Relating phenotypic and genetic variation to urbanization in avian species: a case study on House Sparrows (*Passer domesticus*)

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Relating phenotypic and genetic variation to urbanization in avian species: a case study on house sparrows (*Passer domesticus*)

Fenotypische en genetische variatie in relatie tot urbanizatie bij vogelsoorten: de huismus (*Passer domesticus*) als case-study

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Dankwoord

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General introduction
General introduction

As metropolitan areas are currently sprawling at unprecedented rates, urban species are expected to comprise a significant component of global biodiversity in the future (Chace and Walsh 2006). While this phenomenon has nourished a fast-growing scientific interest in urban ecology, our understanding of ecological and evolutionary consequences of urban sprawl is yet rudimentary (Marzluff et al. 2001). Urbanization affects the distribution and abundance of species as it tends to homogenize urban avifauna by reducing both species richness and evenness. While this has been true for most native species, opposite trends have been reported for densities of non-native ones (Marzluff et al. 2001).

For long, the house sparrow (Passer domesticus) has represented one of the rare exceptions to this pattern of declining native biodiversity in urbanized areas. Traditionally, house sparrows have been forming commensal\(^1\) relationships with mankind allowing them to invade numerous habitats in various parts of the world (Summers-Smith 1963). While they initially thrived well in response to urbanization, in recent decades this species has suddenly suffered massive declines most pronounced in highly urbanized city centers (Chamberlain et al. 2007, De Laet and Summers-Smith 2007). The universal decline of house sparrows in a constantly changing urban environment is not merely an intriguing biological phenomenon per se, but could potentially be a forerunner of a larger ecological problem, ultimately affecting entire urban ecosystems.

The combination of its almost obligate relationship with humans (Anderson 2006), its appearance in a range of diverse habitats and the sudden but heterogeneous collapse of house sparrow numbers provides an excellent opportunity to explore how phenotypic and genetic characteristics of this species respond to urban gradients. Here, I investigate whether two morphological measurements could function as bioindicators for the detection and quantification of presumed stress\(^2\) associated with increased urbanization. Such identification and implementation of cost-efficient indicators of environmental and/or genetic stresses before populations become irreversible affected (‘early warning systems’ \textit{sensu} Clarke et al. 1986, Clarke and McKenzie 1992, Clarke 1995) would be highly beneficial for conservation purposes. Next, using highly polymorphic microsatellite markers I explore how, and to what extent, genetic

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1 Commensalism is defined as the interaction between two species in which one species benefits from the relationship while the other is unaffected by it (Anderson 2006).

2 We define ‘stress’ as the consequence of any factor that alters an organism’s energy budget in terms of energy acquisition or allocation to maintenance and reproduction (Grime 1989, Beyers et al. 1999).
variation covaries with urbanization in contemporary house sparrow populations and whether putative detrimental genetic effects have already emerged.

**AN OVERVIEW OF THE HOUSE SPARROW DECLINE**

Until recently house sparrows were among the most common birds in Europe (Summers-Smith 1988). Numbers of breeding pairs in the UK in the early 1970’s were estimated between 12 and 15 million (Crick et al. 2002). To my knowledge, little information on house sparrow numbers is available for Flanders. Although rather rough approximations, the number of breeding pairs were estimated at 715,000 pairs in the mid 1970’s and 500,000 at 1985 (VLAVICO 1989). Throughout history house sparrows have been regarded as pest species damaging standing crops, transmitting diseases and negatively affecting native avifauna after (un)voluntary human introductions (Anderson 2006). However, nowadays it is widely acknowledged that this species has suffered major declines, shifting its ‘pest status’ to a ‘near threatened’ one (Birdlife International 2006). Yet, while the majority of observed trends describe a substantial decline, this pattern is far from consistent. For instance, census counts suggest that not only the onset of the rural decline preceded the urban one, but that the strength and outcome of the decline, too, differ between both regions. In the rural area the decline has stabilized whereas the urban decline has been more dramatic and seems to be still in progress, transforming (some parts of) city centers into ‘sparrow free’ areas (Robinson et al. 2005; De Laet and Summers-Smith 2007). Finally, in suburban areas (i.e., consisting of outskirts of the city and its suburbs and characterized by lower building density and larger quantity of vegetation; Heij 1985), numbers have been less affected showing only minimal signs of population reductions (figure 1).

This coarse-grained heterogeneity in population dynamics becomes even more complicated as deviating trends have been reported both between and within city centers (Summers-Smith 2003). One of the best documented declines is the one in Kensington Gardens where autumn counts go back as far as 1925 (Sanderson 1996, Vincent 2005). While the local population size was estimated at 2603 birds in 1925, numbers gradually declined to 885 in 1948, most likely due to a reduction in food availability following the replacement of horses by motorized vehicles (Summers-Smith 1988, Anderson 2006). In the 1980’s, this gradual population decline was complemented by a sudden population collapse which resulted in a census count of only eight (!) birds in 2000. Other long-term
**General introduction**

Fig. 1. House sparrow population trends based on census counts between 1970 and 2000 in three different habitats. Rural abundances are based on the BTO Farmland Population Indices; suburban ones represent census data from four suburban villages in the UK (Stockton, Crewkerne, Tranent and Guisborough); urban abundances are based on counts performed in London, Edinburgh, Glasgow, Hamburg and Dublin (source: De Laet 2007).

monitoring schemes showed broadly similar trends in the greater London area (De Laet and Summers-Smith 2007). Likewise, house sparrows have shown a 90% decline in the center of Edinburgh (Dott and Brown 2000) and numbers have been declining by more than 50% in Hamburg during the last three decades (Mitschke et al. 2000). Notably, most populations in Berlin and Paris seem to lack such a decline (Summers-Smith 2003) while, in general, sparrow populations in Scotland and Wales even seem to have increased in numbers (Crick et al. 2002) (table 1). Such a complex pattern complicates the identification of an overall driving force behind the urban collapse but rather suggests a combination of causal factors. Moreover, this urges for detailed studies conducted at a small scale to allow subtle differences within habitats to be detected.

**PUTATIVE CAUSES OF THE HOUSE SPARROW DECLINE**

**Predation**

The two most cited candidate predators that could substantially affect house sparrow numbers are the domestic cat (*Felis catus*) and the sparrowhawk (*Accipiter nisus*). Depending on the distance from human settlements, sparrows comprised between 9.2% and 35% of the diet of sparrowhawks, where lower percentages were associated with more remote areas (Tinbergen 1946, Opdam 1979, Frimer 1989). During field work conducted within the framework of this
thesis, multiple predation events of house sparrows by sparrowhawks have been observed (see discussion Chapter 1). While in the past, the possibility of sparrowhawks as a causal factor of the sparrow decline has generally been dismissed (Newton et al. 1997, Thomson et al. 1998), recent papers (MacLeod et al. 2006, Bell et al. 2010) advocate such conclusions may have been premature. Firstly, sparrowhawks, like many other raptors, have shown a marked increase in population numbers over the last 30 years thanks to legal protection and banning of pesticides like DDT (Lensink 1997, Anderson 2006). Secondly, the onset of the urban house sparrow decline corresponds to the timing at which sparrowhawks have started to colonize the urban habitats (Anderson 2006, Bell et al. 2010). Prior to the urban invasion of sparrowhawks, large periods of predator release may have allowed natural selection to select against behavioral strategies associated with anti-predator defence in urban house sparrows (Blumstein and Daniel 2005) rendering them incapable of instantaneously adjusting their behavior in response to a sudden predator introduction (Steadman 2006). Hence, this sudden arrival of a new predator may have disproportionately increased the vulnerability of urban house sparrows. Thirdly, difference in timing between urban and rural sparrowhawk recovery matches patterns of urban and rural house sparrow decline (Bell et al. 2010). Based on a logistic model, Bell et al. (2010) showed that house sparrow numbers were on average stable or increasing prior to the return of sparrowhawks, but declined

<table>
<thead>
<tr>
<th>City</th>
<th>Overall trend</th>
</tr>
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<tbody>
<tr>
<td>Berlin</td>
<td>Stable</td>
</tr>
<tr>
<td>Bristol</td>
<td>Decline</td>
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<tr>
<td>Dublin</td>
<td>Decline</td>
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<tr>
<td>Edinburgh</td>
<td>Decline</td>
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<tr>
<td>Hamburg</td>
<td>Decline</td>
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<tr>
<td>Lisbon</td>
<td>Increase</td>
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<tr>
<td>London</td>
<td>Decline</td>
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<tr>
<td>Manchester</td>
<td>Stable</td>
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<tr>
<td>Moscow</td>
<td>Decline</td>
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<td>Norwich</td>
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<tr>
<td>Paris</td>
<td>Stable</td>
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<td>Prague</td>
<td>Decline</td>
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<tr>
<td>Rotterdam</td>
<td>Decline</td>
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<tr>
<td>St. Petersburg</td>
<td>Decline</td>
</tr>
<tr>
<td>Warsaw</td>
<td>Slight decline</td>
</tr>
</tbody>
</table>

Table 1. Overview of European population trends (source: Shaw et al. 2008).
strongly afterwards. While the authors focused on predation per se, increased occurrence of predators can also have indirect detrimental effects on house sparrow behavior and physiology. For instance, foraging theory predicts that birds will tend to build up fat reserves at times of unpredictable food supply to lower the risk of starvation (Lima 1986, Houston et al. 1993). MacLeod et al. (2006) performed a comparative study among six islands, halve of which contained no sparrowhawks while in the other halve this raptor was resident. While sparrows in ‘predator-free’ islands behaved in a predictable fashion and gained body mass early at the day and during winter, such behavior was absent in predator dominated islands. MacLeod et al. (2006) argued that house sparrows did not regulate fat reserves in a manner to minimize the starvation risk but rather responded to mass-dependent predation risk, with a trade-off between flight performance and starvation risk that rendered them highly susceptible to changes in the predictability of food supply. While both studies are correlative and hence only provide suggestive evidence for a link between the house sparrow decline and predator incidence, these results emphasize that predator effects might be variable and complex.

Domestic cats have also been identified as potential predators of house sparrows (Churche and Lawton 1987; Woods et al. 2003) as it has been presumed that cats may kill up to 27 million birds in the UK in a span of 5 months only (Woods et al. 2003). In addition, Baker et al. (2005) reported predation rates of cats in an English village that were within the order of magnitude to have potentially detrimental demographic effects. However, such generalization assumes that preys brought in by cats constitute a random sample of the total house sparrow population. Mortality may not be driven by compensatory mechanisms such as age or disease in which case preys would merely represent a “doomed surplus” (Errington 1946), individuals which would have died anyway. In such instances, predation will be incapable of affecting overall population trends. Support for such a scenario was recently provided by Moller and Erritzoe (2000) who showed that immunocompetence of preyed birds were lower than that of non-preyed birds undermining the importance of cat predation as a causal component of the sparrow decline.

Lack of nest sites and change in habitat structure

House sparrows show a marked preference for dense vegetation (Wilkinson 2006). The decline of house sparrows has recently been linked to areas with high socio-economic status (Summers-Smith 2003, Vincent 2005, Shaw et al. 2008) as in the past, affluent areas have witnessed a substantial
transformation in habitat structure with loss of greenery and brownfield sites and ‘tidier’ gardens with higher proportion of paving and non-native ornamental shrubs (Shaw et al. 2008). Such increasing development and concomitant loss of foraging habitat has had an impact on the availability of both weedy areas as well as invertebrate food. Insects provide chicks an essential protein boost a few days after fledging and the absence of insect food can have devastating effects on breeding success when availability is limited (see below) (Vincent 2005). Surveys have further shown a strong correlation between house sparrow abundance and the level of social deprivation with lowest numbers found in wealthy residential areas (Robinson et al. 2005). It has been speculated that such habitat change could be compounded by a lack of available nesting sites. House sparrows typically prefer holes or small crevices near roofs as a nesting site but modern or renovated buildings often lack such opportunities (Vincent 2005, Shaw et al. 2008). A nationwide survey held in the UK indicated house sparrows were avoiding newer buildings (build after 1985) or those that had undergo extensive roof repairs the last decade (Shaw et al. 2008).

Diseases

While the trajectory of a massive and sudden decline in house sparrow numbers is suggestive for an epidemic disease, little is known about the impact of diseases on this species, and in bird populations in general. One of the few well described examples of how epidemics can regulate population numbers is the study of mycoplasmal conjunctivitis in house finches (*Carpodacus mexicanus*) in the eastern half of North America. House finches experienced dramatic declines after the arrival of the epizootic but numbers have stabilized recently (Hochachka WM and Dhondt 2000). A myriad of pathogens have been identified in house sparrows (see Anderson 2006 for a detailed list) of which bacteria from the genus *Salmonella* and single-cell parasites from the genus *Isospora* have been frequently reported (Anderson 2006). While there has been anecdotal evidence that epidemic outbursts do occur in nature and can locally result in substantial population losses or lower nestling condition (Kruszewicz 1991), to date there is no strong support that disease is the culprit of the large-scale house sparrow collapse.

Food shortage

In contrast to the proximate causes of the urban decline which are mostly hypothetical, those of the rural decline are well established. Substantial evidence has accumulated that the rural house sparrow decline can be attributed to lack of
overwinter food availability. Among other farmland bird species, house sparrows have suffered severely from agricultural intensification such as the switch from spring to autumn sowing, thereby reducing the availability of winter stubbles, and more efficient grain storage (Chamberlain et al. 2000). Support was given by a study on the population ecology of rural house sparrows in Oxfordshire (Hole 2001, Hole et al. 2002). Their comparative study on stable populations and a declined one (numbers decreased with 81% since 1971) indicated no variation in breeding performance among study plots while a significantly decreased monthly overwinter survival rate was only apparent in the declining population. Experimental support was further provided by the positive response of survival rates to supplementary feeding in the population that suffered a severe decline in house sparrow numbers while rates remained unaffected in the other ones. While such a scenario is unlikely to account for the urban house sparrow decline, evidence has mounted that in the urban area summer, rather than winter, food supply may be problematic, more specifically the availability of invertebrates for young chicks. Vincent (2005) measured reproductive success along an urbanization gradient in Leicester, UK and reported high rates of chick and whole-brood starvation during June and July in the highly urbanized habitat. A stochastic simulation revealed that in two out of three years juvenile recruitment did not reach the threshold required to sustain population viability due to low chick survival and body mass at fledging (a well-established predictor of post-fledging survival (Hole 2001)). High levels of vegetable material in the diet, low aphid densities or high levels of NO₂ from traffic pollution were highly associated with reproductive failure (Peach et al. 2008). They argued that reduced reproductive output due to low aphid abundance in the immediate vicinity of nesting sites was a plausible underlying mechanism of the urban decline. Other studies in the center of Hamburg have provided further support that brood starvation due to a shortage of insects may cause population declines (Bower 1999, Mitschke et al. 2000).

Hole (2001) further argued that populations subjected to such strong food constraints might become ‘demographic sinks’, i.e. depending on emigration from nearby source populations with a demographic excess. He further contended that due to the sedentary nature of house sparrows, insufficient movement between adjacent populations may result in local extinction causing increased isolation of remnant populations. Such a self-reinforcing spiral of local-extinction and loss of connectivity could then “spread as a’contagion’ through the landscape” (Hole 2001).
Environmental pollution

Several authors have evaluated the association between electromagnetic pollution from mobile phone base stations and house sparrow abundance (Everaert and Bauwens 2007, Balmori and Hallberg 2007, Balmori 2009). While direct effects on avian reproduction or survival cannot be excluded, a more likely effect of mobile phone radiation is the reduction of the reproductive capacity of insects (Balmori 2009). Census counts revealed low abundance of house sparrows with increasing intensity of environmental radiation and a general avoidance of high radiation areas (Everaert and Bauwens 2007, Balmori and Hallberg 2007). However, as the decline has been most severe in highly urbanized areas and the number of mobile base station masts has been increased especially in the most densely populated habitats, such confounding associations may give rise to the observed correlation without any causal link between them. Other environmental pollutants related to increased traffic such as vehicle exhaust emissions (NO₂) have been linked to sparrow declines as well (see above). In addition, the shift from leaded to unleaded petrol coincided with the onset of the sparrow decline in the eighties (Robinson et al. 2005). Lead-free petrol contains the known carcinogen and volatile oxygenate methyl tertiary-butyl ether (MTBE, Summers-Smith 2003, Robinson et al. 2005, Vincent 2005) and while both MTBE and NO₂ are unlikely to affect higher organisms in a direct way (Summers-Smith 2003), it may have an indirect impact through its detrimental effect on aphid densities (Summers-Smith 2003, Vincent 2005, Peach et al. 2008). However, to date such effects on insects are not yet fully understood (Bignal et al. 2004) and it remains unclear why other species such as tits that also feed on aphids were not affected (Robinson et al. 2005), apart from the fact that species may differ in their plasticity to shift to alternative prey (Vincent 2005).

While speculations over the exact cause(s) remain an open debate, there is little, if any, doubt about the existence of an urban house sparrow decline. The driving agent(s) behind the declines will undeniably increase experienced levels of stress and may therefore be accompanied with phenotypic changes (physiological, immunological) at the individual level. In addition, as population sizes become smaller, populations will inevitably be exposed to increasing levels of genetic threat. As such this may result, together with phenotypic changes, in the presence of a phenotypic and genetic signature in the population. In the following sections I briefly review two phenotypic biomarkers of stress (fluctuating asymmetry and ptilochronology) which have become popular tools in ornithological studies due to their ease of application. Subsequently I outline
some of the genetic consequences and threats which declining populations may face.

**INDIVIDUAL-BASED BIOMARKERS OF STRESS**

One of the main focuses of many ecologists and conservation biologists is to find a quantitative metric that allows the early identification of populations under severe stress. The possibility to detect detrimental changes before populations have become irreversibly affected (‘early warning system’, Clarke et al. 1986) will make such tools an invaluable asset to conservation managers as it will allow them to assess whether or not situations urge for remedial action. Ptilochronology and fluctuating asymmetry are two stress indices which have been used and evaluated extensively in the literature. Conservation biologists have conveniently used these measures to document differences between individuals and populations, allowing them to approach their health in a non-invasive manner and link these stress indicators to environmental quality, reproductive output and survival probabilities in an attempt to assist management policies.

**Ptilochronology**

The concept of ‘ptilochronology’ was first introduced by Grubb (1989) and is best described as the study of growth bar size in feathers to infer individual body condition. Much similarity exists with dendrochronology or the analysis of tree-rings which uses patterns of light and dark rings to estimate the age of trees. Likewise, growth bars are alternating dark and light bars, perpendicular to the rachis of a feather (figure 2).

![Fig. 2. Alternating dark (black arrow) and light (white arrow) growth bars on a tail feather.](image)

While the exact underlying mechanism of such a pattern remains to be determined (Grubb 2006), it has been proposed that a shift from a dark to a light band is associated with a reduced blood pressure during sleep and concomitant
nutritional change of the feather follicle (Riddle 1908, Jovani et al. 2010). Jovani et al. (2010) showed that the transition from light to dark and vice versa was irrespective of the photoperiod under which the feather was grown, but rather mimics changes in physiological activity.

Growth bar size has been used as a proxy of an individual’s nutritional condition where narrower growth bars reflect periods of poor nutrition (Grubb 2006). Birds are considered to be very vulnerable to predation during moult and are expected to reduce such periods to the minimum (Grubb 2006). After allocating energy to vital processes such as thermoregulation and immune response, birds in better nutritional condition will be able to translate their surplus of energy into a faster regeneration rate compared to those in a poor condition. Measuring growth bars on original or induced feathers therefore allows researchers to obtain a blueprint of the energetic regime endured by the bird at the time the feather was grown. This has opened a range of opportunities and growth bar analyses have been used in conjunction with various topics like habitat quality, social behavior and reproductive effort (Grubb 2006). However, some essential premises need to be met to allow inferring body condition from growth bars. Firstly, exactly one pair of growth bars must resemble a 24h-period of feather growth (Murphy and King 1991). This assumption was confirmed in an elegant experiment of Brodin (1993) who used radioactive cystine as a food supplement, which is incorporated into the keratin matrix of feathers as they grow, and allowed him to reconstruct the growth patterns on a daily basis. Recently, other authors have also ascertained the relationship by measuring growing feathers on a daily basis in a variety of species (Grubb 2006, Jovani et al. 2010). Secondly, variation in growth bar size between individuals needs to accurately reflect relative differences in net energy intake. Such a negative association between growth bar size and food deprivation has been supported by various laboratory and field experiments (Grubb 1989, Grubb and Cimprich 1990). Body mass, or derivatives thereof, have likewise been used as proxies for body condition. Grubb (2006) argued that such indices may be more ambiguous than daily feather growth because they are more prone to confounding factors such as daily fluctuations or adaptive foraging strategies. As mentioned before, body mass in house sparrows may sometimes better predict predation pressure rather

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3 “Nutritional condition is defined as the state of body components controlled by nutrition and which, in turn, influences an animal’s fitness. I consider nutritional condition to be synonymous with condition, body condition and nutritional state” (Grubb 2006).
General introduction

than individual condition (MacLeod et al. 2006), while in willow tits socially subordinate, and not dominant, birds maintained the highest weight (Ekman and Lilliendahl 1993). Subordinates gained more weight in response to lower food predictability but became much lighter if dominant birds were removed. In both cases, inferring nutritional condition from body mass would most likely result in spurious outcomes.

Fluctuating asymmetry

Symmetry is a ubiquitous feature of living animals although perfect symmetry will often only be approximated and seldom reached. Although many different forms of symmetry exist in nature, bilateral and radial symmetry have been studied most extensively in the past (Graham et al. 2010). Under the most benign conditions, a developmental trajectory results in the exact genetically predetermined phenotypic value, but under stressful conditions mechanisms that need to stabilize this pathway loose efficiency and this results in aberrant phenotypes (Clarke 1995). Developmental stability refers to the potency of an organism to correct for minor deviations from its pathway and to restore it to the original one. A way of estimating developmental stability is to measure the converse, developmental instability\(^4\) by examining the extent of random variation between left and right sides in a bilateral symmetrical trait. As both sides are under the control of a single genome and have experienced the same environment, the phenotypic outcome of both sides should be identical. However, environmental or genetic perturbations can cause random (i.e. with respect to side) fluctuations around the developmental pathway and the inability of individuals to withstand such perturbation may result in deviations from left-right symmetry without directional component at population level, termed “fluctuating” asymmetry (FA)(Palmer and Strobeck 1986, Palmer 1996, Polak 2003, Graham et al. 2010). The underlying mechanism of developmental homeostasis still remains largely unknown (Lens and Eggermont 2008), but some preliminary work suggests perturbations may affect inter-cell communication and rates of cell growth and elongation (Palmer 1994, McAdams and Arkin 1999, Fiering et al. 2000). Since the activity range of such random cellular processes is

\(^4\) Developmental homeostasis refers to the stabilized flow along a developmental pathway within a particular environment. Developmental instability (=developmental noise) is the process that disrupts development along the developmental trajectory within a particular environment and is what is being measured as fluctuating asymmetry. Canalization is the process by which a target phenotype develops under different genetic and environmental conditions (Polak 2003).
rather limited (McAdams and Arkin 1999), effects at each side would be largely independent and give rise to left-right asymmetries (Lens et al. 2002). Besides fluctuating asymmetry other forms of bilateral asymmetry can be distinguished in an animal or plant. Directional asymmetry reflects a consistent larger value at one side such as the asymmetry in flatfish. This should however not be confused with plasticity in vertebrate bones causing between-side differences due to differential use. Antisymmetry arises when asymmetry is the norm but the largest value varies among sides, for example the larger signaling claw in male fiddler crabs which appears either on the right or left side but with equal frequency (Leary and Allendorf 1989, Polak 2003). Both antisymmetry and directional asymmetry are the outcome of a normal development and are therefore not applicable as a stress indicator (Leary and Allendorf 1989). Experimental and empirical work has suggested that increasing levels of stress, both genetic as well as environmental, have resulted in a concomitant increase in levels of fluctuating asymmetry (Palmer and Strobeck 1986, Lens et al. 2002, Lens and Eggermont 2008) and therefore fluctuating asymmetry has been a popular tool to draw inferences about developmental homeostasis (Palmer 1996). Fluctuating asymmetry derived much of its appeal from having an ideal outcome, e.g. perfect symmetry, and even more important, from the belief that it constitutes a more sensitive stress indicator than more traditional fitness estimates (Clarke and McKenzie 1992, Clarke 1995, Lens et al. 2002). The opportunity to identify populations at risk before the onset of stress-mediated changes in fitness has gained wide approval by many conservation biologists (Leary and Allendorf 1989, Lens et al. 2002). Unfortunately, results on relationships between fluctuating asymmetry, stress and fitness have not always been consistent in the past which may hamper the utility of fluctuating asymmetry as a universal biomarker of stress. Instead, associations with stress have been highly variable and seem to be species, stress and trait specific (Bjorksten et al. 2000, Lens et al. 2002, Knierim et al. 2007, Hopton et al. 2009). For instance, traits under sexual selection on average show enhanced levels of FA, while natural selection will enhance the efficiency of buffering mechanisms controlling the developmental pathways of traits directly related to functional performance and thereby reduce levels of fluctuating asymmetry (Balmford et al. 1993, Kodric-Brown 1997). Despite recent advance, statistical issues may also have been an important source of the observed inconsistencies in literature. True effect sizes in FA relationships have been typically very small (Moller and Jennions 2002) while measurement error may bias FA estimates. Therefore several authors recommend to increase the number of measurements per trait and to combine multiple traits in a single analysis in
attempt to acquire an acceptable level of power (Van Dongen 1999, Lens et al. 2002).

**GENETIC THREATS OF SMALL POPULATIONS**

As populations become smaller and more isolated as a result of deterministic factors such as food shortage or predation, stochastic demographic and/or environmental processes may become more important and further reduce reproduction rates or increase mortality rates (Lande 1988, Smith et al. 2006). Furthermore, because not all individuals contribute equally to the next generation in natural populations, census numbers are sometimes more than ten times larger than the genetic effective population size\(^5\) allowing genetic factors to affect populations while demographic population risks remain negligible (Spielman et al. 2004, Smith et al. 2006, but see Lande 1988). Here I focus on stochastic genetic processes that may reinforce the rate of a population decline and hence contribute to the so-called extinction vortex (figure 3) (Gilpin and Soule 1986). Firstly, as populations become smaller, some alleles will become fixed via genetic drift while others will be lost, reducing the overall genetic diversity. Preserving genetic diversity is one the major goals of the World Conservation Union (IUCN) and based on two arguments: (i) genetic variation allows populations to respond and adapt to changing environments, and is presumed to be positively associated with fitness (Reed and Frankham 2003); (ii) in small populations genetic drift may outperform selective forces and allow detrimental mutations to become fixed. As a consequence, mean population fitness can decrease which further decreases population size and increases the extent of genetic drift. Such a synergistic interplay between mutation accumulation and population size produces a downward spiral called a mutational meltdown (Lynch et al. 1995) and has recently been suggested as a potential candidate for population bottlenecks in natural populations (Rowe and Beebee 2003). In contrast to previous factors (e.g. drift and mutation) which will affect populations only in the long-term, inbreeding imposes an immediate threat to population persistence (Hedrick 2001, Keller and Waller 2002, Smith et al. 2006). Small populations promote mating between close

\(^5\) The effective population size is defined as the size of an idealized Wright-Fisher population which would result in exactly the same value of a specific genetic property as in the population in question. Different measures of effective population size have been described in the literature but two most commonly used are inbreeding effective population size and variance effective population size. Inbreeding effective population size predicts the rate of decrease in heterozygosity while the variance effective population size measures the variance of change in gene frequency resulting from one generation of genetic sampling (Wang 2005).
relatives which increases the probability that two deleterious homologous genes within an individual will become homozygous and thus phenotypically expressed. Inbreeding depression refers to the concomitant loss of fitness in response to inbreeding and has been demonstrated for many species (Keller and Waller 2002). Perhaps counter-intuitively, lower levels of inbreeding depression are expected in populations which have been small for a long time as most of the detrimental alleles will have been purged by natural selection. However, such a safety-mechanism will be less efficient or remain absent in populations which have suffered a recent and sudden collapse (Hedrick 2001, Reed 2009). As the pattern of the house sparrow decline rather corresponds to the latter, this species may be particularly vulnerable to immediate genetic threats. In addition, at least on islands, inbreeding has been shown to be high in wild house sparrow populations causing a reduction in recruitment rates of progeny when parents were closely related (Jensen et al. 2007).

Dispersal patterns shape the genetic structure of populations. As mentioned earlier, house sparrows are remarkably sedentary as they revisit the same breeding colony year after year, forage close to roosting and breeding sites and disperse over short distances (Vincent 2005, Anderson 2006). Dispersal is almost exclusively done by juveniles and they tend to settle within a few kilometers of their natal colony (Anderson 2006). Anderson (1994) measured dispersal distance as the difference between natal nest site and the location on which an individual was actually breeding. Mean natal dispersal distances were somewhat larger of females (arithmetic mean = 1.15 km - geometric mean = 0.29 km) compared to those of males (arithmetic mean = 0.69 km - geometric mean = 0.17 km). Using banding records Paradis et al. (1998) determined dispersal distances of 531 house sparrows that were banded as nestlings and recovered during a subsequent breeding period. The arithmetic mean dispersal distance was 1.7 km and the geometric mean was 0.21 km.

**STUDY AREA**

This study was carried out between 2003-2009 in and around the city of Ghent and the village of Zomergem, c. 12 km north-west of Ghent, in Belgium. A grid, each cell measuring 300m x 300m, was placed over the study area in Arcgis v9.2. Using a digital layer which contained an exact outline of all houses the ratio between built-up surface and grid size was calculated for each cell. I divided these ratios into three classes ranging from smaller than 0.10, 0.11-0.30 and larger than 0.30. Throughout this thesis we will refer to these classes as respectively rural,
suburban and urban ones. Depending on the research hypotheses formulated, I have applied different sampling schemes to select populations. For the first two chapters I applied a stratified randomized design and selected four 50-ha rural plots, five 50-ha suburban plots (eastern and northern populations were omitted for logistic reasons), and four 50-ha urban plots. For all thirteen selected plots I additionally obtained digitized maps of landcover using a combination of high resolution aerial photographs and ground truthing (figure 4). For a detailed description of each study plot I refer to Chapter 1. In the last three chapters, I used a different approach. Here, I selected the most central urban study plot and sampled populations with increasing distance from this center. To do this, I plotted six circles with increasing radius (each increment of approximately 700m) over our study area and sampled, as much as possible, two populations on each of these circles. This design was replicated in the rural area (figure 5). An

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**Fig. 3.** Schematic view of the extinction vortex. Open arrows decrease population size, while shaded arrows stimulate population recovery (source: Smith et al. 2006).
overview of all locations sampled is given in table 3. While sampling individuals we obtained the approximate population size for all suburban and rural populations (table 3). Within the city center of Ghent we have used a more detailed mapping-based survey technique to record house sparrow numbers. This survey was repeated three times during this study (June 2005, 2006 and 2009). The area was divided into four blocks and each block was surveyed as a ‘whole area search’. During these counting sessions all accessible routes, streets, parks and backyards (if possible) were covered. While such short-term monitoring schemes do not allow us to draw firm conclusions on whether or not house sparrows have shown marked reductions, we were able to obtain a rough estimate of house sparrow density within the area. The number of birds/ha was estimated as 0.57 sparrows/ha which is comparable to those of other large cities, such as London and Hamburg, which have well documented house sparrow declines (0.1-0.8 birds/ha) (Summers-Smith 2000). Population sizes varied slightly over the years but a preliminary exploration revealed no clear association between population numbers and meteorological data (mean temperature of the preceding summer (June-August), winter (December-February) and spring (March-May)) (see table 2). Figure 6 shows the spatial distribution of house sparrow populations within the city center. Population sizes were larger along the edge of the city center although even in these areas numbers were often much smaller compared to those of suburban or rural populations (see table 3) and especially in the innermost and southern part of the city center between-population distances were largest.

<table>
<thead>
<tr>
<th>Year</th>
<th>Temperature (°C)</th>
<th>Year</th>
<th>Temperature (°C)</th>
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<th>Temperature (°C)</th>
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<th>Temperature (°C)</th>
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<tbody>
<tr>
<td>Summer</td>
<td>2004 22.23 (0.65)</td>
<td>Winter</td>
<td>2005 6.23 (0.79)</td>
<td>Spring</td>
<td>2005 15.92 (1.16)</td>
<td>June</td>
<td>2005 23.50 (2.50)</td>
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<tr>
<td>2005</td>
<td>22.53 (0.93)</td>
<td>2006</td>
<td>4.92 (0.57)</td>
<td>2006</td>
<td>13.77 (1.44)</td>
<td>2006</td>
<td>22.5 (0.86)</td>
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<td>2008</td>
<td>21.29 (0.52)</td>
<td>2009</td>
<td>4.83 (0.84)</td>
<td>2009</td>
<td>15.71 (1.01)</td>
<td>2009</td>
<td>22.25 (1.79)</td>
</tr>
</tbody>
</table>

Table 2. Mean temperature (SE) of the preceding summer, winter and spring of each census period (June).
**General introduction**

Fig. 4. Example landcover maps for two locations per urbanization class - 'Urban' (upper) and 'Suburban' (lower).
Fig. 4. Example landcover maps for two locations per urbanization class - ‘Rural’.
General introduction

Fig. 5. Sampling design used for Chapters 1-2 (A) en Chapter 3-5 (B). Inner contour encompasses Ghent city centre and outer contour encompasses surrounding municipalities. Filled circles represent urban populations, open circles suburban ones and filled triangles rural populations. Abbreviations refer to populations described in table 3.
Fig. 6. Survey of house sparrow numbers within the city center of Ghent performed on June 2005 (left), 2006 (middle) and 2009 (right). The diameter of each point is proportional to the number of house sparrows. Abbreviations refer to populations described in table 3.
Table 3. An overview of all study plots, their abbreviation used throughout this thesis, the designated urbanization class (U= urban, SU= suburban, R= rural), the approximated population size (based upon observations while sampling), the type of data obtained from each plot, and the respective chapter(s) in which they occur.

<table>
<thead>
<tr>
<th>Location</th>
<th>Abbreviation</th>
<th>Urbanization class</th>
<th>Population size (individuals)</th>
<th>Home range Ch.1</th>
<th>Ptilochronology Ch.1,2</th>
<th>FA Ch.2</th>
<th>DNA Ch.3,4,5</th>
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OBJECTIVES AND THESIS OUTLINE

The ultimate aim of this thesis is to broaden our current knowledge on phenotypic and genetic variation in avian species along an urban-rural gradient. Quantifying such variation is a prerequisite to understanding how and to what extent urbanization affects avifauna and how organisms respond to different environments (Blondel et al. 2006). I contrasted different methods and measures to explore how this variation along different urbanization classes is best captured. I selected house sparrows as a model organism as this species has a long tradition of being a successful urban resident, although it experienced a sudden urban collapse several decades ago, and occupies the entire range of the urbanization gradient, i.e. they can be found in both sparsely populated rural areas as well as in densely populated and highly built-up areas. In addition, the reported spatial variation in demography and presumed levels of stress (Vincent 2005, Shaw et al. 2008, Peach et al. 2008) offer a unique opportunity to investigate whether i) the
extent of daily movements are associated to levels of urbanization and/or levels of nutritional stress, ii) urbanization covaries with phenotypic characteristics of environmental and genetic stress, iii) different urbanization classes represent different genetic entities and iv) populations along an urbanization gradient are characterized by small-scale spatial variation in population structure.

To answer these questions we integrated data on behavioral radio-tracking (chapter 1) with morphological stress indicators (chapter 1-3) and genetic markers (chapter 3-5) in house sparrows sampled along an urbanization gradient. This thesis is essentially a compilation of five chapters which were either published or submitted to various journals. Therefore, to some extent duplication may be present throughout this thesis (genetic protocols, figures of the study sites, ...) although I have tried to keep this to a minimum. Table 3 visualizes the complementary data sets collected from each location.

In Chapter 1 I use radio-telemetry to explore how and to what extent home range sizes vary among different urbanization classes. I subsequently combine this information with high-resolution maps of landcover and indirect estimates of body condition (obtained from ptilochronological data). In Chapter 2 I evaluate the utility of fluctuating asymmetry as a biomarker for environmental stress. Here, I perform a correlative study between an a priori defined nutritional stress (Chapter 1) and levels of developmental instability. In addition, I discuss potential pitfalls related to the application of such individual-based proxies of stress. Analyses are conducted at individual and population level and replicated for two morphological traits. Chapter 3 highlights the association between fluctuating asymmetry and genetic diversity. The heterozygosity hypothesis states that high levels of genomic diversity result in a broader range of biochemical substances which prohibit developmental pathways over a wider range of conditions from being distorted through random perturbations. In this chapter I assess whether there is local or genome-wide evidence for such a hypothesis and whether the strength of association is related to the level of urbanization. In Chapter 4 I investigate the genetic population structure of 26 house sparrow populations and explore how genetic variation is hierarchically partitioned among populations and regions. Chapter 5 describes the fine-scale relatedness structure in both the urban and rural area. I therefore use simulated distributions of known relatedness categories to estimate proportions of close kin and assess the geographical variation in kinship distributions. In a closing (General Discussion) chapter, I integrate the results presented in chapters 1-5, discuss potential implications for the conservation of house sparrows, and highlight topics that need further investigation.
General introduction

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General introduction


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Shaw LM, Chamberlain D and Evans M. 2008. The house sparrow *Passer domesticus* in urban areas: reviewing a possible link between post-decline


Constraints on home range behaviour affect nutritional condition in urban house sparrows (*Passer domesticus*)

Carl VANGESTEL, Bart P. BRAECKMAN, Hans MATHEVE, and Luc LENS


©Chantal Deschepper
Chapter 1

ABSTRACT

In human-dominated landscapes, (semi)natural habitats are typically embedded in tracts of unsuitable habitat. Under such conditions, habitat characteristics and grain size of the surrounding landscape may affect how much food, and at what cost, is available for sedentary species with low home range plasticity. Here we combine behavioural radio-tracking, feather ptilochronology and landscape analysis to test how nutritional condition varies with home range size in 13 house sparrow [Passer domesticus (Linnaeus, 1758)] populations along an urban gradient. Urban individuals occupied smaller home ranges than conspecifics from rural areas, most distinctly if key cover was highly scattered. In urban plots, patch connectivity, home range sizes and activity areas were positively correlated, indicating that individual ranging behaviour was related to the spatial distribution of suitable habitat. Urban house sparrows also showed the smallest feather growth bars, which were positively related to home range size at plot level. In contrast, growth bar widths and home range sizes were negatively related in rural populations, while in suburban ones, both variables varied independently. We conclude that individuals from progressively more built-up areas show a restricted ability to adjust their daily ranging behaviour to the scattered distribution of critical resources. This may complement other putative causes of the widespread population decline of urban house sparrows.

INTRODUCTION

In human-dominated landscapes, patches of natural or semi-natural habitat are typically embedded in tracts of unsuitable habitat. Depending on the quality and area of these patches and the grain size of the surrounding landscape, individuals of habitat-restricted species may move frequently among spatially isolated habitat patches (patchy populations sensu Harrison, 1991; Ovaskainen & Hanski, 2004) or populations may become spatially-dissected and show characteristics of a metapopulation structure (Hanski & Gilpin, 1991; Hanski, 1999). Habitat fragmentation, which is ultimately driving these population processes, is considered a key pressure on biodiversity (e.g. Vitousek et al., 1997; Eriksson & Ehrlén, 2001) often in synergy with other human-driven effects such as climate change (Travis, 2003; Opdam & Wascher, 2004). Species may respond to habitat fragmentation by genetic or physiological adaptations, or by adapting their ranging behaviour to the distribution of critical resources (Opdam & Wascher, 2004). Both on theoretical and empirical grounds, more sedentary species (With, Gardner & Turner, 1997; Sekercioglu, Daily & Ehrlich, 2004; Murgui, 2009) are expected to be most affected by shifts in resource distribution. For instance, non-vagile behaviour and reluctance to cross habitat gaps are considered prime reasons why neotropical insectivorous birds (Sekercioglu et al.,
Constraints on home range behaviour affect nutritional condition

2004) and British butterflies (Thomas, 2000; Warren et al., 2001) are most extinction-prone in human-altered landscapes.

Intense human activity in strongly urbanized landscapes typically creates fine-grained and highly-complex mosaics of semi-natural and built-up areas (Rebele, 1994; Blair, 2004). In Europe, such landscape mosaics traditionally constituted population strongholds for urban species such as the house sparrow \textit{(Passer domesticus \textit{(Linnaeus, 1758))}, which, in contrast to many other species, was long assumed to respond positively, rather than negatively, to urban sprawl (Earle, 1988). During the last decades, however, urban house sparrows have shown steep population declines for reasons that are not yet fully understood (Tucker & Heath, 2004; Balmori & Hallberg, 2007). Rural populations, too, declined precipitously in much of Europe (Gregory & Baillie, 1998; Fuller, Tratalos & Gaston, 2009), however, urban ones have not yet shown recent signs of recovery (De Laet & Summers-Smith, 2007) and inverse relationships between the degree of urbanization and individual body mass, size and condition (e.g. Liker et al., 2008) support the believe that urban areas currently constitute a stressful environment for this species. Because house sparrows rank among the most sedentary species of birds (Heij & Moeliker, 1990; Hole et al., 2002; Anderson, 2006), it can be hypothesized that low plasticity in home range behaviour prevents individuals from adaptively responding to rapid changes in the distribution of critical resources in urban areas, which may lead to reduced nutritional condition in areas with strongly scattered resources. Home ranges, i.e. the area with a defined probability of occurrence of an individual during a specific period of time (Millsapugh & Marzluff, 2001), determine how many and which individuals have access to limited resources and, as such, are positioned at the interplay between individual energetic demands and the spatial distribution of such resources (McNab, 1963; Mitchell & Powell, 2004).

Here we test if, to what extent, and in which direction body condition varies with home range size in 13 Belgian house sparrow populations along an urban gradient, by tracking individuals equipped with light-weight radio-transmitters and measuring alternating dark and light growth-bars on individual tail feathers (ptilochronology \textit{sensu} Grubb, 1995). Relationships between the size and structure of home ranges and estimates of body condition reflect whether, and to what extent, individuals can adjust their daily ranging behaviour to the distribution of critical resources, i.e. to minimize energetic or predation costs associated with movements across a hostile matrix (McNab, 1963; Relyea, Lawrence & Demarais, 2000; Bruun & Smith, 2003; Mitchel & Powel, 2004).
Chapter 1

Estimates of daily feather growth rate obtained from ptilochronological measurements offer a powerful tool for assessing the body condition of free-ranging birds (Grubb & Cimprich, 1990; Carbonell, Perez-Tris & Telleria, 2003; Hay et al., 2004). The method is based on the assumptions that each pair of growth bars constitutes a 24-hour period of feather growth, that narrower growth bars reflect periods of poor nutrition, and that any change in nutritional status is indicated in the width of the growth bars (Grubb, 1995).

**Material and Methods**

**Study design**

House sparrows were studied in urban and adjacent suburban areas of the city of Ghent, Belgium (156 km²; 237,000 inhabitants of which 82,584 within the 7.64 km² city centre) and in a rural area near the village of Zomergem (38.8 km²; 8,150 inhabitants) ca. 12 km NW of Ghent (figure 1.1). The ratio of built-up area to total area of grid cells in a GIS model (each grid cell measuring 90,000 m² on the ground) in Ghent and Zomergem ranged between 0.0.10 (henceforward referred to as ‘rural’), 0.11-0.30 (‘suburban’) and larger than 0.30 (‘urban’) (Arcgis ver. 9.2). By applying a stratified randomized design, we selected four 50 ha rural plots, five 50 ha suburban plots (adjacent eastern and northern populations were omitted for logistic reasons) and four 50 ha urban plots in which all patches of suitable habitat were digitized by a combination of high resolution aerial photographs and ground truthing (see Appendix 1.1 for a detailed description of the different study plots).

**Radio-telemetry**

Radio-telemetry was carried out from October to December during three consecutive years (2004-2006). A total of 26 females and 23 males were tracked, distributed as follows: 2004: 8 females, 8 males; 2005: 8 females, 6 males; 2006: 10 females, 9 males (table 1.1). Six tagged birds were replaced during radiotracking, three of which were killed by Sparrowhawks [Accipiter nisus (Linnaeus, 1758)] and three others carrying a failing transmitter. House sparrows were caught using mistnets operated during daytime, standard measurements and blood samples were taken and one adult female and one adult male per study plot were equipped with a light-weight Pip-3 radio-transmitter (Biotrack, UK) glued on a harness (Rappole & Tipton, 1991). The combined weight of transmitter and harness did not exceed 3% of the minimum body mass measured across all
Fig. 1.1. Spatial location of urban (filled circles), suburban (open circles) and rural (filled triangles) study plots within and near the city of Ghent (Belgium). The inner contour encompasses the city centre of Ghent, the outer contour encompasses surrounding municipalities, the dashed line encompasses suburban area of equivalent size as the city centre. Vi=Visserij, Sp=Spanjaardstraat, Em=Emile Braunplein, Ph=Patershol, Du=Duifhuisstraat, Wa=Watersportbaan, Ho=Houtemlaan, Pm=Papiermolenstraat, Pe=Petendockstraat, Ei=Eiland, Ro=Rostraat, Ha=Haagstraat, Fm=Frans De Milde Dreef. Suitable habitat (grey shading), radio-telemetric point fixes (black dots), OEC range (dashed line) and SIP range (solid line) are shown for a single individual (upper left box). See text for details. Abbreviations refer to populations described in table 3 (Introduction).
populations. After release, each tagged individual was allowed 24 hours of
habitation before radio-tracking started with TR4 receivers (Telonics, Arizona,
USA) and three-element Yagi antennas. Prior to the start of this study, 13
individuals had been captured, tagged with the same type of transmitter and
tracked at 15 minute intervals to determine the minimal sampling interval
between two independent point fixes (Swihart & Slade, 1985). Based on the
Schoener’s ratio \( r^2 / t^2 \) (Schoener, 1981) calculated in RANGES 8 (Kenward et al.,
2008), time-to-independence was conservatively estimated at 90 min, and this
interval was retained during this study. Individuals were tracked during 41 ± 9
(SD) days on average, and a total of 1926 point fixes were recorded, with an
average of 40 ± 5 (SD) fixes per individual. All fixes were plotted on a high
resolution, digital map of the study area. To assess the minimum number of point
fixes required to obtain accurate home range estimates, incremental area plots
were drawn for each individual. As all home ranges contained more than the
threshold value of 20 point fixes required to reach a stable size, all were retained
in subsequent statistical analysis.

**Table 1.1.** Home range estimates of 49 radio-tagged house sparrows sampled in urban,
suburban and rural areas near Ghent, Belgium (see text for details). Patch connectivity (PPI)
and nutritional stress (growth bar size) are given for each study plot.

<table>
<thead>
<tr>
<th>Study plot</th>
<th>N_{females}</th>
<th>N_{males}</th>
<th>OEC [ha]</th>
<th>SIP [ha]</th>
<th>PPI</th>
<th>Growth bar size [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emile Brauplein</td>
<td>2</td>
<td>2</td>
<td>0.039±0.0078</td>
<td>0.0032-0.0049</td>
<td>0.58±0.75</td>
<td>0.037-1.63</td>
</tr>
<tr>
<td>Patersholt</td>
<td>3</td>
<td>2</td>
<td>0.091±0.043</td>
<td>0.053-0.13</td>
<td>0.86±0.56</td>
<td>0.43-1.64</td>
</tr>
<tr>
<td>Spanjaardstraat</td>
<td>2</td>
<td>2</td>
<td>0.03±0.038</td>
<td>0.0034-0.086</td>
<td>0.29±0.28</td>
<td>0.060-0.67</td>
</tr>
<tr>
<td>Visserij</td>
<td>2</td>
<td>1</td>
<td>0.013±0.011</td>
<td>0.012-0.014</td>
<td>0.11±0.029</td>
<td>0.078-0.14</td>
</tr>
<tr>
<td>Suburban</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frans De Miledreef</td>
<td>1</td>
<td>1</td>
<td>0.099±0.041</td>
<td>0.070-0.13</td>
<td>0.39±0.019</td>
<td>0.38-0.40</td>
</tr>
<tr>
<td>Houtemlaan</td>
<td>2</td>
<td>2</td>
<td>0.10±0.19</td>
<td>0.0041-0.39</td>
<td>0.73±1.24</td>
<td>0.62-2.59</td>
</tr>
<tr>
<td>Duifhuisstraat</td>
<td>2</td>
<td>2</td>
<td>0.032±0.046</td>
<td>0.0046-0.10</td>
<td>0.29±0.11</td>
<td>0.19-0.42</td>
</tr>
<tr>
<td>Papiermolenstraat</td>
<td>2</td>
<td>2</td>
<td>0.032±0.027</td>
<td>0.0053-0.059</td>
<td>0.32±0.29</td>
<td>0.028-0.71</td>
</tr>
<tr>
<td>Watersportbaan</td>
<td>2</td>
<td>2</td>
<td>0.35±0.53</td>
<td>0.012-1.13</td>
<td>2.38±3.75</td>
<td>0.30-7.99</td>
</tr>
<tr>
<td>Rural</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petendonckstraat</td>
<td>2</td>
<td>1</td>
<td>0.22±0.24</td>
<td>0.036-0.49</td>
<td>1.17±1.48</td>
<td>0.13-2.86</td>
</tr>
<tr>
<td>Eiland</td>
<td>2</td>
<td>2</td>
<td>0.10±0.056</td>
<td>0.046-0.18</td>
<td>1.24±0.72</td>
<td>0.80-2.32</td>
</tr>
<tr>
<td>Haagstraat</td>
<td>3</td>
<td>1</td>
<td>0.052±0.046</td>
<td>0.018-0.10</td>
<td>0.77±0.21</td>
<td>0.53-0.90</td>
</tr>
<tr>
<td>Rostraat</td>
<td>2</td>
<td>2</td>
<td>0.036±0.031</td>
<td>0.015-0.081</td>
<td>0.70±0.21</td>
<td>0.42-0.93</td>
</tr>
</tbody>
</table>

**Home range estimates**

We conducted a telemetric pilot study (see above) to select home range
models (reviewed by Worton, 1989) that best fit the ecology of house sparrows.
Since individuals largely restricted their movements within a limited number of
disjunct locations, resulting in discrete clumps of point fixes, we applied cluster
analysis with nearest-neighbour distances in RANGES 8 (Kenward et al., 2008) to
estimate objective multinuclear outlier-exclusive core areas (OEC) for each individual (rationale and details in Kenward et al., 2001). OEC values quantify the amount of habitat effectively used by the individuals during radio-tracking, and several authors recommended to focus on the central part of home ranges in comparative studies as outer contours are based on fewer data and therefore more strongly affected by single outliers (Seaman et al., 1999). Next, we calculated single inclusive polygons (SIP) encompassing all OEC clusters. A habitat preference analysis performed in RANGES 8 (Kenward et al., 2008) indicated that 74.8% of all radio-telemetric observations of house sparrows fell within the habitat categories “hedge” and “dense bush” (see Summers-Smith, 1963; Wilkinson, 2006 for similar findings on habitat preference) compared to only 2.6% within “urban park” and “lawn” (two other habitat types that are considered important for house sparrows; Chamberlain et al., 2007; Murgui, 2009) (Appendix 1.1). To estimate the total area of preferred habitat within each plot as a function of patch size and distance from the home range, we therefore summed the total area of “hedges” and “dense bushes” within a radius of 300 m around the centroid of each home range (value based on the size of the largest home range) and calculated a distance-weighted, area-based isolation metric \( \sum_{i=1}^{n} \frac{A_i}{D_i} \), with \( i=1,...,n \) suitable habitat patches, \( A_i = \) area of suitable habitat patch, and \( D_i = \) nearest neighbour distance, \( PPI, \) patch proximity index sensu Bender, Tischendorf & Fahrig, 2003).

**Ptilochronology**

Individual nutritional condition of 206 house sparrows was inferred from high-resolution measurement of a standardized number of growth bars on their left and right fifth rectrices (counting outward) that were plucked upon capture. Assumptions underlying the technique of ptilochronology (Grubb, 1989) are that each pair of growth bars denotes a 24 hour time interval and that healthy birds grow their feathers faster and hence show larger growth bars. Growth bar width is therefore believed to reflect the daily nutritional regime a bird experienced while growing its feather (Grubb, 1989; 1995) and can be used as a proxy of nutritional condition. Because ptilochronology provides a measure of body condition spanning a number of days, it eliminates the problem of condition varying with time of day, as occurs with body mass (Gosler, 1996). Causal relationships between access to food resources during feather development and ptilochronological feather marks have been supported by controlled laboratory and field experiments in a wide suite of bird species (Grubb, 1989; Grubb & Cimprich, 1990; but see Murphy & King, 1991). After collection, each rectrix was
pinned on a separate white card and the total feather length was measured to the nearest 0.01 mm with a digital calliper. Next, we marked each feather at a distance of 7/10 from its proximal end, and subsequently marked the proximate and distal ends of five consecutive growth bars with an ultrafine mounting pin. Each marked card was then scanned (Océ OP1130) and growth bar sizes were automatically measured with image analysis software (KS400 Zeiss). Analysis of a subsample of 108 feathers showed high statistical repeatability between two independent measurements of the same rectrix ($r=0.94$, $p=0.0001$). To avoid biases in flight performance (hence home range behaviour) by plucking tail feathers of radio-tagged individuals, nutritional condition was estimated from growth bars collected on 9 ± 1 (SE) individuals of the same age and sex that were caught at the same location during the same season as the tagged individual. Growth bar widths of individuals within each of these clusters were strongly, positively correlated (Intraclass correlation coefficient = 0.2; $p<0.001$). As growth bar widths of original and regrown feathers from the same follicle were positively correlated in a pilot study on 15 individuals ($r=0.63$, $p=0.01$), original rather than induced feathers were measured in order to increase sample sizes.

**Statistical analysis**

We used a non-parametric exact sign test to compare home range sizes between males and females tracked in the same plot during the same month, and a Benard-van Elteren test (Benard & van Elteren, 1953) to quantify year effects using study plot as a block. Heterogeneity in average PPI among urbanization classes was tested with an ordered heterogeneity test (Rice & Gaines, 1994). At the individual level, effects of urbanization on relationships between OEC values and estimates of nutritional condition were examined with general linear mixed models, including sex and year as fixed covariates and applying a sequential stepwise backward procedure to select final models that contained significant effects only. General linear mixed models were used to assess relationships between individual home range size and urbanization, SIP and PPI, respectively. A similar approach was used to analyse effects of urbanization on nutritional condition, with tarsus length included as co-variable to correct for body size. Plot was modelled as a random variable to avoid pseudoreplication (Hurlbert, 1984). Random effects were tested with likelihood ratio tests (Verbeke & Molenberghs, 2000), while fixed effects were tested with traditional F-tests, correcting degrees of freedom for statistical dependence by Satterthwaite formulas (Littell et al., 1996). Significance values of post hoc comparisons were adjusted using a step-down Bonferroni correction (Holm, 1979). Prior to analysis, home range areas
were natural log transformed. All statistical analyses were performed in Statxact 5 (Cytel Software Corporation, 2001) and SAS (version 9.2., SAS Institute 2008, Cary, NC, USA).

RESULTS

Hedges and dense bushes were most strongly scattered in urban plots, intermediately scattered in rural plots and most strongly connected in suburban plots ($P_{c;3}=0.9975$, $k=3$, $p=0.001$). Home range estimates ranged between 0.0032 - 0.49 ha (OEC) and 0.028-2.86 ha (SIP), respectively (table 1.1). SIP ranges did not significantly differ between sexes ($S_N=11$, $p=0.83$) nor between years ($\chi^2=3.5$, df=2, $p=0.17$). OEC ranges differed between sexes ($S_N=4$, $p=0.0062$) and years ($\chi^2=10.2$, df=2, $p=0.0061$), with larger values in males than in females. SIP ranges (corrected for year and sex effects where relevant) did not significantly vary among plots in urban, suburban and rural areas. OEC ranges did not vary among plots in urban plots (all $p>0.05$), however, they did so among urban plots ($\chi^2=17.3$, df=1, $p=0.001$).

Home range sizes differed significantly between urban classes (SIP $F_{2,12.3}=6.38$, $p=0.013$; OEC $F_{2,45}=4.46$, $p=0.017$). Rural home ranges were significantly larger than urban ones (SIP $t_{45}=2.97$, Bonferroni corrected $p=0.015$; OEC $t_{12.2}=3.57$, Bonferroni corrected $p=0.011$), while urban-suburban home ranges (SIP $t_{45}=1.21$, $p=0.03$; OEC $t_{16.8}=1.58$, $p=0.14$) and suburban-rural home ranges (SIP $t_{45}=1.88$, $p=0.066$; OEC $t_{16.5}=2.01$, $p=0.061$) did not mutually differ. OEC ranges were positively correlated with SIP ranges in urban ($F_{1,39}=23.4$, $p<0.001$) and suburban ($F_{1,39}=31.81$, $p<0.001$) populations, but not in rural ones ($F_{1,39}=0.8$, $p=0.38$). In urban populations, OEC ranges increased as patches of hedges and dense bushes became more scattered ($F_{1,18}=4.47$, $p=0.048$) (figure 1.2), but this relationship was not significant in suburban ($F_{1,38.9}=1.36$, $p=0.25$) and rural populations ($F_{1,40.7}=0.86$, $p=0.36$). After correction for body size, mean growth bar widths significantly differed between urban (16.52 ± 0.12mm), suburban (16.98 ± 0.088mm) and rural populations (16.77 ± 0.10mm) ($F_{2,6.68}=4.87$, $p=0.049$). Individuals from urban populations showed significantly smaller growth bars compared to those from suburban populations ($t_{8.27}=3.09$, Bonferroni corrected $p=0.042$), while other pairwise comparisons did not reach statistical significance. After correction for year and sex effects (all $p>0.05$), relationships between OEC ranges and growth bar widths differed significantly between urban, suburban and rural populations ($F_{2, 20.3}=5.84$, $p=0.0099$).
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**Fig. 1.2.** Association between patch connectivity and individual home range size in urban house sparrows.

**Fig. 1.3.** Relationship between individual home range size and body condition in urban (filled circles, solid line), suburban (open circles, short-dashed line) and rural (filled triangles, long-dashed line) populations of house sparrows within and near the city of Ghent (Belgium). Ptilochronological data were not available for some birds (see text for details).
Constraints on home range behaviour affect nutritional condition

Relationships were positive in urban populations ($F_{1,7,43} = 10.18$, $p = 0.014$), negative in rural populations ($F_{1,27} = 4.34$, $p=0.047$), and not significantly related in suburban ones ($F_{1,28} = 1.37$, $p = 0.25$) (figure 1.3). Since OEC ranges were independent of body size ($F_{1,43}=1.07$, $p=0.31$), relationships with growth bar width were not confounded by variation in body size.

**DISCUSSION**

Urban house sparrows occupied significantly smaller home ranges than conspecifics from rural areas, most distinctly in urban plots with highly scattered and isolated patches of key cover habitat. In urban plots, levels of patch connectivity and the size of the ranging and activity areas of radio-tagged house sparrows were all positively correlated, indicating that their ranging behaviour was related to the spatial distribution of suitable habitat. Urban house sparrows also showed the smallest growth bars in their tail feathers, which were positively related to estimates of home range size. In contrast, growth bar widths and home range sizes were negatively related in rural house sparrow, while in suburban populations, both variables varied independently of each other.

Based on experimental evidence that growth bars in bird feathers reflect nutritional condition during feather growth (Grubb, 1989; Grubb & Cimprich, 1990), results of this study imply that urban house sparrows experience nutritional stress when hedges and dense bushes become progressively scattered and isolated. In urban areas, distances between key cover habitat were significantly larger (this study), and mean flock sizes significantly smaller (C. Vangestel, unpubl.data) than in suburban areas, and both factors are known to increase individual predation risk (Lima, 1987; Kleindorfer, Solloway & O'Connor, 2009). Although we did not systematically quantify predation risk in this study, a total of six house sparrows were killed by Sparrowhawks (*Accipiter nissus*) when moving among feeding sites, while several other unsuccessful attacks were observed (C. Vangestel, unpubl. data). Suitable cover against aerial (and possibly ground) predators is hence likely to offer direct fitness benefits in urban house sparrows, similar to what is observed in species of more natural habitats (Lima & Dill, 1990; Zollner & Lima, 2005; Amo, Lopez & Martin, 2007). Indirectly, the presence of suitable cover habitat has been shown to increase feeding time and feeding efficiency in trade-off with vigilance in house sparrows and other species (Lima, 1987; Whitfield, 2003; Barta, Liker & Monus, 2004). Such mechanism may underlie the positive relationship between body condition and availability of hedges and dense bushes which, themselves, are not particularly food-rich for
seed-eaters. House sparrows from rural and suburban populations were in better nutritional condition than urban individuals, likely because of the stronger clustering of suitable habitat patches and/or lower costs of moving among them.

Apart from body condition per se, the distribution of critical resources such as suitable cover may also affect individual ranging behaviour and the strength or direction of relationships between home range size and body condition (Relyea et al., 2000; Dussault et al., 2005). In rural populations, body condition was inversely related to home range size, which is expected if individuals inhabiting high quality areas try to minimize the size of their home ranges to make them more economic or safer to patrol (McNab, 1963; Relyea et al., 2000; Bruun & Smith, 2003; Mitchel & Powel, 2004). In urban populations, however, the strong scatter of suitable habitat patches most likely hampered such adaptive ranging behaviour, resulting in positive, rather than negative, relationships between body condition and home range size. Further (indirect) evidence for spatial constraints on ranging behaviour stemmed from the high level of variability in activity range among urban plots (hence high correlation in home range size at plot level), as opposed to suburban and rural plots. Likewise, Canadian Moose (Alces alces) consistently occupied smaller, not larger, home ranges when food was restricted and of low quality during winter (Dussault et al., 2005), presumably because their mobility was temporarily constrained by deep snow. In areas with more modest snow fall, moose occupied larger winter home ranges as predicted from optimal foraging theory (Lynch & Morgantini, 1984; Tella et al., 1998; Whitaker et al., 2007).

In suburban areas, where hedges and dense bushes were most strongly aggregated, body condition was independent of home range size. Under favourable ambient conditions, body condition may vary independently of home range size for two (non-exclusive) reasons. First, home range sizes may not primarily be driven by nutritional requirements but mainly reflect interspecific (e.g. predator avoidance) and/or intraspecific (e.g. flocking behaviour, territorial defence) interactions (Dussault et al., 2005). Second, if home ranges are small, energetic costs derived from patrolling or feeding movements are likely to be low as well, and proxies of condition that are based on energetic constraints, such as developmental rate (ptilochronology, this study) or developmental precision (fluctuating asymmetry; Lens & Van Dongen, 2002) are generally buffered in absence of (strong) energetic stress (Lens & Eggermont, 2008). Likewise, in two studies that studied effects of dominance status on feather growth rates in passerines (Grubb, 1989; Carrascal et al., 1998), differences in feather growth
only occurred during harsh periods but not when food was supplemented or the climate was milder.

In highly-sedentary species such as the house sparrow, small-scale changes in the distribution of critical resources such as key cover habitat (this paper) or protein-rich invertebrate food during early nestling stages (Peach et al., 2008), may complement other putative causes of urban population decline, such as loss of connectivity between traditional population sources and population sinks (Hole et al., 2002), increased predation risk by domestic predators (Shaw, Chamberlain & Evans, 2008), the deteriorating effects of vehicle emissions (Bignal, Ashmore & Power, 2004) or electromagnetic pollution from mobile phone masts (Balmori & Hallberg, 2007; Everaert & Bauwens, 2007; Balmori, 2009). High resolution data on ranging behaviour and body condition, as presented in this study, may help to understand the underlying causes of population declines in species that are subject to such multiple and synergistic stressors of natural and anthropogenic origin, and to plan actions that may slow down and eventually reverse such declines.

ACKNOWLEDGEMENTS

We are grateful to S. S. Walls, B. Cresswell and two anonymous reviewers for providing helpful comments that greatly improved the manuscript. We also wish to thank D. Ojeda De Vicente, J. Tavara Neves, M. De Vos and S. Boitsois for field assistance. This study was financially supported by research project 01J01808 of Ghent University to LL.

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APPENDIX 1.1. Landscape variables within a 300 m radius around the centroid of house sparrow home ranges and habitat preference expressed as the number of point fixes within a given habitat, in each of 13 study plots near Ghent, Belgium. Values expressed as mean percentage ± SD, preference values indicated between brackets.

<table>
<thead>
<tr>
<th></th>
<th>Built-up area (%)</th>
<th>Bush (%)</th>
<th>Lawn (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urban</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emile Braunplein</td>
<td>61.07 ± 1.77 (5.83 ± 2.95)</td>
<td>1.00 ± 0.046 (84.63 ± 11.81)</td>
<td>1.22 ± 0.088 (0)</td>
</tr>
<tr>
<td>Paterschol</td>
<td>42.12 ± 2.38 (30.40 ± 29.44)</td>
<td>3.03 ± 0.40 (66.86 ± 29.61)</td>
<td>6.77 ± 1.45 (0)</td>
</tr>
<tr>
<td>Spanjaardstraat</td>
<td>31.9 ± 1.14 (0.58 ± 1.15)</td>
<td>2.23 ± 0.092 (59.70 ± 28.94)</td>
<td>5.80 ± 0.25 (4.3 ± 3.62)</td>
</tr>
<tr>
<td>Vissersij</td>
<td>41.43 ± 0.35 (2.30 ± 3.98)</td>
<td>1.92 ± 0.035 (67.16 ± 10.94)</td>
<td>8.43 ± 0.22 (2.56 ± 4.44)</td>
</tr>
<tr>
<td><strong>Suburban</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frans De Milderдеef</td>
<td>27.85 ± 9.12 (0)</td>
<td>3.87 ± 0.65 (63.35 ± 40.65)</td>
<td>13.18 ± 1.12 (0)</td>
</tr>
<tr>
<td>Houtemlaan</td>
<td>28.22 ± 4.47 (0.65 ± 1.30)</td>
<td>3.96 ± 0.064 (85.05 ± 9.36)</td>
<td>14.19 ± 2.24 (0.93 ± 1.85)</td>
</tr>
<tr>
<td>Duitfuslaan</td>
<td>24.95 ± 1.13 (0)</td>
<td>3.71 ± 0.20 (77.53 ± 12.99)</td>
<td>9.39 ± 0.40 (0.65 ± 1.30)</td>
</tr>
<tr>
<td>Papiemolenstraat</td>
<td>24.47 ± 2.46 (0.68 ± 1.35)</td>
<td>2.53 ± 0.082 (57.92 ± 8.83)</td>
<td>18.15 ± 1.90 (10.05 ± 16.87)</td>
</tr>
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<td>Watersportbaan</td>
<td>16.7 ± 2.26 (1.50 ± 3.00)</td>
<td>5.80 ± 0.50 (88.45 ± 13.01)</td>
<td>16.74 ± 1.22 (0)</td>
</tr>
<tr>
<td><strong>Rural</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petendonkstraat</td>
<td>3.36 ± 0.057 (7.50 ± 5.34)</td>
<td>2.17 ± 0.11 (50.37 ± 27.36)</td>
<td>7.64 ± 0.55 (2.87 ± 2.48)</td>
</tr>
<tr>
<td>Eiland</td>
<td>2.67 ± 0.25 (2.05 ± 1.50)</td>
<td>1.96 ± 0.38 (42.85 ± 9.93)</td>
<td>6.32 ± 1.03 (6.67 ± 5.09)</td>
</tr>
<tr>
<td>Haagstraat</td>
<td>2.97 ± 0.17 (28.57 ± 28.60)</td>
<td>1.05 ± 0.062 (42.50 ± 22.87)</td>
<td>7.97 ± 0.74 (2.20 ± 2.15)</td>
</tr>
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<td>Rosstraat</td>
<td>1.75 ± 0.057 (33.45 ± 8.22)</td>
<td>1.76 ± 0.016 (30.30 ± 5.37)</td>
<td>3.07 ± 0.035 (3.28 ± 1.30)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Hedge (%)</th>
<th>Meadow (%)</th>
<th>Scrub (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urban</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emile Braunplein</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Paterschol</td>
<td>0.031 ± 0.0077 (0)</td>
<td>0</td>
<td>1.77 ± 0.039 (0)</td>
</tr>
<tr>
<td>Spanjaardstraat</td>
<td>0.071 ± 0 (0)</td>
<td>0</td>
<td>4.17 ± 1.97 (0)</td>
</tr>
<tr>
<td>Vissersij</td>
<td>0.065 ± 0 (13.03 ± 9.58)</td>
<td>0</td>
<td>2.10 ± 0.16 (0)</td>
</tr>
<tr>
<td><strong>Suburban</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frans De Milderdeef</td>
<td>0.23 ± 0.081 (32.05 ± 41.64)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Houtemlaan</td>
<td>2.76 ± 0.47 (11.2 ± 8.32)</td>
<td>0</td>
<td>2.59 ± 1.70 (0)</td>
</tr>
<tr>
<td>Duitfuslaan</td>
<td>0.0056 ± 0.057 (14.65 ± 7.87)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Papiemolenstraat</td>
<td>0.0086 ± 0.052 (10.45 ± 11.03)</td>
<td>5.16 ± 1.12 (0)</td>
<td>1.81 ± 0.74 (0)</td>
</tr>
<tr>
<td>Watersportbaan</td>
<td>0.0086 ± 0.073 (4.95 ± 8.37)</td>
<td>0</td>
<td>1.29 ± 1.11 (0)</td>
</tr>
<tr>
<td><strong>Rural</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petendonkstraat</td>
<td>0.0087 ± 0.00886 (16.16 ± 8.55)</td>
<td>50.93 ± 7.28 (2.97 ± 3.29)</td>
<td>1.86 ± 0.10 (0.80 ± 1.39)</td>
</tr>
<tr>
<td>Eiland</td>
<td>0.0056 ± 0.0070 (36.32 ± 6.15)</td>
<td>63.59 ± 1.70 (0)</td>
<td>1.41 ± 0.61 (0)</td>
</tr>
<tr>
<td>Haagstraat</td>
<td>0.003 ± 0.0011 (13.97 ± 12.62)</td>
<td>75.61 ± 1.51 (2.27 ± 0.06)</td>
<td>0.043 ± 0.0046 (0)</td>
</tr>
<tr>
<td>Rosstraat</td>
<td>0.0054 ± 0.000649 (8.75 ± 6.45)</td>
<td>88.21 ± 0.13 (0.53 ± 1.05)</td>
<td>0.32 ± 0.010 (0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Tree (%)</th>
<th>Brief description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urban</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emile Braunplein</td>
<td>1.28 ± 0.18 (8.95 ± 12.18)</td>
<td>Mainly commercial buildings</td>
</tr>
<tr>
<td>Paterschol</td>
<td>2.58 ± 0.43 (0.58 ± 1.30)</td>
<td>Flats with lawns in between and terraced houses</td>
</tr>
<tr>
<td>Spanjaardstraat</td>
<td>4.03 ± 0.12 (0.90 ± 1.80)</td>
<td>Flats, terraced houses with small gardens, old abbey</td>
</tr>
<tr>
<td>Vissersij</td>
<td>5.06 ± 0.048 (0)</td>
<td>Terraced houses with gardens</td>
</tr>
<tr>
<td><strong>Suburban</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frans De Milderdeef</td>
<td>0.11 ± 2.82 (4.55 ± 1.06)</td>
<td>Mixture of terraced, semidetached and detached houses with gardens, railway</td>
</tr>
<tr>
<td>Houtemlaan</td>
<td>0.070 ± 2.06 (0.60 ± 1.20)</td>
<td>Deprived area, semidetached houses with gardens</td>
</tr>
<tr>
<td>Duitfuslaan</td>
<td>0.082 ± 0.59 (0.65 ± 1.30)</td>
<td>Residential area, detached houses with large gardens</td>
</tr>
<tr>
<td>Papiemolenstraat</td>
<td>0.13 ± 2.70 (18.38 ± 17.07)</td>
<td>Semidetached and detached houses with gardens and derelict land</td>
</tr>
<tr>
<td>Watersportbaan</td>
<td>0.060 ± 1.03 (0)</td>
<td>Flats, commercial area, recreational area with lawns</td>
</tr>
<tr>
<td><strong>Rural</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petendonkstraat</td>
<td>12.17 ± 0.99 (11.03 ± 15.14)</td>
<td>Farm buildings and detached houses with gardens, and grass with livestock,</td>
</tr>
<tr>
<td>Eiland</td>
<td>9.14 ± 1.01 (1.70 ± 2.13)</td>
<td>Farm buildings, grass with livestock, arable farmland</td>
</tr>
<tr>
<td>Haagstraat</td>
<td>6.15 ± 0.084 (2.27 ± 3.93)</td>
<td>Farm buildings and detached houses with gardens, and grass with livestock</td>
</tr>
<tr>
<td>Rosstraat</td>
<td>1.82 ± 0.0093 (5.53 ± 2.91)</td>
<td>Farm buildings and detached houses</td>
</tr>
</tbody>
</table>

OEC, outlier-exclusive core area; SIP, single inclusive polygons; PPI, patch proximity index.
Chapter 2

Does fluctuating asymmetry constitute a sensitive biomarker of nutritional stress in house sparrows (*Passer domesticus*)?

Carl VANGESTEL & Luc LENS

*Ecological Indicators, 2011, 11, 389–394*

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Chapter 2

ABSTRACT

Small random deviations from left-right symmetry in bilateral traits, termed fluctuating asymmetry (FA), are theoretically predicted to increase with environmental stress and believed to constitute a potential biomarker in conservation. However, reported relationships between FA and stress are generally weak and variable among organisms, traits and stresses. Here we test if, and to what extent, FA increases with nutritional stress, estimated from independent feather growth measurements, in free-ranging house sparrows (Passer domesticus). Ptilochronological feather marks showed significant heterogeneity among study plots, indicating that house sparrow populations were exposed to variable levels of nutritional stress during development. However, individuals from more stressed populations did not show increased levels of fluctuating asymmetry in tarsus or rectrix length, nor was there evidence for significant between-trait concordance in FA at the individual or the population level. Lack of support for FA in tarsus and rectrix length as estimator of nutritional stress in house sparrows may indicate that developmental instability is insensitive to nutritional stress in this species, poorly reflected in patterns of fluctuating asymmetry due to ecological or statistical reasons, or highly context-specific. Such uncertainty continues to hamper the use of FA as a biomarker tool in conservation planning.

INTRODUCTION

Conservation planners are in need of mechanistic insights into how habitat or landscape change may drive populations of conservation concern (Soule, 1991; Arendt, 1996). Yet, traditional endpoints of population viability, such as survival and reproduction, are mostly cumbersome, time-consuming and expensive to measure and hence remain unknown in most applied conservation studies. To overcome this problem, a broad range of individual-based proxies of fitness have been applied, such as body size, (residual) body mass, fat-free mass, quality or quantity of hormones and rate or precision of growth processes (Jakob et al., 1996; Takaki et al., 2001). Because the level of stress sensitivity of these, and other, biomarkers can only be assessed post hoc, candidate markers should preferably be applicable to diverse biological systems and organisms (Huggett, 1992), and stress-mediated changes should preferably be measurable before direct components of fitness, such as survival and reproductive success, are compromised (‘early warning system’; Clarke et al., 1986; Clarke and McKenzie, 1992; Clarke, 1995).

Fluctuating asymmetry (FA, small random deviations from left-right symmetry in bilateral traits: Ludwig, 1932) is widely believed to meet both conditions. FA refers to a pattern of bilateral variation in a sample of individuals, where the mean of right minus left values of a trait is zero and the variation is normally distributed around that mean (Palmer, 1994; Polak, 2003). As corresponding body sides share a single genome and are exposed to identical environmental conditions during development,
deviations from left-right symmetry in bilateral traits cannot be due to genetic or environmental effects (Reeve, 1960). Rather, observed asymmetries are believed to reflect the inability of individuals to buffer their development against small, random perturbations (‘developmental noise’; Palmer, 1994; Auffray et al., 1999). Mechanisms underlying developmental noise are still poorly understood but likely involve perturbations of cellular processes or random variation in physiological rates (Palmer, 1994; McAdams and Arkin, 1999; Fiering et al., 2000). Despite this lack of mechanistic understanding, FA has multiple potential assets as early warning system for conservation planners. First, FA is one of few morphological attributes for which the norm, i.e. perfect symmetry, is known (Palmer, 1996). Second, FA is believed to integrate synergistic interactions among different stressors (Mayer et al., 1992; Clarke, 1993) and comprise a more sensitive marker of stress than more traditional fitness measures (Clarke and McKenzie, 1992; Lens et al., 2002). Third, FA study does not require laborious recaptures, destructive sampling, sophisticated equipment or the fulfillment of stringent model assumptions.

Yet, despite many informative cases where bilateral asymmetry increased when individuals were exposed to exo- or endogenous stress (reviewed by Moller and Swaddle, 1997), the utility of FA as a biomarker of stress remains highly controversial as relationships with stress and fitness are generally weak (Leung and Forbes, 1996; Gorur, 2006) and highly variable among organisms, traits and stresses (Lens et al., 2002; Freeman et al., 2005; Knierim et al., 2007; Carcamo et al., 2008; Hopton et al. 2009). For example, a review of 21 experimental tests of relationships between FA and environmental stress showed a consistent increase in FA in one-third of the case studies, a trait- or stress-specific increase in another third, and no effect in the remainder of the studies (Bjorksten et al., 2000). Moreover, while population asymmetry parameters have been supported in a number of cases (Clarke, 1998), between-trait concordance of FA at the individual level is generally weak or absent (Leamy, 1993; Clarke, 1998; Gangestad and Thornhill, 1999). Given this heterogeneity in relationships with FA and the fact that results from laboratory studies cannot consistently be extrapolated to free-ranging populations that may be subject to synergistic stresses of environmental, genetic and anthropogenic origin (Baker, 1995; Manel and Dehouzie, 1995, Ambo-Rappe et al., 2008), the suitability of FA as a bioassay in conservation planning requires further field validation.

As human populations become increasingly urban (e.g. ca 10% of humans lived in cities in 1900 whereas ca 70% are predicted to do so by 2050; Brown et al., 1998), cities cover progressively more area at rates that often exceed those of urban population growth (O’Meara, 1999). While house sparrows were originally believed
to respond positively, rather than negatively, to urban sprawl (Earle, 1988), urban house sparrow populations across Europe have steeply declined in recent times for reasons that are not yet fully understood (Shaw et al., 2008) but may be related to lack of protein-rich food for nestlings (Peach et al., 2008), loss of population connectivity (Hole et al., 2002), increased predation by domestic species (Shaw et al., 2008) or deteriorating effects of vehicle emissions on invertebrate abundance (Bignal et al., 2004). By studying individual variation in home range size and tail feather growth (a measure of nutritional stress; Grubb, 1989, 2006), Vangestel et al. (2010) showed that house sparrows inhabiting strongly built-up habitat developed more slowly than individuals from more open habitats, especially in areas with highly scattered cover habitat and small home ranges. Apart from providing a mechanistic explanation for such retarded development in urban house sparrow populations, the study by Vangestel et al. (2010) also offers a strong case to evaluate FA as a potential biomarker for stress effects in free-ranging populations, given that (nutritional) stress effects are being assessed independently (see Eggert and Sakaluk, 1994; Lens et al., 2002 for a discussion on stress validation in FA studies). Building on this and other studies (e.g. Nilsson, 1994), we here test whether house sparrows from populations exposed to higher levels of nutritional stress show higher levels of growth asymmetry. Relationships between nutritional stress and fluctuating asymmetry are studied in two phenotypic traits that can be measured accurately in a non-invasive way (tarsus length and rectrix length) and statistically tested at an individual- and population level.

**Material and methods**

**Study site and species**

House sparrows were studied in and around the city centre of Ghent (northern Belgium) and in a rural area near the village of Zomergem, ca. 12 km NW of Ghent. In this region, 13 plots were selected that maximize variation in the ratio of built-up to total grid cell area (range: 0.02-0.61, median value: 0.25, each cell measuring 90,000 m² on the ground; Arcgis version 9.2.). Within these plots, a total of 684 adult house sparrows were trapped by standard mist netting between 2003 and 2008. Upon capture, we sexed and aged each individual, placed one unique metal ring (Belgian Ringing Scheme) and a unique combination of three colour-rings, and measured body mass (to the nearest 0.1g), wing length (to the nearest 0.01mm) and length of the left and right tarsus (to the nearest 0.01mm; three repeated measurements sequenced left-right-left-right-left-right or vice versa and with digital slide calliper reset to zero between two consecutive measurements). Between-capture repeatability of right
Fluctuating asymmetry as a biomarker of nutritional stress?

and left tarsus measurements estimated from 91 re-trapped individuals equalled r=0.97 (p=0.001) for right tarsi and r=0.95 (p=0.001) for left tarsi. Before release, we collected a small sample of body feathers for DNA analysis and the left and right fifth rectrix (counting outward) of 213 individuals for analysis of feather growth.

**Feather growth (ptilochronology) analysis**

Individual nutritional condition was estimated from the width of alternating pairs of light and dark growth bars on left and right rectrices. Each pair of growth bars reflects a 24-hour period of feather growth (Grubb, 1989, 2006; Grubb and Cimprich, 1990) and controlled laboratory and field experiments confirmed causal relationships between access to food resources during feather development and growth bar width (Grubb, 1989; Grubb and Cimprich, 1990; but see Murphy and King, 1991). To measure growth bars, each rectrix was pinned on a single cardboard strip and the total feather length was measured to the nearest 0.01 mm with a digital slide calliper. Next, we marked the proximate and distal ends of five consecutive pairs of light and dark growth bars (starting at a distance of 7/10 from the proximate feather tip) and the proximal and distal end of each feather, with a very fine mounting pin inserted through the rachis. Each strip was then scanned (Océ OP1130) and growth bar widths and feather lengths were automatically measured with image analysis software (KS400 Zeiss). Individual feather growth rates were calculated by averaging growth bar widths on left and right rectrices. After measuring all feathers, each feather was detached and pinned to a new cardboard strip twice to independently re-score growth bar widths (subset of 108 feathers) and feather lengths (all feathers) following the methodology described above. Independent feather measurements showed high statistical repeatability for growth bar width (r=0.94, p=0.001) and rectrix length (r=0.89, p=0.001).

**Fluctuating asymmetry analysis**

To estimate fluctuating asymmetry in tarsus and rectrix length at an individual and population level, we carried out mixed regression analysis by modelling $Y = X\beta + Z\mu + e$ where $\beta$, $\mu$ and $e$ are unknown vectors of fixed effects parameters, random effects and random errors, respectively, while $X$ and $Z$ are known model matrices. Restricted maximum likelihood parameter estimation (REML) was used to obtain individual FA estimates that are unbiased with respect to measurement error (Van Dongen et al., 1999b). First, we separated ME from variance components of the random side effect (FA), and tested for the presence of directional asymmetry (fixed side effect; DA) by F-statistics, adjusting the denominator degrees of freedom by Satterthwaite's formula (Verbeke and Molenberghs, 2000). Second, we tested the
significance of FA by comparing the likelihood of models with and without random side effect. Third, we calculated unbiased FA values for each individual as subject-specific deviations of the fixed regression line in the mixed regression model (Palmer and Strobeck, 1986; Van Dongen et al., 1999b). Fourth, we compared the kurtosis levels of the signed FA values to detect antisymmetry (Knierim et al., 2007). Finally, we calculated absolute values of the signed FA values (unsigned FA estimates, further referred to as “FA”) for hypothesis testing.

**Statistical analysis**

A general linear mixed model was applied to study between-plot variation in growth bar width, FA, and relationships between both variables. Individual-based estimates or mean values per study plot were used when testing hypotheses at individual and population level, respectively. Standardized FA values were modeled as repeated measurements in multiple-trait analyses. Random effects models were applied to study individual- and population-level correlations in trait asymmetry. We therefore calculated intraclass correlation coefficients (ICC) from the ratio of the between-individual or between-population variance to the total variance (Lens and Van Dongen, 1999). To test whether the strength of between-trait correlations in FA varied with the level of nutritional stress, we divided the range of growth bar widths into four quartiles and tested whether ICCs differed among these quartiles. Month, year and study plot were included as random effects to account for a clustered data structure while trait size was added as a fixed covariate. All statistical analyses were performed with program SAS (version 9.2., SAS Institute 2008, Cary, NC, USA).

**Results**

The distribution of signed FA in rectrix length did not show a significant directional component, whereas measurements of right tarsi were consistently larger than those of left ones (table 2.1). Signed FA levels in tarsus and rectrix length were

**Table 2.1.** Variance components and the distribution of signed and unsigned FA values for two phenotypic traits in 13 house sparrow populations near Ghent, Belgium. F-statistics refer to directional asymmetry, chi-square statistics refer to separation of FA from measurement error.

<table>
<thead>
<tr>
<th>trait</th>
<th>N</th>
<th>F-test (numerator d.f., denominator d.f.)</th>
<th>VFA</th>
<th>VME</th>
<th>χ²-test (d.f.=1)</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>feather</td>
<td>213</td>
<td>3.00 (1,177)NS</td>
<td>0.07</td>
<td>0.12</td>
<td>1815.40***</td>
<td>1.50</td>
</tr>
<tr>
<td>tarsus</td>
<td>684</td>
<td>194.13 (1,660)***</td>
<td>0.04</td>
<td>0.01</td>
<td>3933.10***</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Not significant (NS) P > 0.05; * P < 0.05; ** P < 0.01; *** P<0.001

VFA, variance in signed FA; VME, variance in measurement error.
not significantly correlated, differences in the size of left and right trait sides (signed FA) could be significantly separated from variation due to measurement error (repeated measures within each side) for both traits (LRT comparing models with and without random side-effect: all p<0.05). Signed FA of rectrix length in sparrow populations showed a leptokurtic distribution (table 2.1). When plotting individual unsigned FA values of rectrix length (y-axis) on tarsus length (x-axis), most data points were clumped in the lower left corner of the scatter plot (figure 2.1). Such pattern is distinctive for FA where most individuals typically exhibit low levels of asymmetry.

**Table 2.2.** Population- and individual-level correlations in signed and unsigned FA of tarsus and rectrix length in 13 house sparrow populations near Ghent, Belgium.

<table>
<thead>
<tr>
<th></th>
<th>signed FA</th>
<th></th>
<th></th>
<th>unsigned FA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>correlation</td>
<td>Z-statistic</td>
<td>p-value</td>
<td>correlation</td>
<td>Z-statistic</td>
<td>p-value</td>
</tr>
<tr>
<td>Population Asymmetry</td>
<td>-0.19</td>
<td>-0.65</td>
<td>0.52</td>
<td>0.14</td>
<td>0.48</td>
<td>0.63</td>
</tr>
<tr>
<td>Individual Asymmetry</td>
<td>-0.01</td>
<td>-0.07</td>
<td>0.94</td>
<td>-0.01</td>
<td>-0.09</td>
<td>0.93</td>
</tr>
</tbody>
</table>

**Fig. 2.1.** Pairwise relationship between unsigned FA in tarsus and rectrix length in 158 house sparrows sampled from 13 populations.

Growth bar widths significantly varied among plots (F_{12,170}=1.93, p=0.03). FA in tarsus length and rectrix length, in contrast, did not significantly vary among plots, also when both traits were modeled as repeated measurements at the individual level (tarsus: F_{12,599}=1.27, p=0.23; rectrix: F_{12,178}=0.68, p=0.77; multiple-trait FA: F_{12,536}=1.10, p=0.36) (figure 2.2). Estimates of FA in tarsus and rectrix length were not
significantly correlated with growth bar widths at the level of individual house sparrows (tarsus: $F_{1,142}=0.28$, p=0.59; rectrix: $F_{1,134}=0.01$, p=0.92; multiple-trait FA: $F_{1,117}=0.09$, p=0.76) nor at the population level (tarsus: $F_{1,11}=1.35$, p=0.27; rectrix: $F_{1,11}=0.39$, p=0.54; multiple-trait FA: $F_{1,11}=1.26$, p=0.29, figure 2.3).

Likewise, levels of FA in rectrix and tarsus length were not significantly correlated at the population level (i.e. no evidence for a Population Asymmetry Parameter) or the individual level (i.e. no evidence for an Individual Asymmetry Parameter) (table 2.2), nor did the strength of between-trait correlations in FA at the individual level vary with nutritional stress ($\chi^2=5.9$, d.f.=3, p=0.12) (table 2.3).

| Table 2.3. Individual-level correlation in signed and unsigned FA of tarsus and rectrix length in relation to nutritional stress. Individuals were assigned to one of four quartiles based on the width of their rectrix growth bars, with classes I to IV reflecting decreasing levels of nutritional stress. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | signed FA       |                |                |                |                |                |                |                |                |                |                |                |                |
|                                | correlation     | Z-statistic    | p-value        | correlation    | Z-statistic    | p-value        | correlation    | Z-statistic    | p-value        |                |                |                |                |
| class I                        | -0.11           | -0.63          | 0.53           | -0.10          | -0.58          | 0.56           |                |                |                |                |                |                |                |
| class II                       | -0.09           | -0.47          | 0.64           | -0.18          | -0.94          | 0.35           |                |                |                |                |                |                |                |
| class III                      | -0.02           | -0.13          | 0.90           | 0.12           | -0.84          | 0.40           |                |                |                |                |                |                |                |
| class IV                       | -0.03           | -0.14          | 0.89           | -0.16          | -0.89          | 0.37           |                |                |                |                |                |                |                |

**DISCUSSION**

Ptilochronological feather marks showed significant heterogeneity among study plots, indicating that house sparrow populations were exposed to variable levels of nutritional stress during feather growth. Contrary to our expectation, individuals from more stressed populations did not show increased levels of fluctuating asymmetry in tarsus or rectrix length, nor was there evidence for significant between-trait concordance in FA, also when testing correlations at the population level. Low congruence between both presumed markers of developmental stress in free-ranging populations of house sparrows may have multiple (non-exclusive) causes.

First, relationships between nutritional stress and FA may have been present but masked by underlying population processes. Despite their highly sedentary lifestyle (Anderson, 2006), house sparrows show limited bidirectional (postnatal) dispersal among adjacent populations (Vangestel et al., unpubl.). Dispersal away from
Fluctuating asymmetry as a biomarker of nutritional stress?

Fig. 2.2. Between-plot variation (mean value ± SE) in growth bar size (a), tarsus FA (b), and rectrix FA (c).
Fig. 2.3. Pairwise relationships between average growth bar size and standardized FA values for tarsus length (filled circles) and rectrix length (open triangles).

an individual’s natal site is expected to uncouple patterns of FA in tarsi, which are fully-grown before fledging and related to nutritional conditions during the nestling phase, with patterns of FA in traits that reflect nutritional conditions experienced after dispersal. Apart from dispersal, nest studies on house sparrows earlier revealed increased rates of nestling mortality when levels of insect abundance were critically low (Peach et al., 2008). If nest mortality is selective in relation to FA, i.e. if “low quality” individuals show higher levels of FA and higher mortality rates, high nutritional stress experienced as a nestling may result in higher, rather than lower, proportions of individuals with more symmetric tarsi in adult cohorts. Likewise, absence of selective mortality under nutritionally favourable conditions may cause higher relative proportions of low-quality (i.e. more asymmetric) adults. Under such scenario, the lowest levels of FA would be expected in intermediately-stressed populations where the majority of individuals are born with low to intermediate levels of FA and highly asymmetric ones suffer from increased mortality. Unlike tarsi, however, rectrices re-grow after each (natural or induced) episode of feather moult, and rectrix FA can therefore be expected to be less biased due to differential survival or dispersal and better reflect environmental stress effects at the site of capture. Contrary to our expectation, however, growth bar width was not significantly
correlated with rectrix FA either. Finally, predicted relationships between FA and growth bar size were based on presumed nutritional stress effects on adults, while nestlings might be stressed differently as they depend more strongly on insect food. Given the results of the nest studies mentioned above, however, it is unlikely that nestlings in all populations were exposed to equal nutritional conditions.

Second, relationships between nutritional stress and FA may have been present but masked due to statistical problems. Rectrix FA in house sparrows showed a low signal (FA) to noise (ME) ratio, resulting in low statistical power to detect differences between treatments or relationships with other variables (Van Nuffel et al., 2007). Heterogeneity in signal-to-noise ratio of tarsus and rectrix FA and/or variation in “windows of opportunity” (Clarke, 1998) between nutritional stress effects on tarsi (nestling, pre-dispersal) and rectrices (post-fledging, post-dispersal) may be responsible for the low level of between-trait correlation in individual and population level FA measured in our study. Such low concordance is in line with most empirical studies on natural, free-living populations (Clarke, 1995; Gangestad and Thornhill, 1999; Aparicio and Bonal, 2002; Cuervo and Restrepo, 2007; but see Lens and Van Dongen, 1999; Van Dongen et al., 1999c) despite the fact that organism-wide asymmetry is implicitly assumed in most evolutionary models of FA (Dufour and Weatherhead, 1996). More general, Moller and Jennions (2002) showed that effect sizes in FA studies do not differ from those in ecological and evolutionary studies where the amount of variance explained by the factor of interest is often as low as 5-7%. Under such conditions, hundreds of samples might be required to reject null hypotheses with high statistical power (i.e. 80% or more). Since sample sizes in this study were more modest (i.e. within the range expected for ecological studies on free-ranging populations), small effects may have remained undetected and non-significant effects should be interpreted with caution.

Apart from low statistical power, FA also comprises a relatively weak estimator of the underlying developmental instability (Van Dongen, 1998; Whitlock, 1998), despite recent developments in the statistical analysis of DI (Van Dongen et al., 1999a, 1999b; Gangestad et al., 2001; Waldmann, 2004). This is particularly true for individual-level FA estimation, where the level of developmental instability (variance) is estimated from two data points (left and right trait values) only (Whitlock, 1996). Due to sampling variability, house sparrows with equal intrinsic ability to buffer their development against random perturbations may either show low or high levels of FA, which inevitably causes a downward bias in relationships with FA (Whitlock, 1996; Van Dongen, 1998). However, given the fact that a substantial number of house sparrows were measured in each study plot (mean number ± SE per plot: FA rectrix
Chapter 2

15 ± 2 ind; FA tarsus 49 ± 7 ind) and that each of these individuals constituted an independent sampling unit in population-level comparisons (Whitlock, 1996), high sampling variability cannot explain the low Population Asymmetry Parameter (Soule, 1967) measured in our study.

Third, consistent relationships with fluctuating asymmetry may be absent because FA in rectrix and tarsus length does not constitute a sensitive indicator of nutritional stress effects in house sparrows. As the sensitivity of homeostatic processes has been hypothesized to be positively related to the level of stress endured during ontogeny (Parsons, 1990, 1992; Kodric-Brown, 1997; Shykoff and Moller, 1999), FA may be insensitive as index of environmental stress in populations exposed to low or intermediate levels of nutritional stress. Here, house sparrows are unlikely to face strong energetic challenges, and all individuals - including low quality ones - may display low levels of FA. If this is the case, relationships with FA would be expected to be more consistent in highly-stressed populations, due to the unmasking of low-quality individuals. However, the strength of relationships with FA did not depend on nutritional stress in our study. Alternatively, the development of tarsi and rectrices may be strongly buffered in house sparrows, such that high energetic constraints do not result in loss of developmental stability. Empirical evidence is growing that traits with high functional value are subject to strong stabilizing selection, because small deviations from the expected phenotype may already result in substantial fitness loss (Balmford and Thomas, 1992; Thomas, 1993), and to low levels of asymmetric development (Balmford et al., 1993; Clarke, 1995; Aparicio and Bonal, 2002; Karvonen et al., 2003). By studying traits that were directly or indirectly related to mobility (i.e. hopping on the ground and maneuvering during flight), we may unintentionally have constrained the stress sensitivity of FA indices in our study.

In conclusion, results of our study on house sparrows do not support the use of FA in tarsus and rectrix length as a reliable estimator of nutritional stress inferred from feather growth rate (see Moller, 1996; Polo and Carrascal, 1999 and references therein for similar findings), nor do they support the existence of organism-wide asymmetry. Rather, our results suggest that developmental instability is either insensitive to nutritional stress in this species, or poorly reflected in patterns of fluctuating asymmetry due to (population) ecological or statistical reasons, or highly trait- and context-specific. Such uncertainty continues to hamper the use of FA as a robust and universal bio-indicator in urban planning.
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Chapter 2


Fluctuating asymmetry as a biomarker of nutritional stress?


Developmental stability covaries with genome-wide and single-locus heterozygosity in house sparrows

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ABSTRACT
Fluctuating asymmetry (FA), a measure of developmental instability, has been hypothesized to increase with genetic stress. Despite numerous studies providing empirical evidence for associations between FA and genome-wide properties such as multi-locus heterozygosity, support for single-locus effects remains scant. Here we test if, and to what extent, FA co-varies with single- and multilocus markers of genetic diversity in house sparrow (Passer domesticus) populations along an urban gradient. In line with theoretical expectations, FA was inversely correlated with genetic diversity estimated at genome level. However, this relationship was largely driven by variation at a single key locus. Contrary to our expectations, relationships between FA and genetic diversity were not stronger in individuals from urban populations that experience higher nutritional stress. We conclude that loss of genetic diversity adversely affects developmental stability in P. domesticus, and more generally, that the molecular basis of developmental stability may involve complex interactions between local and genome-wide effects. Further study on the relative effects of single-locus and genome-wide effects on the developmental stability of populations with different genetic properties, is therefore needed.

INTRODUCTION
Developmental stability refers to the ability of an organism to achieve a phenotypic endpoint, predetermined by its genotype and the environment, along a developmental pathway in the face of random perturbations [1]. Because developmental stability has been shown to decrease with environmental and genetic stress and to correlate with fitness traits such as fecundity, competitive ability, parasite resistance and survival (see reviews in for example [1, 2, 3]), it has received much attention in ecology and conservation biology. Furthermore, as developmental instability may increase morphological variation and reveal cryptic genetic variation (e.g. [4]), it can affect evolutionary processes, and possibly speciation, too [5].

Population and individual levels of developmental stability are most commonly estimated by corresponding levels of fluctuating asymmetry (FA), i.e. small, random deviations from perfect left-right symmetry in bilateral traits [6]. Developmental stability and FA are inversely related to one another as high levels of FA reflect poor developmental stability. Developmental theory assumes that left and right trait sides reflect two independent replicates of the same developmental event and should therefore develop symmetrically in the absence of random perturbations [3]. While empirical studies revealed positive relationships between FA and genetic stress (reviewed by [7,8]), numerous inconclusive examples nourish the debate over the generality of these
relationships [9,10]. Relationships between FA, stress and fitness have not always been consistent in the past but seem to be highly variable and species, stress and trait specific [2,3]. Heterogeneity in the strength or direction of relationships with FA may result from complex genotype-environment interactions [11]. For example, the fact that relationships between FA and heterozygosity were only significant under suboptimal rearing conditions in the freshwater fish Gambusia holbroooki [12], suboptimal foraging conditions in the forest bird Turdus helleri [13], and suboptimal growing conditions in the flowering plant Lychnis viscaria [14], suggests that developmental stability may be traded-off against other vital life-history traits when individuals become energetically challenged [12,13,15].

Based on developmental and genetic theory, at least two hypothetical mechanisms underlying the genetic basis of developmental stability have been put forward [7,9]: (i) the heterozygosity hypothesis states that individuals with high levels of protein heterozygosity are developmentally stable as a result of dominance or overdominance effects [8,16,17,18]. Genetic dominance refers to increased expression of deleterious recessive alleles in homozygote individuals [19], whereas genetic overdominance refers to superior biochemical efficiency of individuals that are heterozygous for genes at marker loci (‘true overdominance’) or at non-neutral genes tightly linked to the latter (‘associative overdominance’) [7,20,21,22]. Both genetic dominance and ‘associative’ overdominance imply genetic disequilibria, however, the ecological conditions under which these disequilibria occur, can differ. Genetic dominance is most strongly associated with non-random association of diploid genotypes in zygotes (identity disequilibria) which is common under partial inbreeding [17]. Associative overdominance, in turn, is more strongly associated with non-random associations of alleles at different loci in gametes (linkage disequilibria), which typically occurs under recent population bottlenecks followed by rapid population expansion or intermixing of genetically differentiated populations; (ii) the genomic co-adaptation hypothesis states that balanced co-adapted gene complexes result in higher developmental stability because natural selection favours alleles at many different loci that ‘harmoniously’ interact during the developmental process to produce stable phenotypes [7,9,16]. Strong selection or outbreeding has been shown to break up such co-adapted gene complexes [7,9].

While numerous studies have provided empirical evidence for associations between developmental stability and genome-wide processes such as multi-locus heterozygosity, evidence for single locus effects (local effect hypothesis sensu [16,17,23]) is still scant. A study of inactive/null alleles at lactate dehydrogenase
(LDH) loci in rainbow trout (*Oncorhynchus mykiss*) showed reduced levels of developmental stability in heterozygotes, probably due to a reduction in enzyme activity despite potential beneficial effects of chromosomal heterozygosity [24]. A study on blowflies (*Lucilia cuprina*) showed that developmental stability in bristle numbers (but not wing characters) initially decreased upon exposure to a new pesticide but restored after modification of the genetic background through natural selection [25]. While loss of developmental stability was first explained by a disruption of co-adapted gene complexes, further study revealed direct effects of single resistance and modifier genes [26]. Recently, transcriptional knockdown techniques demonstrated the involvement of heath shock protein genes in the molecular control of developmental stability in *Drosophila melanogaster* and *Arabidopsis thaliana* [27,28].

Here we study how developmental stability in a metric trait co-varies with indices of genome-wide and single-locus genetic diversity in microsatellite markers, within and among 26 house sparrow (*Passer domesticus*) populations along an urban gradient. Despite the wealth of analytic tools developed for non-coding neutral markers and their presumed suitability to test relationships with genetic diversity, few studies have applied such markers to model single- and multi-locus relationships with developmental stability [29,30,31]. Based on the following ecological and genetic evidence, relationships between developmental stability and genetic diversity are predicted to be stronger in more urbanized areas. First, urban house sparrows are more strongly, energetically challenged than suburban and rural individuals [32,33]. A previous study confirmed that a similar stress gradient was apparent within our study area [34]. Second, urban populations are on average smaller than suburban and rural ones (C. Vangestel, unpublished data). Under reduced population sizes, variation in inbreeding, estimates of genome-wide diversity based on restricted numbers of markers [21], and statistical power to detect relationships with developmental stability, are expected to increase. Individual-level FA and genetic estimates of multi-locus diversity show high sampling variability. The former represents variances based on two data points (e.g. left and right) while the latter attempts to estimate genome-wide characteristics using only a limited number of markers. As such, both estimates may become very noisy and are therefore regarded as weak estimates of complex biological processes such as respectively developmental stability [35,36] and genome-wide diversity [37]. Consequently, associations between both estimates can be expected to be low (see [37] for a general discussion) while joint analysis of average values between groups can still be done with reasonable accuracy as long as the number of sampled individuals is
Developmental stability covaries with heterozygosity high. As the strength of relationships between developmental stability and genetic diversity may hence vary with the hierarchical level of statistical analysis [31,39,40], hypotheses are tested at the level of populations and individuals.

**MATERIAL AND METHODS**

*Ethics Statement*

All procedures involving animals were reviewed and approved by the Animal Ethics Committee of Ghent University (Permit Number ECP 08/05).

*Study site*

House sparrows were sampled along an urban gradient ranging from the city centre of Ghent (northern Belgium) and its suburban periphery to the rural village of Zomergem, located ca. 12 km NW of Ghent. Urbanization was measured as the ratio of built-up to total grid cell area (each cell measuring 90,000 m² on the ground) and ranged between 0-0.10 (‘rural’), 0.11-0.30 (‘suburban’) and larger than 0.30 (‘urban’) (Arcgis version 9.2.). We selected 26 plots along this gradient (Figure 3.1) in which we captured a total of 690 adult house sparrows by standard mist netting between 2003 and 2009 (equal sex ratios in majority of plots; see Table 4.1 for a detailed outline of the number of individuals sampled per year per location). Upon capture, each individual was sexed and aged and body mass (to the nearest 0.1g), wing length (to the nearest 0.5mm) and length of the left and right tarsus (to the nearest 0.01mm; three repeated measurements sequenced left-right-left-right-left-right or vice versa and with digital slide calliper reset to zero between two consecutive measurements) were measured. Before release, we collected a small sample of body feathers for DNA analysis and the left and right fifth rectrix (counting outward) for feather growth analysis [34].

*Fluctuating asymmetry analysis*

To estimate FA in tarsus length at an individual and population level, we carried out mixed regression analysis with restricted maximum likelihood parameter estimation (REML) to obtain unbiased individual FA estimates [41]. “Side” was modelled as a fixed effect, while “individual” and “individual*side” were modelled as random effects. Individual FA estimates were obtained from the individual random effects (“individual*side”). First, we modelled separate variances in measurement errors (ME) for each bird bander as the level of
accuracy between banders might differ. Second we tested for the presence of directional asymmetry (fixed “side” effect; DA) by F-statistics, adjusting the denominator degrees of freedom by Satterthwaite’s formula [42]. The distribution of signed FA in tarsus length showed a significant directional component in all bird banders as measurements of right tarsi were consistently larger than those of left ones. These differences were attributed to the specific handling of a bird when measuring both tarsi and therefore do not compromise the FA values as the mixed regression model corrects for this systemic bias by estimating subject-specific deviations from the fixed regression slope. Third, we tested the significance of FA by comparing the likelihood of models with and without random “individual*side” effect. Variation in length between repeated
measurements within each side (ME) was significantly separated from variation between both trait sides (signed FA) ($\chi^2 = 8454.7$, d.f.=1, p<0.001) and resulted in strong signal-to-noise ratios (all $\sigma_{FA}^2/\sigma_{ME}^2 >9.4$). Fourth, we calculated unbiased signed FA values (subject specific slope deviations from the fixed regression represented the amount of asymmetry after correcting for DA and ME). Finally, we calculated absolute values of the signed FA values (unsigned FA estimates, further referred to as “FA”) for hypothesis testing. These individual estimates were used for individual-based analyses while population mean values were used for analyses conducted at the population level. Fifth, we compared the kurtosis levels of the signed FA values to detect antisymmetry [43]. Visual inspection of signed FA values did not indicate the presence of antisymmetry as platycurtotic distributions were absent.

**DNA extraction, PCR and genotyping**

Genomic DNA was extracted from ten plucked body feathers using a Chelex resin-based method (InstaGene Matrix, Bio-Rad) [44]. Polymerase chain reactions were organized in four multiplex-sets and included both traditional ‘anonymous’ microsatellites as well as those developed based on expressed sequence tags. For all loci full sequence length, chromosome location on the zebra finch genome and the nearest known zebra finch gene are given in an appendix 3.1. (genome locations were assigned using WU-BLAST 2.0 software). The first multiplex reaction contained Pdoµ1 [45], Pdo32, Pdo47 [46] and TG04-012 [47]; the second one contained Pdoµ3 [45], Pdoµ5 [48], TG13-017 and TG07-022 [47]; the third multiplex reaction contained Pdo10 [48], Pdo16, Pdo19, Pdo22 [46] and TG01-040 [47]; the last set consisted of Pdo9 [48], TG01-148 and TG22-001 [47]. PCR reactions were performed on a 2720 Thermal Cycler (Applied Biosystems) in 9 µL volumes and contained approximately 3 µL genomic DNA, 3 µL QIAGEN Multiplex PCR Mastermix (QIAGEN) and 3 µL primermix (concentrations were 0.1 µM (Pdoµ1), 0.12 µM (TG01-148), 0.16 µM (Pdo10, Pdo19, Pdo22, Pdo32, TG04-012) and 0.2 µM (Pdoµ3, Pdoµ5, Pdo9, Pdo16, Pdo47, TG01-040, TG07-022, TG13-017, TG22-001)). The applied PCR profile contained an initial denaturation step of 15 min at 95°C, followed by 35 cycli of 30 s at 94°C, 90 s at 57°C and 60 s at 72°C. Finally, an additional elongation step of 30 min at 60°C and an indefinite hold at 4°C was allowed. Prior to genotyping samples were quantified using a ND1000 spectrometer (Nanodrop technologies) and adjusted to a final concentration of 10 ng/µL. Negative and positive controls were employed during extraction and PCR to rule out contamination of reagents and ensure adequate primer aliquot working, respectively. PCR products were
visualized on an ABI3730 Genetic Analyzer (Applied Biosystems), an internal LIZ-600 size standard was applied to determine allele size, known standard samples were added to align different runs and fragments were scored using the software package GENEMAPPER 4.0. Only individuals for which at least 10 markers successfully amplified were selected for subsequent analyses.

**Genetic data analysis**

Because the genotyping of noninvasive DNA samples is potentially prone to artefacts [49,50] we tested for scoring errors due to stuttering or differential amplification of size-variant alleles that may cause drop-out of large alleles using MICRO-CHECKER [51]. The same program was also used to assess the observed and expected frequency of null alleles by comparing frequencies of observed and Monte Carlo simulated homozygotes [52]. All microsatellite loci (n=16) were checked for Hardy-Weinberg and linkage equilibrium with GENEPOP 4.0 [53,54]. Mean unbiased expected heterozygosity across all populations (Hₑ [55]) was computed for each locus using FSTAT 2.9.3.2. [56]. Individual genetic diversity was estimated by (i) standardized multilocus heterozygosity (hereafter called MLH [57]), (ii) Ritland inbreeding coefficients ([58]) and (iii) squared differences in allele size (d², [59,60]).

(i) MLH was calculated as the ratio of the proportion of typed loci for which a given individual was heterozygote over the mean heterozygosity of those loci [57], thereby eliminating possible confounding effects of unbalanced datasets. Individual MLH estimates were calculated using Rhh [61], an extension package of R (http://www.r-project.org), which also provides two additional heterozygosity-based indices, i.e. heterozygosity by loci (HL [62]) and internal relatedness (IR [63]). HL weighs the contribution of each locus and is calculated as $\text{HL} = \frac{\sum E_h}{\sum E_h + \sum E_j}$, where $E_h$ and $E_j$ represent the expected heterozygosities of the homozygous and heterozygous loci, respectively. IR on the other hand incorporates allele frequencies to estimate levels of homozygosity. IR = $(2H - \sum f_i)/(2N-\sum f_i)$, where H represents the number of homozygous loci, N the total number of loci and $f_i$ the frequency of the ith allele in the genotype. Positive values reflect high levels of homozygosity while negative values are indicative for high heterozygosity. As all three indices were strongly correlated (all $|r|>0.97$; p<0.001) (Figure 3.2a) and results remained unaffected when based on MLH, IR or HL (despite differences in the relative weight given to alleles or loci when estimating heterozygosity [62]), only results of analyses with MLH are reported.
Developmental stability covaries with heterozygosity

Fig. 3.2. Correlation matrix between multi-locus (a) heterozygosity-based indices (MLH, HL and IR; see text for details) and (b) genetic diversity indices (MLH, \(d^2\) and \(\hat{f}\)).

(ii) Ritland estimates were obtained from the software program MARK (available at http://genetics.forestry.ubc.ca/ritland/programs.html) and calculated as

\[
\hat{f} = \sum_{i,l} \left( \frac{S_{il} - P_{il}^2}{P_{il}} \right) / \sum_{i} (n_i - 1),
\]

where \(i\) and \(l\) represent alleles and loci, respectively; \(S_{il}\) equals 1 if both alleles are allele \(i\) or 0 otherwise, \(P_{il}\) is the frequency of allele \(i\) at locus \(l\) and \(n_i\) denotes the number of alleles at locus \(l\) [58]. This unbiased method-of-moment estimator is thought to be particularly useful for highly variable markers since precision of the estimate is proportional to the number of alleles per locus [64].

(iii) Mean squared distances between two alleles within an individual were calculated as mean

\[
d^2 = \sum_{i=1}^{n} \frac{(i_1 - i_2)^2}{n},
\]

where \(i_1\) and \(i_2\) are the length in repeat units of allele 1 and allele 2 at locus \(i\) and \(n\) is the number of loci analyzed. By dividing all \(d^2\) values by the maximum observed value at that locus, effects of highly variable loci were accounted for [63,65]. This estimator is thought to allow inference about the time since coalescence of two alleles, given that alleles of more similar length are more likely related by common ancestry [60,65] and has proven to be a valuable measure in the event of recent admixture of highly differentiated populations and superior fitness of hybrid descendant due to heterosis. Under such conditions, \(d^2\) is hypothesized to be the most optimal fitness predictor as it integrates the migration signature into its estimate [65], unlike the other heterozygosity-based indices.
To test whether the number of genetic markers used in our study was sufficient to make valid inferences on genome-wide heterozygosity, we divided our marker set in two random subsets and calculated individual multilocus heterozygosity indices for each subset with the program Rhh [61]. In order to make the claim of genome-wide heterozygosity tenable, both subsets should yield comparable estimates of individual multilocus heterozygosity. Hence, individual multilocus heterozygosity estimates should be positively correlated and this procedure was repeated 1000 times to obtain confidence intervals for mean heterozygosity-heterozygosity correlations.

**Statistical analysis**

We used Pearson correlation coefficients to quantify the strength of associations between $d^2$, MLH and $\hat{f}$, and general linear models with Gaussian error structure to study between-plot variation in genetic diversity and relationships with tarsus FA. Observer was added as a covariate to account for possible confounding effects of between-observer heterogeneity and analyses were tested at two hierarchical levels: among individuals and among populations (using mean values). Individual-based analyses were conducted in two ways. First, associations between FA and genetic diversity were estimated for each population using an ANCOVA model (population, genetic diversity and their interaction were modelled as fixed factors). An average within-population effect was estimated using a contrast statement. Second, individuals were pooled across all populations. While in the former model differences between populations are ignored, results from the latter model should resemble those of the population-level analysis if strong population effects are present. Initially, models were run for all markers combined (genome-wide effects). Next, the procedure was repeated per locus (unstandardized heterozygosity and $d^2$ estimates) and the relationship between genetic variability and strength, measured as total variance explained, of these (single-locus) genotype-FA associations was assessed. Positive Spearman rank correlation coefficients imply that more heterozygous markers are more informative [23]. Finally, we applied a general linear mixed model to test whether associations between FA and genetic diversity varied with urbanization. Genetic diversity, urbanization, their interaction and bird bander were included as fixed factors. Study plot and the interaction with each index of genetic variation were modelled as random effects. Degrees of freedom were estimated by Satterthwaite formulas to account for statistical dependence [66]. Per multi-locus genetic diversity index a Bonferroni correction [67] was applied to account for multiple comparisons. All statistical
analyses were performed with program SAS (version 9.2., SAS Institute 2008, Cary, NC, USA).

RESULTS

Genetic diversity

All loci were highly polymorphic and most locus by population combinations were in Hardy-Weinberg equilibrium, yet some deviations reached significance after Bonferroni correction (three populations for Pdo47, two populations for PdoµS and one for resp. Pdo32, Pdo9 and TG13-017). There was no evidence that scoring errors due to large allele drop-out or stutter contributed to this nonequilibrium. To ascertain that these deviations did not influence our results we ran all analyses with and without these five markers. Removing these loci did not alter any of the overall conclusions, hence only results based on the total dataset are reported. There was no evidence for linkage disequilibrium between any pair of loci. Standard statistics for each marker are presented in Table 3.1. Estimates of genetic diversity were significantly correlated at the individual level, most strongly between \( \hat{f} \) and MLH (\( \hat{f} \)-MLH: \( r=-0.77, p<0.001 \); \( d^2 \)-MLH: \( r=0.52, p<0.001 \); \( d^2 - \hat{f} \): \( r=-0.40, p<0.001 \)) (Figure 3.2b). MLH was a weak predictor of genome-wide heterozygosity as independent random sets of loci resulted in low (but significant) positive heterozygosity-heterozygosity correlations (mean \( r= 0.16, 95\% \) CI= [0.10-0.22]). MLH and \( \hat{f} \) significantly varied among populations (resp. \( F_{25,502}=2.35, p=0.0003 \) and \( F_{25,502}=2.00, p=0.0031 \)) while \( d^2 \) showed a near-significant trend (\( F_{25,502}=1.52, p=0.053 \)).

Multi-locus association between FA and genetic diversity

Genetic diversity estimated by MLH and \( \hat{f} \) was significantly associated with tarsus FA modeled across all individuals. Highly homozygous individuals showed higher levels of FA compared to more heterozygous ones. However, MLH and \( \hat{f} \) explained only little variation in FA (MLH: \( F_{1,517}=6.59, p=0.01, R^2=0.049 \); \( \hat{f} \): \( F_{1,517}=4.70, p=0.03, R^2=0.046 \)) (Table 3.2). When tested in each population separately, a similar (non-significant) trend occurred (MLH: \( F_{1,467}=1.73, p=0.19 \); \( \hat{f} \): \( F_{1,467}=0.70, p=0.40 \)). As opposed to the weak associations measured at the individual level, mean values of MLH and \( \hat{f} \) were strongly associated with mean levels of FA across all populations (MLH: \( F_{1,24}=12.31, p=0.001, R^2=0.34 \); \( \hat{f} \):
Chapter 3

$F_{1,24}=7.88$, $p=0.009$, $R^2=0.25$) (Figure 3.3; Table 3.2). In contrast, $d^2$ was not correlated with FA at the individual nor population level (all $p>0.15$).

Table 3.1. Locus specific descriptive statistics for 16 microsatellite markers.

<table>
<thead>
<tr>
<th>Locus</th>
<th>$N$</th>
<th>$N_A$</th>
<th>$H_o$</th>
<th>$H_e$</th>
<th>$f_{null}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG01-040</td>
<td>537</td>
<td>6</td>
<td>0.40</td>
<td>0.44</td>
<td>0.028 (0.01)</td>
</tr>
<tr>
<td>TG01-148</td>
<td>486</td>
<td>3</td>
<td>0.42</td>
<td>0.38</td>
<td>-0.023 (0.02)</td>
</tr>
<tr>
<td>TG04-012</td>
<td>549</td>
<td>5</td>
<td>0.53</td>
<td>0.59</td>
<td>0.039 (0.016)</td>
</tr>
<tr>
<td>TG07-022</td>
<td>493</td>
<td>5</td>
<td>0.37</td>
<td>0.41</td>
<td>0.026 (0.012)</td>
</tr>
<tr>
<td>TG13-017</td>
<td>547</td>
<td>8</td>
<td>0.52</td>
<td>0.64</td>
<td>0.074 (0.015)</td>
</tr>
<tr>
<td>TG22-001</td>
<td>478</td>
<td>11</td>
<td>0.34</td>
<td>0.41</td>
<td>0.054 (0.013)</td>
</tr>
<tr>
<td>Pdoµ1</td>
<td>550</td>
<td>20</td>
<td>0.80</td>
<td>0.85</td>
<td>0.024 (0.009)</td>
</tr>
<tr>
<td>Pdoµ3</td>
<td>515</td>
<td>19</td>
<td>0.83</td>
<td>0.85</td>
<td>0.015 (0.007)</td>
</tr>
<tr>
<td>Pdoµ5</td>
<td>523</td>
<td>22</td>
<td>0.76</td>
<td>0.82</td>
<td>0.033 (0.011)</td>
</tr>
<tr>
<td>Pdo9</td>
<td>442</td>
<td>31</td>
<td>0.65</td>
<td>0.75</td>
<td>0.052 (0.013)</td>
</tr>
<tr>
<td>Pdo10</td>
<td>596</td>
<td>18</td>
<td>0.78</td>
<td>0.82</td>
<td>0.021 (0.011)</td>
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<tr>
<td>Pdo16</td>
<td>549</td>
<td>17</td>
<td>0.81</td>
<td>0.84</td>
<td>0.016 (0.01)</td>
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<tr>
<td>Pdo19</td>
<td>573</td>
<td>9</td>
<td>0.60</td>
<td>0.62</td>
<td>0.008 (0.011)</td>
</tr>
<tr>
<td>Pdo22</td>
<td>578</td>
<td>16</td>
<td>0.73</td>
<td>0.72</td>
<td>-0.005 (0.011)</td>
</tr>
<tr>
<td>Pdo32</td>
<td>491</td>
<td>20</td>
<td>0.59</td>
<td>0.75</td>
<td>0.093 (0.015)</td>
</tr>
<tr>
<td>Pdo47</td>
<td>562</td>
<td>17</td>
<td>0.68</td>
<td>0.83</td>
<td>0.078 (0.013)</td>
</tr>
</tbody>
</table>

Number of individuals genotyped ($N$), number of distinct alleles per locus ($N_A$), observed ($H_o$) and expected ($H_e$) heterozygosity and null allele frequency ($f_{null}$)

**Single-locus association between FA and genetic diversity**

Single-locus effects at the individual level were in concordance with those based on multiple loci, i.e. individual genotypes failed to explain variation in FA at each locus (all $R^2\leq0.06$). When analyzing each microsatellite locus separately, the association between heterozygosity and FA at population level was strongest at loci Pdoµ1, Pdo16 and TG04-012, whereas mean differences in allelic size were strongest at locus Pdo16. After sequential Bonferroni correction for multiple testing, the association at locus Pdoµ1 remained significant (Table 3.3).

As all loci were in linkage equilibrium and heterozygosity-heterozygosity correlations were low, FA was modeled as a multiple regression with mean heterozygosity at each locus as independent variable. A model with all loci explained 85% of the variance in FA, whereas 43% of the variance was explained by a model with locus Pdoµ1 only, and 53% by a model with loci Pdoµ1 and
Developmental stability covaries with heterozygosity

Fig. 3.3. Inverse relationship between standardized multilocus heterozygosity and fluctuating asymmetry across 26 house sparrow populations.

Pdo16 only. After removing one or both loci, FA-MLH relationships remained significant (Pdo1 removed: $F_{1,24}=9.97$, $p=0.0043$, $R^2_{MLH}=0.29$ ; Pdo1+Pdo16 removed: $F_{1,24}=6.94$, $p=0.015$, $R^2_{MLH}=0.22$). The strength of single-locus FA-$d^2$ relationships (16 loci) were positively correlated with expected heterozygosity at population level ($r_s=0.54$, $p=0.03$) but not at individual level ($r_s=-0.21$, $p=0.43$). In contrast, FA-MLH relationships did not significantly vary with genetic diversity (all $p>0.58$) (Figure 3.4).

**Effects of urbanization on FA-genotype relationships**

The strength of FA-MLH relationships tested at the individual level significantly varied with urbanization ($F_{2,513}=4.25$, $p=0.01$): both variables were inversely related in rural populations ($t_{513}=-3.73$, $p=0.002$), but unrelated in urban ($t_{513}=-0.45$, $p=0.65$) and suburban ($t_{513}=0.95$, $p=0.34$) ones. In contrast, the strength and direction of FA-$\hat{f}$ ($F_{2,41.6}=0.79$, $p=0.46$) and FA-$d^2$ ($F_{2,307}=2.05$, $p=0.13$) relationships did not vary with urbanization.
Table 3.2. Relationship between fluctuating asymmetry and three multi-locus genetic diversity estimates at three hierarchical levels of statistical analysis. Significant tests are indicated in bold.

<table>
<thead>
<tr>
<th></th>
<th>d²</th>
<th>slope (SE)</th>
<th>F</th>
<th>num, den</th>
<th>p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual level across all individuals</td>
<td>-1.20 (0.83)</td>
<td>2.11</td>
<td>1, 517</td>
<td>0.15</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>Individual level within population</td>
<td>-1.02 (0.94)</td>
<td>1.18</td>
<td>1, 467</td>
<td>0.28</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Population level</td>
<td>-3.93 (3.12)</td>
<td>1.59</td>
<td>1, 24</td>
<td>0.22</td>
<td>0.062</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>MLH</th>
<th>slope (SE)</th>
<th>F</th>
<th>num, den</th>
<th>p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual level across all individuals</td>
<td>-0.49 (0.19)</td>
<td>6.59</td>
<td>1, 517</td>
<td><strong>0.01</strong></td>
<td>0.049</td>
<td></td>
</tr>
<tr>
<td>Individual level within population</td>
<td>-0.30 (0.23)</td>
<td>1.73</td>
<td>1, 467</td>
<td>0.19</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Population level</td>
<td>-1.88 (0.54)</td>
<td>12.31</td>
<td>1, 24</td>
<td><strong>0.001</strong></td>
<td>0.339</td>
<td></td>
</tr>
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<table>
<thead>
<tr>
<th></th>
<th>Ritland estimates</th>
<th>slope (SE)</th>
<th>F</th>
<th>num, den</th>
<th>p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual level across all individuals</td>
<td>0.63 (0.29)</td>
<td>4.70</td>
<td>1, 517</td>
<td><strong>0.03</strong></td>
<td>0.046</td>
<td></td>
</tr>
<tr>
<td>Individual level within population</td>
<td>0.33 (0.39)</td>
<td>0.70</td>
<td>1, 467</td>
<td>0.40</td>
<td>-</td>
<td></td>
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<tr>
<td>Population level</td>
<td>2.18 (0.78)</td>
<td>7.88</td>
<td>1, 24</td>
<td><strong>0.009</strong></td>
<td>0.247</td>
<td></td>
</tr>
</tbody>
</table>

F=F-test, num,den= numerator and denominator degrees of freedom, R²= amount of variation in FA explained by heterozygosity.

**DISCUSSION**

Estimates of genetic diversity and developmental stability, averaged across individuals, significantly co-varied in the direction expected by population genetic theory, whereas individual estimates were only weakly associated. Both genome-wide and locus-specific estimates of genetic diversity strongly correlated with developmental stability at the population level, and this correlation was mainly driven by genetic variation at two key loci only.

Whether relationships between developmental stability and genetic variability are driven by genome-wide heterozygosity or local effect of key loci, remains a topic of much debate [7,9]. Relationships between proxies of developmental stability and genetic variability have typically been based on limited numbers of loci only, which were implicitly assumed to represent genome-wide properties. Such assumption, however, is only justified when repeated random subsets of markers give rise to strong heterozygosity-heterozygosity correlations [61], and this premise is often violated in randomly mating populations [61,68,69]. As levels of heterozygosity among markers within individuals were only moderately correlated in this study, our results do not fully
Developmental stability covaries with heterozygosity support the role of genome-wide heterozygosity underlying relationships with developmental stability. Rather, single-locus effects at a few key loci, such as Pdoµ1, are more likely to drive these relationships.

**Table 3.3.** Relationship between fluctuating asymmetry and single-locus genetic diversity at the individual (across all individuals) and population level. Statistical significance levels before (bold) and after (underlined) Bonferroni correction for multiple tests refer to a critical alpha-value of 0.05.

<table>
<thead>
<tr>
<th>Locus</th>
<th>He</th>
<th>F num, p</th>
<th>R²</th>
<th>F num, p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG01-040</td>
<td>0.45</td>
<td>3.53 1,495 0.06 0.045</td>
<td>6.8 1,495 0.01 0.051</td>
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<td>TG01-148</td>
<td>0.41</td>
<td>3.05 1,374 0.08 0.049</td>
<td>1.77 1,374 0.18 0.046</td>
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<td></td>
</tr>
<tr>
<td>TG04-012</td>
<td>0.61</td>
<td>0.09 1,474 0.77 0.039</td>
<td>0.48 1,474 0.49 0.039</td>
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<td></td>
</tr>
<tr>
<td>TG07-022</td>
<td>0.43</td>
<td>0.29 1,433 0.59 0.031</td>
<td>0.21 1,433 0.65 0.031</td>
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</tr>
<tr>
<td>TG13-017</td>
<td>0.66</td>
<td>0.91 1,469 0.34 0.033</td>
<td>0.38 1,469 0.54 0.032</td>
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</tr>
<tr>
<td>TG22-001</td>
<td>0.49</td>
<td>0.04 1,369 0.84 0.027</td>
<td>2.40 1,369 0.12 0.033</td>
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<tr>
<td>Pdoµ1</td>
<td>0.87</td>
<td>0.46 1,495 0.50 0.041</td>
<td>2.86 1,495 0.09 0.046</td>
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<tr>
<td>Pdoµ3</td>
<td>0.89</td>
<td>0.22 1,400 0.64 0.025</td>
<td>0.55 1,400 0.46 0.026</td>
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<tr>
<td>Pdoµ5</td>
<td>0.85</td>
<td>0.71 1,442 0.40 0.038</td>
<td>1.10 1,442 0.29 0.039</td>
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</tr>
<tr>
<td>Pdo9</td>
<td>0.79</td>
<td>3.15 1,378 0.08 0.043</td>
<td>0.62 1,378 0.43 0.036</td>
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<td></td>
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<tr>
<td>Pdo10</td>
<td>0.84</td>
<td>0.14 1,477 0.71 0.039</td>
<td>1.51 1,477 0.22 0.042</td>
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<tr>
<td>Pdo16</td>
<td>0.87</td>
<td>0.35 1,482 0.56 0.042</td>
<td>0.02 1,482 0.88 0.041</td>
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<tr>
<td>Pdo19</td>
<td>0.64</td>
<td>10.81 1,481 0.001 0.060</td>
<td>6.01 1,481 0.01 0.051</td>
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<tr>
<td>Pdo22</td>
<td>0.74</td>
<td>0.04 1,500 0.84 0.042</td>
<td>1.19 1,500 0.27 0.044</td>
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<tr>
<td>Pdo32</td>
<td>0.78</td>
<td>1.01 1,446 0.31 0.028</td>
<td>0.08 1,446 0.78 0.026</td>
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<tr>
<td>Pdo47</td>
<td>0.85</td>
<td>0.5 1,476 0.48 0.037</td>
<td>0.78 1,476 0.38 0.037</td>
<td></td>
<td></td>
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<table>
<thead>
<tr>
<th>Locus</th>
<th>He</th>
<th>F num, p</th>
<th>R²</th>
<th>F num, p</th>
<th>R²</th>
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<tr>
<td>TG01-040</td>
<td>0.45</td>
<td>0.09 1,24 0.76 0.004</td>
<td>0.47 1,24 0.50 0.019</td>
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<td>TG01-148</td>
<td>0.41</td>
<td>0.34 1,24 0.57 0.014</td>
<td>0.78 1,24 0.38 0.032</td>
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<td>TG04-012</td>
<td>0.61</td>
<td>0.93 1,24 0.34 0.037</td>
<td>5.09 1,24 0.03 0.175</td>
<td></td>
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<tr>
<td>TG07-022</td>
<td>0.43</td>
<td>0.02 1,24 0.90 0.001</td>
<td>0.99 1,24 0.33 0.040</td>
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<tr>
<td>TG13-017</td>
<td>0.66</td>
<td>2.85 1,24 0.10 0.106</td>
<td>0.21 1,24 0.65 0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG22-001</td>
<td>0.49</td>
<td>2.38 1,24 0.14 0.090</td>
<td>3.13 1,24 0.09 0.115</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pdoµ1</td>
<td>0.87</td>
<td>3.26 1,24 0.08 0.120</td>
<td>18.17 1,24 0.001 0.431</td>
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</tr>
<tr>
<td>Pdoµ3</td>
<td>0.89</td>
<td>2.04 1,24 0.17 0.078</td>
<td>0.02 1,24 0.88 0.001</td>
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</tr>
<tr>
<td>Pdoµ5</td>
<td>0.85</td>
<td>0.12 1,24 0.73 0.005</td>
<td>0.53 1,24 0.47 0.022</td>
<td></td>
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</tr>
<tr>
<td>Pdo9</td>
<td>0.79</td>
<td>0.42 1,24 0.52 0.017</td>
<td>0.01 1,24 0.96 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pdo10</td>
<td>0.84</td>
<td>0.59 1,24 0.45 0.024</td>
<td>0.08 1,24 0.78 0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pdo16</td>
<td>0.87</td>
<td>6.65 1,24 0.02 0.217</td>
<td>7.33 1,24 0.01 0.234</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pdo19</td>
<td>0.64</td>
<td>0.23 1,24 0.64 0.009</td>
<td>0.58 1,24 0.45 0.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pdo22</td>
<td>0.74</td>
<td>1.34 1,24 0.26 0.053</td>
<td>0.67 1,24 0.42 0.027</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pdo32</td>
<td>0.78</td>
<td>1.06 1,24 0.31 0.042</td>
<td>2.28 1,24 0.14 0.087</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pdo47</td>
<td>0.85</td>
<td>2.31 1,24 0.14 0.088</td>
<td>1.95 1,24 0.18 0.075</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F=F-test, num, den= numerator and denominator degrees of freedom, R²= amount of variation in FA explained by heterozygosity.
Fig. 3.4. Relationship between locus specific variability (H_e) and strength of the association between FA and genetic diversity. Left panes represent analyses at the individual level, right panes those at the population level. Associations are shown for two diversity indices: d² (upper panes) and observed heterozygosity (lower panes).

Recent studies challenged the view that high levels of linkage disequilibrium are uncommon in natural populations, especially in small, bottlenecked or recently-mixed populations [70,71,72]. In addition, the selection of markers in genetic studies may be biased if based on the criterion of maximum variability [37], resulting in a slight overrepresentation of genes under balancing selection that retain enhanced levels of gene diversity due to heterosis. In our study, both markers that showed the strongest single-locus effects on developmental stability also displayed very high levels of heterozygosity. Likewise, fitness traits responded most strongly to the genetic constitution of the four most variable loci in a study on *Acrocephalus arundinaceus* [70]. Despite the fact that results from our study provide strong evidence for single-locus effects, genome-wide effects cannot entirely be ruled out as associations between FA and MLH persisted after removal of the two presumed key loci.

Unlike MLH and Ritland estimates, mean d² only weakly predicted patterns in developmental stability at the population level. Results from this study hence
Developmental stability covaries with heterozygosity

Support the conclusion that heterozygosity-based measures usually outperform those based on allelic distances like \( d^2 \) to estimate inbreeding [73] and the negative appraisal of the use of squared distances between alleles to model relationships with fitness or its proxies [74]. Under recent admixture of genetically divergent populations [65,75] or high variability at microsatellite loci [65,74,75,76], however, the use of mean \( d^2 \) may still be justified. While some studies showed stronger genotype-fitness associations with increasing variability of the genetic marker under study [70,77], others failed to detect such relationship [69] despite the theoretical prediction of such an effect [23]. Our results show that the effect of marker variability and relationships with proxies of fitness can be marker-dependent. The positive relationship in mean \( d^2 \), but not in both other markers, may be explained by the fact that highly variable loci are thought to mutate in a step-wise mode, which is the underlying model for \( d^2 \)-based measures [65,78,79]. Yet, even at the most variable loci, mean \( d^2 \) did not reach equal explanatory power compared to single-locus heterozygosity.

It has been hypothesized that the strength of relationships between developmental stability and genetic diversity may depend on other types of stressors [2] and that relationships with genetic stress or fitness may be more apparent under adverse conditions, i.e. when individuals are energetically challenged [12,13,15,80]. Results of this study are not in concordance with this hypothesis since FA-heterozygosity associations were strongest in rural, not urban, populations. If juvenile mortality rates were higher in urban populations and selective in relation to FA, highly asymmetric and homozygous adults might be locally underrepresented, possibly changing the direction and/or strength of associations between FA and genetic variability. While nest studies on house sparrows revealed increased rates of nestling mortality when levels of insect abundance were critically low [33], levels of FA were not significantly lower in urban compared to suburban or rural populations in our study area [81] and observed proportions of homozygous individuals matched the expected ones as populations were in Hardy-Weinberg equilibrium. Hence, lack of support for interactive effects of nutritional stress and genetic diversity in the direction predicted, more likely resulted from low statistical power of individual-level analyses, although we cannot rule out that levels of stress during trait ontogeny in our study area were lower than those reported in the literature [33] as we did not quantitatively gauge the amount of perceived stress. Unfortunately, the restricted number of urban populations prevented us from testing the interactive stress hypothesis at the population level which would have assisted us in
differentiating between low statistical power and an absence of stress as a possible explanation of the observed individual-level patterns.

In conclusion, results of this study provide strong evidence that relationships between developmental stability and heterozygosity can be driven by local effects at a few key loci and/or by genome-wide effects, the relative contribution of which may depend on relative frequencies and fitness effects of deleterious genes [23]. Despite the fact that local linkage disequilibrium with key loci is regarded as the most promising mechanism to explain associations between developmental stability and heterozygosity, empirical support for the local effect hypothesis remains scant. Further research is therefore needed to unravel the relative effects of single-locus and genome-wide processes on developmental stability of populations with different genetic properties. While developmental stability earlier proved to be weakly associated with nutritional stress [81], relationships with heterozygosity appear stronger at the population level, irrespective of the underlying genetic basis. This study emphasizes again that the accuracy of developmental stability as a proxy for heterozygosity at the individual level remains low and the application of individual FA estimates in general should be abandoned.

ACKNOWLEDGEMENTS

We are indebted to H. Matheve, T. Cammaer and C. Nuyens for field assistance and A. Krupa and G. Horsburgh for laboratory assistance. We are greatful to two anonymous reviewers for providing helpful comments that greatly improved the manuscript. Genotyping was performed at the NERC Biomolecular Analysis Facility funded by the Natural Environment Research Council, UK. Fieldwork and genetic analyses were funded by research grants G.0149.09 of the Fund for Scientific Research - Flanders (to S. Van Dongen and LL) and research grant 01J01808 of Ghent University (to LL).

REFERENCES


Developmental stability covaries with heterozygosity


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Chapter 3


Appendix. Details of the 16 microsatellites used in this study, their location on the zebra finch (Taeniopygia guttata) genome and the position of the nearest known zebra finch gene.

<table>
<thead>
<tr>
<th>Loas</th>
<th>EMBL accession number</th>
<th>Full sequence length</th>
<th>Location in the zebra finch genome</th>
<th>Nearest gene on ZF map</th>
<th>Tgt name (Burt, Roslin)</th>
<th>Description gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG01-040</td>
<td>DV576233</td>
<td>832</td>
<td>Chr 1A: 42,620,542</td>
<td>Contig20.15</td>
<td>Chr 1A: 42,621,707-42,624,796 reverse strand</td>
<td>DUSP6</td>
</tr>
<tr>
<td>TG01-148</td>
<td>CK301512</td>
<td>849</td>
<td>Chr 1: 65,237,140</td>
<td>Contig3.570</td>
<td>Chr 1: 65,244,245-65,244,574 reverse strand</td>
<td>PDC9-1</td>
</tr>
<tr>
<td>TG04-012</td>
<td>CK306810</td>
<td>657</td>
<td>Chr 4A: 17,044,573 (chr unknown)</td>
<td>Contig15.1086</td>
<td>Chr 4A: 16,986,073-17,044,190 forward strand</td>
<td>ARHGEF9</td>
</tr>
<tr>
<td>TG07-022</td>
<td>DV948210</td>
<td>715</td>
<td>Chr 7: 11,940,140 &amp; Chr 7: 11,970,627 Contig5.1386</td>
<td>Chr 7: 11,915,850-11,965,575 reverse strand</td>
<td>IFIH1</td>
<td>Interferon-induced helicase C domain-containing protein 1</td>
</tr>
<tr>
<td>TG13-017</td>
<td>CK313422</td>
<td>853</td>
<td>Chr 13: 18,542</td>
<td>Contig147.4</td>
<td>Chr 13: 18,142-21,075 reverse strand</td>
<td>EGR1</td>
</tr>
<tr>
<td>TG22-001</td>
<td>CK317533</td>
<td>654</td>
<td>Chr 22: 1,428,098 (chr unknown)</td>
<td>Contig117.57</td>
<td>Chr 22: 1,429,499-1,460,562 reverse strand</td>
<td>BNP3L</td>
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<tr>
<td>Pdo1b1</td>
<td>AM287188</td>
<td>191</td>
<td>Chr 1A: 34,300,915</td>
<td>Contig20.275</td>
<td>Chr 1A: 34,269,533-34,271,134 forward strand</td>
<td>DYNK2</td>
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<tr>
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<td>AM287190</td>
<td>277</td>
<td>Chr 8: 1,399,316 (chr unknown)</td>
<td>Contig62.252</td>
<td>Chr 8: 1,951,927-2,011,591 reverse strand</td>
<td>PLAPG4A</td>
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<td>Pdo1b3</td>
<td>YS126</td>
<td>390</td>
<td>Chr 4: 48,501,861</td>
<td>Contig11.668</td>
<td>Chr 4: 48,579,775-48,683,197 reverse strand</td>
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<tr>
<td>Pdo1b4</td>
<td>AF354423</td>
<td>518</td>
<td>Chr 24: 5,327,333 (chr unknown)</td>
<td>Contig70.208</td>
<td>Chr 24: 5,258,154-5,276,865 forward strand</td>
<td>ATP21A</td>
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<tr>
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<td>AF354424</td>
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<td>Contig3.829</td>
<td>Chr 1: 58,299,864-58,682,577 reverse strand</td>
<td>ENOX1</td>
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<td>294</td>
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<td>Contig34.193</td>
<td>Chr 2: 96,803,202-96,810,698 forward strand</td>
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<td>341</td>
<td>Chr 2: 33,983,679</td>
<td>Contig3.1020</td>
<td>Chr 2: 33,910,198-33,986,663 reverse strand</td>
<td>not available</td>
</tr>
<tr>
<td>Pdo1b8</td>
<td>AM159001</td>
<td>146</td>
<td>Chr 4: 9,128,968</td>
<td>Contig2.145</td>
<td>Chr 4: 9,131,494-9,150,548 forward strand</td>
<td>IL15</td>
</tr>
<tr>
<td>Pdo1b9</td>
<td>AM159011</td>
<td>361</td>
<td>Chr 1: 41,865,024</td>
<td>Contig5.931</td>
<td>Chr 1: 41,781,858-42,61,992 reverse strand</td>
<td>GP1C6</td>
</tr>
<tr>
<td>Pdo1b10</td>
<td>AM159027</td>
<td>282</td>
<td>Chr 5: 54,452,777</td>
<td>Contig1.2403</td>
<td>Chr 5: 54,573,873-54,712,474 reverse strand</td>
<td>BRF1</td>
</tr>
</tbody>
</table>

* EST based microsatellites, † Anonymous microsatellites, isolated via traditional cloning
Source species: * zebra finch, † house sparrow

§ Genome locations in the zebra finch were assigned using the WU GSC BUST software provided by the Washington University server following [82]
Chapter 4

Characterizing spatial genetic structure in contemporary house sparrow populations along an urbanization gradient

Carl VANGESTEL, Joachim MERGEAY, Deborah A. DAWSON, Tom CALLENS, Viki VANDOMME and Luc LENS

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Chapter 4

ABSTRACT

House sparrows (Passer domesticus) have suffered major declines in urban as well as rural areas, while remaining relatively stable in suburban ones. The difference in timing, magnitude and progress of this urban and rural decline has suggested that the rural area on the one hand and the urban and suburban ones on the other hand, can be regarded as two distinct and independent units. Here we use neutral DNA markers to study if and how genetic variation can be partitioned in a hierarchical way among different urbanization classes. Principal coordinate analyses seemed to confirm the hypothesis that urban/suburban and rural populations comprise two distinct genetic clusters although the difference remained weak. Comparison of $D_{	ext{est}}$ values at different hierarchical scales provided evidence for population differentiation through genetic drift. Isolation by distance was not observed in either the urban/suburban or rural area, but there was evidence of such a pattern when all populations were included into a single analysis. Hence, the association between genetic and geographical distance was most likely driven by genetic differences between the urban/suburban and rural areas. The results shown here can be used as baseline information for future genetic monitoring programmes and provide additional insights into contemporary house sparrow dynamics along an urbanization gradient.

INTRODUCTION

Population connectivity, mediated by dispersal, influences many key ecological and evolutionary features (Ronce, 2007). It affects population growth rates, spatial distribution of genetic diversity across populations, local adaptation and global population dynamics (Ronce, 2007; Lowe and Allendorf, 2010). Connectivity among populations is one of the key determinants of long-term population viability and profoundly affects resilience to demographic, genetic and environmental disturbances (Hanski, 1999). Although conservation biologists sometimes refer to connectivity as a phenomenon per se, genetic and demographic connectivity are two fundamentally distinct concepts which cannot be used interchangeably (Lowe and Allendorf, 2010). Genetic connectivity, the extent to which gene flow affects evolutionary processes (Lowe and Allendorf, 2010) does not always provide insights into demographic connectivity, the degree to which immigration affects growth, survival and birth rates (Taylor and Dizon, 1999; Lowe and Allendorf, 2010), and vice versa. A solid understanding on population substructure and exchange of migrants between populations has thus become a major goal of many ecological studies as it can substantially facilitate management decisions. In the past such information was limited to costly and time-consuming long-term demographical studies, but with the emergence of modern advanced molecular techniques useful and complementary end-results can often be obtained simply by dint of sampling a fraction of the population at a single moment in time. In addition, according to the management objectives in question, use of genetic studies may complement other studies aiming
to delineate appropriate biological conservation units (Taylor and Dizon, 1996, 1999; Avise, 1995).

House sparrows (*Passer domesticus*), once a thriving ubiquitous species (Anderson 2006), have suffered a dramatic decline in abundance and distribution in built-up and rural habitats during the last decades (Hole et al. 2002; Chamberlain et al. 2007; De Laet and Summers-Smith 2007). Within the built-up environment we can differentiate between urban populations, which have suffered massive declines, and suburban ones which have remained rather stable. Evidence has accumulated that these urban and rural reductions vary considerably both spatially as well as temporally (De Laet and Summers-Smith 2007; Shaw et al. 2008). The onset of the rural decline precedes the urban one and while in the former numbers have stabilized, albeit at a lower level, they continue to plummet in highly urbanized city centres (De Laet and Summers-Smith 2007). Agricultural intensification, inversely related to survival rate as a consequence of reduced food availability, has generally been accepted as the proximate cause of the rural decline (Hole et al. 2002). In contrast, a myriad of putative hypotheses have been put forward in an attempt to explain the decline in highly urbanized areas but none of them seem to be unanimously persuasive. Urban crashes have been linked to loss of source-sink dynamics (Hole 2001; Wilson 2004), increased predation pressure (Shaw et al. 2008; Bell et al. 2010), detrimental effects of vehicle emissions (Bignal et al. 2004) and electromagnetic pollution from mobile phone masts (Balmori and Hallberg 2007; Everaert and Bauwens 2007; Balmori 2009). To date, the most compelling evidence stems from a nest-box study conducted along an urban gradient, which suggested reduced reproductive output due to insufficient invertebrate availability and subsequent deficit in local recruitment as a major contributor of urban decline in house sparrow numbers (Peach et al. 2008).

House sparrows are among the most sedentary of all passerine birds (Heij and Moeliker 1990; Anderson 2006). Estimates of mean postnatal dispersal distance for this species are as low as 1-1.7km (Cheke 1972; Paradis et al. 1998) and adults tend to remain faithful to the colony in which they first breed (Summers-Smith 1988). This sedentary behaviour combined with the difference in timing, magnitude and progress of the decline have led to the general belief that urban and suburban populations on the one hand and rural ones on the other hand, comprise two distinct and independent units and hence call for separate conservation strategies (Anderson 2006; De Laet and Summers-Smith 2007; Shaw et al. 2008). This implicitly assumes that both habitats are characterized by a high degree of isolation and are to a large extent self-sustainable (Wilson 2004). This view has been challenged by Heij (1985)
who concluded in his study that both urban and rural populations were highly dependent on suburban immigrants for their long-term viability. In addition, several other studies revealed no (large) genetic dissimilarities between populations in the absence of geographical barriers (Fleisher 1983; Parkin and Cole 1984; Kekkonen et al. 2010). However, all these studies share a common feature as they describe dispersal and/or genetic structure well before the onset of the decline. Interestingly, Hole (2001) did report significant genetic structure and loss of connectivity between neighbouring farms in a rural area well after the decline has commenced. Local extinction without recolonization may transform a contiguous distribution of house sparrow populations into a patchy one, a pattern currently observed (especially) in many urban areas (Shaw et al. 2008; Vangestel unpublished data). As house sparrows most likely used to disperse according to a stepping-stone pattern (Kekkonen et al. 2010), loss of intermediate ‘stepping-stones’ combined with their intrinsic sedentary nature (Anderson 2006) may have reduced or even inhibited contemporary dispersal between adjacent colonies (Hole 2001). Hole (2001) was among the first to suggest that such loss of (intermediate) populations may gradually increase distances between remaining source-sink populations up to a point at which metapopulation dynamics become constrained and populations genetically diverge. He argued that this may ultimately result in an extinction-vortex that spreads throughout the landscape. Particularly less vagile species such as house sparrows are expected to show increased vulnerability to such landscape alterations (Hole 2001; Sekercioglu et al. 2002) and a tendency towards greater population structure (Stangel 1990). Hence, together with the observed large-scale reductions in urban house sparrow numbers (De Laet and Summers-Smith 2007), we could expect that contemporary urban house sparrow populations are particularly susceptible to processes like genetic drift and that exchange of migrants between contemporary populations may have become problematic in this species.

As such, historical estimates of genetic connectivity do not necessarily resemble current ones as dispersal rates may have changed substantially over the course of time. Yet, no attempt has been made to obtain detailed information on small-scale genetic structure in three different urbanization classes for this species in its post-decline era. This paucity of information advocates for an urgent need to bridge this lacuna. In the current study we use genetic variation across microsatellite loci to identify small-scale genetic differentiation and changes in genetic diversity along an urban-rural gradient and relate this to putative demographic changes. These results may further contribute to our general understanding of contemporary population dynamics of house sparrows in an urban-rural landscape.
MATERIAL AND METHODS

Study site and species

House sparrows were studied in and around the city centre of Ghent (northern Belgium) and in an adjacent rural area near the village of Zomergem, ca. 12 km NW of Ghent. The degree of urbanization was measured as the ratio of built-up to total grid cell area (each cell measuring 90,000 m² on the ground) and ranged between 0-0.10 (henceforward referred to as ‘rural’), 0.11-0.30 (‘suburban’) and larger than 0.30 (‘urban’) (Arcgis version 9.2). Within this range we selected 26 plots (figure 4.1), in which we captured a total of 690 adult house sparrows by standard mist netting between 2003 and 2009 (table 4.1) and attempted to obtain an equal sex ratio of males and females within each plot. Although samples were collected over a time span of 6 years there is no evidence that large changes in allele frequencies have occurred during this period so samples were pooled across years. Upon capture, we took standard morphological measurements (see Vangestel et al. 2010 for details) and collected a small sample of body feathers for DNA analysis.

DNA extraction, PCR and genotyping

We applied a Chelex resin-based method (InstaGene Matrix, Bio-Rad) (Walsh et al. 1991) to extract genomic DNA from a total of ten plucked body feathers. Sixteen microsatellite markers (both traditional ‘anonymous’ microsatellites as well as those developed based on expressed sequence tags (table 4.2)) were selected based on their polymorphism and stutter profile. Polymerase chain reactions were organized in four multiplex-sets and compatibility between primer pairs was checked using AutoDimer (Vallone and Butler 2004). The first multiplex reaction contained Pdoµ1 (Neumann and Wetton 1996), Pdo32, Pdo47 (Dawson et al. in preparation) and TG04-012 (Dawson et al. 2010); the second one contained Pdoµ3 (Neumann and Wetton, 1996), Pdoµ5 (Griffith et al. 1999), TG13-017 and TG07-022 (Dawson et al. 2010); the third multiplex reaction contained Pdo10 (Griffith et al. 2007), Pdo16, Pdo19, Pdo22 (Dawson et al. in preparation) and TG01-040 (Dawson et al. 2010); the last set consisted of Pdo9 (Griffith et al. 2007), TG01-148 and TG22-001 (Dawson et al. 2010). PCR reactions were performed on a 2720 Thermal Cycler (Applied Biosystems) in 9 µL volumes and contained approximately 3 µL genomic DNA, 3 µL QIAGEN Multiplex PCR Mastermix (QIAGEN) and 3 µL primer mix (concentrations were 0.1 µM (Pdoµ1), 0.12 µM (TG01-148), 0.16 µM (Pdo10, Pdo19, Pdo22, Pdo32, TG04-012) and 0.2 µM (Pdoµ3, Pdoµ5, Pdo9, Pdo16, Pdo47, TG01-040, TG07-022, TG13-017, TG22-001)). The applied PCR profile contained an initial denaturation step of 15 min at 95°C, followed by 35 cycles of 30 s at 94°C, 90 s at 57°C and 60 s at 72°C.
Finally, an additional elongation step of 30 min at 60°C and an indefinite hold at 4°C was allowed. Prior to genotyping sample DNA concentrations were quantified using a ND1000 spectrometer (Nanodrop Technologies) and adjusted to a final concentration of 10 ng/µL. Negative controls were employed during extraction and PCR to rule out contamination of reagents. PCR products were visualized on an ABI3130 Genetic Analyzer (Applied Biosystems), an internal LIZ-600 size standard was applied to determine allele size and fragments were scored using the software package GENEMAPPER 4.0.

Table 4.1. Schematic overview of the number of individuals sampled per year per location. Abbreviations refer to populations described in table 3 (Introduction).

<table>
<thead>
<tr>
<th>Population( ^{(a)} )</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>OW-R</td>
<td>5</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DB-R</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EI-R</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GS-R</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS-R</td>
<td>6</td>
<td>10</td>
<td>3</td>
<td></td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HY-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>MS-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td>PD-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>PS-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>RS-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>VM-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>SD-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>FM-SU</td>
<td>2</td>
<td>16</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR-SU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>HL-SU</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>7</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>MB-SU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>SC-SU</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>SS-SU</td>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>5</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>WB-SU</td>
<td>2</td>
<td></td>
<td></td>
<td>12</td>
<td></td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>WO-SU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td>ML-SU</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>EB-U</td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td></td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>PH-U</td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>5</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>VI-U</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>SP-U</td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>9</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

\( ^{(a)} \) Last letter refers to urbanization class (R=rural; SU=suburban; U=urban)
Fig. 4.1. Map showing urban (filled circles), suburban (open circles) and rural (filled triangles) study plots within and near the city of Ghent (Belgium). Inner contour encompasses Ghent city centre, outer contour encompasses surrounding municipalities. Abbreviations refer to populations described in table 3 (Introduction).
Microsatellite analysis

We used MICRO-CHECKER (Van Oosterhout et al. 2004) to identify scoring errors that could be attributed to stuttering, differential amplification of size-variant alleles causing large allele drop-out or the presence of null alleles. All microsatellite loci were checked for Hardy-Weinberg and linkage equilibrium with GENEPOP version 4.0 (Raymond and Rousset 1995, Rousset 2008) and a family-wise error rate of 0.05 was obtained by employing a sequential Bonferroni correction as suggested by Weir (1990).

Patterns of genetic diversity along urbanization classes.

Genetic diversity for each locus-population combination was quantified using three statistics: allelic richness \(\left(A_{[\hat{g}]}\right)\), observed \(\left(H_{o}\right)\) and unbiased expected heterozygosity \(\left(H_{u};\right.\) Nei 1978). Measures of heterozygosity were computed in FSTAT version 2.9.3.2 (Goudet, 1995), while allelic richness was calculated using ARES version 1.2.2 (Van Loon et al., 2007). This latter measure is highly dependent on sample size (Kalinowski 2004) and patterns of allelic diversity may be obscured or even reversed when samples of different sizes are compared (Van Loon et al., 2007). We used the rarefaction method (Hurlbert 1971) to compensate for potential confounding effects of such variation in sample numbers. Rather than using the smallest observed sample size as a reference sample size which might result in a loss of information or decrease in accuracy (Van Loon et al. 2007), the algorithm implemented in the ARES package allows extrapolation to a user-predefined sample size. The average number of gene copies \(\left(g\right)\) per population in our dataset was 40 which we therefore used as the common sample size. The significance of spatial heterogeneity in genetic diversity indices between the three urbanization classes was calculated using 1000 permutations. We used DEMEtics version 0.8.0 (Gerlach et al. 2010) to calculate pair-wise levels of genetic differentiation among all populations and overall levels of genetic differentiation within each urbanization class. We preferred to calculate \(D_{\text{est}}\) instead of the more traditional \(F_{\text{st}}\) values because the former is independent of the level of loci polymorphism and therefore yields more sensible and easily interpretable estimates of population differentiation (Jost 2008). A permutation approach with 1000 iterations was used to assess the level of statistical significance. We tested whether isolation by distance contributed substantially to genetic differentiation by correlating genetic distance, \(D_{\text{est}}/(1- D_{\text{est}})\), with geographical distance (Bohonak 2002).
Table 4.2. Locus specific summary statistics for 16 microsatellite markers.

<table>
<thead>
<tr>
<th>locus</th>
<th>$N$</th>
<th>$N_A$</th>
<th>$H_o$</th>
<th>$H_e$</th>
<th>$D_{est}$</th>
<th>$f_{null}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG01-040$^a$</td>
<td>537</td>
<td>6</td>
<td>0.40</td>
<td>0.44</td>
<td>0.019</td>
<td>0.028 (0.01)</td>
</tr>
<tr>
<td>TG01-148$^a$</td>
<td>486</td>
<td>3</td>
<td>0.42</td>
<td>0.38</td>
<td>-0.005 (-0.016, 0.007)</td>
<td>-0.023 (0.02)</td>
</tr>
<tr>
<td>TG04-012$^a$</td>
<td>549</td>
<td>5</td>
<td>0.53</td>
<td>0.59</td>
<td>0.002 (-0.016, 0.020)</td>
<td>0.039 (0.016)</td>
</tr>
<tr>
<td>TG07-022$^a$</td>
<td>493</td>
<td>5</td>
<td>0.37</td>
<td>0.41</td>
<td>0.022 (0.012, 0.031)</td>
<td>0.026 (0.012)</td>
</tr>
<tr>
<td>TG13-017$^a$</td>
<td>547</td>
<td>8</td>
<td>0.52</td>
<td>0.64</td>
<td>0.028 (0.008, 0.049)</td>
<td>0.074 (0.015)</td>
</tr>
<tr>
<td>TG22-001$^a$</td>
<td>478</td>
<td>11</td>
<td>0.34</td>
<td>0.41</td>
<td>0.020 (0.005, 0.034)</td>
<td>0.054 (0.013)</td>
</tr>
<tr>
<td>pdoµ1</td>
<td>550</td>
<td>20</td>
<td>0.80</td>
<td>0.85</td>
<td>0.067 (0.032, 0.102)</td>
<td>0.024 (0.009)</td>
</tr>
<tr>
<td>pdoµ3</td>
<td>515</td>
<td>19</td>
<td>0.83</td>
<td>0.85</td>
<td>0.128 (0.078, 0.178)</td>
<td>0.015 (0.007)</td>
</tr>
<tr>
<td>pdoµ5</td>
<td>523</td>
<td>22</td>
<td>0.76</td>
<td>0.82</td>
<td>0.289 (0.243, 0.335)</td>
<td>0.033 (0.011)</td>
</tr>
<tr>
<td>pdo9</td>
<td>442</td>
<td>31</td>
<td>0.65</td>
<td>0.75</td>
<td>0.236 (0.193, 0.279)</td>
<td>0.052 (0.013)</td>
</tr>
<tr>
<td>pdo10</td>
<td>596</td>
<td>18</td>
<td>0.78</td>
<td>0.82</td>
<td>0.072 (0.037, 0.107)</td>
<td>0.021 (0.011)</td>
</tr>
<tr>
<td>pdo16</td>
<td>549</td>
<td>17</td>
<td>0.81</td>
<td>0.84</td>
<td>0.061 (0.025, 0.097)</td>
<td>0.016 (0.01)</td>
</tr>
<tr>
<td>Pdo19</td>
<td>573</td>
<td>9</td>
<td>0.60</td>
<td>0.62</td>
<td>0.039 (0.021, 0.057)</td>
<td>0.008 (0.011)</td>
</tr>
<tr>
<td>Pdo22</td>
<td>578</td>
<td>16</td>
<td>0.73</td>
<td>0.72</td>
<td>0.034 (0.015, 0.053)</td>
<td>-0.005 (0.011)</td>
</tr>
<tr>
<td>pdo32</td>
<td>491</td>
<td>20</td>
<td>0.59</td>
<td>0.75</td>
<td>0.069 (0.038, 0.100)</td>
<td>0.093 (0.015)</td>
</tr>
<tr>
<td>pdo47</td>
<td>562</td>
<td>17</td>
<td>0.68</td>
<td>0.83</td>
<td>0.156 (0.118, 0.194)</td>
<td>0.078 (0.013)</td>
</tr>
</tbody>
</table>

$^a$EST based microsatellites
Number of individuals genotyped ($N$), number of distinct alleles per locus ($N_A$), observed ($H_o$) and expected ($H_e$) heterozygosity, genetic differentiation ($D_{est}$) and null allele frequency ($f_{null}$).

Inference of population structure

We explored multiple avenues for investigating the genetic population structure. First, we performed an individual-based Bayesian analysis implemented in STRUCTURE version 2.2 (Pritchard et al. 2000) to delineate clusters ($K$) of individuals based on their multilocus genotypes. Without a priori knowledge of source populations the algorithm attempts to find groups of individuals that minimize deviations from Hardy-Weinberg and linkage disequilibrium (Pritchard et al. 2000). Ten independent runs of $K=1$-26 were run at 200 000 Markov Chain Monte Carlo repetitions and a burn-in period of 100 000 under the admixture model with correlated allele frequencies and no prior placed on the population of origin. We used the modal value of the ad hoc quantity $\Delta K$, which is based on the second order rate of change of the likelihood function ($\ln Pr (X|K)$, as a model choice criterion to detect the true $K$ (Evanno et al. 2005). This approach overcomes difficulties in estimating the true $K$ in case likelihood values increase with stepwise values of $K$ (Pritchard et al. 2007). When $\Delta K$ failed to reveal an unambiguous signal we chose the most parsimonious model having the highest mean value of $\ln Pr (X|K)$ as the most optimal model (Pritchard et al. 2007). We tested an alternative clustering algorithm
using INSTRUCT (Gao et al. 2007) which is an extension of STRUCTURE but does not require Hardy-Weinberg equilibrium within clusters and jointly estimates levels of population inbreeding and population structure. Besides estimating a population inbreeding coefficient, identical settings as those for STRUCTURE were used in INSTRUCT. Replicate cluster analyses were aligned using CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007) and displayed using the graphical program DISTRACT version 1.0 (Rosenberg 2004). Next we applied a principal coordinate analysis (PCoA) to summarize a matrix of pairwise $D_{est}$ values using GENALEX version 6.4 (Peakall and Smouse 2006). This kind of multivariate dimension-reducing method is exploratory in nature and graphical displays of the most important principal axes were used in an attempt to visualize genetic similarity among populations within urbanization classes (Jombart et al. 2009). Finally, we applied an analysis of molecular variance (AMOVA) as implemented in ARLEQUIN version 3.5.1.2 (Excoffier et al. 2005) to examine how genetic variation was partitioned over three hierarchical scales: within populations, between populations within urbanization classes, and among urbanization classes. As neither the number of individuals nor loci determine the power of a nested AMOVA but rather the number of populations that can be permuted among higher hierarchical levels, we first applied the multinomial theorem to our sampling scheme to ascertain the minimum P-value was small enough (Fitzpatrick 2009).

**Detection of recent bottleneck events**

Strong reductions in effective population size (e.g. bottlenecks) result in contrasting rates at which allelic diversity and heterozygosity at Hardy-Weinberg equilibrium ($H_e$) are lost (Cornuet and Luikart 1996). During a bottleneck particularly rare alleles are lost rapidly and while its impact on $H_e$ is minimal (Hedrick et al. 1986) it strongly affects the expected heterozygosity at mutation-drift equilibrium ($H_{eq}$) as the latter is highly dependent on the absolute number of alleles (Ewens 1972). In bottlenecked populations this transient disruption of mutation-drift equilibrium therefore generates an “excess in heterozygosity” ($H_e > H_{eq}$) (Luikart and Cornuet, 1998) and a distortion in the characteristic allele frequency distribution of non-bottlenecked populations. We conducted a graphical test as the one implemented in BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996; Piry et al. 1999) to detect for such recent bottleneck events. This test differentiates whether or not allele frequency distributions show a mode shift from low to intermediate allele frequency. We did not use a formal test for ‘heterozygosity excess’ as the distribution of $H_{eq}$ obtained through coalescence-based simulations, and thus the results, often
profundely hinge on the chosen model of microsatellite evolution (Hawley et al. 2006) and this is currently unknown in our study area.

**Detecting recent migration events**

To quantify the extent of recent migration events between urbanization classes we pooled all urban populations into a single urban one and performed a similar grouping for suburban and rural populations. For each individual we used a partial Bayesian assignment technique (Rannala and Mountain 1997) to identify its area of origin. This algorithm computes and subsequently uses the posterior probability density of unknown population allele frequencies (Bayesian approach) rather than the observed sample allele frequencies when calculating individual multilocus genotype probabilities (frequentist approach). Cornuet et al. (1999) further extended this methodology by adding a formal statistical ‘exclusion test’. For each population, a likelihood distribution for Monte Carlo simulated genotypes is generated and the population is excluded as a possible origin if the likelihood for the genotype of the sampled individual falls in the tail-end of this distribution. As such, a source population can be identified when all but one population is being excluded and assigning individuals to an area different from their sampling location was interpreted as a migration event. This method has the advantage over all others that the true population of origin does not need to be sampled (Cornuet et al. 1999). Assignment test will be statistically rigorous when putative source populations are strongly differentiated but imprecise for weakly differentiated populations (Paetkau et al. 2004). Assigning recent migrants to its population of origin is conducted at an ecological timescale rather than an evolutionary one and hence assumptions like drift-migration equilibrium are less stringent (Peery et al. 2008). Analyses were performed in GENECLASS2 version 2.0 (Piry et al. 2004) and the focal individual was each time removed from the sample when allele frequencies were estimated (the leave-one-out procedure).

**RESULTS**

**Summary statistics**

All loci were highly polymorphic across populations and number of alleles per locus ranged from 3 (locus TG01-148) to 31 ( locus Pdo9). Most locus by population combinations were in Hardy-Weinberg equilibrium, yet some deviations reached significance after Bonferroni correction (three populations for Pdo47, two populations for Pdoq5 and one for resp. Pdo32, Pdo9 and TG13-017). There was no evidence that scoring errors due to large allele drop-out or stutter contributed to this
nonequilibrium. To ascertain that these deviations did not influence our results we ran all analyses with and without these five markers. Removing these loci did not alter any of the observed patterns, hence only results based on the total dataset are being presented here. There was no evidence for linkage disequilibrium between any pair of loci. Standard statistics for each marker are presented in table 4.2.

Genetic diversity among urbanization classes

Mean number of alleles per locus ranged from 4.8-7.9, allelic richness from 5.86-9.62 and expected heterozygosity from 0.60-0.71. Permutation tests did not detect significant differences in these diversity indices between urbanization classes (all p>0.05). Pairwise levels of population differentiation ranged from 0.002 to 0.076 (mean $D_{est} \pm SE: 0.037 \pm 0.008$). When respectively all urban, suburban and rural populations were pooled pairwise $D_{est}$ values were 0.001 between the urban and rural group, 0.002 between the suburban and rural one and 0.001 between the urban and suburban group. Overall differentiation between urban populations was 0.025, 0.028 for suburban populations and 0.033 for rural ones. There was support for an isolation-by-distance population structure as a Mantel test revealed a positive correlation between genetic and geographic distance (Mantel r=0.19; p=0.001). These trends were however absent when considering the rural and urban/suburban habitats separately (both p>0.05). Mean population-specific descriptors and population pairwise estimates are given in table 4.3.

Population structure inference

Individual-based Bayesian analyses failed to identify multiple clusters along the urbanization gradient and were consistent with aforementioned low levels of genetic divergence between populations (figure 4.2). $\Delta K$ did not show a clear mode when clustering was performed using STRUCTURE but posterior probabilities started to gradually decline for K>7, hence we chose K=1 as the most parsimonious and optimal model (figure 4.3 A-D). When levels of inbreeding were accounted for, posterior probabilities incremented with increasing K’s and reached a plateau at approximately K=13. To overcome this problem we used $\Delta K$ to delineate the most appropriate model as suggested by Evanno et al. (2005), but no clear mode could be detected although lower cluster numbers had the smallest $\Delta K$ values (respectively K=1 and K=2) (figure 4.3 E-H). The lack of support for strong population structure corroborated with observed values of mean membership coefficients that were highly admixed for all K, i.e. very few individuals showed strong and unique affinity to a specific cluster (figure 4.2). The principal coordinate analysis revealed a tendency to
Table 4.3. (part 1) Summary of genetic descriptors at within- and between-population level. Abbreviations refer to populations described in table 3 (Introduction).

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Cluster urban and suburban populations on the one hand and rural ones on the other hand. The two main PCoA axes together explained 50% of total genetic variation (figure 4.4). Finally, a hierarchical AMOVA indicated that no genetic variation could be assigned to differences between urbanization classes, very little between populations within urbanization classes (2.2%) and most of the variation (97.8%) resided within populations. The minimum p-value for three groups of resp. 13, 9 and 4 populations was much smaller than 0.001 so absence of population structure at higher hierarchical levels could not be attributed to a lack of power.
Chapter 4

Table 4.3. (part 2) Summary of genetic descriptors at within- and between-population level. Abbreviations refer to populations described in Table 3 (Introduction).

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<td>0.055</td>
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</tbody>
</table>

(a) Last letter refers to urbanization class (R=rural; SU= suburban; U= urban)
N: number of individuals; Np: number of private alleles; Nq: mean number of alleles per locus; A(40): total allelic richness across all loci at g= 40 gene copies; H: unbiased expected heterozygosity (Nei, 1978); F: fixation index (Weir & Cockerham, 1984)
Level of significance: ns=non significant; * <0.05; ** <0.01; *** <0.001

Recent demographic changes and migration events

Only EB-U, the most central urban population, showed signs of a recent bottleneck. Here, allelic richness was low while heterozygosity was retained at a level equivalent to that of other populations. The graphical test indicated a clear mode at intermediate frequency alleles rather than at low frequency alleles while in all other populations the allele frequency distribution was typically L-shaped (figure 4.5).
An exhaustive characterization of recent migration events between different urbanization classes was made impractical due to weak genetic structure at this scale. Only 10.1% of all individuals (31 rural, 32 suburban and 7 urban birds) could be assigned to a single and unique source. 61% of these 31 rural birds were residents while 39% were suburban migrants. The suburban subset consisted of 78% residents and 22% rural immigrants, while the urban group contained only immigrants (43% originated from the suburban area and 57% from the rural one). Most of the other birds showed mixed ancestry (73.9%) while a small fraction (15.9%) resembled immigrants from unknown sources (table 4.4).

**DISCUSSION**

Although house sparrows are not extremely vagile by nature (Anderson 2006), analysis of microsatellite data did reveal high historical connectivity or recent common ancestry between populations along an urbanization gradient. Principal coordinate analyses provided evidence for a moderate distinction between suburban/urban and rural populations but a hierarchical MANOVA could not corroborate this. While tests for bottlenecks in most urban populations could not substantiate the well-described massive decline of house sparrow numbers in large European cities (Crick et al. 2002, De Laet and Summers-Smith 2007), a bottleneck signature was present at the most central urban population. Preliminary exploration of dispersal patterns suggests that peripheral urban populations may function as sinks in which putative genetic erosive effects are countered by gene flow from source populations. House sparrows have experienced dramatic declines which have been particularly pronounced in large European city centers (De Laet and Summers-Smith 2007). Contrary to our expectations, neutral microsatellite data did not support these trends although census counts revealed smaller urban population sizes compared to their rural and suburban counterparts (unpublished data). Either urban population sizes have remained stable in the recent past, albeit at a lower level, or we were unable to reveal the genetic consequences of a bottleneck. Genetic signatures of population declines are highly ephemeral and become invisible if insufficient or too much time has elapsed since the onset of the population decline (Luikart et al. 1998; Keller et al. 2001). Unfortunately, lack of historical data prevents us from assessing the severity and timing of putative urban bottlenecks and to
Fig. 4.2. Population structure inferred from microsatellite polymorphism. All samples show evidence of highly mixed ancestry supporting a lack of population structure for 690 house sparrows along an urbanization gradient. The plot shows the average ancestry of 10 independent runs for K=3 clusters (plots for other K values show similar patterns). Upper panel: INSTRUCT analysis (Gao et al. 2007), lower panel: STRUCTURE analysis (Pritchard et al. 2000). Abbreviations refer to populations described in table 3 (Introduction).
Genetic structure in contemporary house sparrow populations

Fig. 4.3. Graphical display of the four steps to detect true number of cluster as outlined by Evanno et al. (2005). A and E: L(K), mean posterior likelihood (±SD) over 10 runs for each K value. B and F: L'(K), first order rate of change in the posterior likelihood (±SD). C and G: L''(K), absolute value of the second order rate of change in the posterior likelihood (±SD). D and H: ΔK, mean absolute values of L''(K) over standard deviation of L(K). A-D: INSTRUCT analysis (Gao et al. 2007), E-H: STRUCTURE analysis (Pritchard et al. 2000).
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Fig. 4.4. Scatterplot of the first two PCoA axes of genetic variation (based on pairwise Dest values) between house sparrow populations. Symbols represent different urbanization classes: filled circles (urban), open circles (suburban) and filled triangles (rural). Abbreviations refer to populations described in table 3 (Introduction).

Table 4.4. Number of individuals that could be assigned to a single site (N_{unique}) and their most likely site of origin (assigned site). Individuals were excluded from a site if its genotype likelihood was less than 0.01 when compared to a likelihood distribution of 10000 simulated genotypes. Percentages are given in brackets.

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>N</th>
<th>Assigned site</th>
<th></th>
<th></th>
<th>N_{unique}</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Rural</td>
<td>Suburban</td>
<td>Urban</td>
<td></td>
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<tr>
<td>Rural</td>
<td>345</td>
<td>19 (0.61)</td>
<td>12 (0.39)</td>
<td>0</td>
<td>31 (0.09)</td>
</tr>
<tr>
<td>Suburban</td>
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<td>7 (0.22)</td>
<td>25 (0.78)</td>
<td>0</td>
<td>32 (0.13)</td>
</tr>
<tr>
<td>Urban</td>
<td>89</td>
<td>4 (0.43)</td>
<td>3 (0.57)</td>
<td>0</td>
<td>7 (0.08)</td>
</tr>
</tbody>
</table>

unambiguously exclude such confounding effects. Failure to detect genetic bottlenecks can also be attributed to immigration of rare alleles into the population (Keller et al. 2001) and offers a plausible explanation given the small scale on which this study was conducted. While genetic diversity may be depleted during the initial phase of a bottleneck, admixture events may result in a subsequent increase of diversity, obscuring and compromising tests to detect bottleneck events (Keller et al.)
2001). Dispersal estimates indicated moderate site-tenacity in suburban and rural areas, reflecting the well-known sedentary behavior of house sparrows (Anderson 2006). More interestingly however was the complete lack of any urban immigrant in these areas while the urban subgroup consisted of exclusively suburban and rural emigrants, at least suggesting the urban habitat might function as a ‘dispersal sink’ (Dias 1996) and thereby masking true bottleneck signatures. In contrast, the innermost urban population did not seem to be affected by such an influx of ‘neighboring alleles’ as loss of genetic diversity was not entirely restored. Peripheral

![Figure 4.5](image)

**Fig. 4.5.** Allele frequency distributions of 16 microsatellites for each population conformed to the L-shaped frequency distribution expected for neutral loci at mutation-drift equilibrium (Luikart et al. 1998), with one exception for the most central urban population EB-U (mode shift indicated by arrow). Abbreviations refer to populations described in table 3 (Introduction).
urban populations may be fed by immigration but apparently the core one lacks these dynamics. Such a scenario may be even more pronounced in larger metropolitan cities as distances between core populations and potential sources will increase by several orders of magnitude. The low amount of genetic structure at this scale however did not allow us to conduct a formal and exhaustive assessment of migration rates between urban, suburban and rural areas as the sensitivity of the analysis hinges on the amount of differentiation between putative sources. Hence, we contend that these results should be cautiously interpreted and urge for the need to conduct an in-depth evaluation of the extent of (unidirectional) exchange of migrants between the urban area and its surroundings.

Our data indicated low levels of differentiation and equal measures of genetic diversity along the urbanization gradient. It may be too premature to conclude that this would indicate high dispersal rates between current populations. Indirect evidence suggests that genetic drift is not fully countered by migration. Genetic differentiation between different urbanization classes (after pooling all populations) was an order of magnitude smaller than overall $D_{est}$ values between populations within each urbanization class. If migration between populations were high, we expected both measures to be of similar size. These results rather suggest that genetic drift is strong enough to withstand the homogenizing effect of dispersal. Even under the assumption that historical estimates of genetic connectivity resemble current ones, we could conservatively claim demographic independence based upon genetic independence but not the opposite (Taylor and Dizon 1999). The influence of only a few immigrants may be sufficient to replenish genetic variation and reduce inbreeding depression (‘genetic rescue’ sensu Ingvarsson (2001)), yet such numbers will often have only trivial demographic effects (Taylor and Dizon 1996). A study on reproductive success of house sparrows along an urban gradient revealed insufficient local recruitment in two out of three years as it did not reach the predicted threshold required for population stability (Peach et al. 2008). Vincent (2005) showed that this deficit was not counterbalanced by immigration as population sizes declined overall by 28% during this three year. Unfortunately, no estimates on genetic differentiation were available while our study lacked demographic data. We contend that in order to optimize management schemes, additional studies integrating quantitative data on genetic and demographic connectivity are warranted.

Connectivity or lack thereof plays a crucial role in evolutionary processes. Although evolutionary theory predicts that high levels of gene flow mitigate local adaptive responses, it does not necessarily eliminate the adaptive potential of a population. The evolution of differences in body mass has been documented in birds
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due to non-random dispersal with respect to the phenotype (Garant et al. 2005; Storz 2005; Postma and van Noordwijk 2005). A study on house sparrows reported a consistent reduction in body size and condition of free-living urban house sparrows compared to their rural conspecifics (Liker et al. 2008). Liker et al. (2008) suggested that these differences in body mass were genetically determined as differences did not disappear when birds were placed in a common environment. In the face of substantial dispersal rates between both environments such genetic differences may still persist if dispersal is non-random with respect to body mass. In such a scenario, smaller birds will then be either forced or attracted to move to highly built-up areas, while heavier birds tend to settle in the surrounding areas. However, in contrast to Liker et al. (2008), we could not confirm differences in body mass per se between urban and rural birds (data not shown) although we reported a reduction of nutritional condition in urban house sparrows earlier (Vangestel et al. 2010).

This is the first study, to our knowledge, that investigates the population genetic structure of contemporary house sparrow populations after the onset of the decline. While we could identify to a certain extent urban/suburban and rural genetic clusters, small-scale patterns were less distinct. As pointed out by previous researchers (Kekkonen et al. 2010) our results could function as a baseline granting us the opportunity to monitor and evaluate future genetic changes in urban house sparrow populations.

Acknowledgement

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REFERENCES


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Chapter 5

Spatial heterogeneity in genetic relatedness among house sparrows along an urban gradient: a plea for individual-based analysis

Carl VANGESTEL, Joachim MERGEAY, Deborah A. DAWSON, Viki VANDOMME and Luc LENS

*Molecular Ecology, submitted*
Chapter 5

ABSTRACT
Understanding factors that shape patterns of kinship in sedentary species is important for evolutionary ecologists as well as conservation biologists. Yet, how patterns of relatedness are hierarchically structured in space remains poorly known, also in common species. Here we use information from 16 polymorphic microsatellite DNA markers to study how small-scale kinship structure varies among house sparrows (Passer domesticus) along an urban-rural gradient. Average levels of relatedness were higher among urban individuals than among individuals from rural areas, suggesting lower rates of dispersal in more built up habitats. Comparison of observed levels of relatedness with simulated distributions of known kinship values showed that central urban individuals had the highest proportion closely-related conspecifics in their immediate neighbourhood. Spatial auto-correlograms supported this small-scale genetic structure and further indicated stronger effects of genetic drift and/or limited dispersal in urban populations. Results of this study underscore the importance of individual-level analyses as a complementary approach to traditional population-level analyses when studying genetic population structure over small spatial scales.

INTRODUCTION
Assessing the proportion of shared alleles that are identical by descent as a measure of genetic relatedness between pairs of individuals (dyads) has proven informative in a wide range of scientific disciplines (Blouin 2003). For instance, captive or in situ breeding programs can be optimized by implementing information on the average degree of relatedness into artificial selection schemes (Lynch and Walsh 1998), quantitative geneticists can use the association between genetic and phenotypic similarity to study the extent of trait-inheritance in natural populations (Ritland 1996), and knowledge of kin structure can help evolutionary ecologists to unravel the evolutionary ecology of cooperative behavior in social species (Danchin et al. 2008). Temporal or spatial variation in genetic relatedness can further inform ecologists on spatial dispersal scales or non-random and sex-dependent dispersal events (Sweigart et al. 1999; Van de Casteele and Matthysen 2006; Zamudio and Wieczorek 2007), on local population dynamics (Ruzzante et al. 2001), and on the most appropriate management strategies for species of conservation concern.

Often, conservation geneticists infer rates of dispersal and geneflow from patterns of population genetic structure - with ‘populations’ invariably comprising the smallest units of interest. Individual-level genetic analyses are much less common in conservation biology (Blouin 2003), despite the fact that such analyses may reveal small-scale patterns of genetic structure that remain cryptic
in population-level ones (Zamudio and Wieczorek 2007). Increased aggregation of close kin in small, isolated populations may foster loss of genetic variation and inbreeding depression, and as such, compromise population persistence (Keller and Waller 2002). Monitoring spatial heterogeneity in genetic relatedness can therefore be informative when aiming to identifying populations susceptible to (future) genetic threats. Analytical tools to infer pairwise relatedness from genetic data in the absence of pedigree information have become readily available (Blouin 2003), and access to large numbers of polymorphic microsatellite DNA markers mitigates the high sampling variance generally associated with estimates of relatedness (Lynch and Ritland 1999; Sweigart et al. 1999).

Here we study fine-scale patterns of genetic relatedness among house sparrows along an urban gradient. Urban house sparrows have shown strong local declines during recent decades (Chamberlain et al. 2007; De Laet and Summers-Smith 2007), while local extinction of small populations has transformed uniformly distributed, panmictic populations (Kekkonen et al. 2010) into patchy population networks, most strongly so in highly urbanized regions (Shaw et al. 2008; Vangestel unpublished data). Juvenile house sparrows disperse in a ‘stepping-stone’ manner, postnatal dispersal distances are typically short and adult birds exhibit high breeding site fidelity (Heij and Moeliker 1990; Anderson 2006; Kekkonen et al. 2010). Such a sedentary lifestyle, in combination with local population extinction, can be expected to distort historical levels of population connectivity and to result in accumulation of close relatives within small geographical ranges. Yet, despite its long history as ecological model species in behavioural and experimental studies (Lendvai et al. 2007; Nakagawa et al. 2007), surprisingly little is known on patterns of kinship structure in natural populations of the house sparrow.

As part of a pilot study along an urban gradient near the city of Ghent, Belgium, urban house sparrow populations were genetically separated from rural ones through a Principal Coordinate Analysis, and genetic erosive effects were shown to be restricted to the most central urban population (Vangestel et al., unpublished). These results supported the hypothesis that urban and rural house sparrow populations act as independent demographic entities (De Laet and Summers-Smith 2007). Building on these results, we here estimate individual levels of pairwise relatedness to quantify genetic structuring at two hierarchically-ordered spatial scales, i.e. a local scale (peripheral vs. central populations) and a regional scale (urban vs. rural populations), and address the following research
questions: (i) Do observed distributions of relatedness coefficients differ from simulated ones under the assumption of panmixia?; (ii) Are average levels of relatedness lower among individuals from different locations than among individuals from the same location?; (iii) Do mixed distributions of various kinship categories differ between locations?; and (iv) Does genetic distance correlate with geographical distance.

**MATERIAL AND METHODS**

**Study site and species**

Data were collected in the greater Ghent area (156 km²; 237.000 inhabitants, of which 82.584 reside within the 7.64 km² city centre) and in an adjacent rural area near the village of Zomergem (38.8 km²; 8.150 inhabitants) ca. 12 km north-west of Ghent (figure 5.1). Ratios of built-up area to total area of grid cells (each GIS grid cell measuring 90 000 m² on the ground; Arcgis v9.2) in Zomergem and Ghent were 0-0.10 (rural) and >0.10 (urban), respectively (Vangestel et al. 2010). To study small-scale variation in patterns of kinship, rural and urban areas (regional scale) were subdivided in a ‘central’ and ‘peripheral’ zone (local scale) each (figure 5.1). In the urban area, the central zone comprised the area with the highest ratio of built-up area to total area of grid cells (>0.30; compared to 0.11-0.30 for the peripheral zone), whereas central and peripheral zones in the rural area were of comparable size and spatial configuration as the urban zones, yet did not differ in ratio of built-up area (both 0-0.10) (Vangestel et al. 2010). Between 2003-2009, a total of 690 adult house sparrows from four ‘central’ and nine ‘peripheral’ populations in urban and rural areas were captured with mist-nets (figure 5.1). Populations were sampled over variable time-spans. However, as the length of the sampling period was not correlated with average \( r_p=-0.02, p = 0.93 \) or variation \( r_p=0.08, p = 0.69 \) in pairwise relatedness, genotypes were pooled over years. Upon capture, standard morphological measurements were taken and a small sample of body feathers for DNA analysis was collected (see Vangestel et al. 2010 for details).

**DNA extraction, PCR and genotyping**

We applied a Chelex resin-based method (InstaGene Matrix, Bio-Rad) (Walsh et al. 1991) to extract genomic DNA from ten body feathers per individual. Sixteen microsatellite markers (both traditional ‘anonymous’ microsatellites and markers based on expressed sequence tags) were selected based on their
Fig. 5.1. Central (filled symbols) and peripheral (open symbols) study plots within an urban (circles) and rural (triangles) area near the city of Ghent, Belgium. The inner contour encompasses the city centre of Ghent, the outer contour encompasses the surrounding municipalities. Abbreviations refer to populations described in table 3 (Introduction).
polymorphism and stutter profile (see details in Vangestel et al. submitted). Polymerase chain reactions were organized in four multiplex-sets and compatibility between primer pairs was checked with AutoDimer (Vallone and Butler 2004). The first multiplex reaction contained \textit{Pdo1} (Neumann and Wetton 1996), \textit{Pdo32}, \textit{Pdo47} (Dawson et al. in preparation) and \textit{TG04-012} (Dawson et al. 2010); the second one contained \textit{Pdo3} (Neumann and Wetton, 1996), \textit{Pdo5} (Griffith et al. 1999), \textit{TG13-017} and \textit{TG07-022} (Dawson et al. 2010); the third multiplex reaction contained \textit{Pdo10} (Griffith et al. 2007), \textit{Pdo16}, \textit{Pdo19}, \textit{Pdo22} (Dawson et al. in preparation) and \textit{TG01-040} (Dawson et al. 2010); the last set consisted of \textit{Pdo9} (Griffith et al. 2007), \textit{TG01-148} and \textit{TG22-001} (Dawson et al. 2010). PCR reactions were performed on a 2720 Thermal Cycler (Applied Biosystems) in 9 µL volumes and contained approximately 3 µL genomic DNA, 3 µL QIAGEN Multiplex PCR Mastermix (QIAGEN) and 3 µL primermix (concentrations were 0.1 µM (\textit{Pdo1}), 0.12 µM (\textit{TG01-148}), 0.16 µM (\textit{Pdo10}, \textit{Pdo19}, \textit{Pdo22}, \textit{Pdo32}, \textit{TG04-012}) and 0.2 µM (\textit{Pdo3}, \textit{Pdo5}, \textit{Pdo9}, \textit{Pdo16}, \textit{Pdo47}, \textit{TG01-040}, \textit{TG07-022}, \textit{TG13-017}, \textit{TG22-001})). The PCR profile included an initial denaturation step of 15 min at 95°C, followed by 35 cycles of 30 s at 94°C, 90 s at 57°C and 60 s at 72°C; followed by an additional elongation step of 30 min at 60°C and an indefinite hold at 4°C. Prior to genotyping, samples were quantified using a ND1000 spectrometer (Nanodrop Technologies) and adjusted to a standard concentration of 10 ng/µL. Negative controls during extraction and PCR were included to rule out contamination of reagents. PCR products were visualized on an ABI3730 Genetic Analyzer (Applied Biosystems), an internal LIZ-600 size standard was applied to determine allele size, and fragments were scored using the software package GENEMAPPER v4.0. All loci under study were autosomal as inferred from the chromosomal location of their homologs on the genome of the zebra finch, \textit{Taeniopygia guttata} (Vangestel et al. unpublished data).

We used MICRO-CHECKER software (Van Oosterhout et al. 2004) to identify scoring errors that could be attributed to stuttering, differential amplification of size-variant alleles causing large allele drop-out, or the presence of null alleles. All microsatellite loci were checked for Hardy-Weinberg and linkage equilibrium with GENEPOP version 4.0 (Raymond and Rousset 1995, Rousset 2008) and a family-wise error rate of 0.05 was obtained by applying sequential Bonferroni correction (Weir 1990).

\textbf{Spatial kinship analysis}

Kinship analyses can be assigned to two types of models: those that estimate relatedness between individuals as a continuous measure of genome-
wide identity by descent, and those that assign dyads to discrete relationship categories (Weir et al. 2006). To date, there is no single best relatedness estimator that outperforms all others. Rather, their performance appears context-specific and to rely on the underlying true genetic population structure (Van de Casteele et al. 2001, Wang 2011). As recommended by Van de Casteele et al. (2001), we performed Monte Carlo simulations using COANCESTRY Version 1.0 (Wang 2011) to calculate correlation coefficients between different estimates and true simulated relatedness values (using observed allele frequencies). Based on these simulations, we selected the Queller-Goodnight moment estimator \( r_{QG} \) (Queller and Goodnight 1989) that yielded a strong correlation between true and estimated values \( r=0.82, p<0.001; \) figure 5.2) within the range reported in previous studies (Van de Casteele et al. 2001 and references therein). The asymmetric Queller and Goodnight (1989) index between individuals \( x \) and \( y \) (using the former as the reference individual) was calculated as \( \hat{r}_{QG} = \frac{\sum_i \sum_k (p_{y} - p_{kl})}{\sum_i \sum_k (p_{x} - p_{kl})} \), where \( p_{kl} \) equals the population allele frequency of allele \( k \) at locus \( l \) over all individuals as if they were a single population (no substantial effect on relatedness estimates were found when analyses were performed in the urban or rural area separately), \( p_{x} \) equals the frequency of allele \( k \) in the reference individual (1 or 0.5 for homozygotes and heterozygotes, respectively), and \( p_{y} \) equals the frequency of allele \( k \) in the individual compared (1, 0.5 or 0 for heterozygotes, homozygotes or the absence of allele \( k \), respectively). When summed over both individuals, this index transforms into a symmetrical one (Queller and Goodnight 1998).

For each population, we calculated the frequency distribution of relatedness coefficients between all \( n(n-1)/2 \) dyads using GenAlEx version 6.41 (Peakall and Smouse 2006), while confidence intervals for mean within-population relatedness were estimated via bootstrapping. Likewise, we generated a null distribution of relatedness coefficients among unrelated individuals by randomly permuting genotypes over all populations for 999 times. As corresponding upper and lower 95% intervals represent the range of relatedness values to be expected under random mating, mean values of empirical distributions above the simulated 95% upper limit are considered indicative of non-panmictic conditions. Next, we estimated relatedness coefficients between all possible dyads within central/peripheral zones or urban/rural areas, respectively. Mean coefficients of relatedness that exceed the 95% upper limit thereby provide evidence for an increase in relatedness due to
the presence of small-scale genetic structure at the particular spatial scale involved.

![Figure 5.2](image.png)

**Fig. 5.2.** Association between relatedness estimates of simulated kinship categories (mean value=black dot, standard error=error bars) and expected theoretical relatedness values (dashed lines). 100 dyads were simulated for each of four relationship categories (U=unrelated, HS=half siblings, FS=full siblings and PO=parent-offspring).

Given local population structuring, we further expected individuals from common environments (i.e. central/central) to be more strongly related than dyads containing individuals from mixed environments (i.e. central/peripheral). We therefore compared differences in mean relatedness between both dyad types and performed a bootstrap test of 1000 repetitions in COANCESTRY Version 1.0 (Wang 2011) to assess the level of statistical significance. To test for ‘urban center’ effects, we compared mean levels of relatedness in urban and rural centers and assessed whether there was regional heterogeneity in differences between dyads of common (central/central) versus mixed (central/peripheral) origin. Finally, individuals of urban and rural areas were pooled and mean levels of relatedness of both zones were compared.

We compared the kin structure of common and mixed dyads by quantifying the relative contribution of unrelated (U), half-sibling (HS), full-sibling
Spatial heterogeneity in genetic relatedness distributions

(FS) or parent-offspring (PO) types of relatedness (true r respectively 0, 0.125, 0.5 and 0.5; Blouin 2003). Based on observed allele frequencies within respectively the urban and rural area, we simulated 1000 pairs of each type with COANCESTRY Version 1.0 (Wang 2011) and estimated the proportion of each type to the empirical distribution using a finite Bayesian mixture analysis in which the observed distribution was modeled as a mix of normal distributions (all simulated distributions reached normality). Proportions were drawn from a uniform Dirichlet distribution generating 100,000 posterior samples after discarding the initial 50,000 (burn-in) samples. Next, proportions of HS, FS and PO types were pooled into a single ‘close kin’ type, and differences in the proportion of ‘close kin’ between common and mixed dyads were considered significant if the 95% credibility interval did not contain zero. All analyses were performed in WinBUGS Version 1.4 (Lunn et al. 2000). For the Bayesian analysis, relatedness coefficients were estimated within each area separately.

Finally, we assessed patterns of fine-scale genetic structure by quantifying the association between matrices of pairwise genetic/spatial distances (Smouse and Peakall 1999; Peakall et al. 2003; Vekemans and Hardy 2004) through spatial auto-correlation analysis in GenAlEx version 6.41 (Peakall and Smouse 2006). Under a restricted dispersal model, auto-correlograms are predicted to yield positive correlations at short spatial distances, followed by a gradual decrease to zero with increasing geographical distance and a subsequent random fluctuation of positive and negative values of the correlation coefficient (Smouse and Peakall 1999). The first x-intercept is thereby regarded to estimate the extent of nonrandom genetic structure, i.e. to reflect the point at which random stochastic drift replaces geneflow as the key determinant of genetic structure. As this intercept may depend on the true scale of genetic structure, the chosen distance class size and the sample size per distance class (Peakall et al. 2003), we performed a second auto-correlation analysis in which we plotted pairwise genetic distances against increasing inclusive distance classes. Here, the distance class at which the auto-correlation coefficient no longer remains significant (using 999 bootstraps) approximates the true extent of identifiable genetic structure (Peakall et al. 2003).
RESULTS

Microsatellite data

Loci were highly polymorphic in all populations and there was no evidence for linkage disequilibrium between any pair of loci. All locus-by-population combinations were in Hardy-Weinberg equilibrium apart from Pdo47 which deviated from Hardy-Weinberg equilibrium in three populations, Pdoμ5 in two populations, and Pdo9, Pdo32 and TG13-017 in one population each. There was no evidence for scoring errors due to large allele drop-out or stutter, and when re-running analyses without these five markers, inclusion or exclusion of Pdoμ5 and Pdo9 had a minor effect on relatedness estimates in population OW, but not in any other marker-by-population combination (figure 5.3). Since removal of either both loci or all genetic data from population OW did not affect any conclusion of our study, results presented in this paper are based on information from all populations and loci.

Hierarchical variation in relatedness

Some, but not all, populations showed mean relatedness coefficients outside the limits of the null distribution generated by randomly permuting genotypes over all populations. Pooling populations in either a central or peripheral zone resulted in larger than expected pairwise relatedness among individuals from the urban center only. When pooling populations in urban and rural areas, only the former showed evidence of a significant higher degree of relatedness than expected under complete panmixia (figure 5.3). While these genetic signatures were not consistent at the smallest spatial scale (population level), patterns at local and regional scales showed that individuals from the urban area were more strongly related, especially so in the central urban zone. Such pattern can be expected under the assumption of (semi)isolation and lack of ample migration to counterbalance the effects of nonrandom mating.

Relatedness in mixed and common dyads

Notwithstanding the low overall level of kinship in our study area, levels of relatedness significantly differed between dyads consisting of individuals from a mixed (central-peripheral) origin compared to those from the central zone only, both in urban and rural populations (all p<0.01). While such local ‘center’ effect was expected to be strongest in the urban area, this was not supported by our data as the two-factor (origin*area) interaction was not significant (p>0.05).
Fig. 5.3. Mean relatedness values and 95% confidence limits are plotted. Gray bars represent 95% upper and lower expected relatedness values under the assumption of panmixia across all populations. These values were contrasted with relatedness estimates (a) within each population, (b) central and peripheral zones within each area, and (c) urban versus rural area, mean values outside these simulated 95% confidence intervals represent non-panmictic conditions. Population OW with significantly higher $r_{QG}$ most likely comprises an outlier due to the performance of loci Pdoµ5 and Pdo9. Abbreviations refer to populations described in table 3 (Introduction).
higher level of relatedness among individuals from the urban compared to the rural center \((p<0.01)\) was consistent with the overall higher relatedness in the entire urban area compared to the rural one \((p<0.01)\) (figure 5.4).

**Bayesian mixture analysis of kinship distributions**

The distribution of pairwise relatedness coefficients peaked at \(r_{GG}=0\) in both urban and rural areas and strongly overlapped with a simulated distribution of unrelated individuals (figure 5.5). The estimated percentage (with 95% credibility interval) of close kin equaled 10.8% (9.1-12.6) in the urban center, while urban dyads of mixed (central-peripheral) origin contained a significantly lower percentage of 8.9% (8.2-9.6) close kin. In contrast, differences in close kin between both dyad types did not reach statistical significance in rural populations, however, estimated proportions were significantly lower than in urban populations (central-central dyads: 5.7% (4.4-7.0); central-peripheral dyads: 5.5% (4.9-6.1)) (figure 5.6).

**Correlations between spatial and genetic distances**

Spatial auto-correlograms provided analogous evidence for small-scale restricted gene flow, i.e. significant, positive genetic correlations among geographically adjacent populations and a subsequent decline with increasing distance (figure 5.7). Auto-correlograms intersected the x-axis between 1500-2000 m for urban individuals, and between 500-1000 m for rural ones. At larger distances, patterns became chaotic as \(r\) tended to fluctuate around zero, hence confirming that the spatial scale of our study was appropriate. Based on their proportion of shared genes, individuals became genetically clustered at distances below 1.5 – 2 km (urban) or 0.5 – 1 km (rural), while populations beyond this threshold were considered as genetically independent. Likewise, multiple distance class plots indicated non-randomly distributed genotypes at small geographical distances and a gradual decrease towards zero with increasing distance size class (figure 5.7). Here, auto-correlation coefficients reached zero values at 3.5 km (urban) and 5 km (rural), respectively.

**Discussion**

Individual-level analysis of relatedness coefficients inferred from microsatellite genotypes revealed small-scale genetic population structure in urban and rural house sparrows that was indicative of highly restricted gene flow beyond distances of 3500 m. Average relatedness was higher among urban individuals, suggesting lower dispersal in more strongly built up habitats.
Comparison of observed levels of relatedness with simulated distributions of known kinship values further showed that central urban sparrows had the highest proportion of closely-related conspecifics in their immediate neighbourhood. Spatial auto-correlograms supported this small-scale genetic structure and indicated stronger effects of genetic drift and (or) more restricted dispersal in urban populations. Results of this study suggest that further loss of stepping stone populations in urbanized areas may result in a substantial decrease in genetic diversity in remnant populations of this highly sedentary species.

**Fig. 5.4.** Genetic population structure at a local (central vs. peripheral) and a regional (urban vs. rural) scale. Mean levels of relatedness and error bars (SE) are plotted for several dyad groups: ‘within UC’ and ‘within RC’ refer to common dyads comprising individuals from Urban Center or Rural Center respectively; ‘between UC-UP’ and ‘between RC-RP’ refer to mixed dyads comprising one individual from Urban Center or Rural Center, respectively, and one individual from Urban Periphery or Rural Periphery, respectively; ‘within U’ and ‘within R’ refer to all possible dyads within the Urban or Rural zone, respectively.
Fig. 5.5. Observed (solid line) versus expected (bars) patterns of relatedness in central urban house sparrows. U=unrelated (true r=0); HS=half siblings (true r=0.125); FS=full siblings (true r=0.5); PO=parent-offspring (true r=0.5).

Fig. 5.6. Posterior mean proportions of close kin obtained from a finite Bayesian mixture analysis with error bars representing 95% credibility intervals. Significant differences between dyad types in the urban or rural area are indicated by “*”; ‘within UC’ and ‘within RC’ refer to common dyads comprising individuals from Urban Center or Rural Center respectively; ‘between UC-UP’ and ‘between RC-RP’ refer to mixed dyads comprising one individual from Urban Center or Rural Center, respectively, and one individual from Urban Periphery or Rural Periphery, respectively.
**Spatial heterogeneity in genetic relatedness distributions**

**Fig. 5.7.** Spatial auto-correlation analysis in a urban and rural area. (a) Single correlogram depicting the auto-correlation as a function of distance. (b) Multiple distance class plot depicting the effect of different distance size classes on the extent of genetic autocorrelation. Error bars were estimated using 999 bootstraps.
Population-level genetic analysis based on Wright’s F-statistics (or derivatives thereof) earlier provided only weak evidence for regional structuring of house sparrow populations in the same study area (Vangestel et al. unpublished). While results of the current study appear to contradict such panmictic pattern, F-related statistics largely reflect evolutionary outcomes and assume equilibrium conditions that are often not met in natural populations (Whitlock and McCauley 1999). In contrast, patterns of kinship reflect ongoing genetic processes (Peakall et al. 2003) though it is currently unclear whether, and to what extent, distributions of relatedness are also affected by genetic disequilibria. Relatedness based analyses identified small-scale population structure as neighboring individuals were more related to each other than more distant ones. Although such pattern was to be expected in species with small postnatal dispersal distances, mean levels of relatedness differed between urban and rural areas. This suggests urban environments pose stronger constraints on house sparrow dispersal than less built-up ones. Earlier, Liker et al. (2009) did not reveal significant variation in kinship within and between winter feeding flocks of house sparrows in NW Hungary, nor were patterns related to geographical distance. Results from our study provide different, non-exclusive explanations for this apparent lack of small-scale genetic structure. First, Liker and colleagues studied house sparrows in a moderately urbanized area were genetic population structuring is predicted to be weak. Second, as mentioned by the authors, despite the fact that their sampling design was most appropriate to relate feeding aggregations to genetic relatedness, the spatial scale of their study (most distant populations separated by ca. 1200 m, majority of the populations within 500 m range) may have fallen below the threshold for detecting genetic structuring. Third, genetic differentiation among populations and feeding flocks may have increased differences in estimates of relatedness between ‘common’ and ‘mixed’ dyads. However, as allele frequencies did not strongly vary among most of our populations, we consider this explanation less likely. Both Liker et al. (2009) and our study report low levels of relatedness among house sparrows. High juvenile mortality (± 50% annually for adults, Anderson (2006)) and/or postnatal dispersal rates are suggested to cause such patterns (Liker et al. 2009), although the latter seems rather unlikely in our study area. High annual mortality rates lower the probability that closely related individuals both survive for a longer period of time (Liker et al. 2009).

Use of auto-correlation analysis, revealed that proximate sparrow pairs were genetically more strongly correlated than randomly chosen pairs, while this was not true for more distant ones. Average estimates of relatedness were higher
in urban populations, suggesting stronger local population structure in highly built-up areas. Strong genetic drift and/or weak geneflow may cause substantial genetic correlation between house sparrows within these areas. Positive genetic structuring was detectable up to 1.5 – 2 km in urban populations, while rural sparrows showed genetic independence at 0.5 – 1 km already. However, as x-intercepts of auto-correlograms (representing shifts from geneflow to genetic drift as main driver of population differentiation) are believed to depend on the sampling scheme applied (Vekemans and Hardy 2004), it is recommendable to use multiple distance size plots to detect the true extent of genetic structure (Peakall et al. 2003), even if sampling schemes are more or less comparable as in our study. Based on this second analysis, distances over which geneflow was still detectable tended to be larger for rural (5km) than for urban (3.5km) populations (no statistical test applied). Since genetic drift was expected to be stronger in urban populations (characterized by lower census population sizes; C. Vangestel, unpublished), this may have caused the observed difference between both correlograms, possibly in synergy with low dispersal rates. In the urban area, but not in the rural one, the proportion of close kin (modeled as a dichotomous variable) differed between common and mixed dyads, suggesting that ‘center’ effects were stronger in the former. However, no such pattern was apparent for mean estimates of relatedness (if modeled as a continuous variable) as ‘center’ effects were present in both the urban and rural area and effect sizes did not differ. As overall level of relatedness was lowest in the rural area, shifting from a continuous to a dichotomous variable may have reduced the level of variation in kinship in this area, thereby causing the apparent discordance between both analyses. While analyzing relatedness as a continuous measure could not confirm the a priori hypothesized stronger urban ‘center’ effect, it did reveal higher relatedness values in the urban area suggesting a higher degree of isolation.

With the exception of central urban dyads, proportions of close kin were smaller than those reported in Liker et al. (2009) where all flock-assemblages except one (0.056) had mean proportion of close kin ranging from 0.11 to 0.19. Such difference may have been real or caused by differences in the study design (i.e. scale over which populations were sampled) or analytical methods applied. As opposed to Liker et al (2009) who assigned dyads to particular classes of kinship based on likelihood algorithms (Milligan 2003), we applied a Bayesian mixture approach that exploited the full range of data and is considered to be less prone to large errors of inference associated with individual kinship estimates (Lynch and Ritland 1999; Sweigart et al. 1999). When applying a likelihood method comparable to Liker et al. (2009), estimated proportions of close kin,
increased to 0.15-0.21 (data not shown), however, without affecting any of the conclusions of our paper.

To date, individual-based analysis of relatedness remains a less popular tool to study small-scale population structure. Examples include the studies by Ruzzante et al. (2001) and Sweigart et al. (1999) who inferred genetic population structure based on estimates of relatedness in brown trout (Salmo trutta) and the wildflower Mimulus guttatus, respectively. Van de Casteele and Matthysen (2006) used kinship information to identify small-scale genetic structure in great tits where clustering algorithms based on Hardy-Weinberg and linkage disequilibria (Pritchard et al. 2000) earlier failed to do so. Likewise, individual-based genetic analyses showed substantial among-patch movement in pikas (Ochotona princeps) while mark-recapture analysis did not pick up such signal (Peacock 1997; see Spong and Creel 2001 for an example where both methods provided consistent results). Despite these positive results, inferring small-scale population structure from relatedness values needs to be done with caution as distributions of coefficients of relatedness are not exclusively shaped by the level of connectivity among adjacent populations. For instance, sibling similarity in dispersal which occurs in several species (Massot and Clobert 2000) can affect the extent of kinship assemblages in a population. For example, parents may control dispersal of their offspring as shown in great tits, resulting in strong sibling similarity of dispersal directions (Matthysen et al. 2005, 2010). Spatial differences in such family behavior may induce geographical variation in fine-scale kinship structure. As house sparrows show a strong preference to reselect previous occupied nesting locations (Vincent 2005), reduced annual variation in family dispersal may promote aggregations of offspring. In marginal habitats such dispersal may be strongly canalized annually into only a limited number of ‘attractive corridors’, while dispersal may be more random in optimal habitats. Spatial differences in quality of feeding areas, too, may affect these processes. Parents appear to make nonrandom behavioral decisions while foraging as shown by the lower incidence of aggressive scrounging towards kin relative to unrelated individuals (Tóth et al. 2009). As such, differences in access to high quality areas for offspring from high and low dominant status parents, may affect on its turn differential dispersal between family groups. At the moment it is unclear whether such kin-based dispersal takes place in house sparrows and to what extent this affects the geographical distribution of relatedness.

This study provides new insights into patterns of spatial heterogeneity in relatedness in a declining species, and underscores the importance of individual-
level genetic analysis as a complementary approach to population-level analyses when studying small-scale population structuring.

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Chapter 5


General discussion
General discussion

In this final chapter I recapitulate the main conclusions, integrate and compare results of different chapters and make suggestions of how these may contribute to conservation management. Lastly, I outline possible future research directions that may help clarify some questions that remain unresolved in this thesis.

Overview of the main conclusions

i. Urban house sparrows occupied, on average, the smallest home ranges along the urban-rural gradient and activity ranges were inversely related to scatter of key vegetation (bushes, hedges). Similar patterns were not observed in suburban or rural areas. Nutritional condition, as assessed from ptilochronological feather marks, was lowest in urban populations and showed a positive relation with home range size in this habitat. Based on these results, scatter of key vegetation in strongly built-up areas is believed to hamper urban house sparrows in occupying optimally-sized home ranges, which on its turn results in lower nutritional condition.

ii. Levels of fluctuating asymmetry in tarsus length did not covary with levels of presumed environmental stress, and results of this study therefore add to the growing evidence that relationships between environmental stress and fluctuating asymmetry are species-, trait- and/or context-dependent. This pleads for prudent use of fluctuating asymmetry as a biomarker of stress in evolutionary-ecological or conservation studies.

iii. As opposed to environmental stress, fluctuating asymmetry was inversely related to estimates of genetic diversity at population level, as predicted from the heterozygosity hypothesis. While relationships at population level accounted for more than 34% of the observed variation, this was not corroborated by analyses conducted at the individual level, most likely due to the large error of inference generally associated with individual-based estimates. Genome-wide estimates of heterozygosity at the population level were also inversely related to levels of fluctuating asymmetry, however, this association was driven to a large extent by patterns at two loci. Based on these results, local linkage disequilibrium with key loci is proposed as the most likely explanation for the observed relationships, possibly in synergy with genome-wide effects.
iv. While census-based density estimates indicated that the size of the urban house sparrow population in this study was of the same order as those reported in other large European cities, genetic analyses did not provide evidence for a genetic bottleneck. Only the most central urban population showed signs of a depauperate genetic richness, although exchange of migrants may have obscured bottleneck signatures in other urban populations. Principal coordinate analyses further revealed that (sub)urban and rural populations tended to cluster into two separate genepools.

v. As hypothesized, kinship analyses revealed non-random spatial patterns of genetic similarity, indicative for small-scale population structure. Results of this study supported a stronger local population structure in urban areas compared to rural ones. Average relatedness and proportion of nearby close kin was highest in highly urbanized areas, possibly due to lower rates of dispersal. Autocorrellograms provided further evidence for the presence of small-scale population structure in house sparrows and strongest effects of genetic drift and/or isolation in urban areas.

Due to logistic and time constraints it was impossible to replicate our study design for multiple urban-rural gradients (i.e. by studying populations in and around multiple cities). Therefore, the observed relationships with urbanization (Chapter 1, 4, and 5) may not be generalized to all urban habitats in a straightforward manner as this would be a classic example of pseudoreplication (Hurlbert 1984). Strictly spoken, statistical inferences are therefore restricted to the sampled populations and can constitute a specific effect of the city of Ghent, rather than a true urban effect. Therefore, future studies are needed which provide true replicates and allow valid generalizations of observed patterns. Notably, sample sizes of such studies need not necessarily to be as high as those used in this thesis. Spatial autocorrelation using only four peripheral populations instead of nine provided enough statistical power to detect similar patterns as the ones reported using the complete dataset (using 118680 pairwise relatedness estimates).
BIOMONITORING TOOLS FOR HOUSE SPARROWS: FLUCTUATING ASYMMETRY OR PTILOCHRONOLOGY?

Due to the presumed stress sensitivity of bilateral trait asymmetry that allows researchers to identify populations under stress before fitness is irreversibly affected, and because FA relationships are expected to be strongest under adverse conditions (which is often the case for threatened species), FA has aroused large expectations in conservation biology. Yet, empirical studies are plagued by large inconsistencies in the strength (and even direction) of relationships between FA, stress and fitness. In this thesis, I related FA to presumed environmental (Chapter 1) and genetic (Chapter 3) stress, and showed that associations were lacking in the former while being relatively strong in the latter.

Fluctuating asymmetry has been suggested to integrate the effect of various synergistic stressors into a single phenotypic endpoint, and while researchers promote this as an asset, it may in turn also be a major source of heterogeneity in reported relationships. For instance, while different stressors might act synergistically in the development of some traits, this may not be the case in others, or the level of synergy may also vary between species or populations for a single trait.

If various stressors affect populations in a complex pattern of antagonistic and synergistic ways the eventual outcome may be hard to interpret without prior knowledge of the specific effect of each stressor. As fluctuating asymmetry in house sparrows was affected by levels of heterozygosity (Chapter 3), positive FA-environmental stress relationships require genetic diversity to covary with levels of nutritional stress (Chapter 1) which is not an evident claim. Loss of genetic variation may be affected by processes independent of the level of nutritional stress. For instance, strong genetic drift or non-random mating within a population may be expected to increase FA also under low levels of environmental stress. As a result, studies relating FA to a particular environmental stressor (such as studied in Chapter 2) may then find it difficult to detect patterns or explain lack thereof. In their study on FA in the flower *Lychnis viscaria*, Siikamaki and Lammi (1998) demonstrated both genetic and environmental effects on levels of FA but both acted in a synergistic way. Marginal populations also turned out to be most homozygous, and while the authors used evidence from a common-garden experiment to conclude that environmental effects were most dominant, they cautioned to interpret FA-environmental stress relationships without prior knowledge of the genetic
properties of each population. In our study, spatial variation in nutritional stress may not have co-varied with levels of genetic stress, possibly confounding relationships between FA and environmental stress. However, such confounding effect would then be expected to also mask relationships between FA and homozygosity in case nutritional stress would have a strong effect on FA. Since this was not the case, nutritional stress seemed not, or at most very weakly, associated with FA in our population of house sparrows.

Hence, while it can be instructive to have an integrated measure of stress, I believe conservation biologists would benefit more from tools linking cause and result in a more straightforward way. Ptilochronology (Chapter 1) might be such a tool, although this technique is certainly not flawless either. Studies that implemented both biomarkers simultaneously tended to detect more consistent and stronger stress associations with growth bar size than with FA (Carbonell and Telleria 1999, Polo and Carrascal 1999, Talloen et al. 2008). Our results (Chapter 1-2) further supported these trends as we found variation in levels of nutritional stress in a predictable way which could in addition be linked to a potential causal agent. In contrast, FA remained unaffected by differences in nutritional state. In addition, feather growth can be induced by pulling and subsequently collecting regrown feathers, which would allow researchers to obtain many replicates from a single individual in order to obtain more accurate estimates. This has proven to be a valid procedure in house sparrows, as follicle history does not appear to influence growth bar size, i.e. series of repetitively induced rectrices all had similar growth bar sizes when exposed to constant stress regimes (Grubb and Pravosudov 1994). In contrast, FA of bilateral traits such as tarsus length provides only a single phenotypic outcome per individual. Furthermore, in small populations in particular, it might be impossible to increase statistical power by incrementing the number of sampled individuals while, as mentioned previously, ptilochronology suffers less from these constraints as repetitive sampling of feathers rather than individuals can be used to increase power.

**DETECTING POPULATION STRUCTURE: INTEGRATING INDIVIDUAL AND POPULATION-LEVEL ANALYSES**

Traditional population genetic analyses, such as F-statistics or genetic distances, still remain the first choice when characterizing population structure (Latch et al. 2006). Yet, new advanced statistical methods have emerged and the possibility to obtain large numbers of markers for non-model species now allows to implement individual multi-locus information when analyzing genetic data.
General discussion

Such individual-based approaches allow greater precision than previous methods that strongly rely on summary statistics (e.g. $F_{ST}$, Pearse and Crandall 2004, Planes and Lemer 2011). The assets of such individual-oriented analyses have been most clearly demonstrated in the context of detecting dispersal events. Several individual-based studies have unruled dispersal patterns which remained undetected by allele frequency-based methods. For instance, Castric and Bernatchez (2004) showed that individual assignment was biased towards spatially proximate locations indicative for an ‘isolation by distance’ pattern in two fish species, while such signatures remained cryptic when traditional measures of genetic differentiation were used. Kraaijveld-Smit et al. (2002) was able to identify philopatry in female antechinus (Antechinus agilis), a small marsupial, and male-biased dispersal using individual-based assignment tests while genetic structure, as assessed from $F_{ST}$ values, was lacking.

In Chapter 5 we presented a specific application of such an individual-based method. While based on the same genetic dataset, individual-level (Chapter 5) and population-level (Chapter 4) genetic analysis revealed marked differences in the ability to detect small-scale genetic patterns. Population-level analyses (Chapter 4) revealed a moderate distinction between urban/suburban and rural populations which was further supported by an ‘isolation by distance’ pattern (presumably driven by the two separate clusters, e.g. urban/suburban versus rural). Within each zone (urban/suburban and rural, respectively) there was no pattern of nonrandom aggregation of genotypes (pairwise $D_{est}$ values did not increase with distance nor was any specific spatial pattern observed). Contrasting $D_{est}$ values at different hierarchical levels (within and between the different urbanization classes) further indicated that genetic drift was not counterbalanced by low rates of migration, however, the evidence was fairly weak. In contrast, spatial variation in individual relatedness estimates (Chapter 5) revealed stronger evidence for local population structure. In addition, autocorrelograms confirmed strong effects of genetic drift in distant populations and suggested that genetic similarity between individuals decreased with increasing distance at a small geographical scale. Stronger drift effects in urban areas, most likely due to small population sizes as observed from census counts (Introduction), caused this pattern to disappear at a smaller scale. Such detailed information was not obtained from traditional population genetic analysis in Chapter 4. Differences in the capacity to detect small-scale population structure might be reflected in the way genetic data is analyzed as both approaches use different kinds of information. $F_{ST}$ values (or derivatives thereof) summarize all the available genetic information over all alleles, loci and individuals into a single.
estimate of genetic differentiation which is easy to interpret and to use in subsequent analyses. However, the gain in both simplicity and ease might come with a cost. Individual-based methods, like the ones used in Chapter 5, take advantage of the multilocus structure of the data and use each multilocus individual genotype as an independent unit of information. This study highlights potential insights that can be obtained by integrating both individual and population based methods to study population structure at a small geographical scale. Results from chapters 4 and 5 do not justify to conclude that one method should be replaced by another. Rather, both approaches should be used in concert as they allow complementary insights.

**Implications for Conservation**

Since the first reports on the urban sparrow decline were published, there has been a genuine and growing interest of the general public into this species. Such public concern has prompted many research institutes to commence studies on house sparrows in an attempt to elucidate the causes of the sparrow collapse. The goal of this thesis was not explicitly conservation oriented, but rather to study fundamental ecological concepts in an urban environment by using house sparrows as a model species. However, insights gained from results presented in this thesis may provide useful information for local conservation initiatives for this species in particular, and for urban biodiversity in general.

As outlined in the introduction, the current urban landscape is characterized by an increase in pavements and non-native ornamental shrubs, often at the expense of native vegetation (Shaw et al. 2008). This has been linked to low aphid abundance, high nestling mortality and lack of sufficient juvenile recruitment in urban sparrows (Vincent 2005, Peach et al. 2008). Noteworthy, great tits (*Parus major*) and other urban species such as blackbirds (*Turdus merula*) provide their young with insects as well. But notwithstanding some studies demonstrated reduced breeding success in great tits and other passerines in the urban habitat (Cowie and Hinsley 1987, Solonen 2001, Chamberlain et al. 2009), these species have not witnessed population declines of the same magnitude as those observed in sparrows (Luniak et al. 1990, Summers-Smith 2005). One of the key differences that distinguish house sparrows from other urban birds, is their extreme sedentary behavior (Anderson 2006) and this might provide a plausible explanation of why this species struggles to persist in modern urban landscapes. In Chapter 1, I described an additional effect of habitat loss that may contribute to the urban decline: further loss of suitable vegetation for
foraging or hiding may scatter remnant patches to such an extent that house sparrows are no longer able to cover critical amounts within their daily home range movements, in particular during the breeding season when daily distances covered decrease substantially (Mitschke et al. 2000, Shaw 2009). This may put the less vagile sparrow at a disadvantage if other species are less reluctant to explore larger areas in times of food deprivation. Loss of vegetation may therefore adversely affect fitness components of urban house sparrow, both as nestling (Peach et al. 2008) and as adult (this thesis). Protection of these small landscape elements seems imperative for any conservation plan that aims to prevent house sparrows from disappearing from urban areas. Not only the area but also the configuration of small landscape elements appears important, i.e. inter-patch distances should be kept short enough. In this respect it might be more beneficial to concentrate on redeveloping fewer locations but which harbour sufficient vegetation in a concentrated way than on numerous, but uniformly and less densely distributed vegetation patches throughout the urban landscape. Chamberlain et al. (2004) already reported the importance of spatial configuration of suitable habitat as the occurrence of bird species in gardens was mainly determined by the surrounding habitat matrix rather than the garden habitat per se.

These findings could be easily incorporated into the concept of a ‘lobe-city’, i.e. green ‘wedges’ or ‘fingers’ that lie in between lobes of heavily built-up area and where each of these radial green fingers connects the rural area with a part of the city center (Rombaut 2008). This idea originated in the first half of the 20th century and provided an alternative to the traditional concentric growth model in which city centers and its inhabitants become more and more isolated from the ecological infrastructure of rural areas (Rombaut 2008). Such growth has shown to have repercussions on both biodiversity in general and on human wellbeing (Marzluff 2001, Shaw 2009). In his doctoral thesis, Tjallingii (1996) advocated the idea of a ‘lobe-city’ to mitigate the detrimental consequences of concentric urban sprawl. Recently, the EU (2004) has promoted the lobe city model as a good example of sustainable urban development, creating opportunities for cities to expand while offering sports and leisure infrastructure and the opportunity to attract biodiversity into the city center. As home range sizes of sparrows in general are comparatively small (Chapter1), implementing a relatively small amount of hedges, thick bushes and native vegetation at the border of these wedges would provide sparrows with the required seeds and cover. It seems feasible that such small-scale networks of vegetation patches could be integrated into any pre-existing developmental plan without too much
difficulty. Most likely, these networks will also attract invertebrates which in its
turn will positively affect the breeding performance of urban house sparrows.
Finally, as ‘city-lobes’ models result into a longer fringe between the built-up lobes
and green spaces compared to the much shorter circumference of the concentric
expanded city (Rombaut 2008), larger numbers of sparrows could be sustained,
which would reduce the impact of demographic and genetic stochastic processes
associated with small population sizes. Although such an urban redevelopment
(‘lobe-city’) will be hard to accomplish in current large cities, it might be an option
for more moderate-sized cities which are expected to grow in the future.

A further incentive to promote such green wedges is their potential to
function as corridors between suburban and urban populations. Suburban areas
are regarded as the most optimal sparrow habitat supporting higher densities
(Chamberlain et al. 2007, Vangestel unpublished data) and birds in better
condition (Chapter 1) than their urban counterparts. Heij (1985) assumed that
suburban house sparrow populations might serve as source populations and
therefore maintain multiple sink populations. However, this study was performed
prior to the collapse of urban populations and it is currently unclear whether
suburban populations still show a demographic excess. In our study, we did find
evidence of small-scale population structure (Chapter 5). Most notably, we found
evidence that population structure was higher in an urban environment
compared to a rural control area. The urban center showed strongest aggregation
of close kin, most likely due to lower dispersal rates and this has, to our
knowledge, never been shown in this species. At the moment it remains hard to
predict if and to what extent these differences in kinship structure have fitness
repercussions and affect population viability.

In conclusion, since urban sprawl is expected to increase, rather than
decrease, in future, it is crucial to look at how we can fully exploit a city’s
potential to conserve biodiversity, particularly as these habitats are home to a
number of species that may become of conservation concern. Ecological studies
should therefore constitute an integral part of urban redevelopment planning.

FUTURE AREAS FOR RESEARCH

Monitoring population trends

To date, no baseline information is available for our study area which does
not allow us to test for negative population trends or to quantify spatial
differences in the strength of such declines. At a meeting held in February 2009 in
General discussion

Newcastle (UK), the Working Group on Urban Sparrows provided a detailed outline of a census methodology to monitor house sparrow abundances in urban habitats using a standardized protocol meant to facilitate comparative analyses between different countries. They proposed a mapping-based survey (similar to the one used in this study; General Introduction) consisting of three consecutive counts during the early breeding season. Yet, even the most standardized census design does not solve the problems intrinsically associated with monitoring urban populations: low detectability due to visual and auditive constraints and large inaccessible area may bias estimates of population size substantially. Monitoring genetic changes may therefore be a useful complement to such surveys. Detecting bottlenecks using tests for ‘heterozygosity excess’ (see Chapter 4) (Luikart and Cornuet 1998, Piry et al. 1999) seem less applicable for our study design as estimates are most likely confounded by immigration. Keller et al. (2001) showed that low levels of immigration were sufficient to neutralize signatures of a genetic bottleneck as genetic outcomes were very different from those predicted from mutations only. In addition, in these models mutation plays an important role in shaping observed patterns of genetic variation while interactions between drift and migration may constitute more important drivers. To overcome these problems, it would be recommendable to sample populations repeatedly in time, preferably separated by a few generations. Such a sampling design allows to disentangle effects of genetic drift from effects of migration, and to estimate changes in effective population size through time (see footnote 5 in General Introduction).

Monitoring phenotypic trends

Museum specimens grant a wonderful opportunity to take a look in the past, when house sparrow populations were still thriving well in both urban and rural habitats. By contrasting such historical populations with their contemporary counterparts we could evaluate whether morphological measurements such as fluctuating asymmetry and ptilochronology, mimic such temporal patterns.

Experimental research

One of the main weaknesses of this study is its correlative nature, which hampers the study of causal relationships. Yet, this study has highlighted several windows of opportunity to transform the correlative design to an experimental one in the future. : (i) The positive correlation between homerange size and nutritional condition suggests that high scatter of suitable vegetation causes failure of urban house sparrows to adjust their home ranges to their energetic
Mechanisms
been
such
chicks,
increasing
experimentally
positive
from
sparrows
(providing
nutritional
growth
and
heterozygosity
assumption
bilateral
borders
because
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variation
between
and
stress
(i)
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This
part
of
the
study
could
be
improved
by
studying
house
sparrows
along
a
well-defined
stress
gradient
with
known
fitness
effects.
Such
a
gradient
has
been
demonstrated
in
a
study
in
Leicester,
UK
(Vincent
2005,
Peach
et
al.
2008)
where
lack
of
invertebrate
food
caused
substantial
mortality
and
loss
of
body
condition
in
juvenile
house
sparrows.
Manipulating
food
availability
(providing
supplementary
insects)
along
this
gradient
would
allow
(i)
to
evaluate
whether
growth
bar
size
and
levels
of
FA
reflect
food
stress
in
house
sparrow
chicks,
and
(ii)
to
verify
whether
there
is
evidence
for
selective
mortality,
e.g.
a
positive
association
between
mortality
rates
and
levels
of
FA.
The
first
steps
of
such
a
collaboration
with
The
Royal
Society
for
the
Protection
of
Birds
(UK)
have
been
taken.

**Mechanisms underlying the ‘heterozygosity theory’**

As
predicted
from
theory,
fluctuating
asymmetry
decreased
with
increasing
levels
of
heterozygosity
either
due
to
local
or
genome-wide
effects
(Chapter
3).
In
this
thesis,
I
was
not
able
to
unambiguously
differentiate
between
both
mechanisms
although
single-locus
effects
seem
to
provide
a
plausible
explanation.
To
evaluate
whether
single
key
loci
could
cause
the
observed
patterns
through
linkage
with
key
loci,
FA-heterozygosity
relationships
should
be
studied
in
a
captive
breeding
population
of
house
sparrows.
In
doing
so,
studying
full
siblings
would
allow
us
to
control
for
variation
in
inbreeding
and
to
exclude
genome-wide
effects
whilst
standardizing
environmental
conditions
as
much
as
possible.
Such
analyses
would
increase
our
understanding
of
the
mechanisms
underlying
relationships
between
heterozygosity
and
fluctuating
asymmetry,
in
particular
if
multiple
phenotypic
traits
could
be
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priori
screened
and
modeled
as
repeated
measurements.
By
using
x-ray
measurements,
standardized
measurements
from
virtually
any
pair
of
bilateral
bones
could
be
collected
and
digital
image
software
could
be
used
to
further
reduce
measurement
errors.
Finally,
future
research
should
also
attempt
to
unravel
the
underlying
cellular
mechanism of developmental stability as such a breakthrough will be crucial to interpret and explain patterns observed in nature.

**Mobility**

Evidence is growing that food shortage during the breeding season is problematic for urban birds. Tracking adult birds during these intense periods of feeding may increase our knowledge on urban activity patterns and allow us to quantify mobility constraints similar or different from those observed during the non-breeding period (Chapter 1). Likewise, it might be enlightening to include other species which forage on insects as well, but did not suffer from a similar population crash.

Tracking juvenile birds might be the only solution to fully understand patterns, distances and rates of dispersal within the urban environment. Genetic methods may not meet all the necessary requirements needed to provide reliable estimates, while low probabilities of resighting /retrapping may hamper the use of traditional capture-recapture methods. Although first year survival rates are presumed to be low (Ringsby et al. 2002) and researchers may therefore face a substantial drop-out from their original sample, all transmitters from predated individuals were retrieved in this study. However, the limited longevity of transmitter batteries continues to challenge the study of post-fledging dispersal, resulting in poor knowledge on the timing of dispersal as well as settlement.

**REFERENCES**


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General discussion


Shaw LM, Chamberlain D and Evans M. 2008. The house sparrow Passer domesticus in urban areas: reviewing a possible link between post-decline


Summary

Urbanization is expected to increase at staggering rates in the near future underpinning the importance of the urban habitat as a source of biodiversity. As these habitats already accommodate several avian species of conservation concern, urban habitats have started to draw the attention of conservation biologists and ecologists. One of these species, the house sparrow, has always been living and thriving well in highly built-up areas. Yet, since a few decades this commensal of man has showed marked reductions in population size and numbers to such an extent that it has been vanished from sight in many large European cities. Under growing public interest many research institutes have promoted studies on house sparrows in an attempt to elucidate the causes of these declines, but to date many of the putative answers still remain hypothetical.

In this thesis I aim to expand our current knowledge on how genetic and phenotypic variation is distributed along an urban-rural gradient in house sparrow populations using high-resolution data on behavioral radio-tracking, morphological stress indicators and neutral microsatellite markers. To quantify presumed environmental or genetic stress we used a combination of fluctuating asymmetry and ptilochronology. The former refers to small random deviations between both sides of a bilateral trait (here we used both tarsi and tail feather length) and estimates the underlying developmental instability. Ptilochronology uses the size of growth bars, alternating dark and light bars perpendicular to the rachis of a feather, as a proxy for nutritional stress where smaller growth bars respond to poor nutritional condition. From a conservation point of view, such biomarkers would be most valuable if they are able to detect subtle differences in genetic and/or environmental stress before adverse fitness effects arise (e.g. ‘early warning systems’).

In Chapter 1 I related home range behavior with high-resolution landcover maps and ptilochronological data. In general, home range sizes of house sparrows were extremely small rendering them very susceptible to small-scale human induced habitat alterations. In addition, home range size varied significantly along an urban-rural gradient where urban house sparrows were characterized by the smallest home ranges. Home range size in the urban habitat was linked to the
spatial distribution of key vegetation (bushes, hedges), e.g. home range size decreased with increased scatter of cover. In contrast, patch connectivity did not affect home range size in neither suburban nor rural areas. Finally, urban sparrows were in poorest nutritional condition and these indices were positively associated with estimates of home range size, e.g. urban birds able to maintain a large home range were characterized by less worse body conditions. In summary, these results suggest home range behavior in urban house sparrows was constrained due to the scattered distribution of critical resources which led to suboptimal home range size and impoverished nutritional condition. Further experimental prove is however warranted.

In Chapter 2 I assess the utility of fluctuating asymmetry as a proxy for environmental stress. Levels of nutritional stress (Chapter 1) did not covary with levels of fluctuating asymmetry, neither at the individual nor population level. This further supports the notion that FA relationships remain inconsistent and the utmost caution is necessary when applying FA as a proxy for environmental stress in conservation biology.

In Chapter 3 I evaluate whether fluctuating asymmetry relates to levels of heterozygosity. According to the heterozygosity hypothesis genetic diversity promotes homeostatic mechanisms to buffer developmental pathways against random perturbations. Our results corroborated this hypothesis as, in contrast to environmental stress, FA was inversely related to levels of heterozygosity. Heterozygosity at population-level was able to explain as much as 34% of the observed variation in FA but a single key locus was the main driver underlying this pattern, although we could not entirely rule out genome-wide effects. These results suggest local linkage disequilibrium with key loci and/or genome-wide effects as the potential molecular basis of developmental stability. Presumably the large error of inference associated with individual estimates of FA precluded individual-based analyses from obtaining an equivalent statistical power as the one obtained in population-based analyses.

In Chapter 4 I explore how genetic variation of house sparrow populations is partitioned along different urbanization classes using 16 neutral microsatellite markers. With the exception of the most central urban population, house sparrow populations in our study area did not reveal strong bottleneck signatures although these could have been masked by ongoing dispersal events. Principal coordinate analyses showed a moderate distinction between either the urban/suburban populations and the rural ones. Hierarchical $D_{est}$ values further suggested genetic drift as an important determinant of spatial genetic variation.
In Chapter 5 I apply an individual-based approach to detect small-scale geographical variation in kinship distributions. As predicted, spatial patterns of genetic similarity were nonrandom distributed. Urban areas were characterized by a higher average relatedness and higher proportion of close kin. Spatial autocorrelograms further supported small-scale population structure but also reported a smaller extent of positive genetic structure in highly urbanized areas, suggesting genetic drift might be stronger here due to small population sizes. These results advocate to integrate both individual and population-based analyses when attempting to visualize small-scale population structures.

Although not the primary aim of this thesis, some of these results may have implications for future conservation schemes. Evidence is mounting that loss of native vegetation may have strong repercussions on house sparrow body condition, both for nestlings as well as adults (Chapter 1). Considerable attention should therefore be given to the protection of these small landscape elements if we aim to stop or even reverse the current urban sparrow decline. In addition, behavioral tracking data (Chapter 1) suggested that the focus should be on both increasing the volume as well as optimizing the spatial configuration of key vegetation patches, e.g. reducing inter-patch distances to the minimal.
Samenvatting

Wereldwijd zien we een toename van de mate van urbanisatie of verstedelijking wat enkel maar het belang en potentieel van het urbane habitat als bron voor biodiversiteit onderstreept. Deze habitats herbergen bovendien nu reeds enkele bedreigde vogelsoorten en hebben bijgevolg de aandacht getrokken van ecologen en conservatiebiologen. Een van deze soorten, de huismus, is van oudsher nauw geassocieerd met de mens en het urbane milieu maar vertoont sinds een aantal decennia een opmerkelijke daling in populatiegroottes en aantallen. Onder invloed van een steeds goeiende publieke interesse hebben tal van onderzoeksinstellingen studies opgestart in een poging de onderliggende oorzaken van de achteruitgang van de huismus te verklaren. Echter, tot op heden is geen enkele van de vooruitgeschoven hypotheses omtrent de achteruitgang van de huismus als onomstotelijk bewezen geacht.

In deze doctoraatschrift bestudeer ik fenotypische en genetische variatie in huismussenpopulaties langsheen een urbane-rurale gradiënt en tracht de hierover nog bestaande lacunes in onze huidige kennis op te vullen. Hierbij integreer ik data over zowel dagelijkse bewegingspatronen, morfologische stressindicatoren als neutrale genetische variatie. Omgevings- en genetische stress werden gekwantificeerd aan de hand van een combinatie van fluctuerende asymmetrie (FA) en ptilochronologie. Fluctuerende asymmetrie refereert naar de kleine willekeurige deviaties tussen beide zijden van een bilateraal kenmerk (zowel tarsus- als staartpenlengte werden hiervoor aangewend) en weerspiegelt de onderliggende mate van ontwikkelingsinstabiliteit. Ptilochronologie gebruikt de grootte van groeibanden, alternenderende lichte en donkere banden loodrecht op de schacht van een veer, als proxy voor nutritionele stress, waarbij kleinere groeibanden representatief staan voor een lagere nutritionele conditie van het individu.

In Hoofdstuk 1 integreer ik dagelijkse verplaatsingsafstanden, ptilochronologie en landschapskarakteristieken. Globaal genomen waren homeranges uitermate klein, waardoor deze soort mogelijk zeer gevoelig is aan keinschalige landschapsveranderingen, al dan niet geïnduceerd door de mens. De omvang van homeranges varieerde langsheen het urbane-rurale continuum, waarbij urbane huismussen gekarakteriseerd werden door de kleinste
Samenvatting

homeranges. Bovendien was enkel in het urbane habitat de grootte van een homerange gerelateerd aan de spatiale distributie van vegetatie (struiken en hagen werden als meest belangrijke vegetatie voor huismussen geïdentificeerd). Urbane huismussen vertoonden de laagste nutritionele conditie en deze was positief geassocieerd met de omvang van de homerange, m.a.w. binnen het urbane milieu bleken huismussen met de grootste homerange gekarakteriseerd te worden door de hoogste nutritionale conditie. Samenvattend, deze resultaten suggereren dat de omvang van urbane homeranges gelimiteerd werd door een sterke fragmentatie van beschikbare vegetatie wat uiteindelijk resulteerde in een verlaagde nutritionele conditie. Verder experimentaal onderzoek is echter aangewezen.

In Hoofdstuk 2 onderzoek ik de bruikbaarheid van FA als maat voor omgevingsstress. Nutritionele stress (Hoofdstuk 1) was niet geassocieerd met de mate van asymmetrie, noch op individueel noch op populatieniveau. Deze resultaten roepen op tot enige voorzichtigheid bij het gebruiken en interpreteren van FA als proxy voor omgevingsstress.

In Hoofdstuk 3 associeer ik FA met de mate van genetisch diversiteit (heterozygositeit). Volgens de *heterozygositeithypothese* promoot genetische diversiteit de homeostatische mechanismen die de ontwikkeling van een kenmerk tegen willekeurige perturbaties bufferen en aldus de mate van asymmetrie minimaliseren. Onze resultaten ondersteunden deze hypothese en toonden aan dat, in tegenstelling tot omgevingsstress (Hoofdstuk 2), genetische stress geassocieerd was met FA. Op populatieniveau verklaarde heterozygositeit 34% van de variatie in FA en meer opmerkelijk was dat deze relatie sterk gedreven werd door het effect van slechts één enkele locus, hoewel genoomwijde effecten niet onomstotelijk verworpen konden worden. Deze resultaten suggereren lokaal linkage disequilibrium of genoomwijde effecten als de potentiele moleculaire basis van ontwikkelingsstabiliteit. Een gebrek aan voldoende statistische power verhinderden analyses op individueel niveau voorafgaande resultaten te bevestigen.

In Hoofdstuk 4 onderzoek ik de neutrale genetische variatie van huismussenpopulaties langs een urbane-rurale gradiënt. Enkel de meest centraal verstedelijkte populatie vertoonde een genetisch signatuur van een plotse daling in populatiegrootte (cfr. ‘flessenhals’), hoewel het niet uitgesloten is dat dergelijke signaturen ook in andere (urbane) populaties aanwezig waren maar gemaskeerd werden door een influx van immigranten. Een principale coördinaten analyse vertoonde een matige scheiding tussen enerzijds de urbane en suburbane
populaties en anderzijds de rurale populaties. Analyses op basis van hierarchische $D_{est}$ waardes wezen tenslotte op de aanwezigheid van een aanzienlijke mate van genetische drift binnen huismussenpopulaties.

In Hoofdstuk 5 gebruik ik een individueel gebaseerde analyse om kleinschalige spatiale verschillen in verwantschapsdistributies te identificeren. Urbane regios werden gekarakteriseerd door een gemiddeld genomen hogere verwantschap en sterkere populatiestructuur. Spatiale autocorrelogrammen ondersteunden verder deze kleinschalige populatiestructuur en suggereerden bovendien een hogere mate van genetische drift in sterk verstedelijkte milieus. Deze individueel gebaseerde analyses onderstrepen nogmaals hun belang en toegevoegde waarde aan meer ‘traditionele’ populatie gebaseerde analyses bij het onderzoeken van kleinschalige genetische populatiestructuur.

Hoewel niet het hoofddoel van deze doctoraatsscriptie kunnen sommige resultaten mogelijk van enig belang zijn voor conservatiebiologen. Er komen steeds meer aanwijzingen naar voren dat het verlies van inheemse vegetatie een cruciale rol speelt in de achteruitgang van de huismus door zijn sterke negatieve impact op de lichaamsconditie van pulli en adulte huismussen (Hoofdstuk 1). Bijzonder aandacht dient dan ook besteed te worden aan de bescherming van deze kleine landschapselementen indien we de huidige daling in het huismussenbestand willen stoppen. Homerange data (Hoofdstuk 1) suggereert dat de focus niet enkel op het volume dient te liggen maar tevens op de spatiale configuratie van landschapselementen.