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

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Introduction, thesis overview and synthesis

General introduction

Speciation, the formation of new species, has been a key topic in biology ever since Darwin postulated the concept of evolution (Darwin 1859). It is currently widely accepted among biologists that species can evolve over time and split into separate species that are reproductively isolated from each other. The classical view of speciation emphasizes that geographic isolation is crucial. Populations living in allopatry diverge, due to selection or drift, and they will in time evolve into novel species that will not hybridize when coming into secondary contact (Mayr 1942). Nowadays, biologists realise that speciation is not restricted to purely allopatric circumstances. Speciation can also happen when secondary contact occurs after a period of allopatry (Dobzhansky 1937), or in parapatry with populations occurring adjacent to each other (Gavrilets 2000), and even in sympatric situations in which geographic isolation is absent (Dieckmann & Doebeli 1999). In all of these situations there are two general prerequisites for speciation to take place: there should be intra-specific variation and a mechanism for reproductive isolation.

There are various kinds of random processes and events that can lead to intra-specific variation such as founder effects and genetic drift (Coyne & Orr 2004). Alternatively, divergent selection may be a driving force leading to intra-specific variation. Natural selection pressures related to habitat structure, food sources, climatic circumstances and predation can vary among groups of animals within a species, resulting in differentiation via ecological adaptations (Schluter 2001). In addition, sexual selection pressures can lead to divergence in traits involved in mate-choice (e.g. Fisherian runaway: Fisher 1930; Lande 1981), which can contribute to the completion of reproductive isolation.

Reproductive isolation can be the result of post- and prezygotic mating barriers. Postmating barriers are the result of genetic mismatches between individuals of diverging populations causing inviability, infertility, ecological inviability or behavioural maladaptiveness in the offspring (Coyne & Orr 2004; Presgraves 2002; Price & Bouvier 2002). Premating barriers prevent mating between individuals from diverging populations. This requires divergence in signals and responses to these signals between diverging groups of animals when they are not fully separated in space or time. Signal and corresponding response differentiation make it possible for individuals to mate assortatively in order to avoid possible maladaptive effects of hybridization (Gottsberger & Mayer 2007; Kruuk *et al.* 1999; Saetre *et al.* 1999). Signals can diverge as a result of natural and sexual selection (Endler & Basolo 1998; Leal & Fleishman 2004; Slabbekoorn 2004). Furthermore, signal differentiation can be influenced by processes like reinforcement and character displacement (Brown & Wilson 1956; Butlin 1989).

It is without a doubt that ecology and sexual selection often play a crucial role in speciation, because they can easily cause divergence and reproductive isolation (Kirkpatrick & Ravigne 2002; Orr & Smith 1998; Rundle & Nosil 2005). Ecology and sexual selection can act in concert if sexual signals are under environmental selection. Visual or acoustic signals, for instance, can diverge between environments when this improves the local detectability of a signal by conspecifics. There are numerous examples in which divergence between

closely-related species in their signals match the environmental conditions (Bergmann 1978; Heuwinkel 1982; Losos *et al.* 1998; Seehausen *et al.* 1999; Maan *et al.* 2006). Evidence from behavioural studies shows that ecologically diverged sexual traits can in turn influence mate preferences that could explain assortative mating patterns in the field (e.g. Irwin *et al.* 2001; Seehausen & Van Alphen 1998).

However, there are still many unresolved questions about ecological speciation and the role signal divergence plays in it. It is for example not clear whether all habitat-related variation in signals is likely to promote assortative mating or how rapidly habitat-dependent responses to diverging signals develop. This is to a large extent due to the relatively slow rate at which speciation events take place, making it rather difficult to observe the processes involved in speciation in nature all the way from the initial phases to the final stages in which diverged groups can be regarded as new species. Consequently, much of what is known so far is based on phylogenies and experiments with sister- or subspecies. Unfortunately it is difficult to infer from these current patterns of (sub)species what has historically happened during the very beginning of their divergence. Studies on populations that are just beginning to differentiate in both ecological and sexual traits can thus provide valuable new information about the initial steps of speciation.

Urban habitat

An excellent opportunity to study the first steps in population divergence is provided by the emergence of the urban habitat, which is an evolutionary very novel habitat that dramatically differs from most other habitats. Urbanization is a recent global phenomenon related to the enormous growth of the world population since the early 20th century and the pattern of more and more people moving from villages and farms to cities. Currently already half of the human population lives in cities and in the next two decades an estimated two billion people are expected to start occupying urban areas not yet existing (Millenium-Ecosystem-Assessment 2005; UN-Habitat 2008).

Urbanization typically concerns a rapid change of natural environments into urban habitat and has a major impact on the original flora and fauna of such areas. Many species cannot cope with the sudden change and characteristics of the novel urban habitat. As a general result, urbanization at a large geographic scale leads to a shift in species composition and a decrease in species number: local and often rare species are replaced by typical urban species that turn out to be the same in many cities independent of the original flora and fauna (Marzluff 2001; McKinney 2006). Interestingly, some bird species have become quite successful urban inhabitants, while still occurring in their natural habitat. These species are particularly useful to study intraspecific population differentiation, because populations occupying urban and non-urban habitat might undergo trait divergence as a result of their exposure to extremely different selection pressures.

Urban and natural habitats differ in a large suite of abiotic and biotic ecological factors (Warren *et al.* 2006). Cities have a microclimate that is warmer and drier than the surrounding regions (Landsberg 1981). There is relatively much soil- and air pollution in cities

as well as the presence of artificial lighting. Cities are characterized by a large amount of solid surfaces like buildings and roads with relatively little vegetation. Another composition of the flora and fauna in cities implies the presence of other food sources and predators including anthropogenic food and domesticated cats (Woods *et al.* 2003). In addition, animals in urban areas will also have to cope with more disturbances by human activities.

Divergence in ecological and sexual traits

Although not yet studied in many species, it is clear that at least some successful urban species have adapted to ecological circumstances of the city (Shochat *et al.* 2006a). As a consequence, urban animals may have diverged adaptively from conspecifics still living in their original habitat in terms of ecological traits, defined as physical and behavioural traits related to the interaction of individuals with their environment. Morphological differentiation between urban and non-urban populations has for instance been shown in several bird species (Badyaev *et al.* 2008; Johnston & Selander 1971; Rasner *et al.* 2004). An example indicating a behavioural adaptation is given by a study showing that magpies (*Pica pica*) built their nests at higher locations in urbanised areas, presumably to reduce the level of disturbance by human activities (Wang *et al.* 2008).

Urbanization can also have an effect on sexual signals like plumage coloration (Slagsvold & Lifjeld 1985) and bird song (Slabbekoorn & Ripmeester 2008). Particularly for the latter there is a growing body of evidence that the urban environment has an effect in a whole range of species. Song plays an important role in inter- and intrasexual communication in many bird species, because they use it to attract mates and defend territories (Catchpole & Slater 2008; Collins 2004). It is therefore essential for the reproductive success of males that their song is well audible. Interference by environmental noise may hinder effective detection and discrimination of acoustic signals (Ryan & Brenowitz 1985). Cities are full of anthropogenic noise originating from automobiles, trains, airplanes, compressors, industrial machines and construction activities. Most of the sounds made by human activity are relatively loud and have their spectral energy concentrated in the lower frequencies. Consequently, the background noise in cities is much louder in the low frequency domain than in most natural environments (Figure 1.1a,b). Songs of many bird species have at least a partial spectral overlap with anthropogenic noise resulting in a reduced signal-to-noise ratio. This could potentially hamper acoustic communication making it more difficult to attract mates and repel rival males. One way to reduce the masking effect of anthropogenic noise is to sing with a higher pitch as this will lead to an increase in signal-to-noise ratio, and thus better audibility. Several bird species have been shown to alter their acoustic signal in this way in response to anthropogenic noise. Urban great tits (*Parus major*), house finches (*Carpodacus mexicanus*) and song sparrows (*Melospiza melodia*) produce songs with a relatively high minimum frequency in territories with high levels of anthropogenic noise (Fernández-Juricic *et al.* 2005; Slabbekoorn & Peet 2003; Wood & Yezerinac 2006). Moreover, a large scale survey across Europe showed that urban great tits consistently sing with a higher frequency than their conspecifics in nearby forests (Slabbekoorn & den Boer-Visser 2006).

Species simultaneously occurring in urban- and nonurban environments can thus differentiate from each other in ecological and sexual traits. This means that processes involved in the initial part of population divergence and speciation can be studied in the novel urban environment that is all around us. Species that are common in urban and natural habitats, in which both ecological and sexual traits are under strong divergent environmental selection, are particularly suited to investigate the processes leading to intra-specific differentiation, which may well be the first step towards speciation.

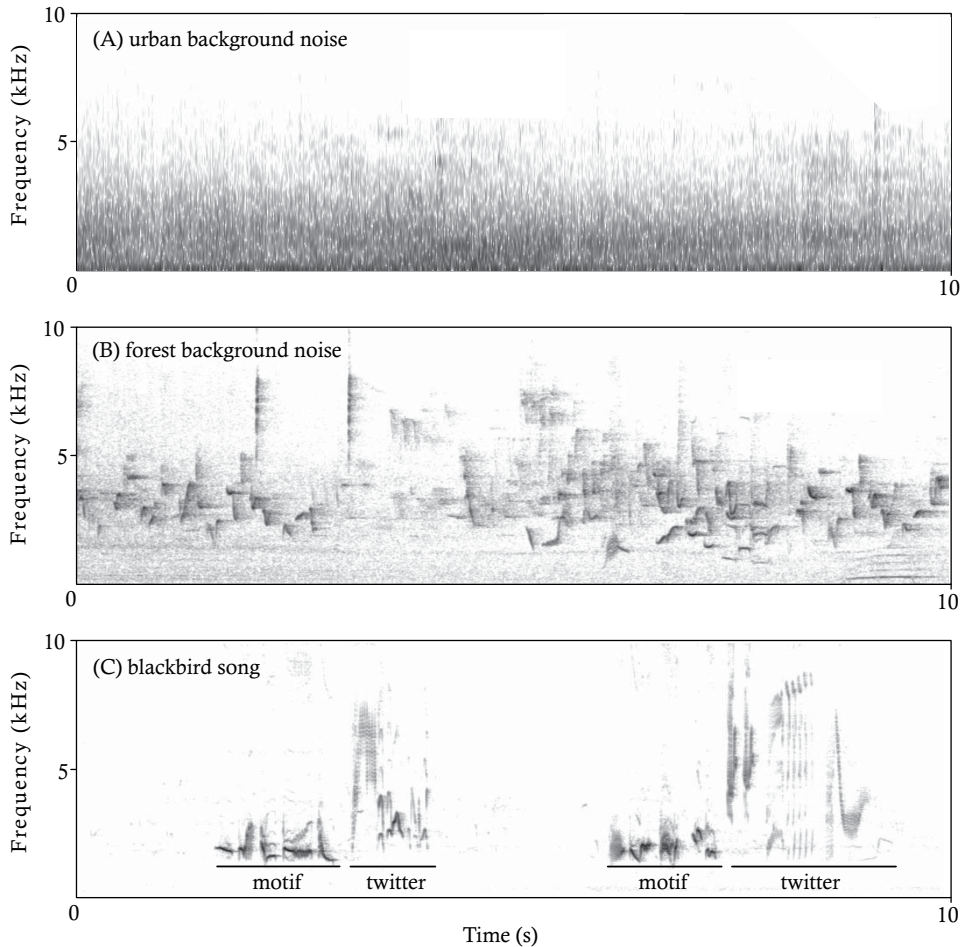


Figure 1.1 Three spectrograms illustrating the difference in masking of blackbird song by urban and forest background noise. The top panel (A) shows a representative example of low-frequency urban background noise. The middle panel (B) is a spectrogram of a recording made in a forest in the Netherlands in which several European birds are singing. It is a typical example of forest background noise consisting of relatively high frequencies. The bottom panel shows two blackbird songs each having a motif part followed by a twitter part.

The study species: blackbirds

A well-suited model species to study habitat-related population differentiation and the role of sexual signals in it is the European blackbird (*Turdus merula*). It is a member of the family Turdidae and the males of this medium-sized thrush have an entirely black plumage with an orange-yellow beak and narrow orange-yellow eye-ring, which are brightly coloured during the breeding season. Females are slightly smaller and have dark-brown feathers with a duller and browner eye-ring and beak. Blackbirds have a widespread distribution covering most of Europe, some regions in Asia, a small area in Northern Africa as well as New Zealand and South Australia where they were introduced in the 19th century (Clement & Hathway 2000). Their original habitat used to be primarily forests, particularly those with deciduous trees and dense undergrowth in which they feed on insects, snails, worms, fruits and berries. Nowadays they are also a successful species in urbanised areas. They first appeared in a few German cities in the early 19th century after which they gradually started to occur in more cities in Western Europe (Luniak *et al.* 1990). Blackbirds are usually abundant in the cities and forest in which they occur. In the Netherlands they are the most numerous breeding species with over one million breeding pairs (Hustings & Vergeer 2002).

There is an increasing number of studies indicating that anthropogenic factors have an effect on blackbirds, leading to a divergence between urban- and forest populations in a variety of traits. Urban blackbirds have a lower ecto- and endoparasite load than their forest conspecifics (Geue & Partecke 2008; Gregoire *et al.* 2002). Furthermore, individuals in the city have a lower tendency to migrate in winter and they start reproduction earlier in the season (Partecke *et al.* 2004; Partecke & Gwinner 2007). This allows urban birds to have more breeding attempts per year, but on the other hand they have a smaller clutch size and fewer fledglings per successful breeding attempt than conspecifics in the forest (Gregoire 2003). Additionally, a lower acute corticosterone stress response has been found in urban birds compared to forest birds, which is presumably a way to reduce the potentially deleterious effects of persistent exposure to stressful situations in the city (e.g. disturbance by human activities) (Partecke *et al.* 2006a). A morphological comparison of individuals from many locations across Europe revealed that there is considerable differentiation in various morphological traits (e.g. wing length, bill size and tarsus length) between adjacent urban and rural areas (Evans *et al.* 2009a).

There is also evidence for genetic adaptations in blackbirds, because a common garden experiment showed that phenotypic variation between urban and forest individuals in at least some of the above mentioned traits is not merely the result of phenotypic plasticity (Partecke *et al.* 2006a; Partecke *et al.* 2004; Partecke *et al.* 2005). A recent study on blackbirds also revealed the existence of genetic differentiation of neutral markers between urban and nearby rural populations (Evans *et al.* 2009b). This suggests that adaptations of various traits to life in the city are causing a reduced gene flow between urban and forest populations.

An important sexual signal in blackbirds is their song, because it functions, as in most bird species, in territorial defence and mate attraction (Catchpole & Slater 2008; Collins 2004). Blackbirds are songbirds and they learn their species-specific song from hearing conspecifics.

Territorial males deliver their song from high song posts like exposed branches, tall bushes and rooftops. They sing during the entire breeding season with a peak in singing activity around dawn and dusk (de Vos & de Meersman 2005). Blackbirds have a large repertoire consisting of dozens of songs (Hall-Craggs 1962). The acoustic structure of their songs is very stereotypic. A song always starts with a motif part having several simple short whistle-like elements of a relatively low frequency directly followed by a twitter part having more variable elements of a softer amplitude and higher frequency (Figure 1.1c; Todt 1970a; Dabelsteen & Pedersen 1985). In between consecutive songs there is usually a short period of silence. This inter-song pause duration and the relative proportion of twitter per song can vary considerably within individuals. Twitter proportion and pause duration have been suggested to reflect a male's level of motivation to fight (Dabelsteen 1984a; Dabelsteen & Pedersen 1990).

Blackbirds are an excellent species to investigate the causes and consequences of song variation and divergence. Firstly, blackbirds sing loud and can usually be approached to a short distance, which makes it relatively easy to get high-quality recordings. Secondly, the clear song structure and high levels of singing activity make blackbirds an appropriate study species for large quantitative acoustic analyses. Thirdly, playback experiments with males in the field are feasible, because their responses to playback are relatively easy to observe and categorise (e.g. Dabelsteen & Pedersen 1985). Surprisingly, no previous studies have addressed the influence of anthropogenic noise on blackbird song. However, it seems very plausible that urban noise can have consequences for songs in cities, and particularly for the motif part, due to its large spectral overlap with anthropogenic noise (Figure 1.1c).

Thesis outline and chapter summaries

In this thesis, I use urban and forest blackbirds as a model system to study habitat-related population differentiation with a focus on the causes and consequences of song divergence. For this purpose, I tested the extent to which urban and forest blackbirds have differentiated from each other acoustically, morphologically and genetically. Furthermore, I investigated which social and environmental factors influence blackbird song at the individual level (i.e. across individuals) as well as the population level (i.e. across habitats) in order to understand which acoustic variation could potentially be relevant in habitat-dependent divergence. A large playback experiment with territorial males was conducted to test whether acoustic divergence influences the responses of urban and forest blackbirds. I will below continue with brief summaries of the chapters 2 to 5 followed by a synthesis of the results of this thesis. In the synthesis I evaluate how my findings on blackbirds contribute to our general understanding of the processes involved in habitat-related population divergence and potentially the first steps towards speciation.

The first question I address is whether urban- and forest blackbirds differ in their songs and if so, whether this affects their response to songs. In **chapter 2, “Habitat-dependent acoustic divergence affects playback response in urban and forest populations of the European blackbird”**, I recorded territorial male blackbirds in the following three

city-forest pairs in the Netherlands: Arnhem and The Veluwe, Breda and The Liesbos, and Leiden and Meijndel (Figure. 1.2). The acoustic analysis showed that there were several significant habitat-dependent spectral and temporal song differences. Firstly, blackbirds in cities produced songs with a higher peak frequency than conspecifics in the forest, for both the motif- and twitter part. Secondly, urban blackbirds had longer twitter proportions per song and longer inter-song pauses.

These observations were the basis for a reciprocal playback experiment in the city-forest pair of Arnhem and The Veluwe (chapter 2). In this experiment we compared responses of urban and forest territorial males towards songs from the forest and the city with either a low or high motif frequency. The results show that male blackbirds indeed pay attention to the motif frequency as well as to the origin (city or forest) of the playback stimulus. Moreover, urban males responded strongest to motifs with high frequencies, but forest males responded more to low motif frequencies. Hence, the response strength of urban and forest males towards songs with a high or low frequency was opposite. This asymmetric response is important as it suggests that song divergence might influence where males with typical urban or forest songs will settle and whether they will be able to maintain a territory.

Additionally, chapter 2 contains a comparison of the early morning background noise levels of the six study locations. This was done to verify the assumption that anthropogenic noise influences the ambient background sound spectra in cities, which would imply that

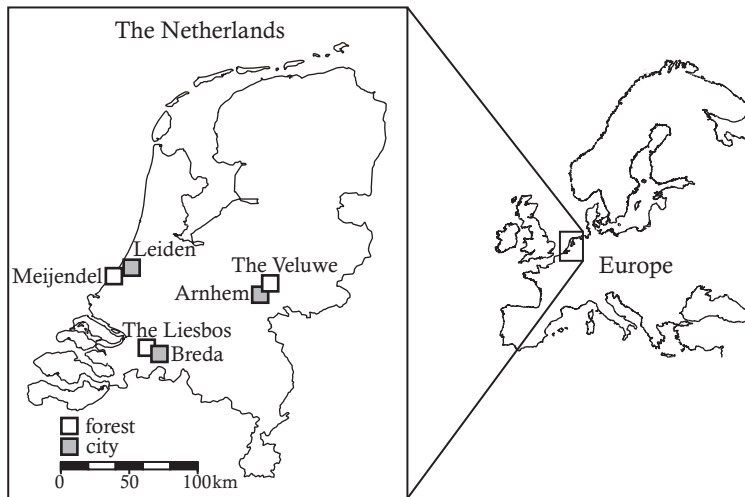


Figure 1.2. Map showing the six study locations in the Netherlands, which consisted of three pairs of a city and a nearby forest. The centre of a city and a forest within a pair were only 5 to 10 kilometres apart and the distances between the three city-forest pairs ranged from 65 to 100 kilometres. The three cities, Arnhem, Breda and Leiden, 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). The three associated forest locations were the Veluwe, the Liesbos, and Meijndel. The Veluwe is a relatively large area near Arnhem of about 100,000 hectares mainly covered with mixed forest and moorland. We conducted our study on the part of the Veluwe located 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. Meijndel is a mixed forest-dune area of about 1,875 hectares located near Leiden.

particularly low-frequencies of bird song will be masked in urban environments. The results confirm this assumption as the background noise was louder in all frequencies in the city than in the forest and importantly, the largest differences in noise amplitude were in the relatively low frequencies up to about 3 kHz.

In chapters 3 and 4 the causes of habitat-dependent song divergence between urban and forest birds are explored, because understanding the mechanisms underlying song divergence will help evaluate their possible consequences for population differentiation. Song birds learn their species-specific songs from hearing conspecifics (Kroodsma 1996). Many song birds have one sensitive phase early in life and are unable to learn new songs later on. Young birds will thus learn the songs that are produced by adults in their environment. These songs match local environmental conditions in case selection on song features over generations has occurred. Individuals will then have locally adapted songs when they stay in the same habitat as they grew up in. However, when dispersing to another habitat they do not have the capability to learn new locally-adapted songs. This does not necessarily imply that individuals that disperse to another habitat cannot adjust what they sing. Some song features might be plastic within individuals allowing them to deal with varying situations. An example of such a phenomenon in birds is the Lombard effect; an increase in song amplitude within individuals when noise levels elevate (Brumm 2004; Brumm & Slabbekoorn 2005; Pytte *et al.* 2003).

There are several reasons why it could be relevant for population divergence whether fixed or plastic song features are involved in habitat-related song differences. Switching to another habitat might be more problematic with fixed song features, because individuals will not be able to adapt their song to novel local conditions. This can reduce the efficiency of singing to attract mates and repel rivals. Furthermore, in contrast to plastic song features, fixed acoustic traits may signal reliably where an individual grew up. Consequently, especially fixed song features can be used in mate choice decisions to assess an individual's possible ecological adaptation to its current habitat. In conclusion, fixed song features are expected to have a larger impact on habitat-dependent gene flow than short-term plastic song features, because they probably have a larger influence on communication between individuals from different habitats (Figure 1.3a,b).

Chapter 3, “Do blackbirds signal motivation to fight with their song?” concerns another playback experiment testing for motivational signalling conducted in the city of Leiden. It has been suggested that there is considerable intra-individual variation (i.e. plasticity) in the temporal song structure of blackbirds reflecting the willingness to fight of individuals (Dabelsteen 1984a; Dabelsteen & Pedersen 1990). Agonistic motivational status might depend on the intensity of territorial interactions. Territory density of blackbirds in cities is higher than in forests (e.g. chapter 4) implying habitat-dependent differences in the number of territorial interactions. Habitat-dependent density differences might therefore translate into habitat-related song differences between urban and forest populations via short-term plastic song features reflecting agonistic state. The idea of the playback experiment was therefore to study the relationship between song variation and willingness to fight in order to understand the possible causes of song divergence at the population level. More specifically, we tested

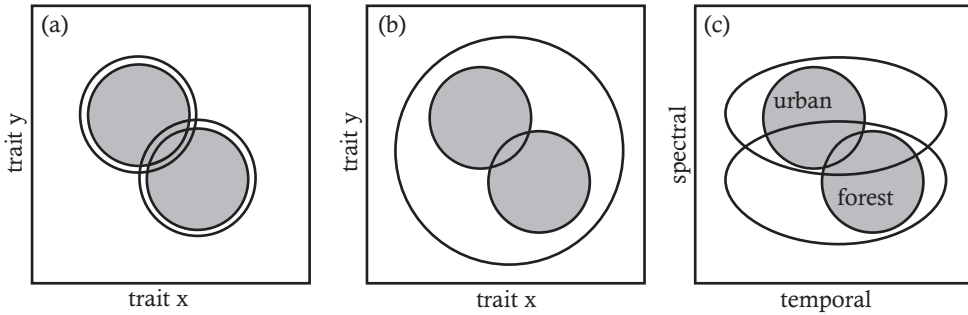


Figure 1.3. Schematic illustration of the possible role of plasticity on song divergence between populations. This illustration is based on figure 3 of Slabbekoorn and Smith 2002a and figure 2 of Podos et al. 2004. Gray areas indicate the realized acoustic variation within a population (i.e. the range of song characteristics produced by all males of one population). The black lines (circles and ellipses) represent the range of potential song variation of a population (i.e. the range of song characteristics that adult males in a population can potentially learn and produce in all conditions they could encounter in both their own and other populations). (A) A situation with song divergence between two populations with fixed song traits. The realized and potential variation within populations is practically the same. The divergence between populations is the result of adaptations or drift over generations as the song characteristics within adults are fixed. (B) A situation with flexible song traits and song divergence between populations. The two populations have a different realized song variation, but the same potential variation. In this situation adult birds have a wider range of potential song variation than they actually produce due to plastic song characteristics. The song divergence between populations is due to different local conditions related to for instance acoustic environmental factors and social circumstances. The adult birds produce songs matching current local circumstances implying that when they disperse to another population they can sing like local birds. (C) Schematic overview of the results on blackbird song divergence between urban and forest populations. The y-axis shows variation in spectral song features and the x-axis shows variation in temporal song features. There is a spectral and temporal difference in realized acoustic variation between urban and forest populations. Potential variation for the spectral features differs between urban and forest populations as there is little plasticity in spectral features. The spectral divergence is likely due to an adaptation to anthropogenic noise in cities accumulated over generations. On the other hand, potential temporal variation is the same for both habitats, since adult blackbirds have a wide range of intra-individual variation in temporal song traits. The temporal divergence is due to differences in current local conditions related to number of rival males and seasonality. The figure illustrates that birds switching to another habitat can probably change their songs temporally, but not spectrally, to cope with novel conditions.

whether song variation predicted a male's response strength to playback and whether there were intra-individual song differences before and after the playback.

Results showed that song characteristics of spontaneous singing territorial males did not predict whether an individual would respond aggressively to playback. However, individuals that responded aggressively increased their twitter frequency when comparing songs produced before and after the playback. This suggests that intra-individual variation in twitter frequency reflects individuals' motivational state to some extent. That no effect of pause duration or twitter proportion was found in this playback experiment contradicts other studies (Dabelsteen 1984a; Dabelsteen & Pedersen 1990). We discuss in this chapter that this could be due to methodological differences related to the setup of the playback experiments.

In chapter 4, **"Habitat-related birdsong divergence: a multi-level study on the influence of territory density and ambient noise in European blackbirds"**, I investigated in the city of Leiden and the nearby forest of Meijndel whether variation in song characteristics between males match local conditions with respect to noise level and territory-density (i.e. number of other nearby singing territorial blackbirds). Such correlations would indicate that

short-term plasticity within individuals might underlie song divergence at the population level instead of fixed song differences between urban and forest birds.

The results of the comparison between urban and forest birds at the population level confirmed the earlier findings of chapter 2. Urban birds sing with a higher frequency in the motif- and twitter parts of their song, have a larger twitter proportion as well as longer intersong pauses than conspecifics in the forest. Furthermore, territory density was higher in cities suggesting more vocal interactions between males. Across individuals, there was no correlation between ambient noise and any of the song characteristics. In contrast, temporal song features, but not spectral ones, were correlated with territory density and seasonality at the individual level. The combination of the influence of these two factors at the individual level appears to be sufficient to explain the observed divergence in temporal song structure at the population level. Hence, these findings on the temporal song features show that short-term individual variation can be responsible for habitat-dependent song divergence at the population level. Furthermore, the divergence in spectral features between habitats seems not or hardly attributable to plastic song differences between urban and forest birds.

Finally, I compared morphological characteristics and genetic neutral markers between free-living birds from two city-forest pairs in **chapter 5, “Morphological and genetic differentiation between urban and forest blackbirds”**. Pairwise comparisons between birds within a city-forest pair showed the existence of significant morphological differences. Males, and possibly also the females, from the city are heavier than conspecifics in the forest. Urban males have shorter bills and tarsi than forest males in one of the city-forest pair, but not in the other. These findings show that morphological differentiation can occur at a very small geographic scale.

The analysis with microsatellites showed that all four populations are genetically significantly different from each other including the urban and adjacent forest populations within pairs. This indicates that population differentiation can occur at short geographic distances. Furthermore, results of genetic assignments tests show that study populations of the same habitat are more similar to each other than populations of different habitats with no difference in genetic similarity between urban populations and forest ones. These findings suggest that it is likely that habitat played an important role in genetic population divergence either via habitat-dependent colonization or habitat-dependent gene flow between existing populations.

Synthesis

Morphological differentiation

I show that there are various morphological differences between urban and adjacent forest populations. This illustrates that morphological differentiation can occur at short geographic distances. Several other studies have also looked at the influence of urban habitat on blackbird morphology. An evaluation of the results of these and studies and my own shows an interesting pattern with populations of urban birds having almost always shorter bills, often longer wings

and sometimes smaller tarsi than populations in nearby non-urban areas. This general pattern does not apply to all urban-rural pairs, because there are various cases in which in one or more traits no difference or even the opposite pattern is observed. This could be the result of founder effects (Grant & Grant 1995; Saccheri *et al.* 2006) or locality-specific habitat differences such as microclimatic circumstances, food availability and predator abundance. Nevertheless, the moderately consistent divergence between urban and non-urban populations in some traits points at possible habitat-dependent selection pressures as a driving force.

In theory, morphological differentiation could lead to song divergence like shown in Darwin finches (Podos 2001; Podos & Nowicki 2004), where relatively large cumbersome beaks and body sizes are related to songs with rather narrow frequency bandwidths and slow rates of syllable repetition due to physical constraints on song production. However, it does not seem likely that morphological divergence is the cause of the acoustic differences observed in our study populations. The direction of acoustic divergence was the same in all city-forest pairs in contrast to the observed differentiation in bill length. Body weight showed a consistent pattern with urban birds being heavier than conspecifics in nearby forests. In many animals size and frequency are negatively correlated due to physical constraints of the vocal apparatus (Bradbury & Vehrencamp 1998; Mager *et al.* 2007). If body weight would affect song characteristics it would therefore most likely cause a decrease in frequency in urban birds rather than the observed rise in frequency. To conclude, morphological differences do not seem to influence habitat-dependent song divergence between urban and forest populations.

Genetic differentiation

I show significant genetic differences at neutral markers between urban and forest populations. There are in birds only a few other examples of genetic differentiation between nearby areas presumably due the ability of birds to disperse over long distances. A cross-fostering experiment with great tits (*Parus major*) from two qualitatively different parts within one forest separated by ~3 km showed significant differences in nestling condition and shape between birds originating from either low- or high density areas within the same forest (Shapiro *et al.* 2006). These were interpreted as genetic adaptations at a small geographic scale to local conditions related to competition for food and nesting availability. Furthermore, a study on urban and rural populations of house finches (*Coproductus mexicans*) located 6-10 kilometres apart showed genetic divergence in bill shape as well as genetic differentiation at neutral markers probably related to habitat differences (Badyaev *et al.* 2008). These studies and our own thus indicate that genetic differentiation between nearby areas is possible in birds in case locations are ecologically sufficiently distinct.

In our study, individuals were more likely to be assigned to populations with the same habitat as an individuals' home habitat than to populations of another habitat. There were no differences between urban or forest individuals in how likely they were to be assigned to the other study location with the same habitat type as their own. A large-scale study across Europe with distances of usually several hundred kilometres between city-rural pairs found a slightly different pattern as the assignment tests in this blackbird study showed that rural

populations are less differentiated from each other than urban populations, which suggests that urban populations often had a rural location as their source population (Evans *et al.* 2009b). They interpreted this as evidence for a scenario in which colonization of cities occurred via independent events across Europe. However, at shorter distances it is more probable that urban birds have colonized new nearby cities in a leapfrog manner (proposed by Luniak *et al.* 1990). Furthermore, at short distances there is more chance of habitat-dependent gene flow after colonization. This is supported by the finding in our small-scale study showing that urban populations are genetically just as similar as forest ones. Habitat-dependent colonization or habitat-dependent gene flow between existing populations therefore appears to influence population divergence when locations with similar habitats are close to each other.

Song plasticity influences acoustic population divergence

The chapters in this thesis concerning blackbird song showed some interesting and novel findings. There is divergence in several song characteristics between urban and forest blackbirds with habitat-related variation in both anthropogenic noise levels and social circumstances appearing to play an important role in this divergence.

There is substantial intra-individual variation in various song characteristics of blackbirds. This intra-individual variation is related to the local and current social circumstances of a male. Males increased their twitter frequency when they responded aggressively to a playback that simulated an intruder. Furthermore, an increase in territory density was correlated with an increase in twitter proportion and shortening of the intersong pause duration. Additionally, twitter proportions and pauses became larger during the breeding season. The results are not fully congruent as temporal structure was not related to motivation to fight in the playback experiment, whereas no effect of territory density or seasonality on twitter frequency was found in the analysis at the individual level. The results from the playback experiment testing for a relationship between aggressiveness and how an individual is singing are also not in line with previous studies (Dabelsteen 1984a; Dabelsteen & Pedersen 1990). These earlier studies suggested that motivation to fight is reflected by the temporal song structure, which is similar to results from our correlational analysis at the individual level. It thus seems that particularly the results from our playback experiment testing for motivational signalling are not congruent with other studies. This might be related to experimental differences in the setup of studies such as collecting data during slightly different moments in the breeding season or measuring whether song variation of territorial males predict their responses to playback instead of measuring whether responses of territorial males depends on variation of playback song simulating intruders.

Nevertheless, it is clear that song variation related to social circumstances plays a large role in the song divergence between urban and forest blackbirds. Especially the temporal divergence between urban and forest males seems to be primarily the result of short-term individual variation related to habitat-dependent differences in territory density and seasonality. This is an important finding with general implications for research on the role of signal divergence in population differentiation. It implies that not all signals that consistently

vary between habitats are equally likely to affect gene flow via a divergence in response (Figure 1.3). Signals differing between habitats due to short-time individual variation are for instance unlikely to act as a signal of local ecological adaptiveness in mate choice. This applies to acoustic signals in birds, but may apply to a wider taxonomic range including for instance frogs and primates (Ryan *et al.* 1990; Sugiura *et al.* 2006; Koda *et al.* 2008; Schwartz *et al.* 2008).

The same idea might apply to other signal modalities, such as olfactory and visual ones, provided they can change relatively rapidly with context varying consistently between habitats, although there are to my knowledge no studies investigating this. More investigations on individual signal flexibility are therefore required for a better understanding of the impact of population-level signal divergence on hybridization and speciation.

Habitat-dependent divergence in song and response to song

Motif frequency is a song characteristic that consistently differs between urban and nearby forest populations, and which is most likely independent of social context as it is not related to territory density or seasonality, nor does it vary with probability of attack or change when individuals respond aggressively towards playback. Anthropogenic noise is very likely to be a driving force underlying the increase in motif frequency sung by birds in cities compared to conspecifics in forests. In general, the background noise is much louder in cities relative to forests, particularly in the lower frequencies up to roughly 3 kHz. This anthropogenic noise is in the range of the motif part and an increase in frequency in urban environments will thus lead to an improved signal-to-noise ratio and therefore better audibility.

In contrast to other species (Slabbekoorn & Peet 2003; Fernández-Juricic *et al.* 2005; Wood & Yezerinac 2006), there is in blackbirds no correlation between frequency use and anthropogenic noise at the individual level. This suggests the presence of species-specific explanations for noise-dependent acoustic divergence at the individual level, which are possibly related to song learning processes as well as constraints related to singing style on auditory feedback and feedback via social interactions. It would therefore be worthwhile to study more taxonomic groups to get a better understanding of noise-dependent acoustic variation.

The reciprocal playback experiment on the effect of motif frequency and origin of a song shows an interesting pattern. Only a few studies have provided playback evidence for an impact on responses to ecologically diverged traits (Brambilla *et al.* 2008; Patten *et al.* 2004). Furthermore, the relevance of the origin of a song has been demonstrated in several other bird species: individuals usually respond stronger to local songs than non-local songs (McGregor *et al.* 1983; Searcy *et al.* 2002; Tomback *et al.* 1983). My playback experiment is the first in which the influence on response of a diverging ecological trait and the origin of a song were tested simultaneously and results show that both are important. Territorial male blackbirds pay attention to the motif frequency, because individuals from the city and the forest respond differently to songs with either a high or low frequency. Furthermore, blackbirds make a distinction between local versus non-local songs. Both the frequency of the diverging motif part and the origin of the song may affect dispersal and could influence

habitat-dependent gene flow through settlement advantages or assortative mating depending on shared ancestral habitat (Baker & Cunningham 1985; Irwin & Price 1999; Slabbekoorn & Smith 2002a). Considering the urban habitat is on an evolutionary timescale relatively novel, the habitat-dependent divergence in song and response to song in blackbirds suggests that in general habitat-dependent signal divergence can rapidly arise and could already play a role in the first stages of population differentiation.

Future research and implications

To conclude, the relatively recent colonization of urban areas by blackbirds has rapidly resulted in evolutionary changes leading to habitat-dependent differentiation. This is not only in ecological traits such as morphology, but also in song, which is an important sexual trait. As discussed above, not all diverging song characteristics are equally likely to lead to a habitat-dependent difference in response. Nevertheless, in at least one of the diverging song traits there is already habitat-dependent differentiation in response to song between urban and forest males, which shows that habitat-dependent responses can also evolve rapidly. In addition, urban and forest populations are nowadays no longer panmictic, despite them usually being adjacent to each other, indicating a possible habitat-related reduction in gene flow between urban and forest populations.

This rapid differentiation of blackbirds in our urbanizing world thus makes it a very suitable species to study processes involved in the initial steps of population divergence in general. It would for instance be worthwhile to have a closer look at the fitness consequences of divergence in song. One relatively simple way to do this would be to compare reproductive success of individuals singing typical urban or forests songs in both habitats. Moreover, a playback experiment with females in the lab could verify whether the observed effect of habitat-dependent song divergence on male responses is also present in females. This is important as, although male responses may provide insight into rate and direction of successful male dispersal, female mate choice decisions also play a key role in gene flow patterns. Experimental exposure of young males, which are learning to sing, to various noise conditions and adult songs can help to improve the understanding of the mechanisms underlying noise-dependent song divergence. The importance of environmental and genetic components in the divergence of ecological traits between urban and forest populations can be studied in cross-breeding experiments.

Importantly, these suggested studies can not only provide fundamental scientific insight, but can also have implications for conservation programs. They might provide insight in the dynamics between populations occupying urban and non-urban areas. Furthermore, such studies can help to evaluate why and how some species adapt and thrive in the novel urban circumstances, whereas other species cannot and therefore disappear. Such knowledge is required to understand which consequences the global expansion of urban areas will have on biodiversity. Hence, it could be used to develop effective actions for conservation purposes.

