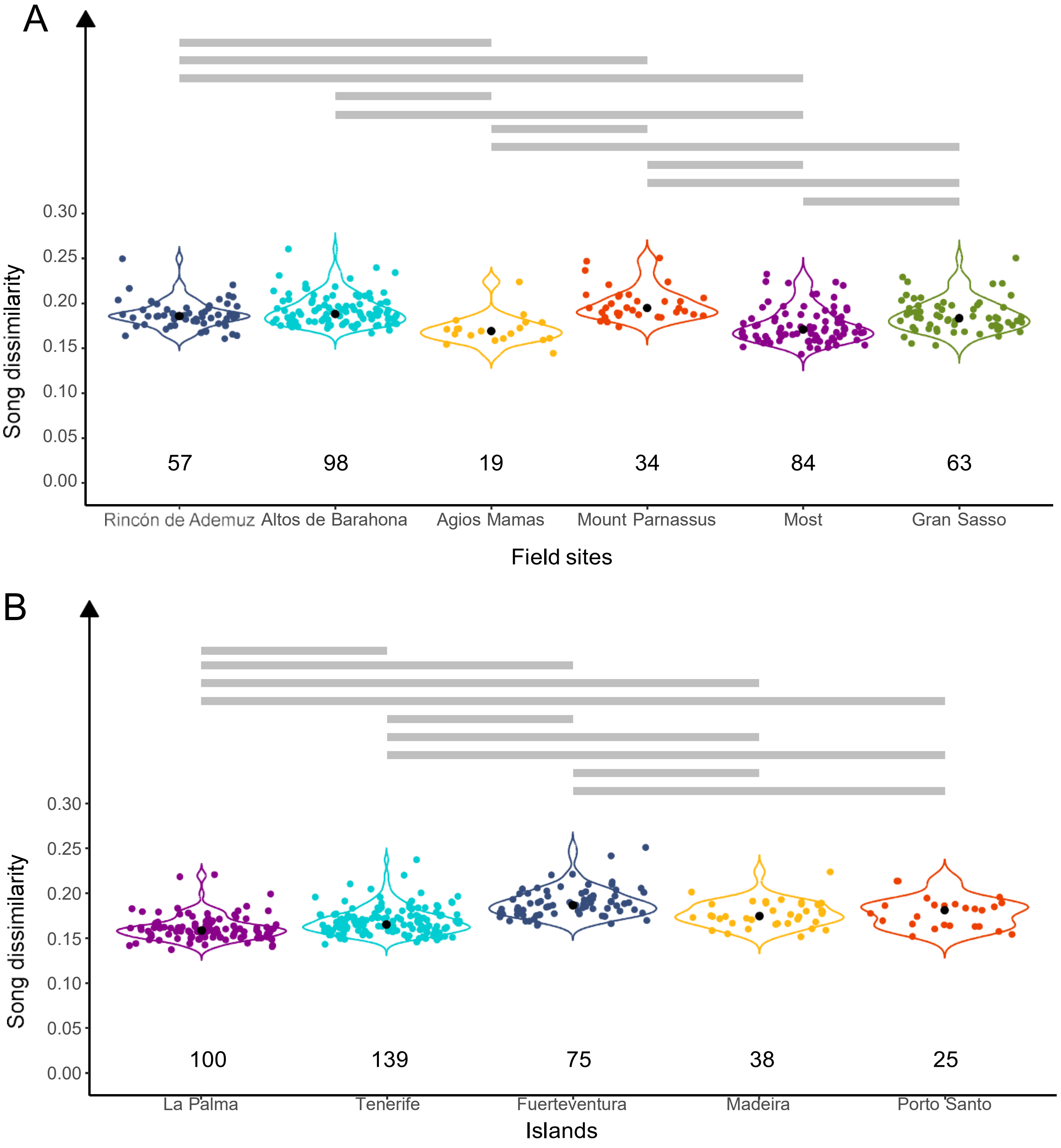
Mount Parnassus (GR)

Gran Sasso (IT)

Most (CZ)

**Suppl. Fig. S1.** Spatial distribution of recorded individuals (indicated by black dots) in studied regions for Tawny Pipits (A) and Berthelot's Pipits (B). Note that scales differ between maps, each scale bar represents 10 km. Geographic coordinates for all individuals are provided in Suppl. Table S1. A GPX file containing individual codes and coordinates, allowing dynamic exploration of the maps in suitable viewers, is provided along with other source data. Map source: OpenTopoMap


B
Madeira (PT)
Porto Santo (PT)
Tenerife (ES)
La Palma (ES)
Fuerteventura (ES)
Santana
Porto da Cruz
Machico
Santa Cruz
Funchal
Caniço
Câmara de Lobos
Vila Baleira
San Cristobal de La Laguna
Santa Cruz de Tenerife
Candelaria
Puerto del Rosario
Santa Cruz de La Palma



**Suppl. Fig. S2.** Variation in mean song dissimilarity at different field sites for Tawny Pipits (A) and at different islands for Berthelot's Pipits (B). Numbers above X axis indicate the total number of song types included in the comparison within a given area; this exceed the number or recorded males in B due to the presence of individuals singing two different song types. Each dot represents the mean song dissimilarity for each song type from all relevant pairwise comparisons within a given geographic scale; black dots represent median values. Horizontal bars above the plots indicate significant differences ($p < 0.005$) in song similarity between areas, based on the results of one-way ANOVA followed by post-hoc Tukey tests (see Suppl. Table S2 for details).

**Supplementary Table S1.** List of recorded pipit males, with geographic coordinates, locality name, year of recording, and no. of song types in the repertoire. Males whose song types are shown in Fig. 2 are indicated. This table is provided as a separate spreadsheet file (XLSX format) and can be provided upon request.

**Supplementary Table S2.** Results of the analyses of variance comparing mean song dissimilarity of song types of Tawny and Berthelot's Pipits across spatial scales and among areas (field sites or islands) where singing males were recorded.

**A:** Summary table for all analyses of variance (ANOVA). Significant differences are marked in bold.

| **Species** | **Source of variation** | ***df*** | ***SS*** | ***MS*** | ***F*** | ***p*-value** |
|---|---|---|---|---|---|---|
| **Tawny Pipits** | | | | | | dicated |
| | *Geographic variation at five spatial scales* | | | | | |
| | Between groups | 4 | 0.024 | 0.006 | 13.67 | **<0.001** |
| | Residual | 1522 | 0.690 | 0.004 | | |
| | *Geographic variation across field sites* | | | | | |
| | Between groups | 5 | 0.024 | 0.005 | 16.37 | **<0.001** |
| | Residual | 349 | 0.106 | 0.003 | | |
| **Berthelot's Pipits** | | | | | | |
| | *Geographic variation at five spatial scales* | | | | | |
| | Between groups | 4 | 0.101 | 0.025 | 68.65 | **<0.001** |
| | Residual | 1720 | 0.632 | 0.000 | | |
| | *Geographic* variation across islands | | | | | |
| | Between groups | 4 | 0.037 | 0.009 | 41.02 | **<0.001** |
| | Residual | 372 | 0.082 | 0.000 | | |

**B:** Results from post-hoc Tukey tests for comparisons of mean song dissimilarity across geographic scales and field sites for Tawny Pipits. Significant differences are marked in bold.

| Tawny Pipits | | | |
|---|---|---|---|
| **Level 1** | **Level 2** | **Mean difference** | ***p*-value** |
| *Geographic variation at five spatial scales* | | | |
| local | same site (3.5 km - 10 km) | -0.002 | 0.602 |
| local | same site (>10 km) | -0.002 | 0.494 |
| local | same country | -0.009 | **0.000** |
| local | between countries | -0.009 | **0.000** |
| same site (3.5 km - 10 km) | same site (>10 km) | 0.000 | |
| same site (3.5 km - 10 km) | same country | -0.007 | **0.000** |
| same site (3.5 km - 10 km) | between countries | -0.007 | **0.000** |
| same site (>10 km) | same country | -0.007 | **0.001** |
| same site (>10 km) | between countries | -0.006 | **0.000** |
| same country | between countries | 0.000 | 0.999 |
| *Geographic variation across field sites* | | | |
| Agios Mamas | Altos de Barahona | -0.021 | **0.000** |
| Agios Mamas | Gran Sasso | -0.015 | **0.007** |
| Agios Mamas | Most | -0.004 | 0.909 |
| Agios Mamas | Mount Parnassus | -0.029 | **0.000** |
| Agios Mamas | Rincón de Ademuz | -0.016 | **0.004** |
| Altos de Barahona | Gran Sasso | 0.005 | 0.295 |
| Altos de Barahona | Most | 0.017 | **0.000** |
| Altos de Barahona | Mount Parnassus | -0.007 | 0.299 |
| Altos de Barahona | Rincón de Ademuz | 0.005 | 0.482 |
| Gran Sasso | Most | 0.011 | **0.001** |
| Gran Sasso | Mount Parnassus | -0.013 | **0.006** |
| Gran Sasso | Rincón de Ademuz | -0.007 | 0.999 |
| Most | Mount Parnassus | -0.024 | **0.000** |
| Most | Rincón de Ademuz | -0.012 | **0.000** |
| Mount Parnassus | Rincón de Ademuz | 0.012 | **0.014** |

**C:** Results from post-hoc Tukey tests for comparisons of mean song dissimilarity across geographic scales and field sites for Berthelot's Pipits. Significant differences are marked in bold.

| **Berthelot's Pipits** | | | |
|---|---|---|---|
| **Level 1** | **Level 2** | **Mean difference** | ***p*-value** |
| *Geographic variation at five spatial scales* | | | |
| local | same island (3.5 km - 10 km) | -0.007 | **0.000** |
| local | same island (>10 km) | -0.006 | **0.000** |
| local | same archipelago | -0.010 | **0.000** |
| local | between archipelagos | -0.022 | **0.000** |
| same island (3.5 km - 10 km) | same island (>10 km) | 0.000 | 0.995 |
| same island (3.5 km - 10 km) | same archipelago | -0.002 | 0.398 |
| same island (3.5 km - 10 km) | between archipelagos | -0.015 | **0.000** |
| same island (>10 km) | same archipelago | -0.003 | 0.122 |
| same island (>10 km) | between archipelagos | -0.015 | **0.000** |
| same archipelago | between archipelagos | -0.012 | **0.000** |
| *Geographic variation across islands* | | | |
| Fuerteventura | La Palma | 0.027 | **0.000** |
| Fuerteventura | Madeira | 0.011 | **0.000** |
| Fuerteventura | Porto Santo | 0.011 | **0.011** |
| Fuerteventura | Tenerife | 0.020 | **0.000** |
| La Palma | Madeira | -0.016 | **0.000** |
| La Palma | Porto Santo | -0.016 | **0.000** |
| La Palma | Tenerife | -0.006 | **0.008** |
| Madeira | Porto Santo | 0.000 | 0.999 |
| Madeira | Tenerife | 0.009 | **0.007** |
| Porto Santo | Teneife | 0.009 | **0.023** |