Geographic variation in coal tit song across continents and reduced species recognition between Central European and Mediterranean populations

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Abstract
Intraspecific variation of passerine song may influence mate choice and consequently provides a potential mechanism for premating reproductive isolation in birds. Notable geographic variation of songs is particularly common in widespread bird species, such as the coal tit Periparus ater (Aves, Passeriformes, Paridae) having a large trans-Palearctic distribution range including allopatric populations. In this study, measurement and analysis of 16 song parameters from different Western Palearctic populations showed a relatively uniform song in all continental European and the island populations of Corsica and Sardinia. Song variation among and within these populations seems so variable that there is a broad geographical overlap of acoustic parameters between populations. However, songs from northwest African and Cypriot populations had lowest minimum frequencies and were thus significantly different from continental European, Corsican and Sardinian populations. To test for intraspecific species recognition and potential acoustic isolation of different Palearctic populations, we carried out field playback experiments on Central European coal tit males with songs from populations of the Eurasian (Germany, Latvia, Russian Far East, Japan) and the Mediterranean region (northwest Africa, Cyprus, Corsica, Sardinia). Eurasian song types elicited similar strong territorial reactions in Central European coal tits as local control songs. In contrast, none of the Mediterranean songs evoked any notable reaction. Thus, there is geographic variation in coal tit song across continents that coincides with reduced species recognition between Central European and Mediterranean populations.

Key words
Bird song, geographic variation, song parameter, coal tit Periparus ater, territorial behaviour, playback experiments, acoustic isolation.

Introduction
Song is an important mean for intra- and interspecific communication in passerine birds. Most often songs have a territorial context and function in mate attraction and stimulation (Catchpole & Slater 1995, Collins 2004). Intraspecific variation of bird songs may reduce species recognition and decrease potential mating success; thus, territorial song is remarkably involved in reproductive isolation mechanisms (Slabbe Koorn & Smith 2002). Territorial songs often show geographical differences, which may affect the intensity of a male’s territorial response towards unknown song types. Due to enhanced geographical and behavioural changes in small remote populations, passerine song dialects on islands are often remarkably distinct from those of conspecific neighbouring continental populations. Examples include the Corsican finch on Corsica Carduelis corsi-
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Cana (Fürschler & Kalko 2007), the Cyprus short-toed tree creeper Certhia brachydactyla dorotheae (Fürschler & Randler 2009) and the Cyprus wheatear Oenanthe cypria ca on Cyprus (Randler et al. 2012). Such island dialects often elicit weaker territorial responses in continental relatives and may enhance acoustic and behavioural isolation between populations (Packert & Martens 2004). However, acoustic and behavioural differentiation processes throughout a large continental distribution range of a species including marginal allopatric populations are less understood.

A striking example of a passerine bird species with a trans-Palearctic distribution range is the coal tit Periparus ater (Aves, Passeriformes, Paridae). The song among Palearctic coal tit populations is considered as relatively uniform (Lohrl & Thielcke 1973, Thielcke 1973, Martens 1996); however, more recently some variation of song parameters was detected across their southern breeding range and one most divergent island population on Cyprus (Tietze et al. 2011). The intensity of territorial responses towards different song types can be assessed with song playbacks from different geographic origin and can give hints on song divergence and potential reproductive isolation (Slabbekoorn & Smith 2002, Tietze et al. 2012, Hagemeyer et al. 2012, Pegan et al. 2015). Playback experiments with Sino-Himalayan coal tit song types may evoke significantly weaker territorial reactions in German populations than local control songs (Tietze et al. 2011). Playbacks on German coal tits with songs of the Afghan coal tit P. a. melanolophus and the Afghan great tit Parus major (cinereus group) showed that coal tits were able to distinguish between hetero- and conspecific song (Thielcke 1969). Reverse playbacks on Nepalese coal tits with German songs showed a reduced territorial response (Martens 1975) indicating that the test birds were able to distinguish between their own and an alien dialect.

In the light of these previous studies, here we tested whether: (i) coal tit songs from the Mediterranean islands and North Africa differed from continental European songs, and (ii) whether they were less effective stimuli to Central European coal tits in comparison to songs from continental Eurasian populations, (iii) whereas continental Eurasian songs should represent continuous populations. They were thus expected to be similar to each other and should still evoke strong territorial aggressions among different Eurasian populations. We tested these assumptions by statistical analysis of quantifiable song traits and by using playback experiments.

Materials and Methods

Song recording and sonographic analysis

Original recordings (Table 1, Fig. 1) were converted to wav format into a sampling rate of 22.1 kHz with 16 bit mono and analysed with Avisoft SASLab Light v3.74 (Specht 1999). For exemplary recording methods and equipment see Martens et al. 1999, Fürschler & Kalko 2007, Fürschler & Randler 2009. The following 16 parameters were measured from all verses per song type and individual (Fig. S1): Length of the verse (Δt), maximum frequency of the verse (fE1max), minimum frequency of the verse (fE1min), length of the first complete element group (ΔtE1), maximum frequency of that element (fE1max), minimum frequency of that element (fE1min), length of the element with the smallest frequency range within the first complete element group (ΔtE2), maximum frequency of this element (fE2max), minimum frequency of this element (fE2min) (Suppl. Fig. S1B), number of elements per verse (nE), number of element groups per verse (nEg), number of elements per element group (nEgE). In addition, means per song type from the frequency range of the verse (Δf = fmax - fmin), mean frequency of the verse [fmean = (fmin + fmax)/2] and verse speed (= nE/Δt) were calculated. In total, 1497 song verses representing 287 song types of 216 males from ten different Western Palearctic populations were measured and analysed (Table 1). Mean values per song type from the song parameters were calculated and analysed via discriminant analysis using SPSS, version 11.5.

Playback experiments

Song verses were chosen from the original recordings and playback stimuli were designed using Avisoft SASLab Light v3.74 (Specht, 1999). 17 song types from various populations of the Palearctic region were tested (Fig. 1, 2 and Table 1): Continental Eurasia: Dresden in East Germany, 2 × North Japan, Russian Far East, Central Latvia; Northwest Africa: 2 × North Morocco and 2 × North Tunisia; Mediterranean Region: 4 × Cyprus and 2 × Corsica and 2 × Sardinia. Playbacks were performed in Central Europe in coniferous and mixed woods, i.e. in the Dresden Heath (East Germany) and in the Bohemian Switzerland (near Jetřichovice, Czech Republic, 50 km Southeast of Dresden) during the breeding season from 26 March to 6 June 2010. A portable Mini Disc Recorder MD MT-15 from Sharp with a Hama 14461 loudspeaker was placed in the centre of a territory (identified by a singing male) directing upwards and adjusted to natural loudness. Each male was tested only once. The different playbacks were displayed in alteration to avoid a timely bias of our analysis. During one experiment the individual bird was displayed two playbacks. The first playback (= test playback) consisted of one test song from one of the populations mentioned above; it included one single original verse repeated ten times interrupted with 3 s silence each. Then a pause for at least 2 min followed between the playbacks. During this period, territorial reactions were observed and the tested bird could calm down. If the test bird was highly excited and singing intensely
the second playback was not started until the bird had stopped singing and departed. Afterwards, the second playback (= control playback) was tested, which consisted of a local control song (Black Forest, South Germany) and included one single original verse repeated ten times interrupted by 3 s silence each. The control was applied

Table 1. Information on song recordings from the Palearctic region used for song analysis or playback experiment, respectively.

<table>
<thead>
<tr>
<th>region</th>
<th>locality</th>
<th>n males</th>
<th>n verses</th>
<th>recordist</th>
<th>year</th>
</tr>
</thead>
<tbody>
<tr>
<td>East Germany</td>
<td>Dresden Heath</td>
<td>36</td>
<td>280</td>
<td>Päckert</td>
<td>2007</td>
</tr>
<tr>
<td>Austria</td>
<td>Salzburg</td>
<td>4</td>
<td>10</td>
<td>Martens</td>
<td>1991</td>
</tr>
<tr>
<td>South France</td>
<td>Covennes</td>
<td>7</td>
<td>49</td>
<td>Förchler, Martens</td>
<td>1993, 2002</td>
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<tr>
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<td>Sardinia</td>
<td>30</td>
<td>183</td>
<td>Förchler</td>
<td>2003</td>
</tr>
<tr>
<td>South Greece</td>
<td>Peloponneseis</td>
<td>16</td>
<td>139</td>
<td>Martens</td>
<td>2010</td>
</tr>
<tr>
<td>Cyprus</td>
<td>Troodos Mountains</td>
<td>17</td>
<td>49</td>
<td>Randler</td>
<td>2008</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td></td>
<td><strong>216</strong></td>
<td><strong>1497</strong></td>
<td></td>
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<table>
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<tr>
<th>SONG PLAYBACKS</th>
<th>subspecies</th>
<th>recording locality</th>
</tr>
</thead>
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<tr>
<td>EURASIA 0</td>
<td><em>P. ater/abietum</em></td>
<td>East Germany, Dresden Heath</td>
</tr>
<tr>
<td>EURASIA 1</td>
<td><em>P. a. insularis</em></td>
<td>North Japan, Sapporo</td>
</tr>
<tr>
<td>EURASIA 2</td>
<td><em>P. a. ater</em></td>
<td>Russian Far East, Sikhote Alin</td>
</tr>
<tr>
<td>EURASIA 3</td>
<td><em>P. a. ater</em></td>
<td>Central Latvia, Saulkrasti</td>
</tr>
<tr>
<td>EURASIA 4</td>
<td><em>P. a. insularis</em></td>
<td>North Japan, Sapporo</td>
</tr>
<tr>
<td>NORTHWEST AFRICA 1</td>
<td><em>P. a. atlas</em></td>
<td>North Morocco, Ifrane</td>
</tr>
<tr>
<td>NORTHWEST AFRICA 2</td>
<td><em>P. a. ledouci</em></td>
<td>North Tunisia, Tunis</td>
</tr>
<tr>
<td>NORTHWEST AFRICA 3</td>
<td><em>P. a. atlas</em></td>
<td>North Morocco, Ifrane</td>
</tr>
<tr>
<td>NORTHWEST AFRICA 4</td>
<td><em>P. a. ledouci</em></td>
<td>North Tunisia, Tunis</td>
</tr>
<tr>
<td>CYPRUS 1</td>
<td><em>P. a. cypriotes</em></td>
<td>Cyprus, Troodos mountains</td>
</tr>
<tr>
<td>CYPRUS 2</td>
<td><em>P. a. cypriotes</em></td>
<td>Cyprus, Troodos mountains</td>
</tr>
<tr>
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<td><em>P. a. cypriotes</em></td>
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<td>CYPRUS 4</td>
<td><em>P. a. cypriotes</em></td>
<td>Cyprus, Troodos mountains</td>
</tr>
<tr>
<td>CORSICA/SARDINIA 1</td>
<td><em>P. a. sardus</em></td>
<td>France, Corsica, Illarata</td>
</tr>
<tr>
<td>CORSICA/SARDINIA 2</td>
<td><em>P. a. sardus</em></td>
<td>Italy, Sardinia, Limbara</td>
</tr>
<tr>
<td>CORSICA/SARDINIA 3</td>
<td><em>P. a. sardus</em></td>
<td>France, Corsica, Illarata</td>
</tr>
<tr>
<td>CORSICA/SARDINIA 4</td>
<td><em>P. a. sardus</em></td>
<td>Italy, Sardinia, Limbara</td>
</tr>
<tr>
<td>CONTROL</td>
<td><em>P. ater/abietum</em></td>
<td>South Germany, Black Forest</td>
</tr>
</tbody>
</table>

Fig 1. Map of the Palearctic region showing the origin of song recordings used for song analysis and playback experiments.
to generally verify the presence of a territorial male at the experimental sites and to give a further estimate of the mean territorial reaction of local males to a German test song. In total, 289 double playback experiments (including test and control playback) were performed, i.e. the playback of each population was tested 17 times. For quantification of the territorial reactions, the following behaviours were recorded, all scoring one point each: approach to at least 10 m, successive approach to 5 to 0 m, vertical approach (i.e. down from the tree top to at least the lower half of the tree), display of territorial song, change of song type and display of calls. Accordingly, the maximum score for the intensity of territorial reactions was 6 points. For statistical analysis, mean reactions between pooled populations (Eurasia, Northwest Africa, Cyprus and Corsica/Sardinia) were tested against each other for significance by Mann-Whitney Rank Sum Test using SPSS 11.5.

Results

Song of Northwest African and Cypriot coal tits differs from European populations

Nine discriminant functions (DF) contributed to the analysis in a stepwise procedure and explained a cumulative 100% of the variance. The first two functions had an eigenvalue higher than one indicating a good among-group differentiation: DF1 (eigenvalue 2.044) explained 52.5% of the variance and Wilks’ λ was 0.08. DF2 with an eigenvalue of 1.151 explained 29.6% of the variance and Wilks’ λ was 0.244 (Fig. 3). DF1 showed the highest correlations to three song parameters (significantly negative correlation): maximum frequencies of the verse \( f_{1 \text{max}} \) (–0.625), of the first element \( f_{1 \text{max}} \) (–0.560) and of the second element \( f_{2 \text{max}} \) (–0.456). A single song parameter correlated most strongly to DF2: minimum frequency of the verse \( f_{\text{min}} \) (0.538). Thus, both discriminant functions were mainly determined by frequency parameters. The predicted group affiliation assigned 56.4% of all song types correctly to the respective population (origin of recording) with Northwest Africa (65.0%), Dresden (73.3%) and Cyprus (84.2%) showing the highest values of correct assignment. Coal tits from the population of Dresden and Northwest Africa showed the highest maximum frequencies of the verse and the first two elements (Fig. 3). Population mean value for the verse maximum frequency was 8.6 kHz for Dresden and 7.5 kHz for Northwest Africa. Coal tits from Northwest Africa and Cyprus had the lowest minimum frequencies with respect to DF2 (population mean values 2.6 kHz and 2.5 kHz). The remaining populations formed one group and can rather be characterised by intermediate maximum and minimum frequency values.

Central European coal tit populations do not respond to Mediterranean songs

The intensity of territorial responses of Central European coal tits towards playbacks from populations of the Eurasian and the Mediterranean region differed greatly depending on the geographic origin of the tested playback (Fig. 4). Verses from Eurasia (Latvia, Germany, the Russian Far East and Japan) elicited strong territorial reactions in Central European coal tits. Most of these Eurasian songs evoked similarly strong reactions as the control song from South Germany. Importantly, none of the twelve Mediterranean verses (Northwest Africa, Cyprus, Corsica and Sardinia) evoked any notable territorial reaction in Central European coal tits. Comparison of mean reaction scores of all five Eurasian songs versus each set of Mediterranean songs (Northwest Africa, Cyprus or Corsica/Sardinia) as well as versus all Mediterranean songs taken together showed highly significant differences (\( P < 0.001 \), Mann-Whitney Rank Sum Test; Fig. 4).

Discussion

The song analysis showed that the allopatric populations from Northwest Africa and Cyprus diverged from other continental European and some Mediterranean populations by frequency differences. Our finding that the most southern populations from Northwest Africa and Cyprus have lower minimum frequencies than the remaining populations indicates some degree of geographical song variation in coal tits. So far, it has only been known that Northwest African coal tits have distinct alarm calls (Löhrl & Theilcke 1973), and that Cypriot coal tits may sing lower-pitched than continental coal tits (Goller 1987, Cramp et al. 1993, Gosler & Clement 2007). A possible explanation for the distinctiveness of the Cypriot and Northwest African song might be their long time of geographic separation. Both populations represent the oldest genetic lineage splits in the Western Palearctic while the island populations from Sardinia and Corsica are more closely related to the Southwest continental European abietum lineage and thus represent more recent colonisations (Tietze et al. 2011, Pentzold et al. 2013). The closer phylogenetic relationships of the latter populations were paralleled by song variation as in our song analysis Corsican and Sardinian populations clustered with those from continental Europe. Surprisingly, Corsican and Sardinian songs did not elicit notable territorial responses in Central European coal tits. It is therefore likely that coal tit songs contain subtle response-eliciting parameters such as slight frequency modulation of elements that was not assessed in our acoustic analysis. In common chiffchaffs Phylloscopus collybita for
Fig. 2. Territorial song verses of coal tit males used for playback experiments. Verses are shown in frequency (kHz) versus time (s). Playback experiments were carried on territorial coal tit males in E Germany.
example, slight element modulations and the inclination, i.e. ascent and descent of the frequency course, of single elements are crucial for species recognition (Bøeker et al. 1980). In areas of sympatry, black-capped chickadees *Poecile atricapillus* and mountain chickadees *P. gambeli* presumably distinguish among conspecific and heterospecific songs by note-frequency ratios and the presence versus absence of an introductory note (Lohr 2008).

Frequency parameters of song as well as temporal parameters can also have an adaptive component and might show a broad variation across a species’ range in dependence of vegetation density and openness of habitat for example (Martens 1996, Rheindt et al. 2004, Tobias et al. 2010). Adaptation might play a role in coal tit song variation, too, because throughout the Mediterranean range coal tits might occupy different breeding habitats than in the northern Eurasian range such as oak forests and even macchia in the South compared to boreal forests in the North (Cramp et al. 1993). Therefore, greater song diversification in the South of the coal tits breeding range (this study and Tietze et al. 2011) might relate to multi-fold effects of long-term separation of allopatric populations (Northwest Africa and Cyprus) and local adaptation to different habitats.

Territorial songs from isolated and island populations often differ from those of continental relatives (Forschler & Kalko 2007) and thus may lack response-eliciting parameters when testing in continental populations. This has been shown by playback experiments for songs of several Azorean and Canarian subspecies of the goldcrest, *Regulus regulus* (Packert & Martens 2004, Packert 2006), songs of Afrocanarian blue tits from La Palma *Parus teneriffae palmensis* (Schottler 1995), songs of the Canary Island chiffchaff *Phylloscopus canariensis* (Thielcke et al. 1978) as well as for *Oe. cypriaca* with playbacks of the closely related black-eared wheatear *Oe. hispanica* (Randler et al. 2012). The more divergent song parameters are, the more species recognition decreases and the more the degree of premating reproductive isolation between population increases (Searcy et al. 1997, Nelson 1998). Central European coal tits reacted significantly weaker to song playbacks from Mediterranean populations (Northwest Africa, Cyprus, Corsica/Sardinia) than to playbacks from continental Eurasian populations. This lack of an adequate territorial response may indicate premating acoustic isolation between population increases (Searcy et al. 1997, Nelson 1998). Central European coal tits may still react strongly to single song types from China and the

![Scatterplot of the first two discriminant functions from the 16 sonagraphic parameters of ten coal tit populations. One dot represents one song type of one individual. Each population is colour-coded.](image-url)
Himalayas (Tietze et al. 2011 – however, territorial response to most Asian song types was greatly reduced). In contrast, within the Palearctic region, a decrease in reaction intensity with geographical distance of song origin was shown between Central European and Eastern Eurasian populations. Squares indicate mean values and bars indicate 95% confidence intervals of the reaction. The control is a local verse from the Black Forest, South Germany. Mean reactions between two pooled populations, i.e. Eurasia versus Northwest Africa, Eurasia versus Cyprus and Eurasia versus Corsica/Sardinia were significantly different as indicated by *** (P < 0.001, Mann-Whitney Rank Sum Test).

The broad among- and within-population variation of Eurasian coal tit song among our study taxa was also confirmed for the large trans-continental Eurasian range of the species including allopatric Himalayan and Chinese subspecies (Tietze et al. 2011). The relative uniformity of coal tit song strongly contrasts the genetic diversification reflected by eight mitochondrial lineages (Tietze et al. 2011). In the Western Palearctic four lineages exist: one in North Africa, one on Cyprus and two on the European continent that form a broad secondary contact zone in Central Europe (Martens et al. 2006, Pentzold et al. 2013). Thus, song differentiation does not parallel genetic differentiation in the coal tit populations, maybe because song characteristics have evolved slower than one could expect from strongly structured phylogeographic genetic patterns. A similar scenario was found in the recently diverged sister species pair the orchard Icterus spurius and Fuertes’ orioles I. fuertesi, which are genetically distinct, but do not show discernible differences in their song (Baker et al. 2003, Hagemeyer et al. 2012). However, when compared to other Palearctic tit species the relative uniform coal tit songs stand out as an exception from the rule. Diversification among genetic lineages corresponds well to distinct song types for example in Cyanistes blue tits such C. caeruleus, C. cyanus and C. teneriffae (Martens 1996, Salzburger et al. 2002a, Packert 2015) and in great tits Parus major sensu lato including Asian P. minor, P. cinereus and P. bokharensis (Packert et al. 2005). The three strongly distinct song types of Eurasian willow tits Poecile montanus sensu lato have a complicated spatial distribution (Martens et al. 1995, Quaisser & Eck 2003, Packert 2015): the presumably ancestral Sino-Japanese song type corresponds well to two strongly diverged Chinese genetic lineages P. m. weigoldicus in the South and P. m. affinis/P. m. stoetzneri in the North (Kvist et al. 2001, Salzburger et al. 2002b), but does rarely occur in populations of the Russian Altai, too. In contrast, the alpine and the lowland song type are widely distributed throughout the genetically uniform North Palearctic range, but in the eastern and western marginal populations only one of the two is present, and the two form a mosaic distribution throughout Europe.

Generally, phylogenetic signal of avian vocalizations, particularly of innate calls and innate traits of passerine territorial song is considered to be high (McCracken & Sheldon, 1997, Packert et al. 2003, 2009; Rheindt et al. 2004, Tietze et al. 2015). For the coal tit, these findings are not confirmed by our results, except that the genetically most diverged Mediterranean lineages from Northwest Africa (subspecies P. a. atlas and P. a. ledouci) and from Cyprus (subspecies P. a. cypriotes) showed differentiation of songs. Taking into account the reduced territorial response of Central European coal tits to Mediterranean song types, it seems that few relevant response-eliciting parameters were not detected in our set of measurements. Trans-Palearctic variation of such subtle acoustic traits like in coal tit songs would be a challenging focus for future bioacoustics research.

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Disclosure Statement: the authors declare no competing financial interests.
References


Supplementary

Supplementary Fig. S1. Song verse fragment of *P. a. sardus* showing some of the measured sonagraphic parameters. A and B represent the same verse. They are shown twice with different parameters of song indicated. For abbreviations, see text.

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**Supplementary**


