

Cultural transmission and its possible effect on urban acoustic adaptation of the great tit *Parus major*

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Abstract

Cultural transmission and its possible effect on urban acoustic adaptation of the great tit Parus major.—Urban great tits (*Parus major*) sing with a higher minimum frequency than their forest conspecifics. Cultural processes may account at least in part for the song divergence in city birds as great tits learn their repertoire from conspecifics and switch to high pitch song types in presence of background noise. However, in small cities, this process of cultural divergence could be constrained because it is likely that these birds have a greater exchange of song types with the outside. We tested this prediction by recording great tit songs in a small city (Toledo, central Spain) and in a nearby forest. We found that background noise and the peak and the maximum frequency of songs were higher in the city but the minimum frequency did not differ. The pause length was also longer in forest birds. Seventy percent of the song types were shared between Toledo and the nearby forest. These results suggest that the small size of Toledo allows a homogenized cultural wealth, preventing the development of a high pitch song as observed in larger cities.

Key words: City size, Anthropogenic noise, Cultural evolution, Meme, Song divergence, Frequency

Resumen

Transmisión cultural y su posible efecto en la adaptación acústica urbana del carbonero común Parus major.—El carbonero común (*Parus major*) urbano canta con una frecuencia mínima mayor que sus conspecificos forestales. Detrás de esta divergencia acústica podrían estar algunos procesos culturales, ya que dichas aves aprenden sus cantos de los vecinos y cambian a tipos de canto con una frecuencia alta en presencia de ruido de fondo. Sin embargo, en las ciudades pequeñas este proceso de divergencia cultural podría estar limitado, ya que en dichas ciudades es esperable un alto grado de intercambio de tipos de canto con el exterior. Nosotros testamos esta predicción grabando cantos de carbonero común en una ciudad pequeña (Toledo, España) y en un bosque cercano. El ruido de fondo fue más alto en la ciudad, al igual que la frecuencia "pico" y máxima de los cantos, pero la frecuencia mínima no difirió. La longitud de la pausa fue mayor en el bosque. El setenta por ciento de los tipos de canto se compartieron entre Toledo y el bosque cercano. Estos resultados sugieren que el pequeño tamaño de Toledo impide el establecimiento de una tradición de cantos particular con una frecuencia alta como se observa en ciudades más grandes.

Palabras clave: Tamaño de ciudad, Ruido antropogénico, Evolución cultural, Meme, Divergencia de canto, Frecuencia

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Introduction

Like many other urban bird species, urban great tits, *Parus major*, sing with a higher minimum frequency than their forest conspecifics (Slabbekoorn & den Boer–Visser, 2006) (see table 1), highlighting the role of environmental conditions on sound production (*i.e.* fig. 1 in Laiolo, 2010). Urbanization produces extreme novel habitat conditions for animals in and also near cities (Warren et al., 2006). Although urban and rural habitats differ in many aspects that could influence animal acoustic communication (Shochat et al., 2006), the loud low–frequency background noise in cities (hereafter referred to as ‘anthropogenic noise’) has shown to be directly related to the elicitation of high minimum frequency city songs (Slabbekoorn, 2013).

The mechanism underlying this spectral shift in urban birds remains unclear (Nemeth et al., 2012; Slabbekoorn et al., 2012). It has been hypothesized that urban birds increase the minimum frequency of their songs to avoid overlap with anthropogenic noise. This acoustic shift could be a microevolutionary response or a short–term response (Brumm, 2006; Patricelly & Blickley, 2006; Slabbekoorn & Ripmeester, 2008). This is an important topic, because if the acoustic shift is a microevolutionary response, and because frequency features of the song may be under sexual selection (Halfwerk et al., 2011; 2012; Des Aunay et al., 2013; but see Eens et al., 2012), the spectral shift would entail genetic inheritance and could promote speciation through reproduction isolation (Slabbekoorn & Smith, 2002; Slabbekoorn & Ripmeester, 2008). However, to be selected, the urban acoustic shift should imply fitness benefits to the singer, but empirical data is lacking (Nemeth et al., 2012; Halfwerk & Slabbekoorn, 2013; but see Slabbekoorn, 2013). Experimental procedures have shown that birds can adjust their acoustic signals as a short–term response to increasing noise levels due to vocal plasticity. These plastic adjustments could be mediated by an active frequency shift (Gross et al., 2010; Bermúdez–Cuamatzin et al., 2011; Hanna et al., 2011; Potvin & Mulder, 2013), by switching song types (Cardoso & Atwell, 2011a; Halfwerk & Slabbekoorn, 2009), by increasing the duration of the vocalizations (Montague et al., 2012; Potvin & Mulder, 2013), or by increasing the amplitude of the songs (Brumm, 2004; Schuster et al., 2012). These plastic adjustments are not mutually exclusive and two or more may operate concurrently or vary among species (Slabbekoorn et al., 2012). Such phenotypic plasticity would impede habitat–dependent selection and therefore speciation (Baker, 2006)

Great tits are a good model to study this topic because they are a successful urban songbird and one of the best–studied species in terms of acoustics (Slabbekoorn, 2013). It is a closed–ended learning species (Rivera–Gutiérrez et al., 2011) that achieves a discrete, crystalized repertoire of songs learned from parents and neighbours (McGregor & Krebs, 1982; Franco & Slabbekoorn, 2009). Thus, the different song types sung by each great tit can be considered cultural traits, referred to hereafter as ‘memes’ (Baker et al.,

2001; Baker & Gammon, 2008; Cardoso & Atwell, 2011a) and the learning process in this species is the basis of their ‘cultural transmission’ (Slabbekoorn, 2013). Among the plastic adjustments cited earlier, great tits have been seen to actively switch to a different meme that transmits better in presence of anthropogenic noise (Halfwerk & Slabbekoorn, 2009; Slabbekoorn, 2013). Thus, cultural evolution (*i.e.* changes in the expression frequencies of the meme pool over time) may play an important role in city–forest song divergence in this species (Cardoso & Atwell, 2011a; Slabbekoorn, 2013). Indeed, the process of cultural transmission has led to the establishment of population–specific repertoires or ‘dialects’, separated by either geographical distance or barriers that prevent dispersal (O’Loghlen et al., 2011; Potvin & Parris, 2012). The mechanisms behind this cultural diversity can be classified into two broad categories: (i) stochastic factors such as meme mutation, drift or immigration, and (ii) selective pressures that favour directional changes in the meme pool frequencies such as choice of female, morphological adaptations (*e.g.*, bill/body size), and environmental conditions that affect sound production (Lynch, 1996; Cardoso & Atwell, 2011a; O’Loghlen et al., 2011; Xing et al., 2013). In relation to the latter, and because the anthropogenic noise in cities mask birdsongs (Slabbekoorn & Peet, 2003), acoustic signals can reach the receivers degraded in cities and consequently do not fulfil their biological function of communication (Brumm & Naguib, 2009). The risk of losing the acoustic message may lead birds to try to match their song with the acoustic properties of the environment to enhance transmission as proposed by the Acoustic Adaptation Hypothesis (AAH) (Morton, 1975). The way great tits seem to do this is by switching memes to a higher minimum frequency or singing them for longer periods (Halfwerk & Slabbekoorn, 2009). This acoustic shaping to the environment influences cultural transmission and therefore cultural evolution as birds copy undegraded songs differentially during their sensitive period of learning (Peters et al., 2012). Thus, under anthropogenic noise, only those memes from the whole repertoire that escape from masking or transmit and reach the receiver more clearly will be correctly and frequently copied (Slabbekoorn, 2013). Over time, this may lead to changes in the expression frequencies of the memes sung in a city, increasing those that transmit better under anthropogenic noise, *i.e.*, those with a high minimum frequency (Luther & Baptista, 2010) because of their better acquisition by young birds during their sensitive period. This cultural evolution may be responsible, at least in part, for the city–forest song divergence found in great tits. Over time, it could be culturally and genetically fixed (Price et al., 2003; Slabbekoorn, 2013). Furthermore, such phenotypic plasticity allows acoustic shifts in frequencies and may account, to some degree, in urban song divergence (Nemeth et al., 2012).

The aim of the present study was to compare the typological and spectral song characteristics (Baker, 2006) in two great tit populations, one in a small city, Toledo (central Spain), surrounded by a large rural and

Table 1. Summary of the studies that assess the effect of the anthropogenic noise in the minimum frequency of songbirds. All shifts are toward high frequencies in urban songs in relation to forest songs. Types of study: O. Observational; E. Experimental; O/E. Both. References: 1. Slabbekoom & Peet (2003); 2. Fernández–Juricic et al. (2005); 3. Slabbekoom & der Boer–Visser (2006); 4. Bermúdez–Cuamatzin et al. (2009); 5. Parris & Schneider (2008); 6. Halfwerk & Slabbekoom (2009); 7. Mockford & Marshall (2009); 8. Nemeth & Brumm (2009); 9. Ripmeester et al. (2010); 10. Gross et al. (2010); 11. Hu & Cardoso (2010); 12. Mendes et al. (2011); 13. Salaberria & Gil (2010); 14. Bermúdez–Cuamatzin et al. (2011); 15. Hanna et al. (2011); 16. Hamao et al. (2011); 17. Potvin et al. (2011); 18. Montague et al. (2012); 19. Potvin & Parrish (2012); 20. Nemeth et al. (2013); 21. Potvin & Mulder (2013).

Tabla 1. Recopilación de los estudios que evalúan el efecto del ruido antropogénico en la frecuencia mínima de los pájaros cantores. En los cantos urbanos todos los cambios son hacia frecuencias altas en comparación con los cantos del bosque. Tipos de estudio: O. Observacional; E. Experimental; O/E. Ambos. (Para las abreviaturas de las referencias, véase arriba.)

| Song birds | | Minimum frequency shift | Type of study | References |
|-----------------------|---------------------------------|-------------------------|---------------|--------------------|
| Great tit | <i>Parus major</i> | Yes | O/E | 1, 3, 6, 7, 13, 16 |
| House finch | <i>Carpodacus mexicanus</i> | Yes | O/E | 2, 4, 14 |
| Grey shrike–thrush | <i>Colluricincla harmonica</i> | Yes | O | 5 |
| Grey fantail | <i>Rhipidura fuliginosa</i> | No | O | 5 |
| Common blackbird | <i>Turdus merula</i> | Yes | O/E | 8, 9, 11, 12, 20 |
| Reed bunting | <i>Emberiza schoeniclus</i> | Yes | E | 10 |
| Rainbow lorikeet | <i>Trichoglossus haematodus</i> | Yes | O | 11 |
| Eastern rosella | <i>Platycercus eximius</i> | Yes | O | 11 |
| Red wattlebird | <i>Anthochaera carunculata</i> | Yes | O | 11 |
| Noisy miner | <i>Manorina melanocephala</i> | No | O | 11 |
| Bell miner | <i>Manorina melanophrys</i> | Yes | O | 11 |
| Pied currawong | <i>Strepera graculina</i> | No | O | 11 |
| Australian magpie | <i>Gymnorhina tibicen</i> | No | O | 11 |
| Grey butcherbird | <i>Cracticus torquatus</i> | No | O | 11 |
| Magpie–lark | <i>Grallina cyanoleuca</i> | No | O | 11 |
| Willie wagtail | <i>Rhipidura leucophrys</i> | No | O | 11 |
| Common myna | <i>Acridotheres tristis</i> | No | O | 11 |
| Red–winged blackbirds | <i>Agelaius phoeniceus</i> | Yes | O/E | 15 |
| Silvereyes | <i>Zosterops lateralis</i> | Yes | O/E | 17, 19, 21 |
| European robin | <i>Erithacus rubecula</i> | Yes | O/E | 18 |

forest area, and the other in a nearby forest. The small size of Toledo distinguishes this study from the former studies performed in large cities (*i.e.*, Slabbekoom & den Boer–Visser, 2006; Hamao et al., 2011 or Salaberria & Gil, 2010). If cultural evolution is responsible, at least in part, for the city song divergence found in this species, the fact that Toledo great tits may still be learning 'forest memes' (*i.e.* with relative low minimum frequency) from surrounding rural and forest areas may constrain the plastic cultural response of choosing 'urban memes' (*i.e.*, with high minimum frequency) from their repertoires; as many crystalized memes in their

repertoires will be those learned from outside of the city. This possible high exchange of memes between Toledo and forest may allow a cultural convergence. We predicted that the differences in the minimum frequency and the typological song characteristics (*i.e.* percentage of meme type used) between Toledo and the forest would be less marked than those described in previous studies in city–forest pairs in large cities. This comparison of the song features between a relatively small city and the nearby forest could help us to understand the mechanisms involving urban song divergence in great tits.

Methods

Study areas

Great tit songs were recorded in two, well-differentiated habitats: city (Toledo) and nearby forest (Montes de Toledo). Toledo is a relatively small city (17 km²) located 71 km south of Madrid (central Spain, 529 m above sea level), with a population of 80,000 inhabitants. Forest songs were recorded in two nearby areas of the Toledo mountains (Montes de Toledo, Toledo province): Quintos de Mora (39° 25' N, 4° 04' W) and San Pablo de los Montes (39° 31' N, 4° 21' W), located at 80 and 60 km south of Toledo, respectively (average elevation of both forest areas is 908 m a.s.l.; see Ferrer et al., 2012). Both forest areas comprise deciduous forests dominated by Pyrenean Oak *Quercus pyrenaica*, usually relegated to the shady area of the mountains and ravine funds, accompanied by Mediterranean scrublands. Both study areas have a continental Mediterranean climate, with mean values of annual rainfall of 350–450 mm for Toledo and 700–800 mm for the forests, concentrated in the months of autumn and spring. Summer drought and daily thermal oscillation are marked in the whole area.

Song recording

We recorded great tit songs between March 5 and April 27 in 2011. Day 1 = March 1st. All song recordings were made between 900 and 1,500 hours. To ensure the independence of our samples, we only recorded lone individuals located at least 100 m apart, a distance greater than that considered to be the territory size in this species (Naef–Daenzer & Keller, 1999). Urban great tits began to sing 22 days earlier than their forest conspecifics, possibly due to the 'heat island' effect of cities (Shochat et al., 2006) and food availability (Isaksson & Andersson, 2007). To correct the differences in the breeding cycle advancement between forest and urban great tits, we considered the day when the first male was heard singing in each study area as the first day. We recorded 56 songs from 37 different great tit males in Toledo. Four males were recorded three times, eleven males were recorded two times, and the remaining males were recorded once. We recorded 106 songs from 63 males in the forest area; three males were recorded four times, nine males were recorded three times, and 16 males were recorded two times.

All songs were recorded using an EDIROL R–09HR digital recorder equipped with a Sennheiser unidirectional microphone and headphones, pointing directly toward the singing individual. Individuals were recorded at a distance of between 10 and 15 m and at least 10 strophes per song were recorded. After each recording, background noise data (dB) were measured every second for 5 minutes with a multidirectional sound level meter (SLM, A-weighted, reference level 20 µPa) positioned in a fixed location (1.5 m above the ground) with the aid of a tripod. As a measure of background noise, we used the mean value over this period.

Song analysis

We randomly selected one song recording for each male to analyse the same number of songs for each male. Audio tracks were first converted from stereo to mono with the Audacity 1.3.14–beta program. Five strophes of the song were then selected and exported to the program RavenPro 1.4 (Charif et al., 2010), where, before analysis, a band filter was used to remove the typical low frequency background noise after visual inspection of the spectrogram to prevent the unintentional removal of any song element. For spectral description (Baker, 2006), we measured: average minimum, maximum and peak frequency (Hz), frequency bandwidth (difference between the maximum and the minimum frequency of the song, Hz), mean strophe length, mean pause length and average number of notes per phrase (N° notes). Peak frequency was measured automatically; the other variables were measured manually using a Hann window and a fast Fourier transformation (FFT), length of 1,024, resulting in a spectral resolution of 43.1 Hz. Minimum and maximum frequencies were measured by precisely placing a selection box in the spectrogram view, similar to Francis et al. (2011). Because this manual methodology can entail a bias in the frequency measure of birdsongs (Zollinger et al., 2012), we tested the reliability of our measures by re-analysing a subset of song recordings (15 belonging to forest sites and 15 from Toledo) with a power spectra using Avisoft SAS Lab Pro 4.15 (Avisoft Bioacoustics, Schönfließ Str. 83, 16548 Glienicke, Germany). Concretely, we recalculated the minimum and maximum frequency of this subset of song recordings by subtracting 20 dB from the peak amplitude value in the power spectrum. Neither minimum nor maximum frequency differed significantly between the two methodologies used (Student *t*-test: *t* = 0.01; d.f. = 58; *p* = 0.99; manually = 3,397.67 ± 66.26 and automatically = 3,398.34 ± 71.54; Student *t*-test: *t* = –0.51; d.f. = 58; *p* = 0.61; manually = 5,571.48 ± 136.95 and automatically = 5,459.90 ± 172.25, for the minimum and the maximum frequency respectively). We also calculated the song–rate (relation between the total number of phrases recorded and the total length of the strophe). For the typological level of description (Baker, 2006), we noted the note type following the criteria of McGregor & Krebs (1982) by visual inspection of the sound spectrograms, and we classified each meme–type depending on the number of notes, type, and order in the phrase. All song analyses were performed by DNE and JBE. Meme–type classifications of both observers were compared to obtain a single classification.

Statistical analyses

We analysed the acoustic habitat–dependent divergences between study areas using General Linear Models (GLMs), with the spectral song characteristics as dependent variables and study area as a categorical predictor with two levels (Toledo and nearby forest). Standardized date was incorporated in all

models as a covariate. The interaction between study area and date was also included in the full models. Number of notes was analysed using a Generalized Linear Model (GLZ) with a poisson distribution. For post hoc analyses, the Tukey HSD test was used. Differential note type used between Toledo and Forest was analysed using other GLZs with a binomial distribution. In this case, the response variable was the ratio between the 'number of one note type used' (numerator) divided by the 'total number of note types used' (denominator) (Zuur et al., 2009), with the study area as categorical predictor and the standardized date as the covariate. The interaction between study area and date was also included in the full models. In the present study, when the interaction between study area and date was not significant, it was eliminated from the final presented models. All these analyses were performed with R (R Core Team, 2014) and the function 'glm' of the package lme4 (Bates et al., 2014). Assumptions of the homoscedasticity, proper distribution used and independence were verified graphically with the residuals of the model, following the recommendations of Zuur et al. (2010).

Results

Table 2 shows mean \pm SE values of background noise and spectral song characteristics of great tits recorded in the study areas. Some of the spectral song variables showed significant differences between areas, with the exception of song-rate, strophe length and minimum frequency (tables 3). Neither spectral song variable varied significantly with the date (table 3).

In relation to the typological level of description, a total of 16 memes were recorded in the two study areas: 13 in Toledo, two of which (15%) were exclusive, and 14 in the forest, three of which (21%) were exclusive. Eleven of the 13 memes were common to both areas (70% of the memes recorded), but only three were sufficiently sampled to make comparisons. The remaining eight memes were recorded for only one or two males in each study area and therefore not considered suitable for comparison. These shared memes were 'two-note type' memes and they were named arbitrarily as meme 'A' (composed of the notes 'a' and 'b', see below), shared by seven males in Toledo and by 11 in the forest, meme 'B' (composed of the notes 'a' and 'c') shared by three males in Toledo and 31 in the forest, and meme 'C' (composed of the notes 'b' and 'c') shared by seven males in both areas (see fig. 1). Apart from presenting a shorter pause length and a quicker song-rate in Toledo than in the forest (GLM: $F_{1,64} = 5.89$; $p = 0.02$ and $F_{1,64} = 5.49$; $p = 0.02$, respectively), these shared memes did not differ in minimum frequency (GLM: $F_{1,64} = 2.96$; $p = 0.09$) or in peak frequency (GLM: $F_{1,64} = 2.56$; $p = 0.11$); only the maximum frequency differed, being higher in Toledo than in the forest (GLM: $F_{1,64} = 19.82$; $p < 0.001$; Toledo = $5,483 \pm 117$ Hz and Forest = $4,956 \pm 57$ Hz). The difference in maximum frequency of these three shared memes between study areas (527 Hz) was slightly smaller than the difference in maximum frequency when all memes were taken into account (598 Hz, see table 2). Thus, these three shared memes account for 88% of the magnitude of the overall maximum frequency divergence between the Toledo–Forest pair.

Table 2. Mean \pm SE (n) and range of the spectral song characteristics of male great tit (*Parus major*) songs in Toledo and in a nearby forest.

Tabla 2. Media \pm ES (n) y rango de las características espectrales del canto del macho de carbonero común (*Parus major*) en Toledo y en un bosque cercano.

| | Toledo | | Nearby forest | |
|-----------------------|----------------------------|-----------------|---------------------------|-----------------|
| | Mean \pm SE (n) | Range | Mean \pm SE (n) | Range |
| Background noise (dB) | 54.13 \pm 0.84 (37) | 45.10–63.66 | 38.97 \pm 0.55 (63) | 30.89–48.90 |
| N° notes | 2.27 \pm 0.1 (37) | 2–4 | 2.19 \pm 0.07 (63) | 2–4 |
| Song-rate (St/s) | 0.19 \pm 0.03 (37) | 0.03–0.37 | 0.15 \pm 0.02 (63) | 0.01–0.35 |
| Pause length (s) | 3.14 \pm 0.38 (36) | 1.30–6.80 | 4.98 \pm 0.35 (61) | 1.02–12.51 |
| Strophe length (s) | 2.83 \pm 0.26 (37) | 1.61–8.03 | 2.67 \pm 0.14 (63) | 1.26–5.64 |
| Min. Freq. (Hz) | 3,389.27 \pm 82.41 (37) | 2,495.1–4,179.6 | 3,256.19 \pm 45.29 (63) | 2,288.1–3,978.1 |
| Peak Freq. (Hz) | 4,413.24 \pm 109.13 (37) | 3,375.0–5,564.6 | 4,198.82 \pm 62.17 (63) | 3,140.6–4,952.4 |
| Max. Freq. (Hz) | 5,813.36 \pm 146.16 (37) | 4,570.3–7,944.9 | 5,215 \pm 89.78 (63) | 4,318.4–7,244.3 |
| Bandwidth (Hz) | 2,483.35 \pm 124.99 (37) | 1,275.6–4,878.5 | 1,925.81 \pm 78.77 (63) | 1,039.1–3,732.8 |

Table 3. Summary of the GLMs analysing the effect of study area (Factor) and date (Covariate) on song characteristics of male great tits (*Parus major*). Estimate is the slope of the relationship between the covariate and the dependent variable. Significant results are highlighted in bold.

Tabla 3. Resultados de los GLM que analizan el efecto del área de estudio (factor fijo) y la fecha (covariable) en las características del canto de los machos de carbonero común (Parus major). "Estimate" es la pendiente de la relación entre la covariable y la variable dependiente. Los resultados significativos se destacan en negrita.

| | Test Factor | p | Estimate | Test Covariate | p |
|-----------------------|--------------------|-------------------|----------|-------------------|------|
| Background noise (dB) | $F_{1,98} = 283.7$ | < 0.001 | 0.02 | $F_{1,97} = 0.16$ | 0.69 |
| N° notes | $Z_{1,98} = 0.48$ | 0.57 | 0.001 | $Z_{1,97} = 5.88$ | 0.89 |
| Song-rate (St/s) | $F_{1,98} = 3.16$ | 0.08 | < 0.001 | $F_{1,97} = 0.38$ | 0.54 |
| Pause length (s) | $F_{1,98} = 9.23$ | 0.003 | −0.003 | $F_{1,97} = 0.89$ | 0.35 |
| Strophe length (s) | $F_{1,98} = 0.78$ | 0.40 | 0.014 | $F_{1,97} = 0.73$ | 0.40 |
| Min. Freq. (Hz) | $F_{1,98} = 1.13$ | 0.29 | 2.86 | $F_{1,97} = 0.29$ | 0.59 |
| Peak Freq. (Hz) | $F_{1,98} = 7.81$ | 0.006 | −8.42 | $F_{1,97} = 1.36$ | 0.25 |
| Max. Freq. (Hz) | $F_{1,96} = 17.32$ | < 0.001 | 35.3 | $F_{1,96} = 1.67$ | 0.20 |
| Bandwidth (Hz) | $F_{1,96} = 15.39$ | < 0.001 | 30.84 | $F_{1,96} = 1.30$ | 0.26 |

The 16 memes observed in great tit songs were made up of a combination of 11 different note types (table 4). To test city–forest differences, we used note types that represent at least 10% of the total (table 4). Three note types were therefore considered and they were named arbitrarily as: 'a' (Peak frequency = 3,500 Hz), 'b' (Peak frequency = 4,500 Hz) and 'c' (Peak frequency = 5,500 Hz). The use of note type 'a' and 'c' differed significantly between Toledo and forest (GLZ. Note type 'a': $Z_{1,98} = -13.77$; $p < 0.001$ and Note type 'c': $Z_{1,98} = 12.91$; $p < 0.001$), while the use of note type 'b' did not differ between study areas (GLZ. Note type 'b': $Z_{1,98} = 0.65$; $p = 0.52$). Note type 'a' was less frequently used in Toledo than in forest (Tukey test, $p < 0.001$; fig. 2A), contrary to note type 'c' used, which was significantly more frequently used in Toledo (Tukey test, $p < 0.01$; fig. 2C).

Discussion

Great tits in Toledo started singing before those in forest. This advancement in the phenology of urban great tits has been reported in other city–forest pair comparisons (Partecke & Gwinner, 2007; Chamberlain et al., 2009). Analysis of song recordings revealed differences in the spectral song characteristics between city and forest birds, probably due to the constraints of background noise in the city, as observed in many other studies (reviewed in Laiolo, 2010). The differences observed include the temporal features of the song, such as shorter pauses in Toledo, which help to create a contrast with the background noise and

enhance the detectability of the signal (Warren et al., 2006; Hanna et al., 2011).

Beyond this temporal feature of the song, the main difference between city and forest songs in other city–forest pair studies is the shift of the minimum frequency toward high frequencies (table 1). Surprisingly, the minimum frequency of great tit songs did not differ between Toledo and forest, but the maximum frequency did so significantly, making bandwidth in Toledo wider. The peak frequency was also significantly higher in Toledo. This is in contrast with the strategy of urban great tits reported previously, which were shown to increase the minimum frequency of their songs (Slabbekoorn & den Boer–Visser, 2006; Mockford & Marshall, 2009; Montague et al., 2012) even in a very close urban great tit population (Salaberria & Gil, 2010), a mechanism that has also been reported in other urban bird species (reviewed in Brumm & Zollinger, 2011; table 1). In our study, the same memes sung in Toledo and forest showed the same frequency shift in the peak and maximum frequency mentioned earlier (fig. 1), suggesting that the singing strategy in Toledo males is a short–term phenotypic response to increase the SNR in presence of anthropogenic noise (Halfwerk et al., 2011). This is also seen in other species (Gross et al., 2010; Verzijden et al., 2010; Potvin & Mulder, 2013). This variation in Toledo songs could be a consequence of an active frequency shift (McMullen et al., 2014), possibly because these frequency features of the great tit songs (peak and maximum frequencies) can be more plastically modulated; or it could be a side effect of louder singing, i.e. the Lombard ef-

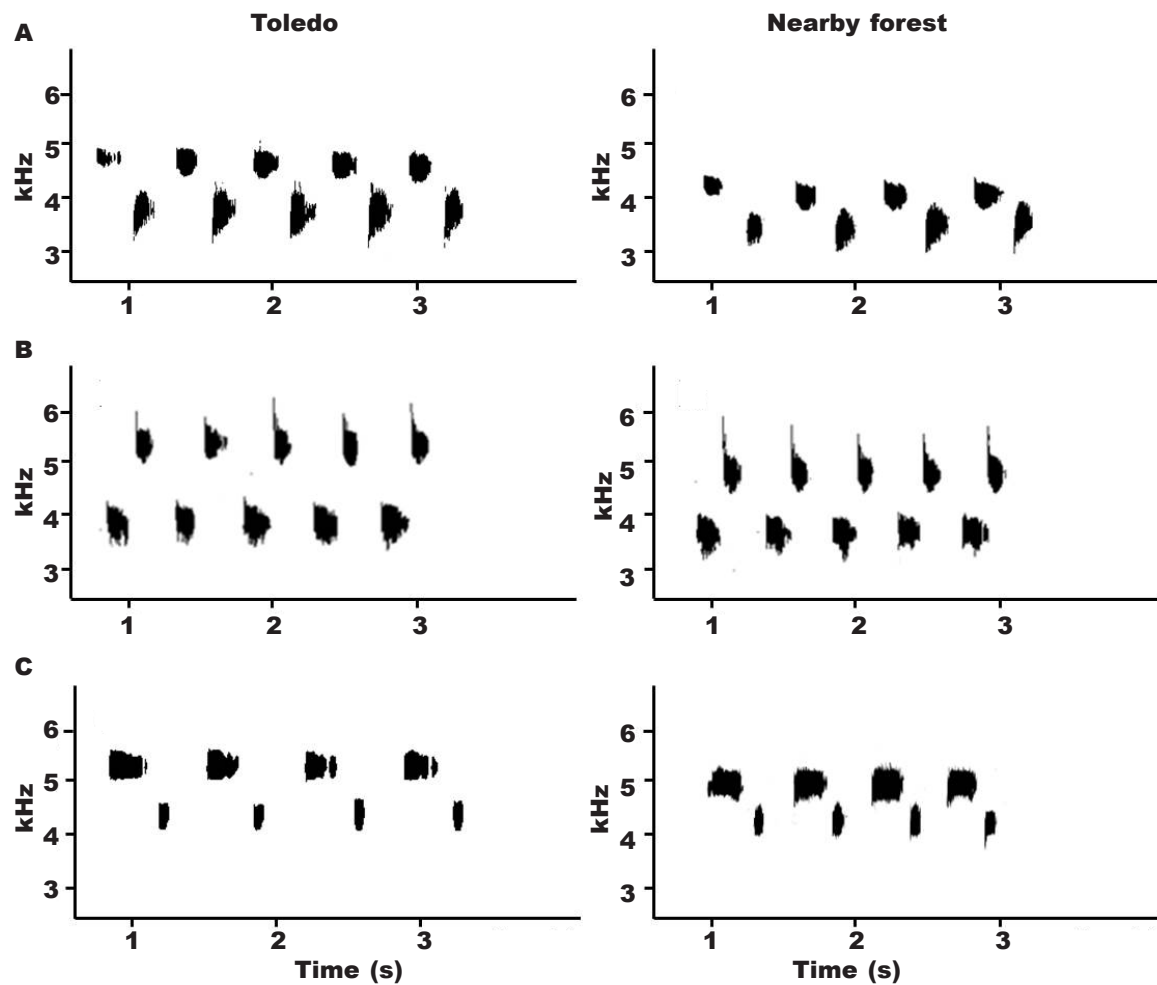


Fig. 1. Sonograms of the shared 'memes' most frequently used, Toledo and nearby forest. Each level represents the same 'meme' elicited in Toledo and nearby forest (A, B, or C).

Fig. 1. Sonograma de los "memes" más frecuentemente utilizados en Toledo y en un bosque cercano. Cada nivel representa el mismo "meme" cantado en Toledo y en el bosque, etiquetado con el nombre dado a los memes compartidos (A, B o C).

fect (Zollinger & Brumm, 2011). It has been shown that the Lombard effect increases the bandwidth of the signal and, to a lesser extent, peak frequency. Changes in minimum frequency, in contrast, are highly independent of the Lombard effect (Cardoso & Atwell, 2011b). However, we could not confirm this mechanism as we did not accurately measure song amplitude (Brumm, 2004). Both active frequency shift and increased amplitude are short-term plastic adjustments that improve detection and increase the SNR in presence of anthropogenic noise (Nemeth & Brumm, 2010; Halfwerk et al., 2011).

The low minimum frequency found in Toledo great tit songs, however, is a surprising result and the main 'urban song difference' reported in this study (table 1). This lack of significant difference in

the minimum frequency between Toledo and forest could be a side effect of the small metropolitan area of Toledo and the process of cultural transmission (O'Loughlen et al., 2011; Potvin & Parris, 2012). Anthropogenic noise might influence cultural transmission by favouring songs that propagate better in the acoustic environment and reach the receiver more clearly (Luther & Baptista, 2010), particularly when birds differentially copy undegraded songs in their learning process (Peters et al., 2012). Thus, a phenotypic plastic response like sing with a higher minimum frequency in cities (Slabbekoorn & Peet, 2003), can be culturally fixed in a scenario where most of the population is under the selection pressure (Price et al., 2003). This is also propitiated because high minimum frequency songs transmit better and

Table 4. Percentage of occurrence (%) of the 11 different note types used by great tits in the two study areas.

Tabla 4. Porcentaje de ocurrencia (%) de los 11 tipos de notas diferentes usadas por el carbonero común en las dos áreas de estudio.

| Note type | Toledo | Nearby forest |
|-----------|--------|---------------|
| a | 18.44 | 38.66 |
| b | 36.18 | 35.53 |
| c | 29.40 | 17.75 |
| d | 3.11 | 3.62 |
| e | 1.31 | 0.82 |
| f | 2.68 | 0.79 |
| g | 0 | 0.81 |
| h | 1.80 | 0 |
| i | 3.97 | 0 |
| j | 0 | 2.01 |
| k | 3.11 | 0 |

reach the receiver neatly (Des Anuay et al., 2013; Potvin & Mulder, 2013) and great tits choose these high memes from their repertoire and sung them for longer duration (Halfwerk & Slabbekoorn, 2009). In big cities, with a large enough surface area to harbour an almost isolated resident population throughout its life span, this scenario may occur. It can therefore be expected that within a few generations, isolated populations present culturally different sets of memes as the result of differences in background noise. This has been observed in San Francisco (California) with the white-crowned sparrow *Zonotrichia leucophrys* (Luther & Baptista, 2010) and seems to be occurring in Europe with the great tit, where a particular set of 'urban memes' are sung in cities (Slabbekoorn & den Boer-Visser, 2006). However, the small metropolitan area of Toledo may allow a higher meme exchange between great tits in Toledo and those in rural and forest areas outside the city, as the high ratio of shared memes suggests; 70% in contraposition with the low ratio of shared memes in other city-forest pair studies (17% in Slabbekoorn & den Boer-Visser 2006 or 8% in Cardoso & Atwell, 2011a). In this scenario, Toledo chicks would learn both types of memes: the low minimum frequency memes from outside the city and the high minimum frequency memes developed in the city. This may lead to a homogenized cultural wealth between Toledo and forest, constraining the development of a particular cultural song tradition in Toledo. However, a long-term study marking individuals in Toledo and the surrounding rural areas would be necessary to assess dispersal between these populations.

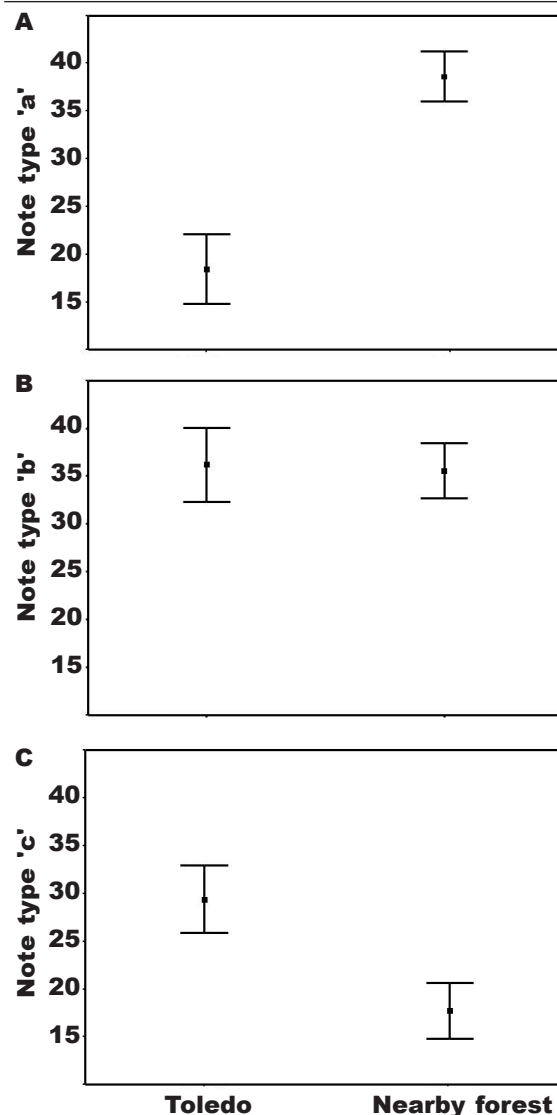


Fig. 2. Percentage of note type used by great tits in Toledo and nearby forest. (Vertical bars indicate the standard error.)

Fig. 2. Porcentaje del uso de cada tipo de nota del carbonero común en Toledo y en un bosque cercano. (Las barras verticales indican el error estándar.)

Although Toledo and forest share 70% of their cultural wealth, a differential note type was used in both study areas (fig. 2). The proportional use of 'high-frequency notes' was higher in Toledo while the proportional use of 'low-frequency notes' was lower (Slabbekoorn & den Boer-Visser, 2006; see fig. 2). This suggests that memes that were less masked by noise were sung more frequently, as proposed by Halfwerk & Slabbekoorn, 2009), and could be a cultural plastic response of Toledo males. The effect of this potential cultural plastic response, however,

was not large enough to differentiate the minimum frequency between study areas.

This study suggests that the degree of isolation of a population could influence the city–forest song divergence in great tits. The size of a city may be an important feature in song divergence, as birds in smaller cities likely have a higher exchange of memes with those in areas outside of the city. In these reduced urban areas, even though great tits are under high anthropogenic acoustic pressure, the minimum frequency shift could be partially constrained due to cultural wealth with a high proportion of low frequency memes from outside the city. It could be that in this situation, great tits sing the same memes louder or shift other frequency features of their songs to enhance sound transmission. Further studies comparing city and forest pairs in different sized cities with different city–forest distances may help to clarify these findings.

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