Song and the city : a comparison between urban and forest blackbirds

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Habitat-dependent acoustic divergence affects playback response in urban and forest populations of the European blackbird.

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Habitat-dependent song variation can play an important role in ecological speciation. Divergence in bird song related to acoustic properties of urban habitat provides an excellent opportunity to study the initial steps of reproductive divergence close to home. In this study, we show spectral and temporal song divergence in European blackbirds in three city-forest pairs. The observed upward spectral shift in frequency is consistent with studies on other species living in areas with loud low-frequency anthropogenic noise. A temporal difference also resulted in an overall higher frequency use in cities: urban songs consisted of longer high-frequency twitters than forest songs in combination with low-frequency motifs of similar duration. A reciprocal playback experiment showed that male blackbirds pay attention to one of the divergent song traits as well as to the origin of the playback stimulus. Furthermore, urban and forest males responded differentially to the same spectral song features, which suggests that the song divergence may influence where males with typical urban or forest songs settle and are able to maintain a territory. Our findings from an urban context show how habitat-dependent song divergence in general has the potential to contribute to a prezygotic reproductive barrier catalyzing the process of ecological speciation.

Introduction

Acoustic signals can play an important role in animal communication and individual variation in signals can often have direct fitness consequences. Birds are relatively well-studied in this respect as male song is often critical for efficient territorial defence and mate attraction (Catchpole & Slater 2008; Collins 2004). Acoustic variation between and within species is typically used by female birds to find a male partner of their own species and of a preferred quality. Females may for example find a locally adapted mate by using geographic variation in acoustic features related to the ecology of a specific habitat (Patten *et al.* 2004; Slabbekoorn & Smith 2002b). Furthermore, local songs and divergent traits may also affect male-male territorial interactions and determine habitat-dependent dispersal probabilities of reproductively successful males. As a consequence, habitat-related geographic variation in acoustic signals among populations of the same species can be critical to the process of ecological speciation (Rundle & Nosil 2005; Schluter 2001; Slabbekoorn & Smith 2002a).

Ecological speciation has been investigated in a variety of taxa and across several naturally occurring ecological gradients, such as water layers in big lakes, altitudinal zones in montane areas, or different types of tropical forests. However, a unique opportunity to study the initial steps of the process of ecological speciation is provided closer to home by the urban habitat (Byrne & Nichols 1999; Shochat *et al.* 2006b; Slabbekoorn & Ripmeester 2008). Cities typically harbour extreme ecological conditions that contrast almost any other surrounding habitat. This yields a variety of species-specific niches, which are often characterized by a distinct set of food sources, vegetation types, nesting sites, hiding places, and predator species (e.g. Badyaev *et al.* 2008; Grimm *et al.* 2008; McKinney 2006). Prominent urban factors such as human disturbance and chemical pollution can also have a significant impact on survival and reproduction of city dwellers (Beale & Monaghan 2004; Tornqvist & Ehrenberg 1992). Acoustically, cities are distinct due to urban-specific transmission properties (Slabbekoorn *et al.* 2007; Warren *et al.* 2006) and the concentration of anthropogenic noise sources, such as cars, trains, and industrial and personal devices (Patricelli & Blickley 2006; Slabbekoorn & den Boer-Visser 2006). These urban conditions are relatively recent and are becoming more and more common, providing ample opportunity to study micro-evolutionary changes in response to novel selection pressures such as anthropogenic noise on city birds.

Several recent studies have shown that anthropogenic noise has an effect on how birds sing (Brumm & Slabbekoorn 2005; Patricelli & Blickley 2006; Slabbekoorn & Ripmeester 2008). Great tits (*Parus major*) from an urban population in the Dutch city of Leiden vary in their frequency use depending on local noise levels in their territories (Slabbekoorn & Peet 2003). Male birds in noisier places produce songs with a higher minimum frequency, which results in less masking by low-frequency traffic noise. The same upward shift in frequency with increasing noise levels has been found in house finches (*Carpodacus mexicanus,* Fernández-Juricic *et al.* 2005) and song sparrows (*Melospiza melodia*, Wood & Yezerinac 2006). Furthermore, a large scale study on great tits also showed a consistent shift at the population level, with birds from ten cities across Europe singing on average with a higher minimum frequency than birds in ten nearby forests (Slabbekoorn & den Boer-Visser 2006). Apparently, the presence of anthropogenic noise can cause a significant divergence in the acoustic mating signals from birds of urban areas compared to the more quiet countryside.

The role for habitat-dependent divergence in mating signals can be particularly important to ecological speciation in case of congruent divergence in other fitness-related traits (Rice & Hostert 1993; Slabbekoorn & Ripmeester 2008; Slabbekoorn & Smith 2002a). The bird species for which urban song divergence has been reported are typically not investigated for urban divergence in other traits. For great tits, we know one study which showed less yellow plumage for urban birds compared to rural ones (Horak *et al.* 2001), and another which showed smaller clutch size for (sub-)urban birds compared to their forest counterparts (Riddington & Gosler 1995). However, a recent study reports on song and bill morphology of two genetically diverged, adjacent populations of house finches in urban and desert habitat. Many urban house finches had longer and deeper bills than conspecifics of the nearby desert, and these birds sang fewer note types with slower trill rates and a wider frequency bandwidth (Badyaev *et al.* 2008). This interesting study investigated the direct relationship between bill morphology and song (c.f. Podos 2001; Slabbekoorn & Smith 2000), but did not include the factor of anthropogenic noise known to affect house finch song (Fernández-Juricic *et al.* 2005).

Several studies on another urban bird species, the European blackbird (*Turdus merula*, Luniak *et al.* 1990), suggest potential for congruent divergence in many more traits. Blackbirds are thriving in cities and forests in a large part of Europe (Clement & Hathway 2000) and divergent patterns are found for breeding onset (Partecke *et al.* 2005), clutch size (Gregoire 2003), migratory tendency (Partecke & Gwinner 2007; Stephan 1999), corticosterone stress response (Partecke *et al.* 2006a) and morphology (Gregoire 2003; Lippens & van Hengel 1962; Partecke 2003, Evans *et al.* 2009a). A common garden experiment revealed that many of these phenotypic differences have at least some genetic component (Partecke *et al.* 2006a; Partecke *et al.* 2004; Partecke *et al.* 2005). Genetic differences at neutral markers between urban and nearby rural blackbird populations have also been found (Evans *et al.* 2009b). Therefore, without assuming or even expecting completion of speciation any time in the near future, urban and forest blackbird populations are a good model system to study the first steps towards reproductive divergence. There are only very few studies that report on both the emergence of distinct habitat-dependent sexual traits and discrimination abilities of these traits (Patten *et al.* 2004; Dingle *et al.* unpublished manuscript), the combination of which may be critical in the initial stages of ecological speciation.

European blackbirds have a large repertoire of songs which all have a stereotypic species-specific structure consisting of a motif part followed by a twitter part (Dabelsteen & Pedersen 1985; Ripmeester *et al.* 2007; Todt 1970b). The motif part has simple elements of a relatively low frequency and loud amplitude, whereas the twitter part is more variable, higher in frequency, and softer in amplitude (Figure 2.1). Typical urban noise could severely hinder communication due to the overlap in frequency with the motifs and the relatively soft amplitude of the twitters. There may be two directions in which urban blackbirds may diverge

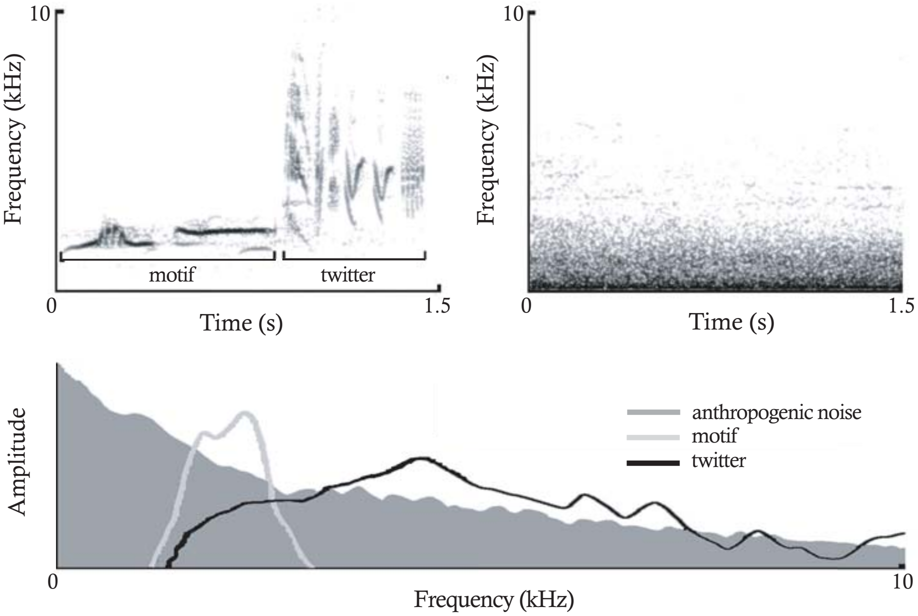

Figure 2.1. Illustration of the potential for masking of blackbird song by urban noise. The top left panel shows a spectrogram of a typical blackbird song consisting of a motif part followed by a twitter part. The top right panel is a spectrogram of typical urban noise originating from car traffic. The bottom panel gives the power spectra of the motif, twitter and urban noise shown in the spectrograms of the left and middle panel. The power spectra of the motif and the twitter are shown at the correct amplitude level relative to each as measured in a song recorded at a distance of 10 meters from a blackbird. The power spectrum of the urban noise is linearly scaled at an arbitrary amplitude level.

spectrally in response to anthropogenic noise: an increase in frequency of the motif and twitter part similar to other species or by a shift in relative duration of the song components, with shorter motif parts and longer twitter parts. If blackbird song turns out to be divergent between urban and forest populations, insight into the potential impact on habitat-dependent gene flow still depends on whether the birds themselves detect the differences and whether they recognize them as meaningful. The impact of habitat-related song divergence in birds has been tested before by using playback experiments (e.g. Irwin *et al.* 2001; Patten *et al.* 2004; Dingle *et al.* unpublished manuscript), but never in the urban context.

In this study, we first compared blackbird songs from three pairs of populations, each consisting of a city population and a nearby forest population. We also assess the ambient noise conditions in the three city-forest pairs to confirm the assumption that there is habitat-dependent background noise. We subsequently investigate whether a divergent song trait is perceived as meaningful in a natural territorial context. We use a playback experiment in which we score the response of both urban and forest males to a divergent song trait, taking the influence of familiarity of songs from the local population into account. A difference in response to urban song features will reveal the potential to affect gene flow into and out of cities for European blackbirds, but will also provide fundamental insight into the role of habitat-dependent acoustic traits in ecological speciation in general.

Material & Methods

Study species and areas

European blackbirds (*Turdus merula*) are songbirds living in forests, rural areas and cities in large parts of Europe (Clement & Hathway 2000). Blackbirds started to settle in urban areas early in the 19th century and they are currently a common species in many European cities whilst still being abundant in their original natural habitat (Luniak & Mulsow 1988). They are abundant in Dutch cities and forests making them the most numerous breeding species in the Netherlands with over a million breeding pairs (Hustings & Vergeer 2002). Male blackbirds have a large repertoire of advertisement songs which they sing to defend their territory and attract females. They sing during the entire breeding season and their singing activity peaks around dawn and dusk (de Vos & de Meersman 2005).

We conducted our study in six locations in the Netherlands, which concerned three pairs of a city and a nearby forest. The centre of a city and a forest within a pair were only 5 to 10 kilometres apart and the distances between the three pairs of bird populations ranged from 65 to 100 kilometres. The three cities, Arnhem (51°58N, 5°54E), Breda (51°35N, 4°46E) and Leiden (52°09N, 4°29E) were relatively old and medium-sized cities for the Netherlands with between 117,000 to 170,000 inhabitants (source: Central Bureau of Statistics of the Netherlands). Recordings were made in both the city centre and adjacent residential areas. The three associated forest locations were the Veluwe (52°01N, 6°00E), the Liesbos (51°34N, 4°41E), and Meijendel (52°08N, 4°20E). The Veluwe is a relatively large area near Arnhem of about 100,000 hectares mainly covered with mixed forest and moorland that we sampled between De Steeg, Zilvense Heide and Koningsheide. The Liesbos is a relatively small, old-growth forest of 200 hectares nearby Breda with mainly deciduous trees and some coniferous trees at the edges. Meijendel is a mixed forest-dune area of about 1,875 hectares located near Leiden.

Song recording and analysis

Recordings were made on weekdays between 4:15 and 9:00 A.M. in April, May and June of 2005 and 2006. Both urban and forest recordings were spread across the season for each of the three city-forest pairs. We only recorded singing males that were not direct neighbours of each other and approached them to a distance of 8 to 15 meters. Recordings were made in wave format files with a sampling rate of 48.0 kHz using a Sennheiser ME67/K6 directional microphone with a foam windshield connected to a Marantz PMD670 digital recorder. The close approach distance and the directionality of the microphone typically guaranteed high quality recordings.

Songs were analyzed with Signal version 3.1.1. Spectrograms were made with a Fast Fourier transformation size of 512, a high-pass filter of 0.6 kHz and the rainbow colour palette setting with a low and high intensity threshold of -35 and -15dB, respectively. Spectrograms plotted on the computer screen showed the frequency range between 0 and 14 kHz on the y-axis with periods of 5 seconds on the x-axis. As a result, visual measurements had a spectral resolution of 27 Hz and a temporal resolution of 7 ms.

We visually measured the duration of the motif, twitter and inter-song pauses as well as the minimum frequency of the motif and twitter by cursor placements on the spectrogram. We included all visible sound traces attributed to bird song in the spectrograms which were standardized by the peak amplitude within each song. The twitter proportion of a song was calculated from the duration of the motif and twitter. Furthermore, the peak frequencies of the motif and the twitter were determined by averaging the loudest frequency present in five time segments of equal duration of either the motif or twitter. We started measuring songs at the beginning of each recording and continued until we had at least ten measurements of each song characteristic per bird.

DB-measurements

We measured the background noise levels in the six locations sampled for song recordings to test the assumption that the urban locations were noisier than the forest locations. Ambient noise measurements were only collected on rainless days with relatively low wind velocities in a short period between the 11th of June and the 2nd of July 2007. Furthermore, all dB-measurements were made in a short time window between 7:00 and 8:00 A.M. This approach provides comparable measurements for all territories at a biologically relevant moment as singing activity of blackbirds is still high at this time of the day. Each location was visited once and per location 16 to 18 sampling points were measured. The sampling points were approximately 200 meters apart from each other and they were a priori selected to cover the areas where songs were recorded.

A Pulsar Model 30 SPL meter was set to spectrum analyzer mode and measured dB(Z) levels of separate octave bands from 125 to 8,000 Hz. The SPL-meter was equipped with a windscreen and made measurements with a duration of one minute per sampling point. During this minute the SPL-meter was held horizontally at a height of about 1 meter above the ground. After every 15 seconds the measurement was paused for several seconds during which the SPL-meter was turned 90 degrees clockwise to get recordings from four directions. Measurements were transferred to a computer via Pulsar Analyser Version 1.1.1.

Playback experiment

Stimuli

Stimulus songs from recordings with a high signal-to-noise ratio were chosen from the dataset of measured songs. Recordings were high-pass filtered at 0.85 kHz and normalized to an equal peak amplitude in Signal version 3.1.1. Songs within the lowest and highest 25 percent of the measured distribution of motif peak frequencies of either Arnhem or the Veluwe were selected as stimulus songs. The song stimuli with either a low- or a high motif peak frequency ($x_{low} \pm$ s.e. = 1965 ± 18.2 Hz, $x_{high} \pm$ s.e. = 2477 ± 24.2 Hz) were subdivided into songs that originated from the city or the forest, creating four different categories of playback stimuli, representing "forest-low", "forest-high", "city-low" and "city-high". These four groups allowed us to distinguish between the effect of the motif frequency and the effect of the stimulus origin, which is important as origin or familiarity is commonly found to influence the response of male territorial birds (e.g. McGregor *et al.* 1983; Searcy *et al.* 2002; Tomback *et al.* 1983).

Each playback stimulus consisted of three different songs of one stimulus category from one male and songs from that individual were not used in other stimuli. The three songs were played back twice in the same sequence within a stimulus. A silence of three seconds was inserted between all six songs present in one stimulus. We created 24 unique exemplars of such six-song stimuli: six exemplars for each of the four stimulus categories.

Playback design

The playbacks were carried out in Arnhem and the Veluwe between 4:15 and 9:30 A.M. from the 17th of April until the 23rd of May 2007. Each playback experiment was done with a different focal male and we avoided testing neighbours that might have been unintentionally exposed to previous playbacks. We selected male blackbirds that were singing without obvious nearby competitors, because singing is indicative of territoriality and, by excluding territorial males occupied in agonistic interactions, foraging, or other activities, we thus reduced variation in the behavioural response related to motivational state.

In total we conducted 72 playback trials: 36 in Arnhem and 36 in the Veluwe. Every focal male was exposed to playback of two stimuli selected from two of the four different playback categories. An advantage of this pairwise setup is that it makes it easier to compare the effects of playback categories, because it reduces the effect of considerable inter-individual variation in playback responses. All 24 unique playback stimuli were used in six playback trials: once in combination with a stimulus from each of the other three stimulus categories in both Arnhem and the Veluwe. Every stimulus category was used the same number of times as the first or second stimulus presentation. The combinations of stimuli played back to birds in Arnhem and the Veluwe were identical as well as the order in which these combinations were presented. This is important as the use of an identical playback scheme in the city and the forest improves the chance to detect a possible asymmetric playback response between urban and forest males.

A trial started by placing the loudspeaker at a distance of about 10 meters from the focal male with the observer another 5 to 10 meters further away. The songs were played via a Sony CD Walkman D-EJ000 connected to a Blaupunkt CB4500 100 W speaker. A Pulsar Model 30 SPL meter was used to set the average playback amplitude at approximately 85 dB(Z), measured at a distance of 1 meter away from the speaker. The behaviour of the focal animal was initially scored for one minute during a pre-playback phase. Subsequently, the first playback stimulus was presented to the bird, followed by three minutes of silence and the second playback stimulus. The behaviour of the responding bird was scored for one minute beginning at the start of each playback.

Behavioural observations were registered using FIT Manager version 3.0 (Held & Manser 2005) on a Palm IIIx handheld computer. We scored the number of strangled songs and the number of flights. Strangled songs are a distinct class of vocalizations, which are much softer in amplitude compared to regular advertisement songs: they lack a clear motif-twitter structure, consist of mainly high frequency elements, and are often accompanied with visual threat displays and short flights (e.g. Dabelsteen 1981). Flights were defined as horizontal movements through the air of at least 10 meters. Singing blackbirds usually do not have a high flying activity, because in undisturbed situations they typically sing at the same perch for several minutes before switching to another song post. A high number of strangled songs and flights are therefore both good indicators of a strongly aggressive territorial response (Dabelsteen & Pedersen 1990; Ripmeester *et al.* 2007). Playbacks were aborted and not used for further analysis when the behaviour of the focal male could not be observed during the entire experimental period.

Statistical analysis

Statistical analyses were conducted in R version 2.5.1. (Ihaka & Gentleman 1996). Linear mixed models (LMM) were made for all of the measured song characteristics, which were entered in the models as the response variable. Measurements for all songs were included in the model and intra-individual variation was dealt with by including the variable "individual" as a random factor. Our paired-sampling scheme of three city-forest pairs was taken into account by nesting the variable "individual" in a random factor called "city-forest pair" having three classes (Arhnem-Veluwe, Leiden-Meijendel and Breda-Liesbos). The variable "habitat type" (city vs. forest) was entered into models as a fixed factor. Model assumptions of normally distributed errors and lack of heteroscedasticity were visually verified. Transformations were applied to response variables when this was required to meet the model's assumptions. Decibel-levels of all the measured octave bands were analyzed in a similar way as the song data.

Generalized linear models (GLM) were used to analyze the data of the playback experiment. The number of strangled songs and number of flights were count data and were entered as response variables in Poisson generalized models with a log link function. We checked for overdispersion in the models and found no indication for extra unexplained variation in the response variables (dispersion parameters < 1.07). Repeated measures on the same focal male were accounted for by including the variable "individual" as a random factor. "Order" was incorporated as an explanatory variable to deal with a possible difference in response strength towards the first or second stimuli in a playback trial. We included "motif frequency" (low vs. high), "stimulus origin" (city vs. forest) and "playback location" (Arnhem vs. Veluwe) as explanatory variables in the model as well as all the two- and three way interactions between these three variables. Stepwise removal of non-significant variables and interactions yielded minimal adequate models for which the significance levels are reported in the results section.

Results

Song

We analyzed 2,788 songs of 165 males (15 to 35 per location). Habitat had a significant effect on all four spectral measurements; blackbirds in the city produced songs with a higher minimum- and peak frequency of both the motif and twitter part (minimum frequency motif; $\text{Lratio}_1 = 18.5$,

$p < 0.0001$, peak frequency motif; Figure 2.2a, $\text{Lratio}_1 = 23.9$, $p < 0.0001$, minimum frequency twitter; $\text{Lratio}_1 = 7.2$, $p = 0.007$ and peak frequency twitter; Figure 2.2b, $\text{Lratio}_1 = 27.5$, $p < 0.0001$). Furthermore, habitat also had an influence on the temporal structure of blackbird song; urban blackbirds had a significantly larger proportion of twitter per song than forest blackbirds ($\text{Lratio}_1 = 14.8$, $p = 0.0001$, Figure 2.2c). This shift was due to significantly longer twitters ($\text{Lratio}_1 = 25.2$, $p < 0.0001$) in combination with motifs of a similar duration ($\text{Lratio}_1 = 0.9$, $p = 0.34$).

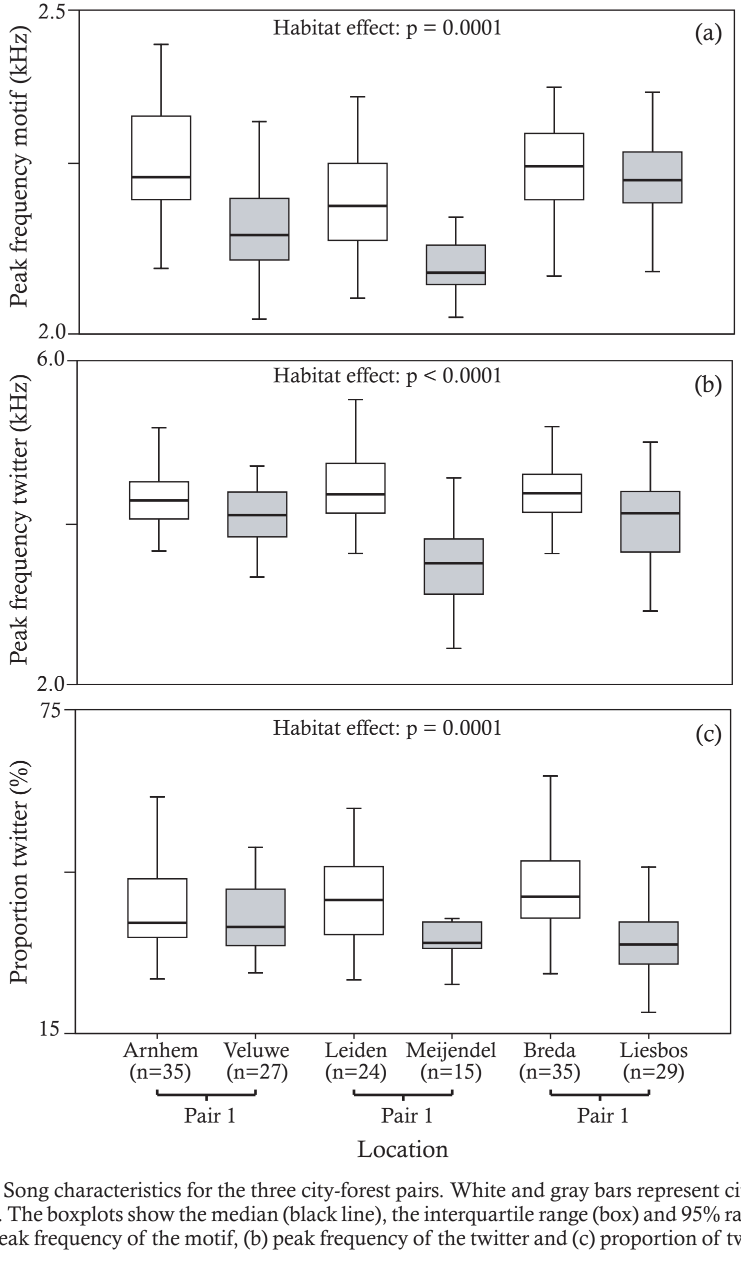

Figure 2.2. Song characteristics for the three city-forest pairs. White and gray bars represent cities and forest, respectively. The boxplots show the median (black line), the interquartile range (box) and 95% range (whiskers) for the (a) peak frequency of the motif, (b) peak frequency of the twitter and (c) proportion of twitter per song.

DB-measurements

Cities were noisier than forests over the entire frequency spectrum. There was a significant effect of habitat on the dB(Z) levels for all octave bands between 0.125 and 8.0 kHz ($p < 0.0001$). The largest differences in noise amplitude between city and forest were found in the relatively low frequencies up to about 3.0 kHz (Figure 2.3).

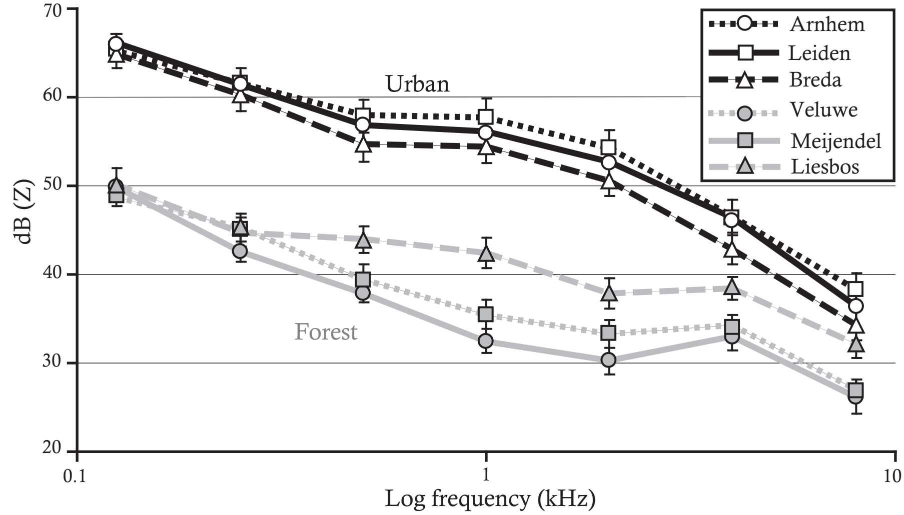

Figure 2.3. Background noise levels of study areas. Shown are the averages and standard errors of the dB(Z) levels per octave band for each of the six study areas. Open white symbols with black lines indicate cities. Forests are represented by filled gray symbols and gray lines.

Playback experiments

The pre-playback phase showed that the baseline levels of number of flights and number of strangled songs were very low as we only observed one bird that uttered two strangled songs and four birds that made a single flight during the pre-playback phase. In contrast, many territorial males responded strongly to our playback of conspecific songs, with congruent patterns for the number of strangled songs and the number of flights. There was considerable variation in response strength related to the four playback categories (Figure 2.4), with emergent effects of motif frequency and stimulus origin (see Table 2.1). The order of playback was significant for both number of strangled songs and number of flights with greater responses to the second stimulus ($\chi^2_1 = 11.2$, $p < 0.001$ and $\chi^2_1 = 4.73$, $p = 0.03$ respectively). Incorporating order as a main effect in the statistical analyses allows us to investigate the impact of the other factors independent of order.

There were three significant interaction effects for the number of strangled songs between motif frequency, stimulus origin, and playback location. The two-way interaction between motif frequency and playback location ($z = 3.0$, $p < 0.001$) showed that the number of strangled songs produced by birds from the forest is higher to playback of low-frequency motifs and that birds from the city on the other hand respond stronger to high-frequency motifs. The two-way interaction between stimulus origin and playback location indicated that more strangled songs were uttered after a male had been exposed to playback stimuli from its own population ($z = 2.52$, $p = 0.01$).

Finally, there was a significant three-way interaction between stimulus origin, motif frequency, and playback location ($\chi^2_1 = 8.92$, $p = 0.003$). This three-way interaction reflects that birds responded with more strangled song to playback stimuli with either a high frequency from their own population or a low frequency from the other population. The test statistics for number of flights also revealed a significant effect for the three-way interaction ($\chi^2_1 = 3.89$, $p = 0.048$) and the same trend, as found for number of strangled songs, for one of the two-way interactions (interaction stimulus origin x playback location; $z = 1.74$, $p = 0.08$).

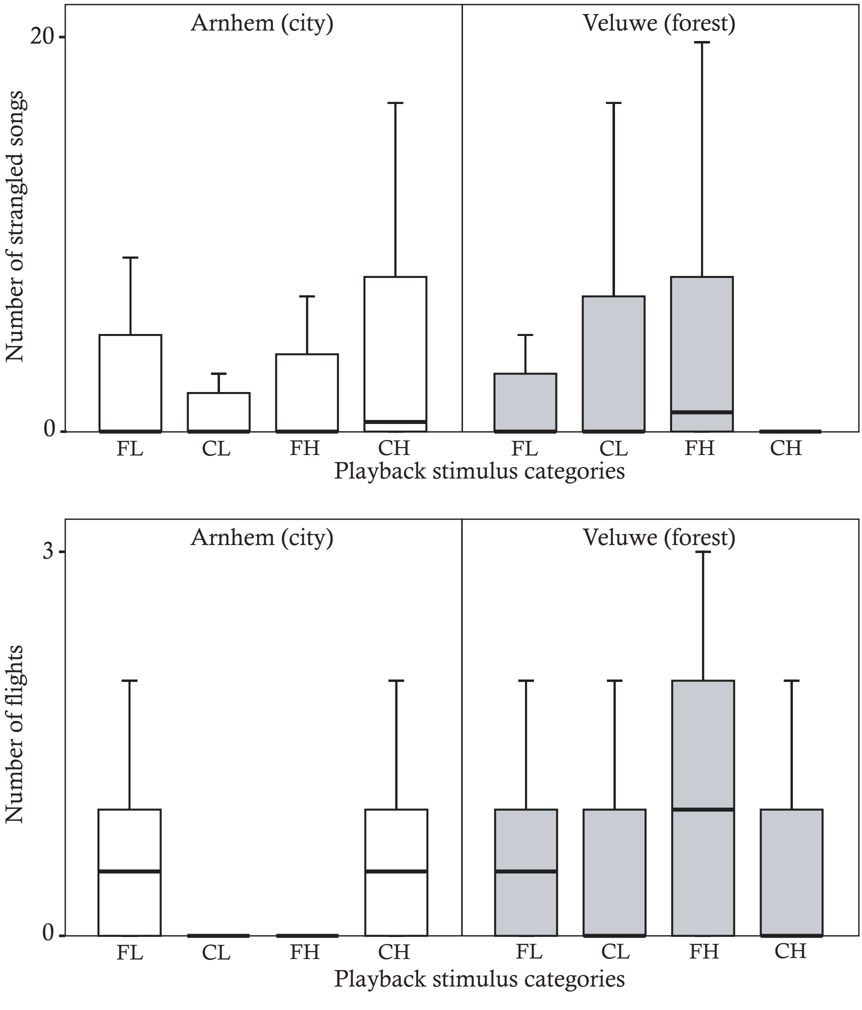

Figure 2.4. The number of strangled songs (top graph) and flights (bottom graph) of males in response to the four different stimulus categories: "forest-low" (FL), "city-low" (CL), "forest-high" (FH) and "city-high" (CH). White and gray bars represent the city and forest playback locations, respectively. The boxplots show the median (black line), interquartile range (gray box) and 95% range (whiskers). Significance levels of factors and interactions are given in Table 1.

Table 2.1. Overview of the results of the Generalized Linear Models on the number of strangled song and number of flights. All factors in the table were retained in the two minimal adequate models of number of strangled songs and number of flights, because in both models the factors playback location, motif frequency and stimulus origin were part of a significant three way interaction and period was a significant main effect.

Explanatory variable	Strangled song		Flights	
	Test statistic	p	Test statistic	p
playback location	z = -1.8	0.066	z = -1.06	0.29
stimulus origin	z = 0.28	0.77	z = -0.97	0.33
motif frequency	z = -1.42	0.16	z = -1.33	0.18
order	$\chi^2 = 11.21$	0.0008	$\chi^2 = 4.73$	0.03
stimulus origin x playback location	z = 2.52	**0.01**	z = 1.74	0.08
stimulus origin x motif frequency	z = 0.89	0.38	z = 1.8	0.07
motif frequency x playback location	z = 3.01	**0.0003**	z = 1.39	0.16
stimulus origin x motif frequency x playback location	$\chi^2 = 8.92$	**0.003**	$\chi^2 = 3.89$	**0.048**

Discussion

We found that songs of urban and forest blackbird populations differ both spectrally and temporally. Urban birds produce songs with a higher minimum and peak frequency for both the motif and the twitter part, while they also have a larger proportion of twitter per song compared to forest birds. We confirmed that background noise in the urban populations was consistently louder across the entire frequency spectrum and particularly in the relatively low frequencies. These findings are in line with the accumulating data on a link between ambient noise levels and avian song spectra (Patricelli & Blickley 2006; Slabbekoorn & Ripmeester 2008). They strongly suggest that the omnipresence of anthropogenic sounds moulds the natural signals of animals living in noisy cities. Furthermore, our playback experiment shows significant interactions between motif frequency, stimulus origin, and playback location. These results reveal an effect of one of the divergent acoustic traits on the behavioural response on top of the expected impact of familiarity with local songs. This means that blackbirds are able to detect habitat-dependent acoustic divergence related to a noisy urban context and attribute meaning to this variation in their mating signal under natural territorial conditions. Furthermore, the fact that both urban and forest birds discriminate between songs dependent on the same divergent trait, but in opposite ways, suggests a potential impact on habitat-dependent dispersal probabilities.

Urban song divergence

European blackbirds in three Dutch cities produce songs of higher frequency compared to birds in nearby forests only 5-10 km away. These results contribute to a growing body of population-level studies with congruent results. A similar spectral divergence was found in great tit songs across Europe in a study on ten city-forest pairs which were 25-100 km apart (Slabbekoorn & den Boer-Visser 2006). The minimum frequency of song in a single urban population of dark-eyed juncos (*Junco hyemalis*) was also shifted upward compared to three out of four forest populations, with the closest forest at a distance of 70 km from the city (Slabbekoorn *et al.* 2007). Acoustic divergence in several other song features related to bill shape and size in house finches were found within a single city-desert pair of two resident populations only 6-10 km apart (Badyaev *et al.* 2008). Furthermore, despite being rare, examples of habitat-dependent acoustic divergence at a small scale are not restricted to urban conditions: two subspecies of the grey-breasted wood-wren (*Henicorhina leucophrys*), living in adjacent zones on a steep altitudinal gradient in the Andes, also exhibit habitat-dependent song divergence, which remains between populations that are only 10-15 km apart (Dingle *et al.* 2008). These studies emphasize the potential of ecology in driving signal changes, across both natural and anthropogenic gradients, even at a very small scale.

Single-population studies reporting a positive relationship between the frequency use and the level of anthropogenic noise are now available for great tits, house finches and song sparrows (Fernández-Juricic *et al.* 2005; Slabbekoorn & Peet 2003; Wood & Yezerinac 2006). The same frequency use-noise level association has also been reported for a natural sound source: low-frequency river noise. This concerned a study on chaffinches (*Fringilla coelebs*) with territories close to and further away from noisy waterfalls and torrents in Scotland (Brumm & Slater 2006). The species mentioned above are small-repertoire singers except for house finches, which are large-repertoire singers like the European blackbird. Although it thus seems that noise-dependent song frequency use is not restricted to species with a particular repertoire size, the behavioural mechanism behind the correlation may vary with singing style.

A behavioural mechanism suggested to be responsible for the association at the level of individual territories has been the selective use of specific song types (Slabbekoorn & den Boer-Visser 2006). Small-repertoire singers typically sing with eventual variety, repeating a single, relatively stereotypic song type a number of times before switching to another. Song type switching behaviour may be based upon auditory feedback of experienced signal-to-noise ratios or through direct social feedback from conspecifics. Especially small-repertoire singers having distinct songs of different frequency ranges may have the potential to prolong singing certain song types under favourable conditions or switch to another one in case of severe masking (Halfwerk & Slabbekoorn in press). The feedback conditions for large-repertoire singers, such as the European blackbirds, contrast dramatically with the replicated successive feedback events of small-repertoire singers that can accumulate to a switching decision. Large-repertoire singers often have over a hundred alternatives of more loosely composed song variants, which are typically sung with immediate variety, using a different song type for every successive song. Specific songs may be repeated only after many others. We cannot rule out the possibility that cumulative experience with song variants affects which song types from a large repertoire are sung under specific noise conditions, but it is not unlikely that other mechanisms could better explain the observed patterns in the current data-set.

An alternative mechanistic explanation to song type selection could be that a rise in pitch may be an inherent consequence of singing louder under noisy conditions. Vocalizing louder in noisy situations is a phenomenon called the Lombard effect and is a well-known response in humans as well as in many other species including birds (Brumm & Slabbekoorn 2005). In humans, talking louder or shouting is typically associated with a rise in fundamental frequency, which is a passive consequence of sound production in case of soft and normal phonation (Alku *et al.* 2002; Gramming *et al.* 1988). However, preliminary data on individual blackbirds raising song amplitude when going from low to high noise conditions suggest only a small noise-level related rise in frequency within individuals of about 10 Hz (Ripmeester *et al.* unpublished manuscript). This is a relatively small shift compared to the observed differences between habitats, which are about 70 to 385 Hz for peak frequency of the motif and twitter, respectively. A shift in frequency as a by-product of an increase in amplitude is therefore unlikely to be the only explanation for the divergence at population level in frequency between urban and forest birds.

Other alternative explanations for noise-dependent song variation in large-repertoire singers that remain could be related to noise- or habitat-dependent song acquisition. Firstly, young birds could predominantly learn songs that are best audible in their local environment and end up with more high-frequency songs in territories with high levels of low-frequency noise (c.f. Slabbekoorn & den Boer-Visser 2006). Secondly, young birds could have a genetic predisposition to hear, memorize, or produce songs of specific frequency ranges more easily. Even though such a genetic explanation is unlikely to account for noise-dependent variation at the individual level, it could explain variation at the population level if urban birds are genetically diverged and have a habitat-specific predisposition related to song (c.f. Nelson 2000). Clearly, further research is required to determine the mechanisms underlying spectral song divergence in urban birds and we especially need ontogenetic studies following noise-dependent developmental trajectories as well as explicit tests of immediate switching or song type selection abilities (c.f. Halfwerk & Slabbekoorn in press).

Frequency shift through temporal adjustment

We also revealed that birds in urban populations produce songs with larger twitter proportions than their forest conspecifics. Despite the relatively low amplitude of twitters, a preliminary study suggests that twitters have a higher signal-to-noise ratio than motifs in urban areas, but not in forests (Ripmeester *et al.* unpublished data). The discrepancy between the relative signal-to-noise ratios of motifs and twitters recorded in urban and forest populations can be explained by habitat-dependent differences in the spectral composition of background noise. Our analyses on the background noise spectra of the study areas show that cities are dominated by low-frequency anthropogenic noise whereas forests have lower levels of low-frequency noise, mainly originating from wind, in combination with more high-frequency sounds from singing birds (c.f. Slabbekoorn & den Boer-Visser 2006). The temporal adjustment towards larger twitter proportions in cities might therefore indeed improve the audibility of blackbird song.

This is the first time that a spectral shift through a temporal adjustment in song structure is reported as a potential adaptation to signalling under noisy conditions. In general, there are several ways in which animals are known to use temporal adjustments in response to high noise levels (Brumm & Slabbekoorn 2005). Several insect, frog, and bird species are capable of a temporal adjustment of the inter-song intervals to exploit the relatively silent gaps in the noise (reviewed by Planque & Slabbekoorn 2008). Animal species as diverse as killer whales (*Orcinus orca*, Foote *et al.* 2004), common marmosets (*Callithrix jacchus*, Brumm *et al.* 2004); and chaffinches (Brumm & Slater 2006) have been found to elongate signal duration with rising noise levels. The temporal adjustment in blackbird songs, with a switch to singing relatively more of the high-frequency component, may be the first of its kind to be described. Although this type of adjustment may lead to masking release, we want to emphasize that there is no evidence yet for a causal explanation related to noise levels. In fact, it is very well possible that ambient noise is not directly affecting this song feature, but that variation is related to another habitat-dependent factor.

A causal explanation for the shift in twitter proportion could be related to habitat-dependent density and the related variation in prevailing social conditions. Higher densities in urban habitat may lead to a higher rate of social interactions with other males, which has been proposed to relate to the production of relatively long twitter parts (Dabelsteen & Pedersen 1985). However, this relationship is not completely clear as a playback study by Ripmeester *et al.* (2007) showed no changes in twitter proportion related to experimental exposure to a simulated singing intruder. Nevertheless, that study did show that the frequency use of the twitter can vary with the agonistic context. The findings concerning intra-individual song variation are interesting but inconclusive with respect to their impact on acoustic differences at the population level. Further study is required to determine if the habitat-dependent variation in duration and frequency of the twitter can be due to habitat-dependent differences in social conditions.

Acoustic impact on behaviour

Several conclusions can be drawn from the playback results with three significant interactions between motif frequency, stimulus origin and playback location. We can say now that urban males respond more to songs with a high motif frequency whereas forest males have a stronger response to a low motif frequency. Furthermore, they respond particularly strong to songs with a high frequency of their own population and songs with a low frequency from the other population. These results demonstrate that male blackbirds can detect variation in the habitat-dependent divergence of motif frequency and that the acoustic variation is perceived as carrying relevant information. The impact of stimulus origin implies that male blackbirds also distinguish between local and foreign songs.

Learning may play an important role in the behavioural response difference to both the diverged trait and the stimulus origin. Learning about the common songs heard in the resident area is assumed to be responsible for female mate preferences for locally common acoustic variants (Irwin & Price 1999). Also male birds can be most responsive to features that are locally most abundant (Slabbekoorn & ten Cate 1998) and might be acoustically tuned through relative exposure rate (den Hartog *et al.* 2008). Therefore, the fact that both urban and forest blackbirds of the current study discriminate between songs based on motif frequency, but respond in opposite ways, may be due to the relative amount of exposure they have had to motifs of a specific frequency range. Urban birds are likely to have heard relatively more songs with high-frequency motifs whereas for forest birds exposure to low-frequency motifs will have been more common.

Several other studies have also provided playback evidence for an impact on response for ecologically diverged traits (Brambilla *et al.* 2008; Patten *et al.* 2004; Dingle *et al.* unpublished manuscript) as well as for songs of different origin (McGregor *et al.* 1983; Searcy *et al.* 2002; Tomback *et al.* 1983). Both factors, the divergent trait and the origin of the song, may affect dispersal and have the potential to promote habitat-dependent gene flow through settlement advantages or assortative mating based on shared ancestral habitat (Irwin & Price 1999; Slabbekoorn & Smith 2002a). Despite the fact that our findings concern a very small geographic scale of just a few kilometres, we believe that song divergence and associated response differences can still be relevant to a potential impact on dispersal. Other studies have reported genetic divergence in fitness-related traits at similarly small geographic scales (Badyaev *et al.* 2008; Blondel *et al.* 1999; Shapiro *et al.* 2006). Moreover, a comparison of urban and forest blackbirds revealed divergence in several physiological and behavioural traits with a genetic basis over a distance of about 40 km (Partecke *et al.* 2006a; Partecke *et al.* 2004; Partecke *et al.* 2005). Given the scale of the current results on blackbird song divergence, we argue that, depending on the ecological contrast, there is considerable potential for parallel divergence in both sexual traits and fitness related traits irrespective of the geographic distance.

In conclusion, we have demonstrated that habitat-dependent acoustic divergence is perceived as meaningful by territorial males in urban and forest populations of the European blackbird. This is to our knowledge the first time that an effect of habitat-dependent song divergence on male response has been demonstrated within a species while controlling for familiarity. The effect was obtained in the context of urban habitat with anthropogenic noise as the most likely environmental selection pressure. Furthermore, not only did we find a habitat-dependent upward shift in frequency use, similar to what has been found for other urban species, but we also report a novel type of frequency shift through a temporal adjustment in song structure. Although further study is required to get insight into causal relationships, singing relatively long high-frequency twitter parts in noisy urban conditions may be beneficial in terms of masking avoidance. The environmentally related song differentiation and associated variation in behavioural response between populations of adjacent but ecologically distinct habitats may influence successful settlement and thereby reproduction of blackbird males. Consequently, these data provide fundamental insights into the potential for song variation to affect gene flow between urban and forest populations of birds as well as into the role of habitat-dependent acoustic traits in the initial stages of ecological speciation in general.

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