

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE
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Genetics of animal populations in urban environments
BACHELOR THESIS

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BACHELOR THESIS ASSIGNMENT

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Thesis title

Genetics of animal populations in urban environments

Objectives of thesis

Habitat loss and fragmentation resulting from urbanization can reduce gene flow and diversity among populations (e.g. Munshi-South et al. 2014, Wilson 2016). This can lead to population isolation and ultimately to local extinction. Although various studies investigated in genetics of different animal species in their natural environment (e.g. Steinfartz et al. 2007) little is known about their gene flow and diversity in urban areas which may create significant refuges in landscapes of intensive agriculture use (Yannic et al. 2021).

Methodology

The goal of the bachelor thesis will critically overview prior studies on the topic. It will investigate how the genetic diversity and gene flow of urban animal populations are affected by urbanization. It will also find out if urban populations can adapt to specific urban conditions.

The proposed extent of the thesis

max. 25 pages

Keywords

city genetics, genetic drift, genetic diversity and structure, habitat fragmentation, urban environment

Recommended information sources

- Mikulíček, P., Pišút, P. 2012. Genetic structure of the marsh frog (*Pelophylax ridibundus*) populations in urban landscape. *European journal of wildlife research* 58: 833-845.
- Munshi-South J, Nagy C, 2014. Urban park characteristics, genetic variation, and historical demography of white-footed mouse (*Peromyscus leucopus*) populations in New York City. *Peer Journal*, 2:e310.
- Steinfartz S, Weitere M, Tautz D, 2007. Tracing the first step to speciation: ecological and genetic differentiation of a salamander population in a small forest. *Molecular Ecology* 16: 4550-4561.
- Wilson A, Fenton B, Malloch G, Boag B, Hubbard S, Begg G 2016. Urbanisation versus agriculture: a comparison of local genetic diversity and gene flow between wood mouse *Apodemus sylvaticus* populations in human-modified landscapes. *Ecography* 39: 87-97.
- Yannic G, Helfe, V, Sermier R, Schmidt BR, Fumagalli L 2021. Fine scale genetic structure in fire salamanders (*Salamandra salamandra*) along a rural-tourban gradient. *Conservation Genetics* 22: 275-292.

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Statutory Declaration

I hereby formally declare that I have written the submitted bachelor thesis independently under the guidance of Ing. Jana Svobodová, Ph. D.. I did not use any outside support except for the quoted literature and other sources mentioned in the paper. I clearly marked and separately listed all of the literature and all of the other sources which I employed when producing this academic work.

I also declare that the printed version coincides with the version submitted through the University Information System.

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Abstract

Species inhabiting urban and near-urban habitats be negatively affected when urbanization and associated environmental changes inflict population isolation, reduced gene flow, local diversity declines, and genetic drift. Declines are partly due to species inhabiting disturbed or fragmented habitats, or their proximity. This a cause for concern because gradually, genetic issues can lead to local extinction. Loss of biodiversity caused by habitat loss and fragmentation may be underappreciated consequence of city expansions. However, it is not yet well understood if occurring changes threaten species generally across taxa. I investigated this question by reviewing a number of studies that either focused on individual species or conducted secondary data analyses. For each species, I estimated the effects of urbanization on genetic diversity and genetic structure as measures of urbanization. Data was collected from scientific journals and articles, and an overview is presented in a table, in a form of direct comparison of species from various taxa. Analysis of research revealed that study results and urbanization effects are not generally applicable across taxa, but should be addressed on a case-by-case basis. Results suggest that, in general, less mobile species living in or in proximity to fragmented or disturbed habitats are more susceptible to environmental changes, and are more likely to suffer from restricted gene flow. The majority (75%) of studied species are affected by urbanization negatively, their genetic diversity and structure, regardless of their class, were affected by urbanization: 53% and 71% respectively. Species' genetic diversity and structure did not respond to urbanization in 41% and 23% of the studies, respectively. 6% responded to urbanization positively. 7,5% of reviewed studies tested species for the development of adaptive genes. In those studied it was discovered that in 80% of cases studied organisms, despite belonging to different classes (mammals and insects), have developed adaptive SNPs.

Keywords: city genetics, genetic drift, genetic diversity and structure, habitat fragmentation, urban environment.

Abstrakt

Druhy obývající městská stanoviště anebo stanoviště v blízkosti měst jsou negativně ovlivněny urbanizací a s tím souvisejícími změnami prostředí. Tyto změny, mimo jiné, způsobují izolaci populací, snížený genový tok, pokles místní diverzity a genetický drift. To vše je, mimo jiné, způsobeno tím, že druhy obývají narušená nebo fragmentovaná stanoviště. To je důvod k obavám z důvodu, že přetrvávající negativní vlivy na genetickou variabilitu druhů mohou postupně vést k místnímu vyhynutí. Ztráta biologické rozmanitosti způsobená ztrátou a fragmentací stanovišť může být nedoceneným důsledkem expanze měst. V současné době však není známo, zda vyskytující se změny ohrožují druhy obecně napříč taxony. Ve své bakalářské práci shrnuju poznatky studií, které se buď zaměřily na jednotlivé druhy, nebo provedly sekundární analýzy dat. Účinky urbanizace na genetickou diverzitu a genetickou strukturu byli pro každý druh použity jako odhad měřítka efektu urbanizace. Data byla shromážděna z vědeckých časopisů a článků, a přehled je uveden v tabulce formou srovnání druhů z různých taxonů. Analýza výzkumů odhalila, že výsledky studie a účinky urbanizace nejsou obecně použitelné napříč taxony, ale měly by být řešeny případ od případu. Výsledky naznačují, že obecně méně mobilní druhy žijící v fragmentovaných nebo narušených stanovištích nebo v jejich blízkosti jsou náchylnější ke změnám prostředí, a je pravděpodobnější, že budou trpět omezeným tokem genů. Většina (75%) studovaných druhů je urbanizací ovlivněna negativně, jejich genetická rozmanitost a struktura, bez ohledu na jejich třídu, byla urbanizací ovlivněna: v 53%, respektive 71% případech. Genetická diverzita a struktura druhů nereagovala na urbanizaci ve 41% a 23% studií. Z toho 6% reagovalo na urbanizaci pozitivně. 7,5% studií testovalo druhy na vývoj adaptivních genů. Bylo zjištěno, že v 80% případů studovaných organismů, přestože patří do různých tříd (savci a hmyz), byli vyvinuty adaptivní SNP.

Klíčová slova: genetika městské krajiny, genetický drift, genetická diverzita a struktura, fragmentace krajiny, městské prostředí.

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1. Introduction and purpose of the thesis

As the growth of human population continues increasing, demand for cities' expansion keeps rising on a global scale (Ordeñana et al. 2010). Urbanization, i.e. the city expansion, the decline of natural land covers and native vegetation, along with their transformation into roads, railways, buildings, residential areas, and other concrete impervious surfaces represents potential threat to the animal species that have been inhabiting those areas (McKinney 2002). As a result, valuable breeding sites where species live and reproduce are destroyed or fragmented into smaller unconnected patches (Kobayashi et al. 2013; Furman et al. 2016). All of that may pose a threat to even disturbance-tolerant species (McKinney 2002). Moreover, disturbance-sensitive species may be threatened with extinction (McKinney 2002; Delaney et al. 2010). Wildlife experience pressure in urban areas in many aspects, among which are: pollution, disease, invasive species, and habitat loss or fragmentation (Ordeñana et al. 2010; (Schmidt et Garroway 2021). The latter affects species' abundances and biodiversity, chances to find a mate, restricts migration, increases isolation, and influences genetic diversity (Rochat et al. 2017). During the last century the percentage of species abundance population has been declining steadily and homo sapiens appears to be largely responsible for it (Rochat et al. 2017).

Prior studies have evaluated the effects of urbanization on species abundance and discovered that negative effects of various levels have been observed in a range of species (Fig. 9., Chapter 10). Studies reveal that while some species are more vulnerable and exposed to the modifications caused by urbanization, others, on the other hand, are more tolerant or even prosperous in urban habitats, for example, gene flow may be aided by man-made parks, waterways, and small forest patches. However, in the majority of cases, urban populations were characterized by lower genetic diversity compared to exurban ones. Tolerance and vulnerability of species are expressed in their genetic material, as well as their behavior and local diversity (Fattorini 2011). As the number of available mates, nutrients and areas of suitable biotopes in a habitat decline, and the number of predators and invasive species increases, the environment may appear more hostile to individual species (Delaney et al. 2010; McKinney 2002). They may start migrating from or avoiding unappealing habitats altogether (Perrier et al. 2018).

Habitat loss along with increasing distances between habitats and decreasing sizes of habitat patches, among other factors, can ultimately contribute to genetic isolation (Fattorini 2011). Reduced gene flow restricts the exchange of genetic material between populations belonging to the same species. The restriction caused the levels of genetic differentiation among studied populations to grow (Delaney et al. 2010; Munshi-South & Kharchenko 2010; Munshi-South et al. 2013). 90% of published studies reviewed in the study by Miles et al. (2019) showed an association of urbanization with genetic drift or gene flow, demonstrating that urbanization has a major influence on species' biology.

Nevertheless, there is a large gap in general knowledge of specific species and urbanization effects on them. Phenomena yet unknown are, for example: the periods of occurred genetic and population changes, responses of individual taxa – positive or negative, and differentiation of anthropogenic causes from natural ones. Populations are affected by a wide range of circumstances, such as biological requirements, behavioral differences, time, size and extent of habitat patch isolation (Rochat et al. 2017). All of which are needed to be taken into account to receive more precise results; omission of one may lead to the distortion of the results (Rochat et al. 2017). And since demographic studies cannot obtain data such as reduced genetic diversity caused by limitations to the gene flow, it is crucial to conduct thorough genetic studies to predict and investigate the risks imposed by urbanization (Kobayashi et al. 2013).

To save biodiversity from future decline a significant step has been taken toward mitigation: increasing amount of research is being conducted. It may serve as valuable data to study and protect susceptible species. The vulnerability of species may be used as bioindicators of ecosystem degradation (Bech et al. 2014). The collected data may also be valuable for other branches of science, such as ecology, evolution and conservation of species.

The main topic of this bachelor thesis is if and how the genetics of animal species residing in the cities or their close proximity are affected and altered in expanding urban environments. Data for the studies were obtained in two ways: by doing sampling, isolating and studying genetic material or by conducting a broad research based on multiple studies. The genetic material was studied using two main genetic markers: single nucleotide polymorphisms – SNPs, and microsatellites.

In my bachelor thesis I aim to investigate city expansion impacts on population genetic patterns of animal species. I introduce to the urbanization phenomenon, review its principles, and summarise and evaluate overview the published data and research available up to date. I discover if, eventually how, the genetic diversity and gene flow of urban animal populations is affected by urbanization. Since the genetic shifts in species can also be caused by multiple effects of different origins (such as epidemics, climate events, environmental barriers, species' traits, ancient demographic processes and historic events), I also aim to investigate if urbanization is the primary cause. I also examine if particular species are able to adapt to urban conditions. I compare specific species studied by various researchers in terms of used markers, sample sizes, and effects of urbanization on genetic diversity and structure of populations of different animal species.

I suppose habitat fragmentation has a negative effect on genetic diversity and gene flow of urban populations. I predict that the closer the species live to intensive urban environments, the lower their genetic diversity is. Respectively, I predict that their genetic diversity will be higher from urban-to-rural gradient.

Despite the fact that immense amount of data is yet needed to be collected, a broad spectrum of animal species has already been studied, providing interesting results that will be demonstrated in this thesis.

2. Introduction to urbanization problematic

As the density of human populations continues to increase globally and more landscapes are altered into urban areas, wildlife populations are becoming more pressured and deprived of their natural habitats. Destruction of these habitats is an outcome of urbanization, one of the most pervasive forces of anthropogenic change over the last century (Munshi-South et Kharchenko 2010). Urbanization is a recent phenomenon, characterized by substantial, continuous, highly dynamic and usually irreversible land transformation from a previously nonurban environment into a cityscape (a heterogeneous environment with a mosaic of suitable and unsuitable habitats) (Beninde et al. 2016). The transformation of nonurban continuous environments into urban heterogeneous landscape patches can be defined as fragmentation. Literally, the breaking apart of habitat; note fragmentation does not imply loss of habitat (Fahrig 1998). However, both habitat loss and fragmentation are perhaps the most fundamental and prevalent threats to biodiversity (Jordan et al. 2009).

Landscape fragmentation is often associated with reduced connectivity among populations. This issue potentially leads to more restricted movements, greater genetic differentiation, higher inbreeding levels, lower genetic diversity (Richardson et al. 2021), and increased occurrence of homozygous genotypes and detrimental recessive alleles (Furman et al. 2016). All of which may increase the risk of abundance declines, local extirpations, and total isolation owing to human-driven landscape change.

To limit the effects of urbanization, parks are often created within metropolitan areas to retain a part of the region's original biodiversity. They are thus a vital component of landscapes. However, little is known about animal populations living in urban natural areas, and to what extent they are affected by urban challenges such as human disturbance, pet predation, the proliferation of pest species and, most importantly, habitat fragmentation (Noël et al. 2007).

Urban areas continue to prevail over rural ones, and given that trend, evolutionary consequences of landscape alterations on animal species are likely to escalate throughout the following years. Total urban land cover across the world is projected to increase by 185% between the years 2000 and 2030 (Seto et al. 2012). Despite this rapid urbanization, some native species have been able to persist at stable or even increasing numbers (Richardson et al. 2021).

Since urbanization is a relatively novel phenomenon, the amount of conducted research is still relatively scarce. Variation in life history traits and heterogeneity in the landscape/city complicates whether, and to what degree, urbanization affects neutral genetic variation across taxa (Fusco et al. 2021).

In the following chapters mechanisms influencing genetic diversity and structure of urban populations will be reviewed.

3. Mechanisms influencing genetic diversity and structure of urban populations

Genetic diversity is gene variation within species comprised of different inherited traits. High genetic diversity may decrease species' susceptibility to various environmental factors, making adaptation and resistance of species to urban challenges easier. Low genetic diversity, on the other hand, may increase species extinction in the long run (Dri et al. 2021). Genetic structure is the number of subpopulations within a population and their extent of isolation. It is a result of urbanization processes as well as natural processes such as selection, genetic drift and migration (Tero et al. 2003; Epperson 1993).

Gene flow is one of the main mechanisms that affect genetic diversity and structure, resulting in the movement of genes from one population to another. Analysis of stream salamander populations in the New York City metropolitan area published by Fusco et al. (2021) demonstrates that gene flow is, moreover, affected by geographic distance and urban disturbance. It is also restricted by barriers produced by the loss of green space in favor of buildings, roads, and dams. The phenomenon is called "isolation by barrier" (IBB). According to Gortat et al. (2015), striped field mouse population inhabiting 17 locations in and around Warsaw, Poland, can also be isolated by city infrastructure. In this case, genetic interactions among particular local populations are modified compared to populations inhabiting natural areas by replacing the isolation-by-distance differentiation pattern with the "isolation-by-infrastructure" pattern.

Genetic drift is a random process leading to genetic divergence over time, and it increases in isolated or small populations (Richardson et al. 2021). Increased genetic drift causes population variation losses and allele frequency change. Munshi-South et Kharchenko (2010) claim genetic drift to be a more important force than selection in urban New York City white-footed mouse populations when it comes to the "formation" or "modification" of the species gene pool.

The bottleneck effect is an extreme form of genetic drift. The latter's effects are accelerated when effective population sizes are reduced. The effective population size is the one with constant proportions, randomly mating individuals, and nonoverlapping generations that produces the same rate of genetic drift as the measured population (Schmidt et Garroway 2021). Reduced adaptive abilities that respond to environmental

changes, decreased levels of genetic diversity, and increased rates of inbreeding depression are also some of the consequences of low effective population sizes.

Although urban inhabitants appear to be affected by landscape fragmentation, Munshi-South et Nagy (2014) suggest, small vertebrates with limited dispersal ability (especially non-volant species – incapable of flying) can avoid genetic bottlenecks if they maintain high population densities in small urban parks.

In the following chapter the key factors of modifications of population genetics in urban environment will be identified.

4. Identifying the causes of modifications of population genetics in urban environment

Attributing landscape genetic effects observed in species to solely anthropogenic fragmentation is not without challenges. Multiple effects of different origins may act simultaneously. In the following chapter I aim to discover if habitat fragmentation caused by urbanization is the main cause for species endangerment and genetic modifications. I identified some factors that affect species' gene flow and genetic diversity. I intend to find out if:

- species' responses are generally applicable across taxa
- natural causes affected species more than city expansion
- urbanization affected species separately or in combination with natural factors
- anthropogenic habitat modifications are indeed the main cause for restricted gene flow and genetic diversity declines

Species responses

Despite the effects of urbanization being tested on a specific class of animals, reptiles, overall results from the research are, though, not generally applicable across taxa, but species-specific. French et al. (2018) summarized (among others) the genetic responses (genetic differentiation, diversity and gene flow) of reptilian fauna to specific urban features. They also assessed the directionality of individual and population level responses to urbanization in reptile species (Lacertid lizard species in Poland; Skink species in Australia; Semi-aquatic turtle species in NC, USA; Snake species in Tennessee, USA; Reptile species in Melbourne, Australia; Oxford, UK; South Bulgaria; and southeastern Spain). Their review identified diverse results that are variable both within and among all scales of ecological organization. This inconsistency is due to the: heterogeneity of urban landscapes, the fact that species responses are also different, and the combination of various factors caused by urbanization interacting simultaneously.

Natural causes

The following studies demonstrate that natural causes may have a bigger impact than urbanization on species. The natural factors are those not caused by urban sprawl or

human actions, for example: natural barriers (e.g. water bodies), epidemics, sex biased dispersal, and behavioral tendencies.

The following studies identified water bodies as the main causes to hamper the gene flow of studied species.

The population structure of an endemic butterfly *Atrytonopsis* on the islands of coastal North Carolina, United States, was found to be unaffected by urbanization. The species' dispersal and, consequently, gene flow, were mainly restricted by major natural landscape features such as open water (Leidner et Haddad 2010).

Quemere et al. (2010) also identified the Manankolana River in Daraina, Madagascar, along with geographical distances, as the primary structuring factor for the lemur - golden-crowned sifaka (*Propithecus tattersalli*). At the same time, the national road across the region did not seem to act as a barrier. However, since the majority of rivers and rice fields are situated along the river, a possibility that it is the human presence that deters sifakas cannot be excluded.

Other species whose dispersal is limited mainly by a river are quendas (*Isoodon fusciventer*) in Perth, Australia. Ottewell et al. (2019) identified the Swan river as a major barrier to gene flow.

Gene flow of common wall lizard (*Podarcis muralis*) was also identified to be hampered mainly by the river Moselle in Trier, Germany. Beninde et al. (2016) support the previous findings by suggesting that this environmental factor acts as the main one, exacerbating the existing isolation of individuals within the city. Whilst the river remains the main factor, persistence of native lineages of common wall lizard inside German urbanized areas might have also been compromised by the adverse effects of invasive species. That is despite the ability of the lizards to cope well with the challenges of urban habitat.

Noël et Lapointe (2010) observed high population differentiation between red-backed salamander (*Plethodon cinereus*) populations on Ile-Bizard and Ile-Perrot islands. In this case, it was also likely due to the separation of these islands from Montréal islands by large water bodies, which restrict the gene flow.

Both organisms studied by Richardson et al. (2021) (the white-footed mice (*Peromyscus leucopus*) and big brown bats (*Eptesicus fuscus*)) in Rhode Island, USA, demonstrated restricted gene flow between the island and mainland populations. Since

the islands and the inhabitants have been separated from the mainland long before the urbanization of Providence (Rhode Island, USA), the differentiation makes sense.

Following studies demonstrate the other natural factors such as epidemics, metabolism-related factors, and behavioral tendencies, and ancient demographic processes may have a greater than urbanization influence on the genetics of the studied species. For example, a behavioral tendency such as philopatry (the tendency to stay in the natal patch) may lead to inbreeding within each wetland and creates slight site-specific differences in genetic variation. (Furman et al. 2016). Another behavioral tendency: sex-biased dispersal (widespread among mammals (Lawson et Perrin 2007)), when not accounted for, may also result in erroneously concluding that urbanization reduces dispersal distances.

A natural widespread occurrence of a disease, the epidemic, may shape the genetic structure of species to a greater extent than urbanization. Smith et al. (2020) conducted a regional analysis to identify if barriers to movement caused by habitat fragmentation were more likely to have shaped the patterns of genetic structure of Bobcat (*Lynx rufus*) populations in California, USA. Results show that bobcat populations declined rapidly and their genetic variation suffered a severe bottleneck effect mainly because of the mange outbreak that took place in 2002.

Changes in nutritional habits of NYC's white-footed mice (*Peromyscus leucopus*) in urban conditions (metabolism-related factors) also seem to have more likely caused the genetic shifts in white-footed mice, rather than the urban park isolation.

In their study Jordan et al. (2009) suggest there is sufficient information to conclude that ancient demographic processes in Indiana, USA, are more likely to have shaped the observed genetic variation of eastern red-backed salamander (*Plethodon cinereus*) rather than more recent habitat change. They also state that if genetic diversity is reduced as a result of geographic expansion or other ancient events, it may be difficult to detect the recent effects of habitat fragmentation.

Combination of natural and anthropogenic causes

Following findings suggest that some species' genetic structure and variability are affected by both anthropogenic and natural factors, such as climatic and historic events. In a study conducted by Harris et al. (2016) modeling results indicate that Long

Island (New York) white-footed mouse (*Peromyscus leucopus*) populations have been isolated by prehistoric climate events, namely, post-glacial sea-level rise. However, identified times of genetic differentiation also coincided with the history of urbanization in New York. Braaker et al. (2017) found three distinct genetic clusters of European hedgehog (*Erinaceus europaeus*) in Zurich, Switzerland. The main separators were the rivers as well as motorways. Moreover, models indicate that the gene flow of hedgehogs was hindered by all urban land cover types except the “green” ones. Homola et al. (2019) also assume that the pattern of genetic differentiation of spotted salamanders (*Ambystoma maculatum*) and wood frogs (*Lithobates sylvaticus*) they observed in Maine (USA) might have been influenced by a combination of contemporary and past processes: postglaciation or postdeforestation recolonization patterns.

Anthropogenic habitat modifications

The following studies attribute the negative changes in the genetics of animal species mainly to urbanization, demonstrating its detrimental effects. Mikulíček et Pišút (2012) studied the gene flow of marsh frogs (*Pelophylax ridibundus*) in Bratislava, Slovakia. The genome of the populations separated by only several hundred meters differed significantly in those localities. They attribute genetic differentiation to anthropogenic factors, specifically busy highways and urbanized areas. Safner et al. (2011) studied the influence of landscape fragmentation on the common frog (*Rana temporaria*) population genetic structure in the northern French Alps. The most genetically differentiated breeding patches were found to be separated by major roads. Moreover, Bevanger (1998) claims that electrocutions and collisions with man-made objects, such as utility structures, are among the main causes for increasing numbers of birds being killed and endangered. Intensive residential and commercial development that date as far back as 1700, have also contributed to creating a mosaic of forested patches within Mount Royal (Montréal, Quebec, Canada). Three red-backed salamander (*Plethodon cinereus*) populations from the Mount Royal (Université, Summit and Oratoire) presented an appreciable level of genetic differentiation despite being separated by only 0.7–1.7 km (Noël et Lapointe 2010).

As demonstrated in multiple studies, it is not always possible to exclude non-anthropogenic factors to determine the reason for species' genetic shifts. For the future

development of studies on urban evolution, Johnson et Munshi-South (2017) make some recommendations: (1) Maximize the number of studied cities to observe the trends. (2) Select cities with specific features (size, climate, socioeconomics). (3) Increase the sample sizes along urban to ex-urban gradients to detect divergence. And (4) conduct experiments to understand the evolutionary mechanisms.

5. Adaptation to urbanization

Adaptation is a physiological or behavioral trait that species develop to survive in their habitat. Data suggests that adaptation to urban habitats occurs due to genotype selection, influencing specific behavioral traits (Müller et al. 2013). Adaptation may occur as a co-evolution of habitat adaptations and associated mating signals (Steinfartz et al. 2007). Species with abilities to inhabit and utilize the resources from both remaining greenspaces and urban infrastructure are characterized as ‘urban adapters’ (McKinney 2002). Hulme-Beaman et al. (2016) suggest the terms ‘synanthropic’ and ‘peri-domestic’ for species that occupy the same habitats as humans without species being dependent on ones.

Urban adapters may achieve high population densities as a result of less severe temperature fluctuations in the cities (the “heat island” effect – the process of heat accumulation in urban environments caused by man-made constructions and human activities (Yang et al. 2016)), a stable food supply, human supplementation, and limited abilities of inhabitants to move across urban landscapes freely.

In the studies conducted by Unfried et al. (2013) and Müller et al. (2013) behavioral adaptations of song sparrows (*Melospiza melodia*) and European blackbird (*Turdus merula*) populations were observed. For example, song sparrows occasionally used bird feeders in urban areas. Harris et al. (2013) and Winchell et al. (2016) observed physiological adaptations in their studies. Urban New York populations of white-footed mice experienced positive selection in biological responses such as xenobiotic metabolism and inborn immune response. And urban anole lizard (*Anolis cristatellus*) populations had longer limbs than those in natural habitats.

Harris et Munshi-South (2016) studied white-footed mice’s (*Peromyscus leucopus*) specific genes involved in adaptive abilities in New York. They focused on protein-coding regions of the genome, using multiple tests of selection. The tests analyzed different parts of genomic structure and associated outliers with environmental variables, which captured the ecological changes imposed by urbanization. Adaptive SNPs were found in the species’ genome. The study suggests changes are occurring as mice adapt to new nutrition resources through metabolism. Despite urban mice populations’ adaptations, they, however, had a two-fold decrease in nucleotide diversity compared to the exurban ones (Harris et Munshi-South, 2016).

6. Urbanization effects on species and their habitats

The effects of urbanization are various, including biotope alteration, population fragmentation, increasing predation (Fattorini 2011), restricted movements, more significant genetic differentiation, higher inbreeding levels, lower genetic diversity, reduced connectivity, isolation of populations (Richardson et al. 2021), or even local extinctions (Dri et al. 2021). And only a minority of invertebrate studies (about 30%), and non-volant (incapable of flying) vertebrate studies (about 12%) show increasing species richness (McKinney 2008).

Fattorini (2011) conducted a study on four insect groups in urban Rome: butterflies (*Lepidoptera*), coprophagous scarabaeids, non-coprophagous scarabaeids and tenebrionids (Fig. 1.). His reconstruction of extinction trends from 1885 to 1999 indicates impressive (15-39%) declines in species richness, with differences according to the ecological characteristics of each insect group. The high percentages of scarabaeid extinctions occurred in the period 1950–1960, the period associated with chaotic and uncontrolled urbanization. Ecological changes that occurred in 1900–1949 were serious for butterflies, because 33% of species were lost in this period, and an additional 2.5% of species disappeared in 1950–1960. So, according to Fattorini, the destruction of suitable biotopes (namely woodlands) is, in most cases, probably the primary cause of extinction.

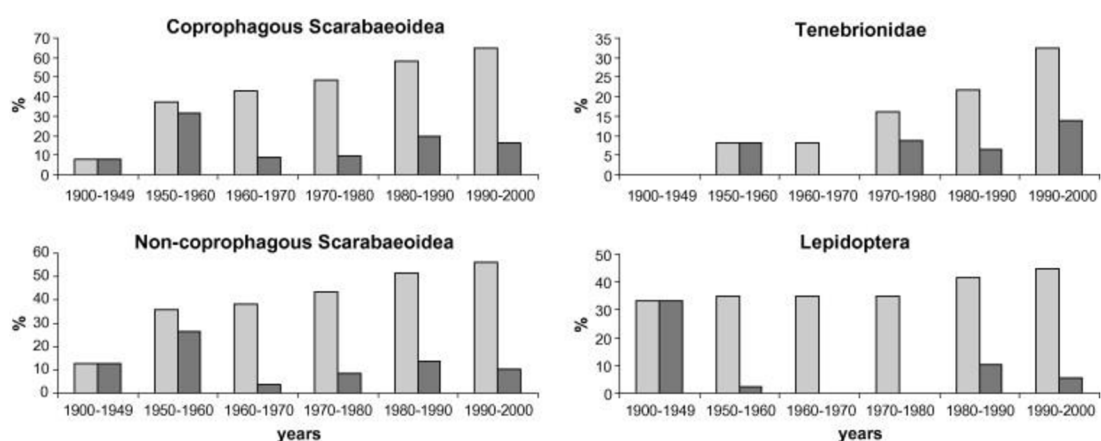


Fig. 1. Temporal trends in percentages of extinct species (light gray) and relative percentages of extinct species (dark gray) for four insect groups in urban Rome (Fattorini, 2011).

McKinney (2008) reviewed 105 studies on the effects of urbanization on the species richness of mammals, reptiles, amphibians, invertebrates and plants. For all groups, species richness tends to be reduced in areas with extreme urbanization (Fig. 2.). The earlier mentioned minority of invertebrate studies (about 30%) and non-volant vertebrate studies (about 12%), where the richness was higher in moderate urbanization levels, could be explained by the relative roles of spatial heterogeneity of habitats and intermediate disturbance dynamics. However, further research is needed to determine the reasons.

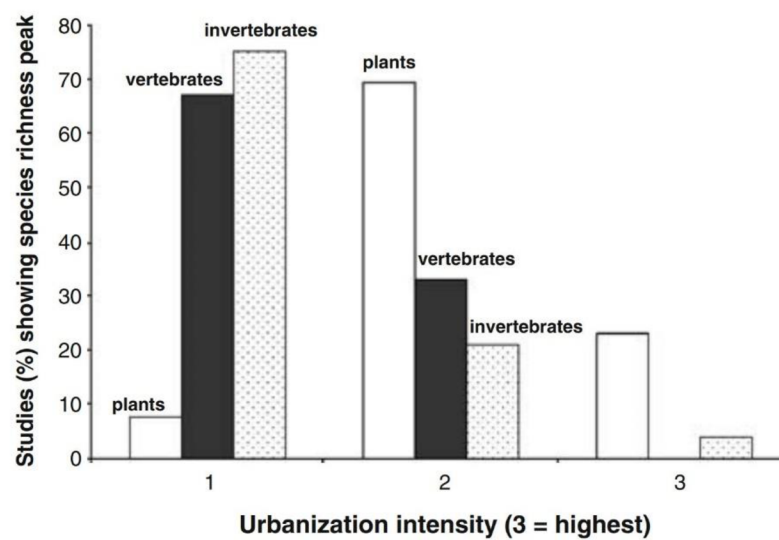


Fig. 2. Percentage of studies, by group, showing species richness peaks at three levels of urbanization (1=lowest level, 3=highest level of urbanization) (McKinney, 2008).

7. Facilitation, neutral and fragmentation models

In the following chapters, the three types of effects of urbanization on species' genetic diversity and gene flow will be reviewed. To generate hypotheses about how urbanization impacts species' movement and connectivity, Richardson et al. (2021) used the three models:

- The fragmentation model: represents the situation when the gene flow of the studied animal species was reduced, impaired or literally fragmented into differentiated genetic subpopulations.
- The facilitation model: is characterized by the facilitation of gene flow of the species caused by urbanization, making species thrive in urban habitats.
- The neutral model: implies that species can survive in urban conditions and suffice within small landscape patches, without their gene pool being affected.

The fragmentation model

In the majority of cases, the fragmentation model is applied, posing a conservation challenge to animals by increasing the resistance of the landscape to dispersal, movement, and gene flow (Unfried et al. 2013). Urbanization does not even have to be of a great extent to cause fragmentation of natural habitats or confer adverse effects on populations of native species (Fusco et al. 2021). Munshi-South et Kharchenko (2010) studied white-footed mice (*Peromyscus leucopus*) extracting DNA from 312 individual tail snips in New York City. Their results show that urban fragmentation produced rapid, substantial genetic structure of white-footed mice populations in NYC. They found that roads, buildings, and human barriers may counteract any potential corridor effect (connection between habitats). That is despite the fact that areas between trapping sites contained corridors of cemeteries, parkway medians, and other manicured vegetation that may have similar permeability to agricultural areas.

In a study conducted by Serieys et al. (2015) two major freeways were fragmenting the habitats and the gene flow of bobcats (*Lynx rufus*) in Santa Monica Mountains National Recreation Area (SMMNRA). Researchers collected blood or tissue by capturing animals or opportunistically from carcasses discovered in the study area. Serieys et al. (2015) discovered that the gene flow was primarily limited by the two freeways (Fig. 3.) built in 1949 (route 101) and 1962 (route 405).

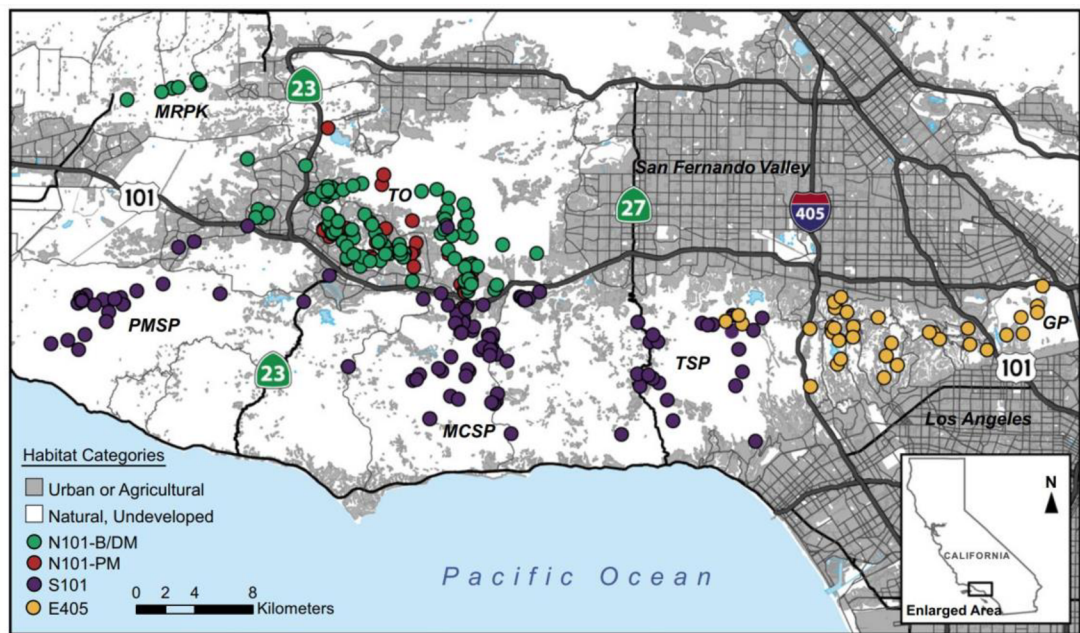


Fig. 3. Map of Santa Monica Mountains National Recreation Area where bobcats were sampled. Colored circles represent individual bobcat sampling locations, and colors correspond with the predominant structure cluster assignment for each individual. Major freeways of interest include the 101 Freeway and the I-405. The 23 (south of the 101 Freeway) and the 27 are secondary roads that intersect the study area (Serieys et al. 2015).

The fragmentation model can even be applied to volant (capable of flying; characterized by flight) species, in this case, song sparrow (*Melospiza melodia*). Despite their high mobility, reduced habitat availability could result in less population connectivity, as the amount of breeding habitat within the range of common dispersal distances was reduced (Unfried et al. 2013).

The facilitation model

Some species despite belonging to different taxa and some of them having less dispersal abilities than others prosper in urban environments. They demonstrate that although urbanization can create dispersal barriers, the movement of various species across urban landscapes may be aided by man-made parks, waterways, and small forest patches. The following studies with examples from invertebrates and birds taxa are to demonstrate that. No mammals' genetic diversity or gene flow reviewed in the studies was facilitated by urbanization processes.

- Invertebrates.

Miles et al. (2018a) studied Western black widow (*Latrodectus hesperus*) in the Western United States. They hypothesized that urban spider populations have higher genetic diversity and less structure than non-urban ones. The contrasts of urban and nonurban patterns prove to be more consistent with gene flow being relatively facilitated, and not reduced among urban locales. Estimates of genetic diversity and phylogeographic history of Western black widow spiders also support the urbanization facilitation model. Not only this demonstrates these organisms are successfully invading urban environments, but they also do so by rapidly spreading across large geographic areas. Miles et al. (2018a) also explained how urban areas specifically drive connectivity: their social network analysis found that there were multiple locales within Phoenix identified as ‘hubs’ of connectivity, whereas sites within Las Vegas each similarly impact gene flow. Alternatively, Albuquerque locales, which overall were significantly more disconnected from the network, included one hub, and this hub connected the other nine Albuquerque locales to the network. This suggests that urban locales not only have more connections, but can also act as hubs that drive connectivity among nonurban locales (Miles et al. 2018a).

- Birds.

Carlen et Munshi-South (2021) studied population genetics of feral pigeons (*Columba livia*) in multiple cities across the Northeastern United States. Their study demonstrates how urbanization across the Northeastern megacity facilitates gene flow in a human commensal: results indicate that pigeons are moving between municipalities more than previously observed, and in the Northeastern United States (Fig. 4.), this movement may be facilitated by extensive urbanization. Björklund et al. (2010) also demonstrate that the great tit (*Parus major*) populations in Barcelona, Spain, contain more genetic variation in city parks than that in nearby forests, and gene flow from urban to nonurban populations is greater than vice versa.

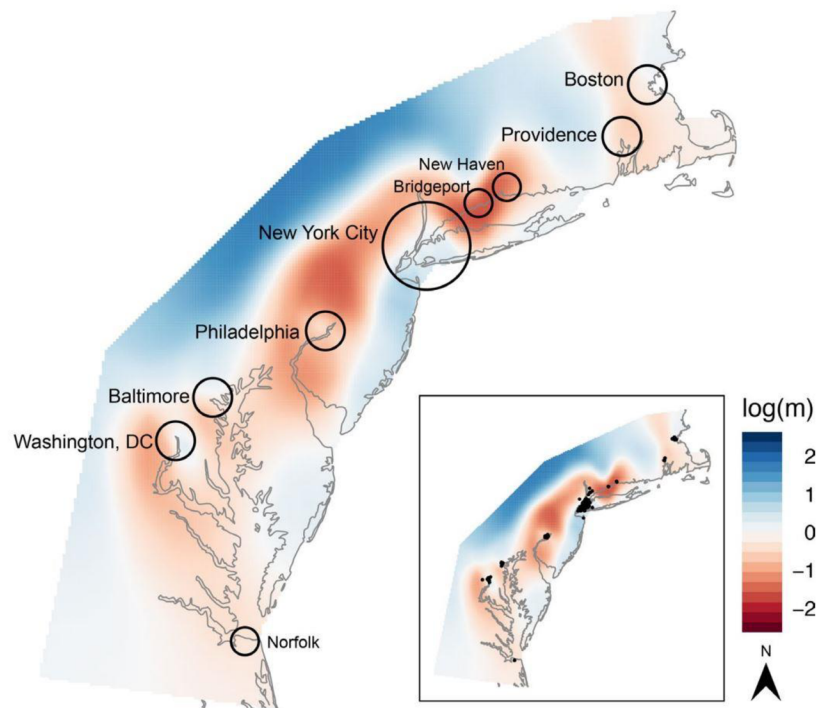


Fig.4. Estimated effective migration surface (EEMS) for pigeons in the Northeastern United States. Coloring of the map represents relative effective migration rates ranging from higher-than-average (blue) to lower-than-average (red) historic gene flow with isolation by distance represented as the null (white). Circles represent the approximate sampling range for each city. Within many cities, there is high-than-average gene flow and isolation by distance, but there is lower-than-average gene flow between cities (Carlen et Munshi-South 2021).

The neutral model

This model implies that species can survive in urban conditions and suffice within small landscape patches, without their gene pool being affected. For example, Furman et al. (2016) estimated gene flow in a population of wood frogs (*Lithobates sylvaticus*) inhabiting both constructed and natural wetlands located in Edmonton, Alberta, Canada. They found no differences in allelic richness among subpopulations, and no genetic structure within the population. Furman et al. (2016) demonstrate in Fig. 5. that site-specific allelic richness estimates indicated that natural and constructed wetlands in Edmonton, Alberta, Canada, contain comparable levels of allelic diversity.

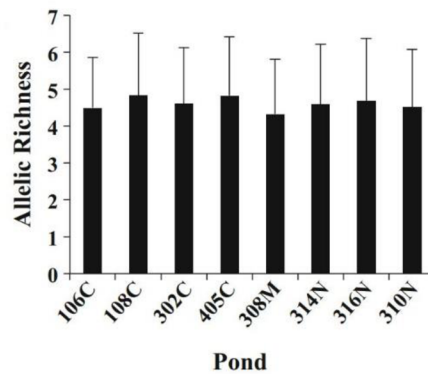


Fig. 5. A rarefied estimate of allelic richness of all wood frog (*Lithobates sylvaticus*) individuals at each wetland. These analyses include three constructed wetlands (C), three natural wetlands (N) located in the North Saskatchewan River valley of Edmonton, and one natural pond surrounded by urban development (M) (Furman et al. 2016).

The fact that the genetic diversity was preserved indicates that it is possible to maintain amphibian populations and preserve gene flow among subpopulations living within urbanized constructed landscapes.

However, these models may not always be applicable, as some species possess greater dispersal capacities than others and have different traits. In the following chapter species are compared and separated into two groups in terms of dispersal abilities: volant versus non-volant ones.

Volant species

- Birds

Following studies support that flying abilities of species might buffer species against the effects of habitat fragmentation. For example, Lohr et al. (2020) hypothesize that the genetic homogeneity observed in Southern boobook (Australian owl) (*Ninox boobook*) in Western Australia is a consequence of long-distance dispersal capacity in addition to their ability as habitat and dietary generalists to make use of highly altered habitats.

Feral pigeons (*Columba livia*) in the Northeastern megacity also maintain high genetic connectivity over a large urbanized region likely due to their ability to move through human-dominated landscapes. Across all sampled cities, pigeons had a weak population genetic differentiation and low inbreeding coefficient ranging (Carlen et Munshi-South 2021).

The analyzed pattern of genetic variation in the great tit (*Parus major*) in 12 parks in central Barcelona, and an adjacent forest populations complies with previous studies, suggesting the genetic diversity was not lower in the parks compared to the forest population (Björklund et al. 2010).

However, Dri et al. (2021) studied 3009 individual counts from 101 bird species in Florianópolis, Southern Brazil, The reduction of habitat area, in this case also, was the cause for a greater local extinction of this species (Fig. 6.).

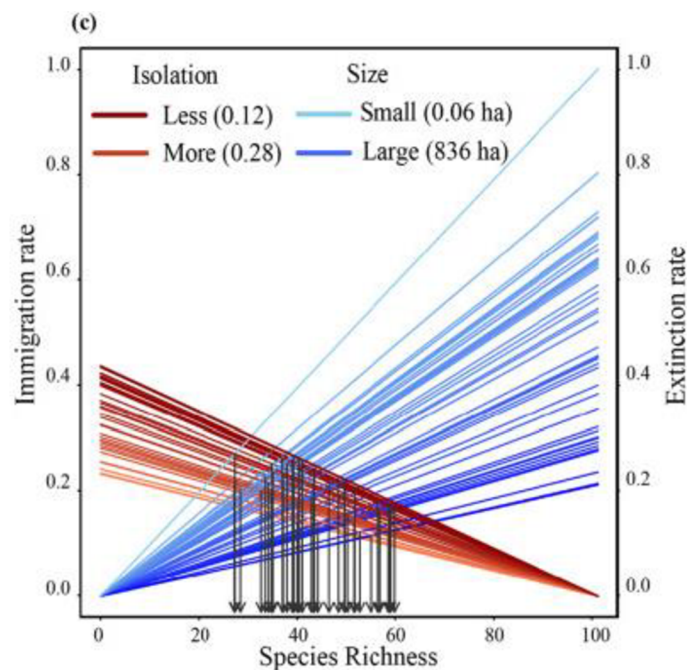


Fig. 6. Larger habitat patches (darker blue) have lower extinction rates and reach the equilibrium between extinction and immigration rates with more species than smaller patches (light blue) (Dri et al. 2021).

- Insects

However, despite the presence of pairs of wings insects in the following studies were not able to maintain their levels of genetic diversity. According to Jha et Kremen (2013), the genetic structure of the yellow-faced bumble bee (*Bombus vosnesenskii*), studied across the southwestern US coast, appears to be mostly limited by commercial, industrial and transportation-related impervious cover in a human-altered habitat. Results of the study conducted by Jha (2015) reveal that bumble bee gene flow is limited by two human-altered land-use types: impervious cover and croplands. Jha (2015) suggests it is likely due to (1) the largely ground-nesting strategy of yellow-faced bumble bee, (2) the lower level of floral resources in these landscapes and (3) bumble bee aversion to dispersal across impervious surfaces.

Rochat et al. (2017) studied the genetic diversity of cabbage white butterfly (*Pieris rapae*) in Marseille, France. They observed that populations of the species were severely affected by the loss of genetic diversity. As the habitat loss increased and connectivity was limited due to the dispersal barriers caused by impervious surfaces, population sizes were reduced.

Fattorini (2011) conducted a personal long-term field research in urban Rome, Italy (Fig. 1.), an extensive literature survey of entomological papers, and examined material preserved in various insect collections. The results suggest an impressive decline with an increasing rate since 1950 in four groups of insects: butterflies, coprophagous scarabaeids, non-coprophagous scarabaeids and tenebrionids.

Non-volant species

The following studies support the hypothesis that species with lesser dispersal abilities are more isolated by urban barriers. For example, despite being large and highly mobile species generally considered to be well adapted to human activity mule deer (*Odocoileus hemionus*) was significantly affected by barriers imposed by urbanization. Furman et al. (2016) observed that genetic structure corresponds with highway boundaries in certain habitat patches.

Harris et Munshi-South (2016) studied white-footed mice (*Peromyscus leucopus*) in New York for their ability to thrive in small urban patches. Their results have also shown mice's genetic structure and diversity to be negatively affected. That is despite

the fact that white-footed mice are relatively tolerant and omnivorous rodents. In urban environments, they, however, besides other factors, suffer from higher rodenticide exposure.

Some of the disturbance-intolerant and less mobile species are - vulnerable to pathogens and pollution - mainly amphibians. Their requirements for both terrestrial and aquatic types of habitats to complete the semi-aquatic life cycle makes them particularly susceptible to habitat losses. However, at the same time, it is their vulnerability, philopatry and short generation times that make them excellent species to study the effects of urbanization. Homola et al. (2019), Munshi-South et al. (2013), Mikulíček et Pišút (2012), Noël et al. (2007), and Noël et Lapointe (2010) demonstrate the effects of habitat fragmentation on amphibians to be negative, with both their genetic diversity and structure being affected.

Schmidt et Garroway (2021), also studied 19 amphibian species in terms of their genetic diversity, allelic richness, effective population size, and population differentiation in Canada and the USA. However, they found that urbanization has minimal effect on amphibian species (Fig. 7.). The explanation appears to lie in the differences among species' responses. Despite amphibians belonging to the same taxa, urbanization effects cannot be generally applied across species.

Species-specific effects of HFI on gene diversity

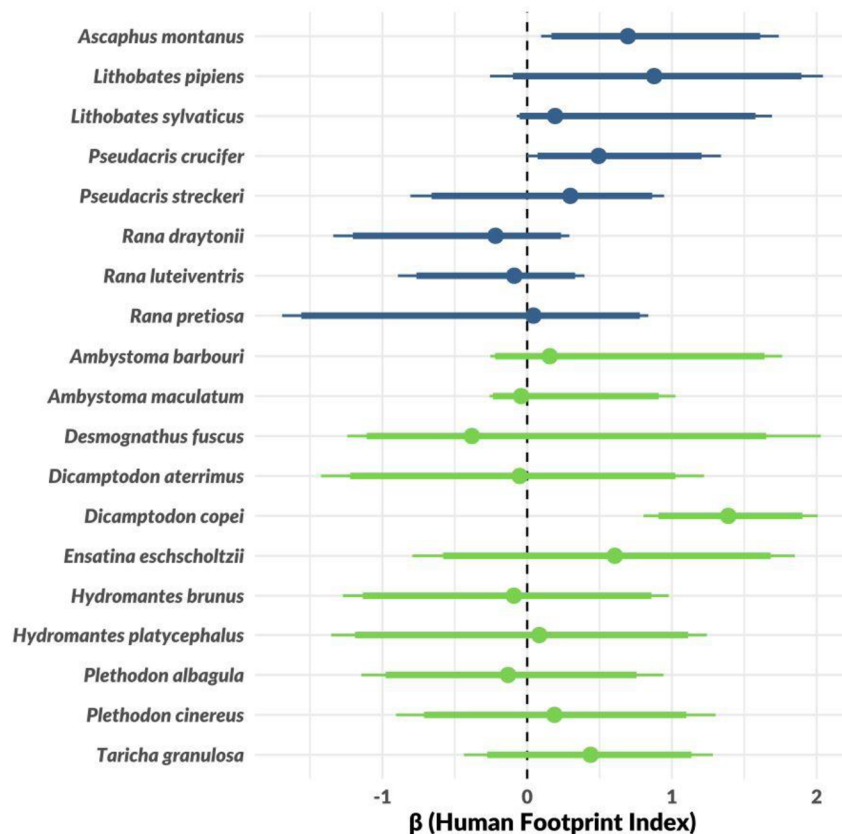


Fig. 7. Species-specific effects of the Human Footprint Index on genetic diversity. The graph shows species-specific coefficients for the effect of the Human Footprint Index on gene diversity. Few species had positive effect sizes (Rocky mountain tailed frog, *Ascaphus montanus*; Cope's giant salamander, *Dicamptodon copei*; and spring peeper *Pseudacris crucifer*), but these were not consistent across metrics of genetic composition or urbanization (Schmidt et Garroway 2021).

Moreover, the genetic diversity of European hedgehogs (*Erinaceus europaeus*), despite limited dispersal abilities, remained unaffected in urban conditions in Zurich, Switzerland. That is likely due to post-glacial expansions of species from glacial refugia not far from the Alps. Another explanation could be the widespreadness of promiscuity and multiple paternity of the European hedgehog; which aids in the maintenance of high genetic variability (Braaker et al. 2017).

8. Distribution of genetic diversity across urban gradient

The following studies compared genetic diversity of species from natural ex-urban sites to the heavily transformed city centers.

Gortat et al. (2015) suggest a possibility that heterogeneity of the urban environment in Warsaw, Poland, allows a high level of genetic variability to be maintained across urbanization gradient. The genetic diversity of striped field mouse (*Apodemus agrarius*) in large suitable habitat patches that were connected with ex-urban regions remained rich. Whereas in other areas, that have been more drastically changed by man, e.g. heavily built-up sites containing small patches, surrounded by busy roads or by large areas of unsuitable habitat, the level of genetic variability is significantly reduced.

Results from the study conducted by Fusco et al. (2021) and Ottewell et al. (2019) also show that studied populations (northern two-lined salamander (*Eurycea bislineata*) and quenda (*Isodon fusciventer*)) can persist within an urban matrix in the areas where urban development was not intense and sufficient habitat was retained.

Despite significant phylogenetic, ecological, and mobility differences between side-blotched lizard (*Uta stansburiana*), Western skink (*Plestiodon skiltonianus*), Western fence lizard (*Sceloporus occidentalis*), and a wrentit (*Chamaea fasciata*), Delaney et al. (2010) compared changes in their gene flow in one study. For all four species, the most significant genetic divergence was found where urban development in California was the oldest and most intensive. All four animals also showed a significant reduction in gene flow associated with intervening roads and freeways, the degree of patch isolation, and the time since isolation.

Results published by Noël et al. (2007) also indicate that allelic richness and heterozygosity of eastern red-backed salamanders (*Plethodon cinereus*) are lower in the urban populations of Quebec, Canada. Exact differentiation tests show that the populations found in the fragmented habitat are genetically differentiated, whereas populations located in the continuous habitat are genetically homogeneous. Urban wood mice (*Apodemus sylvaticus*) populations in Dundee, sampled from sites separated by on average 4 km, were also more genetically differentiated than those in arable habitat, suggesting limited gene flow of wood mice in urbanized areas, despite them being generalists (Wilson et al. 2016).

The studies conducted by Markowski et al. (2021) and Müller et al. (2013) directly compared how birds were affected in urban and non-urban habitats. Markowski et al. (2021) compared the genetic differentiation of the great tit (*Parus major*) populations in the urban park of Poland to the Łagiewniki Forest and the Spała Forest populations. Results revealed that low but significant divergence was found. The authors of the study believe that the genetic differentiation of bird populations is hampered by urban conditions and may be enhanced by urban habitat variation. While Müller et al. (2013) studied twelve locations across the Western Palaearctic to investigate how European blackbird (*Turdus merula*) is affected by urbanization (Fig. 8.). Results demonstrate that urban populations had lower frequencies of the major allele than the paired rural population in 10 of 12 comparisons. Authors hypothesize that the situation differed for Berlin and Tunis due to different selection pressure. That is, however, merely a theory without evidence.

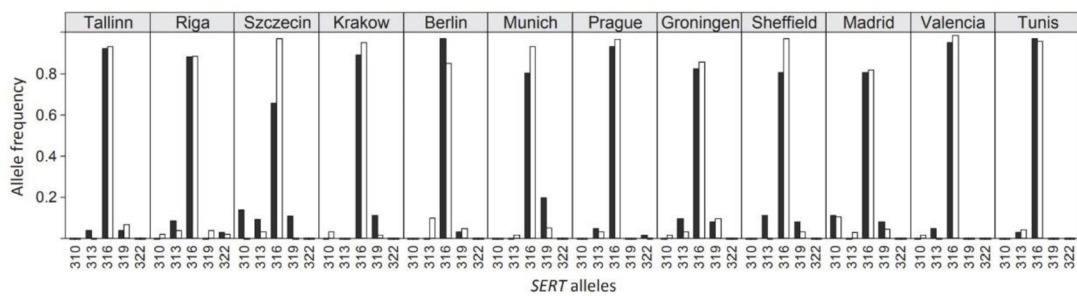


Fig. 8. Allele frequencies of the SERT microsatellite in twelve urban–rural population pairs of the blackbird. Urban sites = filled bars; rural sites = open bars; urban and rural sample sizes: Tallinn (26, 22), Riga (17, 26), Szczecin (32, 31), Krakow (9, 30), Berlin (31, 30), Munich (76, 57), Prague (30, 30), Groningen (31, 31), Sheffield (31, 31), Madrid (31, 33), Valencia (31, 32) and Tunis (35, 59). Urban populations had lower frequencies of the major allele in 10 of 12 comparisons (Müller et al. 2013).

Yannic et al. (2021), Perrier et al. (2018), and Richardson et al. (2021) detected a moderate but significant genetic differentiation in the genetic structure of studied species between sites with different urbanization levels. Their findings suggest limited genetic exchange across the study area. However, species with different dispersal capacities do not appear to be affected to the same extent. For example, Richardson et al. (2021) found that gene flow in big brown bats was less affected by urbanization than that of white-footed mice along the same urban to ex-urban gradient. This supports the expectation that species with greater dispersing abilities maintain higher levels of connectivity, despite their habitat becoming fragmented.

9. Critical evaluation

During my research, literature review, I concluded that the biggest current problem is the lack of data. It was either related to the samples or the scarcity of previous research on the subject.

Some studies used surprisingly small sample sizes (Lohr et al. (2020), Southern boobook (*Ninox boobook*)), only 17 individuals, while others (Vangestel et al. (2011), house sparrows (*Passer domesticus*)), tested their hypotheses on an impressive sample size of 690 individuals. Such differences might have had a crucial impact on the studies' results. It is widely understood that a larger sample size provides more accurate results.

At the same time, I evaluated that most studies use a short sample collection period. Very often, the timespan did not surpass two years. In some cases (Davis et al. 2010), samples were collected for four years (2005-2009), while in other cases (Winchel et al. 2016), between June 12 and June 30, 2012, for only two weeks.

I also noted another complication – possible sampling bias. It occurs when some individuals, due to various reasons, are more likely to be selected than others. The reasons are, for example: fleeing and moving limitations of species, sex-biased dispersal, and limited human power.

To achieve more precise results, I believe the researchers should use consistent sample sizes and timespans, and develop a more randomized sample collecting method.

Based on my research, I also came to the conclusion that comparing genetic diversity levels across species may be problematic, as it depends on how the genetic material was extracted and what method was used to perform the analysis. For the results to be more consistent across studies, I suggest that future research should address using more consistent gene isolation and statistical analysis methods.

Another possible reason for distorted results is kin recognition based on chemical cues (members of the family *Plethodontidae*, Noël et Lapointe (2010)). I believe this theory also should be considered when conducting research.

Since the city genetics discipline is still relatively new, the amount of previously carried out research is not abundant. Nevertheless, the insufficient data issue can be solved by collecting data from hidden literature and museum collections and using it

to trace losses through time. Fattorini (2011) investigated insect extinction in Rome in such manner. In my opinion, future studies would benefit greatly from collecting data from various sources and periods.

I consider some study results and predictions to have certain shortcomings. For instance, a simulation developed by Fahrig (1998) suggests that habitat fragmentation affects population survival only if all of the five conditions she suggests are held for. For this to be the case, however, the number of studies with opposing results would have been substantially smaller.

The use of genetic methods to discover urbanization effects on animal species is certainly a valuable tool that helps detect and predict many risks. Nevertheless, to get more accurate results, I believe future studies should: (1) expand observation periods, determining if the time of the changes in species genome and their abundance correlates with the formation of the cities, (2) investigate the changes in species genome more detailly, to assess if anthropogenic causes are stronger than the natural ones, (3) compare multiple urban and rural habitats, (4) take into account life history traits and requirements of species, and (5) develop a more unified sample collection and gene isolation and statistical analysis methods.

10. Results and discussion

In this bachelor thesis, I studied the effects of cities' expansion on the genetics of vertebrate and invertebrate species. I reviewed sixty studies to find out if, how, and since when the species have been affected. I also attempted to detect if any adaptations occurred.

Urbanization effects on species' genetic diversity and structure were valued with „positive“, „negative“ and „none“ (Fig. 9.). A positive value represents cases, when urbanization affected species with positive outcomes, facilitating urban residents, where they are able to prosper. A negative value represents genetic diversity and gene flow of populations being affected negatively, where species' diversity was reduced, and more diverse structures within populations were formed. The value none represents the state when the genetic material of studied species was not affected in any way, i.e., city expansion did have any influence on species. The „adaptation tested“ column summarizes the species whose genetic material was tested in terms of adaptive abilities to changing urban conditions.

During my research, I analyzed which species are the most vulnerable and how urbanization affects them. I concluded that amphibians are more susceptible than other species. Their requirements for a semi-aquatic lifestyle, in addition to pollution and disease sensitivity all contribute to their decline. In my opinion, future research could achieve more accurate results by taking into account the fact that amphibians are sensible to multiple phenomena apart from urbanization, all of those mentioned above, including climate change.

The most significant conclusion I came to is: the majority (75%) of studied species are, to various extents, affected by urbanization negatively, i.e., more genetic structure formed and/or diversity declined. The majority of species' genetic diversity and structure, regardless of their class, were affected by urbanization negatively: 53% and 71% respectively. No effect on species' genetic diversity and structure was observed in 41% and 23% of the studies, respectively. Finally, only in 6% and 6% studied species the results show that their genetic diversity and structure (resp.) appear to have been affected by urbanization positively.

As demonstrated in the table, the majority of researchers did not test the sampled species for adaptation. Only 7,5% of reviewed studies tested species for the

development of adaptive genes. In those studied it was discovered that in 80% of cases studied organisms, despite belonging to different classes (mammals and insects), have developed adaptive SNPs. They determined species are indeed able to adapt, on both behavioral and genetic levels, to conditions imposed by cities. For example, adaptive SNPs were found in the genome of white-footed mice (*Peromyscus leucopus*) and big brown bats (*Eptesicus fuscus*) by Richardson et al. (2021), white-footed mice (*Peromyscus leucopus*) by Harris et Munshi-South (2016), and red-tailed bumblebees (*Bombus lapidarius*) by Theodorou et al. (2018).

In the following table (Fig. 9.), I made a comparison of specific species studied by various researchers. I compared collected and studied sample sizes and location areas, reviewed the used genetic markers, investigated if the species' adaptive abilities were tested, and summarised the effects of urbanization on species' genetic diversity and structure. Data was collected mainly from Web Of Science, using keywords.

	AUTHORS	LOCATION & AREA	STUDIED SPECIES	SAMPLE SIZE	GENETIC MARKER & NUMBER OF LOCI	ADAPTATION TESTED	EFFECTS OF URBANIZATION ON GENETIC DIVERSITY	EFFECTS OF URBANIZATION ON GENETIC STRUCTURE
1	Homola et al. (2019)	Maine, USA (91,646 km ²)	Spotted salamanders (<i>Ambystoma maculatum</i>)	90	10 microsatellite loci	not tested	negative	negative
			Wood frogs (<i>Lithobates sylvaticus</i>)	87		not tested	negative	negative
2	Fusco et al. (2021)	New York City, USA (783,8 km ²)	Northern two-lined salamander (<i>Eurycea bislineata</i>)	351	15,314 SNP loci	not tested	none	negative
3	Munshi-South et al. (2013)	New York City, USA (783,8 km ²)	Northern dusky salamander (<i>Desmognathus fuscus</i>)	141	5 microsatellite loci	not tested	negative	negative
4	Munshi-South et Kharchenko (2010)	New York City, USA (783,8 km ²)	White-footed mouse (<i>Peromyscus leucopus</i>)	312	18 microsatellite loci	not tested	none	negative
5	Schmidt et Garroway (2021)	Canada and the US (9,985000 & 9,834000 km ² resp.)	19 amphibian species ¹	13,680	raw synthesized microsatellite data ²	not tested	none	none

	AUTHORS	LOCATION & AREA	STUDIED SPECIES	SAMPLE SIZE	GENETIC MARKER & NUMBER OF LOCI	ADAPTATION TESTED	EFFECTS OF URBANIZATION ON GENETIC DIVERSITY	EFFECTS OF URBANIZATION ON GENETIC STRUCTURE
6	Beninde et al. (2016)	Trier, Germany (117,1 km ²)	Common wall lizard (<i>Podarcis muralis</i>)	223	17 microsatellite loci	not tested	none	none
7	Richardson et al. (2021)	Rhode Island, USA (3,144 km ²)	White-footed mouse (<i>Peromyscus leucopus</i>)	215	206,139 SNP loci	tested, found adaptive SNPs	none	negative
			Big brown bat (<i>Eptesicus fuscus</i>)	367	26,736 SNP loci	tested, found adaptive SNPs	none	none
8	Björklund et al. (2010)	Barcelona, Spain (101,9 km ²)	Great tit (<i>Parus major</i>)	248	7 microsatellite loci	not tested	positive	positive
9	Jordan et al. (2009)	Indiana, USA (94,321 km ²)	Eastern red-backed salamander (<i>Plethodon cinereus</i>)	230	6 microsatellite loci	not tested	negative	negative
10	Gortat et al. (2015)	Warsaw, Poland (517,2 km ²)	Striped field mouse (<i>Apodemus agrarius</i>)	490	17 microsatellite loci	not tested	negative	negative
11	Mikulíček et Pišút (2012)	Bratislava, Slovakia (367,6 km ²)	Marsh frog (<i>Pelophylax ridibundus</i>)	494	11 microsatellite loci	not tested	negative	negative
12	Munshi-South et Nagy (2014)	New York City, USA (783,8 km ²)	White-footed mouse (<i>Peromyscus leucopus</i>)	294	18 microsatellite loci	not tested	none	none
13	Noël et al. (2007)	Montreal, Quebec, Canada (431,5 km ²)	Eastern red-backed salamander (<i>Plethodon cinereus</i>)	221	7 microsatellite loci	not tested	negative	negative
14	Unfried et al. (2013)	Seattle, Washington, USA (217 km ²)	Song Sparrow (<i>Melospiza melodia</i>)	469	12 microsatellite loci	not tested	none	negative
15	Furman et al. (2016)	Edmonton, Alberta, Canada (684 km ²)	Wood frog (<i>Lithobates sylvaticus</i>)	182	10 microsatellite loci	not tested	none	none

	AUTHORS	LOCATION & AREA	STUDIED SPECIES	SAMPLE SIZE	GENETIC MARKER & NUMBER OF LOCI	ADAPTATION TESTED	EFFECTS OF URBANIZATION ON GENETIC DIVERSITY	EFFECTS OF URBANIZATION ON GENETIC STRUCTURE
16	Munshi-South et al. (2016)	New York City, USA (783,8 km ²)	White-footed mouse (<i>Peromyscus leucopus</i>)	233	10,000 SNP loci	not tested	negative	negative
17	Ottewell et al. (2019)	Perth, Australia (6,418 km ²)	Quenda (<i>Isoodon fusciventer</i>)	681	12 microsatellite loci	not tested	none	negative
18	Noël et al. (2010)	Montréal, Quebec, Canada (431,5 km ²)	Eastern red-backed salamander (<i>Plethodon cinereus</i>)	416	6 microsatellite loci	not tested	negative	negative
19	Bech et al. (2014)	Central Northern France (632,734 km ²)	Grey partridge (<i>Perdix perdix armoricana</i>)	134	12 microsatellite loci	not tested	none	negative
20	Harris et Munshi-South (2016)	New York City, USA (783,8 km ²)	White-footed mouse (<i>Peromyscus leucopus</i>)	48	154,770 SNP loci	tested, found adaptive SNPs	negative	negative
21	Wilson et al. (2016)	Dundee, Scotland (51,8 km ²)	Wood mouse (<i>Apodemus sylvaticus</i>)	268	9 microsatellite loci	not tested	negative	negative
22	Serieys et al. (2015)	SMMNRA, California, USA (638 km ²)	Bobcat (<i>Lynx rufus</i>)	664	16 microsatellite loci	not tested	negative	negative
23	Carlen et Munshi-South (2021)	Northeastern USA (146,000 km ²)	Feral pigeon (<i>Columba livia</i>)	473	35,200 SNP loci	not tested	positive	positive
24	Fraser et al. (2019)	California, USA (423,970 km ²)	Mule deer (<i>Odocoileus hemionus</i>)	538	14 microsatellite loci	not tested	negative	negative
25	Quemere et al. (2010)	Daraina, Madagascar (2,450 km ²)	Golden-crowned sifaka (<i>Propithecus tattersalli</i>)	230	13 microsatellite loci	not tested	none	none

	AUTHORS	LOCATION & AREA	STUDIED SPECIES	SAMPLE SIZE	GENETIC MARKER & NUMBER OF LOCI	ADAPTATION TESTED	EFFECTS OF URBANIZATION ON GENETIC DIVERSITY	EFFECTS OF URBANIZATION ON GENETIC STRUCTURE
26	Coulon et al. (2004)	France (632,734 km ²)	Roe deer (<i>Capreolus capreolus</i>)	648	12 microsatellite loci	not tested	negative	negative
27	Vandergast et al. (2007)	California, USA (423,970 km ²)	Jerusalem cricket (<i>Stenopelmatus</i>)	260	mitochondrial DNA sequence data	not tested	negative	negative
28	Gardner-Santana et al. (2009)	Baltimore, Maryland, USA (238,5 km ²)	Wild Norway rat (<i>Rattus norvegicus</i>)	277	10 microsatellite loci	not tested	none	none
29	Vangestel et al. (2011)	Ghent, Belgium (156,2 km ²)	House sparrows (<i>Passer domesticus</i>)	690	16 microsatellite loci	not tested	negative	negative
30	Davis et al. (2010)	Ireland & Scotland (84,421 & 77,910 km ² resp.)	Northern colletes (<i>Colletes floralis</i>)	464	9 microsatellite loci	not tested	none	negative
31	Jha (2015)	California, USA (423,970 km ²)	Yellow-faced bumble bee (<i>Bombus vosnesenskii</i>)	860	12 microsatellite loci	not tested	negative	negative
32	MacDougall-Shackleton et al. (2011)	Vancouver Island & Victoria, Canada (31,284 & 19,47 km ² resp.)	Song sparrow (<i>Melospiza melodia</i>)	106	7 microsatellite loci	not tested	negative	negative
33	Jha et Kremen (2013)	Delta, California (2,800 km ²)	Yellow-faced bumble bee (<i>Bombus vosnesenskii</i>)	800	13 microsatellite loci	not tested	none	negative
34	Kajdacs et al. (2013)	Salvador, Brazil (693,8 km ²)	Norway rats (<i>Rattus norvegicus</i>)	146	17 microsatellite loci	not tested	none	negative

	AUTHORS	LOCATION & AREA	STUDIED SPECIES	SAMPLE SIZE	GENETIC MARKER & NUMBER OF LOCI	ADAPTATION TESTED	EFFECTS OF URBANIZATION ON GENETIC DIVERSITY	EFFECTS OF URBANIZATION ON GENETIC STRUCTURE
35	Miles et al. (2018b)	Western United States (4,852,000 km ²)	Western black widow (<i>Latrodectus hesperus</i>)	210	40,533 SNPs from nuDNA and 124 SNPs from mtDNA	not tested	positive	positive
36	Kozakiewicz et al. (2019)	California, USA (423,970 km ²)	Bobcat (<i>Lynx rufus</i>)	271	13,520 SNP loci	not tested	negative	negative
37	Taylor et al. (2011)	Australia (7,617,930 km ²)	Squirrel glider (<i>Petaurus norfolcensis</i>)	259	5 microsatellite loci	not tested	negative	negative
38	Delaney et al. (2010)	SMMNRA, California, USA (638 km ²)	Side-blotched lizard (<i>Utastansburiana</i>)	181	6-8 microsatellite loci	not tested	negative	negative
			Western skink (<i>Plestiodon skiltonianus</i>)	179			negative	negative
			Western fence lizard (<i>Sceloporus occidentalis</i>)	147			negative	negative
			Wrentit (<i>Chamaea fasciata</i>)	69			negative	negative
39	Harris et al. (2013)	New York City, USA (783,8 km ²)	White-footed mouse (<i>Peromyscus leucopus</i>)	112	31,015 SNP loci	tested, not identified	none	negative
40	Goldingay et al. (2013)	Mackay & Brisbane, Queensland, Australia (208,2 & 15,842 km ² resp.)	Squirrel glider (<i>Petaurus norfolcensis</i>)	80 & 265	6 microsatellite loci	not tested	negative	negative
41	Ernest et al. (2014)	California, USA (423,970 km ²)	Puma (<i>Puma concolor</i>)	97	46 microsatellite loci	not tested	negative	negative

	AUTHORS	LOCATION & AREA	STUDIED SPECIES	SAMPLE SIZE	GENETIC MARKER & NUMBER OF LOCI	ADAPTATION TESTED	EFFECTS OF URBANIZATION ON GENETIC DIVERSITY	EFFECTS OF URBANIZATION ON GENETIC STRUCTURE
42	Braaker et al. (2017)	Zurich, Switzerland (87,88 km ²)	European hedgehog (<i>Erinaceus europaeus</i>)	147	10 microsatellite loci	not tested	none	negative
43	Brewer et al. (2020)	Blacksburg, Virginia, USA (51,2 km ²)	Song sparrow (<i>Melospiza melodia</i>)	208	15 microsatellite loci	not tested	none	none
44	Smith et al. (2020)	California, USA (423,970 km ²)	Bobcat (<i>Lynx rufus</i>)	118 & 422	19 & 11 microsatellite loci	not tested	none	negative
45	Straub et al. (2015)	Salzburg, Austria (65,68 km ²)	Fire salamander (<i>Salamandra salamandra</i>)	70	7 microsatellite loci	not tested	none	none
46	Koh et al. (2019)	Singapore (728,6 km ²)	Wild pig (<i>Sus scrofa</i>)	48	55,076 SNP loci	not tested	none	none
47	Miles et al. (2018a)	Western United States (4,852000 km ²)	Western black widow (<i>Latrodectus hesperus</i>)	210	1,900 000 SNP loci	not tested	positive	positive
48	Harris et al. (2016)	New York City, USA (783,8 km ²)	White-footed mouse (<i>Peromyscus leucopus</i>)	191	14,990 SNP loci	not tested	negative	negative
49	Vandergast et al. (2009)	California, USA (423,970 km ²)	Jerusalem cricket (<i>Stenopelmatus</i>)	155	38 anonymous nuclear Inter-Simple Sequence Repeat (ISSR) loci	not tested	negative	negative
50	Van Rees et al. (2018)	Hawaii, USA (28,311 km ²)	Hawaiian gallinule (<i>Gallinula galeata sandvicensis</i>)	152	12 microsatellite loci	not tested	negative	negative
51	Lohr et al. (2020)	Western Australia (2,646000 km ²)	Southern boobook (<i>Ninox boobook</i>)	17	8 microsatellite loci	not tested	none	none

	AUTHORS	LOCATION & AREA	STUDIED SPECIES	SAMPLE SIZE	GENETIC MARKER & NUMBER OF LOCI	ADAPTATION TESTED	EFFECTS OF URBANIZATION ON GENETIC DIVERSITY	EFFECTS OF URBANIZATION ON GENETIC STRUCTURE
52	Trumbo et al. (2019)	Colorado, USA (269,837 km ²)	Puma (<i>Puma concolor</i>)	130	12,000 SNP loci	not tested	none	none
53	Kobayashi et al. (2013)	Chiba prefecture, Japan (5,158 km ²)	Japanese brown frog (<i>Rana japonica</i>)	255	mitochondrial DNA haplotype frequencies	not tested	negative	negative
54	Schmidt et al. (2020)	1008 locations across North America (24,365,000 km ²)	41 mammal species	41,023	raw genotype data from 85 studies	not tested	negative	negative
			25 bird species			not tested	none	none
55	Yannic et al. (2021)	Switzerland (41,285 km ²)	Fire salamander (<i>Salamandra salamandra</i>)	419	9 microsatellite loci	not tested	none	none
56	Lourenço et al. (2017)	Oviedo, Spain (186,6 km ²)	Fire salamander (<i>Salamandra salamandra</i>)	320	15 microsatellite loci	not tested	negative	negative
57	Rochat et al. (2017)	Marseille, France (240,6 km ²)	Cabbage white butterfly (<i>Pieris rapae</i>)	1633	500 SNP loci	not tested	negative	not tested
58	DeMarco et al. (2021)	Southern California, USA (146,347 km ²)	Western gray squirrel (<i>Sciurus griseus</i>)	117	12 microsatellite loci and a 550 bp segment of the mitochondrial control region	not tested	negative	negative
59	Theodorou et al. (2018)	Germany (357,386 km ²)	Red-tailed bumblebee (<i>Bombus lapidarius</i>)	198	110,314 SNP loci	tested, found adaptive SNPs	none	none
60	Perrier et al. (2018)	Montpellier, France (56,88 km ²)	Great Tit (<i>Parus major</i>)	140	97 SNP loci	not tested	negative	negative

Fig. 9.: Overview of the reviewed studies.

¹19 amphibian species - from a list of native ones to North America from the IUCN Red List database: *Ascaphus montanus*, *Lithobates pipiens*, *Lithobates sylvaticus*, *Pseudacris crucifer*, *Pseudacris streckeri*, *Rana draytonii*, *Rana luteiventris*, *Rana pretiosa*, *Ambystoma barbouri*, *Ambystoma maculatum*, *Desmognathus fuscus*, *Dicamptodon aterrimus*, *Dicamptodon copei*, *Ensatina eschscholtzii*, *Hydromantes brunus*, *Hydromantes platycephalus*, *Plethodon albagula*, *Plethodon cinereus*, *Taricha granulosa* (Schmidt et Garroway 2021).

²raw synthesized microsatellite data - were obtained by synthesizing and repurposing archived data, collected for different questions, from online repositories (Schmidt et Garroway 2021).

12. Conclusion

In this thesis I investigated the consequences of city expansion on the genetics of species inhabiting urban and near-urban environments. My primary purpose was to discover the effects imposed by urbanization by reviewing previous studies on the subject matter. I reviewed the genetic markers used in the studies and studied the species in terms of adaptation abilities.

Some of the main inferences will be presented in the following paragraphs.

- Due to constant human population growth, larger numbers of people are aggregated in dense cities. Expanding cities have been leading to habitat loss, fragmentation and deforestation, leading to the decrease of amounts and sizes of habitats.
- Habitat reduction, in most cases (75%), caused a decrease in the genetic diversity and formation of genetic structure in most studied species. Caused modifications in the genetic material of the species lead to their local decline and, potentially, endangerment and local extinction. The majority of species' genetic diversity and structure, regardless of their class, were affected by urbanization negatively: 53% and 71% respectively.
- However, species with no effect on their genetic material or even a positive one were also observed. Specific species were able to not only withstand urbanization, but moreover thrive in urban conditions, i.e., representing one of the three models of urbanization effects (facilitation, neutral and fragmentation models). No effect of urbanization on genetic diversity and structure was observed in 41% and 23% of the studies, respectively. Only in 6% and 6% studied species the results show that their genetic diversity and structure (resp.) appear to have been affected by urbanization positively.
- Most significantly, particular species' reactions to urbanization were dependent on a number of factors. The main reason why some species thrived and others were not affected by urbanization (neutral model) lies primarily in the complexity of species' ecological requirements. Moreover, factors such as species' adaptation abilities, semi-aquatic lifestyle, dispersal capacities and preferences, infection dynamics, number of electric power poles and other man-made structures in and between the habitats, amount of noise and light pollution, all make contributions to genetic modifications to various extents.

- In the majority of cases, samples were collected either by capturing animals or obtaining opportunistic tissues from carcasses discovered in the study areas.
- The most studied taxonomic groups included: birds, mammals, reptiles, insects and amphibians. Of which, the most studied species (almost half) were mammals. They were, most likely, chosen due to their rich variety and capturability.
- According to the reviewed studies, birds were discovered to be more tolerant than other classes. That is presumably due to their dispersal abilities. Mammals, insects, reptiles, and, most of all, amphibians, on the other hand, were more sensitive to urban features.
- Only 7,5% of reviewed studies tested populations for the development of adaptive genes. In 80% of cases studied organisms, despite belonging to different classes (mammals and insects), have developed adaptive SNPs.

My thesis draws attention to the subject of increasing importance. The global trend appears to be a slow but steady genetic diversity decline, demonstrating the importance of studying urbanization effects on species. The gained data could serve as a valuable tool for conservation genetics, to help detect genetic deviations and protect and save the threatened species in time.

Obtained knowledge provides a better understanding of the responses of particular species and taxonomic groups, demonstrating a comparison of a wide variety of species in a table.

This work also provides a comparative overview of species according to their dispersal abilities: volant versus non-volant species.

Moreover, an overview of specific features to have a negative effect is presented in terms of differentiating natural factors (prehistoric climate events, natural barriers (e.g., rivers), invasive species, epidemics (e.g., mange outbreak)) from anthropogenic ones (heterogeneity of urban landscapes, human presence, busy highways and urbanized areas, utility structures).

Furthermore, this work provides insight into the fact that even species from the same class respond differently to the same changes (reptiles, French et al. (2018)). The extent to which urban residents were affected also depended on, among other factors, species' longevity, presence of sex-biased dispersal and philopatry.

This thesis also provides a valuable recapitulation of suggested adjustments future studies could be inspired by.

In my opinion, several steps could be taken to improve research and protect endangered species. For instance, longer observation periods, a wider variety of species, and more detailed studies concerning species biology could make the results more precise. In regards to the protection, I believe that species would, besides other things, benefit from integrating habitats into cities and increasing vegetation cover throughout cities.

Most of the reviewed research indicate species' reduced diversity and gene flow, and increased genetic structure. However, future research is needed to confirm if more taxonomic groups are also affected negatively.

It can be concluded that constant biodiversity decline driven by urbanization, climate change, and other anthropogenic factors is underappreciated by many fields of science. Still, more cases are likely to be documented in the near future.

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