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Patrons de distribution, dispersion par l'Homme et variations intraspécifiques au sein des paysages urbanisés :

Réponses des fourmis à l'urbanisation

Devant le jury composé de :

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A ma mère

RÉSUMÉ

L'urbanisation est un processus complexe tant par les mécanismes historiques, sociaux, économiques et environnementaux qu'il implique que par les multiples modifications qu'il provoque au sein des écosystèmes. Ainsi, les paysages urbanisés sont soumis à des changements spatialement et temporellement abrupts des conditions environnementales comme la température au sol et dans l'air, la fragmentation des habitats et les concentrations de divers polluants. Pourtant, malgré leur apparente inhospitalité pour la biodiversité, les milieux urbanisés sont de véritables écosystèmes où de nombreuses espèces, natives et invasives, sont capables de survivre et même de prospérer. L' « écosystème urbain » est désormais considéré comme un élément important du fonctionnement des environnements densément peuplés, de la santé et du bien être humain ainsi que de la conservation de la biodiversité. De manière plus fondamentale, l'urbanisation, au même titre que le changement climatique et les processus d'invasions biologiques, est une « expérience grandeur nature » nous offrant l'opportunité de comprendre les règles d'assemblage des communautés biologiques, les processus de sélection d'habitat et de dispersion ou encore les processus évolutifs d'adaptation et de diversification. Ce travail s'inscrit dans ce cadre de pensée en se proposant d'étudier les conséquences écologiques et évolutives de l'urbanisation à différents niveaux d'organisations biologiques chez les Formicidae.

Cette thèse comprend 4 chapitres s'organisant autour des thèmes et questions suivants :

Chapitre 1 : **Urbanisation et communautés biologiques :** Comment l'urbanisation affecte-t-elle la distribution des espèces à l'échelle du paysage ?

Chapitre 2 : **Urbanisation et dispersion par l'être humain :** Conceptualisation et modélisation du rôle des activités humaines et des réseaux de transports dans la dispersion d'espèces.

Chapitre 3 : **Urbanisation et interactions biotiques :** L'urbanisation affecte-t-elle les patrons de co-invasions d'un couple invasif hôte-parasite introduits indépendamment ?

Chapitre 4 : **Urbanisation et variations intraspécifiques :** Les populations urbaines et rurales présentent-elles des différences de traits biologiques ?

Nos résultats démontrent qu'en tant que changement multifactoriel, l'urbanisation affecte de manière complexe la composition des communautés biologiques en modifiant de manière idiosyncrasique la distribution des espèces natives et invasives. En effet, chacune des 7 espèces de fourmis étudiées était affecté par une combinaison unique de facteur environnementaux associés à l'urbanisation (p.ex. fragmentation des milieux ouverts, température de surface) mais également au climat (**Chapitre 1**, Gippet et al. 2016 Urban Ecosystems).

Parce qu'ils concentrent des activités humaines, les paysages urbanisés sont le théâtre de multiples invasions biologiques. En effet, l'être humain déplace de nombreuses espèces, leur permettant de franchir des barrières biogéographiques mais aussi de se propager au sein des paysages. Afin de provoquer la discussion autour du concept de *la dispersion des espèces par l'Homme,* nous proposons un nouveau point de vue général centré sur les activités humaines (**Chapitre 2, Partie 1**, Gippet et al. *soumis*) ainsi qu'un modèle de propagation secondaire d'espèce invasive par les activités humaines : MoRIS, dont la principale innovation est d'utiliser la structure du réseau de transport pour influencer la direction des événements de dispersion (**Chapitre 2, Partie 2**, Gippet et al. *In prep*).

En exacerbant le nombre d'introductions et la propagation secondaire des espèces introduites, l'urbanisation est susceptible de mettre en contact des espèces n'ayant jamais co-évolué. Nous montrons ainsi comment l'urbanisation affecte la distribution, à différentes échelles spatiales, d'un couple hôte-parasite (fourmis-champignon : *Lasius neglectus* et *Laboulbenia formicarum*), dont les partenaires sont originaires de deux continents différents. Nos résultats montrent que 58% des colonies de *L. neglectus* sont infectées par *L. formicarum*, que la prévalence du parasite augmente avec le niveau d'urbanisation et suggèrent que le parasite est susceptible de limiter la croissance des colonies et ce d'autant plus dans les milieux urbanisés (**Chapitre 3**, Gippet et al. soumis à *Biological Invasions*).

Enfin, chez l'espèce native *Lasius niger*, présente en population continue sur tout le gradient d'urbanisation, nous mettons en évidence des différences morphologiques, physiologiques et comportementales entre « populations » urbaines et rurales. En effet, alors que les jeunes reines issues de centre-ville sont de plus petite taille que les jeunes reines rurales, leurs ouvrières, nées en *common garden*, ne présentent pas de différence de taille moyenne mais les urbaines sont de taille beaucoup plus variable (à la fois entre colonies et à l'intérieur des colonies) que les ouvrières rurales. Au niveau physiologique, des analyses d'expression de gènes codant pour des protéines de choc thermique (Hsp60, 75 et 90) chez des colonies élevées en *common garden* et soumises ou non à un stress thermique (45°C, 1h) ont révélé des différences d'expression entre populations urbaines et rurales chez les reines mais pas chez les ouvrières. Enfin, en génotypant le sperme contenu dans la spermathèque des jeunes reines, nous montrons que les reines issues de milieu urbain s'accouplent plus souvent avec 2 mâles que les reines rurales. Ces changements phénotypiques ont lieu alors qu'aucune différenciation génétique entre populations urbaines et rurales n'est détectée. Ces résultats novateurs encouragent de futures recherches sur la mise en place d'adaptations locales aux milieux urbanisés, ainsi que sur les dynamiques éco-évolutives agissant en milieux urbains (**Chapitre 4**, Gippet et al. *In prep*).

Mots clés : Urbanisation, sélection d'habitat, dispersion, invasions biologiques, interactions biotiques, variations intraspécifiques, *Lasius neglectus*, *Lasius niger*

Articles acceptés, soumis et en préparation :

Gippet JMW, Mondy N, Diallo-Dudek J, Bellec A, Dumet A, Mistler L and Kaufmann B (2016) I'm not like everybody else: urbanization factors shaping spatial distribution of native and invasive ants are species-specific. Urban Ecosystems *In press*

Gippet JMW, Rocabert C, Mondy N and Kaufmann B. Why ecologists should focus on the "human" in "Human-mediated dispersal". *Submitted to Diversity and Distributions*

Gippet JMW, Rocabert C, Fenet S and Kaufmann B. MoRIS: Model of Routes of Invasive Spread. Human-mediated dispersal, road network and invasion parameters. *In prep*

Gippet JMW, Grangier J, Colin T, Winkler F, Haond M, Bellec A, Dumet A, Mondy N and Kaufmann B. Come together: host-parasite co-invasion is affected by urbanization. *Submitted to Biological Invasions*

SUMMARY

Urbanization is a complex process involving simultaneous changes in several environmental conditions, including ground and air temperature (urban heat island effect), habitat fragmentation and chemical pollution. These changes are often associated with biodiversity loss and changes in ecosystems functioning. However, more than a sink for biodiversity, urban areas constitute true ecosystems where many native and alien species survive and even grow stable populations. The "urban ecosystem" is now considered as an important element in the functioning of densely populated environments, in human health and well-being, as well as in biodiversity conservation. More fundamentally, urbanization constitutes "real life experiment" offering ecologists an opportunity to better understand ecological and evolutionary processes over fine spatial and temporal scale. This thesis investigates ecological and evolutionary consequences urbanization at different biological organization levels using ants (Formicidae) as biological models. First, at the biological community level, we showed that native and alien ant responses to urbanization were species-specific as each species was affected by its own set of environmental changes associated with urbanization (e.g., fragmentation, ground temperature) and climate. Second, we explored the little studied process of human-mediated dispersal by (i) proposing a novel theoretical framework focusing on human activities rather than directly on species and (ii) developing an innovative simulation model of dispersal by transport in terrestrial landscapes, MoRIS (*Model of Routes of Invasive Spread*). Third, we investigated how urbanization affected novel biotic interactions between an alien ant species (*Lasius neglectus*), an alien ectoparasite fungus (*Laboulbenia formicarum*) and native ant species. We showed that urbanization impacted the size of *L. neglectus* colonies, which were smaller in urban area, as well as *L. formicarum* prevalence on *L. neglectus,* which was higher in urban areas. Finally, we investigated intraspecific variations between urban and rural conspecifics of an urban tolerant species: *Lasius niger*. We found that urban young queens were smaller and lighter, less stressed by high temperature and performed multiple mating more often than rural young queens. In common garden, colony founding success were globally similar but urban incipient colonies produced significantly less pupae (and consequently workers). We also found that workers produced in common garden experiments were more variable in size (higher head width variance in both inter- and intra-colony) in urban colonies than in rural colonies. No evidence for genetic differentiation between urban and rural populations was found, suggesting that gene flow was not disrupted between urban and rural populations. All our results converge to a renewed vision of ecological and potential evolutionary dynamics occurring in urban environments. Further investigation will be necessary to assess how ecological processes influence evolutionary trajectories in urban ecosystems, using both ecological (e.g. ant densities and abundances along the urban gradient) and genomic approaches (e.g. using "genotyping by sequencing" methods to identify genes responsible for adaptation to urbanization).

Keywords: Urbanization, environmental filters, human-mediated dispersal, biological invasions, biotic interactions, intraspecific variations, *Lasius neglectus*, *Lasius niger*

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TABLE OF CONTENT

INTRODUCTION

The impacts of *Homo sapiens* on ecological systems are a major topic of research in ecology. While basic science can use changes induced by the activities of Humans to develop and test major theories on ecological and evolutionary processes, applied science focuses on understanding, predicting and often trying to mitigate the effects of human societies on ecosystems.

For nearly a century, science has accumulated significant evidence for the negative impacts of human activities on ecosystems, with concerns for the future of human populations worldwide. However, most studies investigating the responses of biodiversity to future environmental changes have focused on climate change (85% in the past 25 years, Titeux et al. 2016b) and recent studies suggested that it might not be the sole and most threatening change for biodiversity (see Maxwell et al. 2016, Titeux et al. 2016b). As the accuracy of the predictions for biodiversity dynamics depends on the completeness of the models used, there is a growing concern to take into account other major anthropogenic modifications such as land use changes (Foley et al. 2005, Newbolt et al. 2015) biological invasions, pollution and urbanization (Maxwell et al. 2016, Grimm et al. 2008a,b).

This thesis aims to contribute to this effort by investigating the consequences of urbanization on biodiversity at different levels of organization.

Because urbanization is a complex phenomenon, I dedicated an entire part of this introduction (part II) to the description of all major environmental modifications occurring in and around urban areas, before discussing the ecological and evolutionary consequences of urbanization, as well as the issues and challenges of its study.

I) A brief history of urbanization

Urbanization, *the process by which towns and cities are formed and became larger as more and more people begin living and working in central area* (Adams 2015 p87), is a complex phenomenon which arose multiple independent times in human history, from multiple causes. A common explanation of urbanization is that sedentariness (12000 BC), followed by the emergence of farming communities (Neolithic Age and the use of domesticated crops and animals, 10000 BC) and later inventions (such as ox-drawn plow and wheeled cart, metallurgy and irrigation, 6000-4000 BC,), facilitated the establishment of more productive agricultural systems, making possible the concentration in one place of people having nonagricultural activities (Davis 1955). While such explanation could be consistent for many European cities, it does not always correspond to the emergence of urbanization in other part of the world, especially Near-East, China, India, South and Central America, Africa, where other potential causes could be more realistic (e.g. religion, protection against environmental risks or invaders). To date, no globally accepted explanations for urbanization exist (http://www.ancient.eu). Uruk, settled around 4500 BC in ancient Mesopotamia (Irak), is today considered the oldest city in the world. Urbanization appeared all over the world and large populous cities (> 100,000 population) arose on all continents (http://metrocosm.com/history-of-cities). In 1700, less than ten percent of the world's population inhabited urban areas (Gaston 2010 p13) but in the following century (1760-1830), the industrial revolution provoked a radical transformation of human society functioning and started an urban revolution. Since this period, urban populations have sharply risen worldwide, leading to the rapid increase of the number of mega- cities of more than one million inhabitants (16 in 1900, 400 in 2000 and 538 in 2015 (citypopulation.de)). In 2016, 34 cities had more than 10 million of inhabitants, with Tokyo, Japan, being the most populated metropolitan area in the world (38 million of people). In 2008, 50 percent of the human population were urban dwellers and this number is predicted to rise in the next 50 years (Grimm et al. 2008a). Today, most industrialized nations approach 80% of urban population (Gaston 2010 p13).

II) Urbanization: a multifactorial change process

The process of urbanization is particularly complex because it involves a broad variety of environmental modifications. In this chapter, I list and describe the major environmental changes occurring in urbanized landscapes; their consequences on biodiversity will be exposed and discussed in the next chapter.

A) Land cover changes and habitat fragmentation

Urbanization is intrinsically linked to land cover changes. As described above, cities were originally tied to adjacent areas providing agricultural or other resources to urban dwellers. This is not always true in post-industrial societies because the development of transportation networks and technologies allow cities to be spatially disconnected from resources producing areas, e.g. farms, fields, mines, forests or quarries. All urban areas are characterized by high proportions of impervious surfaces constituted by buildings, pavements, roads and other build-up surfaces. Urban green spaces are generally dominated by open vegetation patches (typically lawns) to the detriment of forested areas; they are also characterized by hedges constituted by shrubs or trees of several native and introduced species (Ignatieva and Stewart 2009). Urbanization is also associated with the canalization of watercourses, drainage of wetlands and the global alteration of stormwater flow because of the impervious nature of built-up surfaces (Forman 2014). Urban land cover is consequently a complex mixture of "grey", "green", "yellow" (cultivated fields), "brown" (abandoned industrial sites) and "blue" spaces and the proportion of each land cover type often depends on land use characteristics (Arnold and Gibbons 1996). For example, impervious surfaces are generally strongly dominant in urban cores while residential suburbs have higher proportions of "green" spaces such as lawns, isolated trees and forests fragments (Fig. 1). It also is crucial to distinguish cultivated fields, the "yellow" spaces, from agricultural "green" spaces such as meadows and pastures because such areas are monospecific, regularly plowed and harvested.

The most prominent consequences of these land covers changes are the loss and fragmentation of natural habitats. Forests and wetlands tend to disappear in urbanized landscapes and conserved patches are generally small and spatially distant from each over (Fig. 1, Zedler and Leach 1998, Faulkner 2004, Wang et al. 2008). On the contrary, open vegetated areas, such as lawns, are common in urban and suburban areas and constitute the most of urban "green" spaces; their size and fragmentation is variable depending on the land use context (Fig. 1), among cities and within cities (James and Bound 2009, Smith et al. 2015).

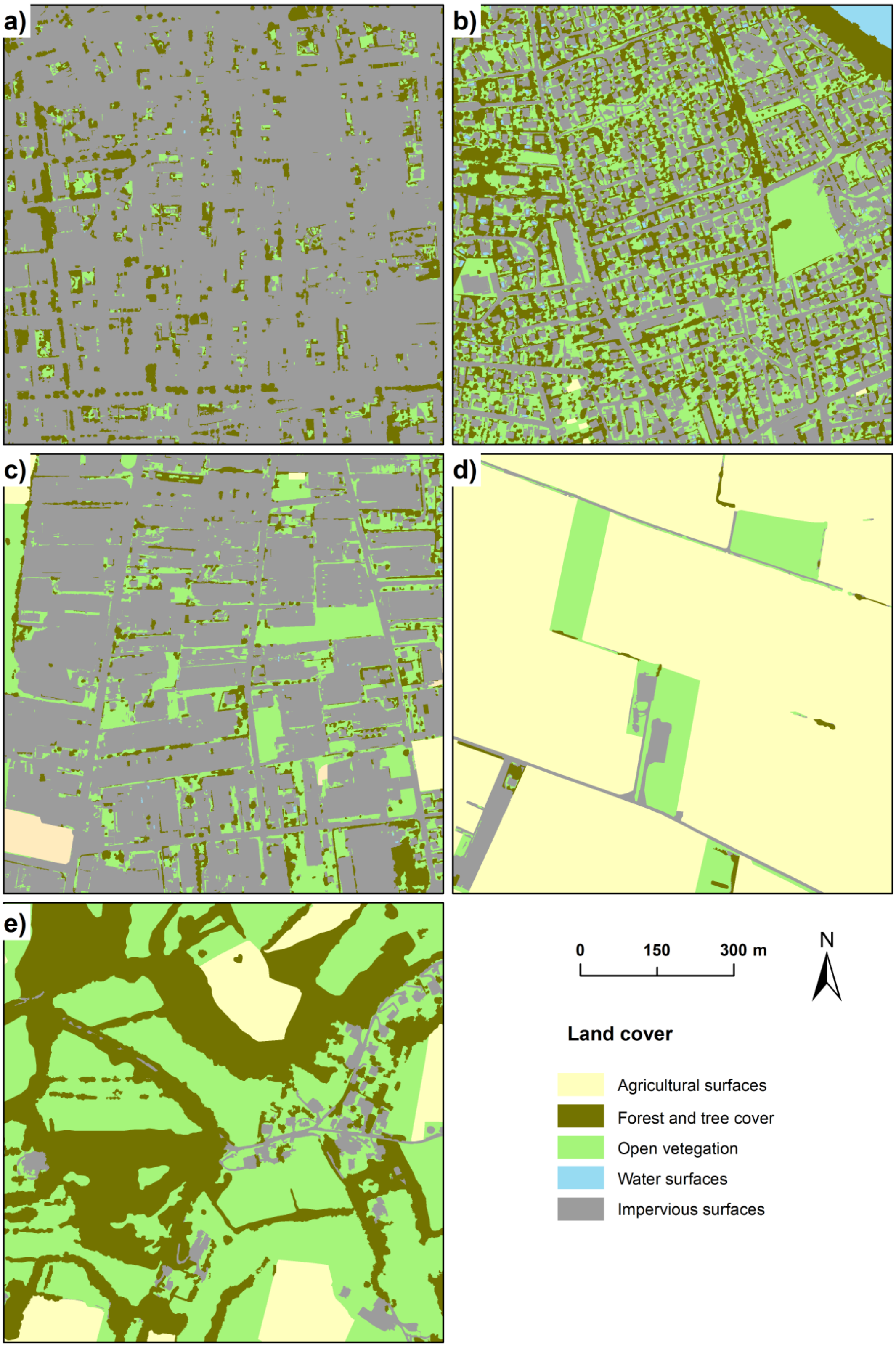

Figure 1: Land cover of five locations along the urban-rural gradient of Lyon metropolis, France. a) Urban core; b) Residential area; c) Industrial area; d) Agricultural area; e) Rural area. Zooms are identical, allowing direct visual comparison of "green" spaces spatial structure. Data from *data.grandlyon.com.*

B) Human activities and movements

Cities host major terrestrial, fluvial, maritime and aerial exchanges clusters such as national and international seaports and airports. The globalization of human activities, combined to the development of transportation networks and technologies, have greatly amplified the movements of humans and merchandises at global, regional and local spatial scales (Hulme 2009).

By concentrating people, urbanized landscapes increase the intensity of human activities such as recreational activities and the transportation of people, food or materials (Gaston, 2010). Transportation networks such as roads and waterways have important consequences on urbanized landscapes structure as urbanization and urban sprawl tends to follow major transport infrastructure. Around city cores, human activities are often spatially structured along major transportation axes, resulting in local hotspots of economic activities enhancing urban spread and city expansion (Fig. 2, Forman 2014 p76-78), however, this is not the only model of city expansion (see Forman 2014 p78). Transportation networks fragment the green component of landscapes as roads and human impacted watercourses replace and incise it (Fig. 1, Forman 2014 p279-286, see Introduction part III-B). Goods and materials transportation results in species introduction outside of their native range as well as native and exotic species spread at local, regional and continental scale (Crespo et al. 2011, Johnson et al. 2001, Introduction part III-B).

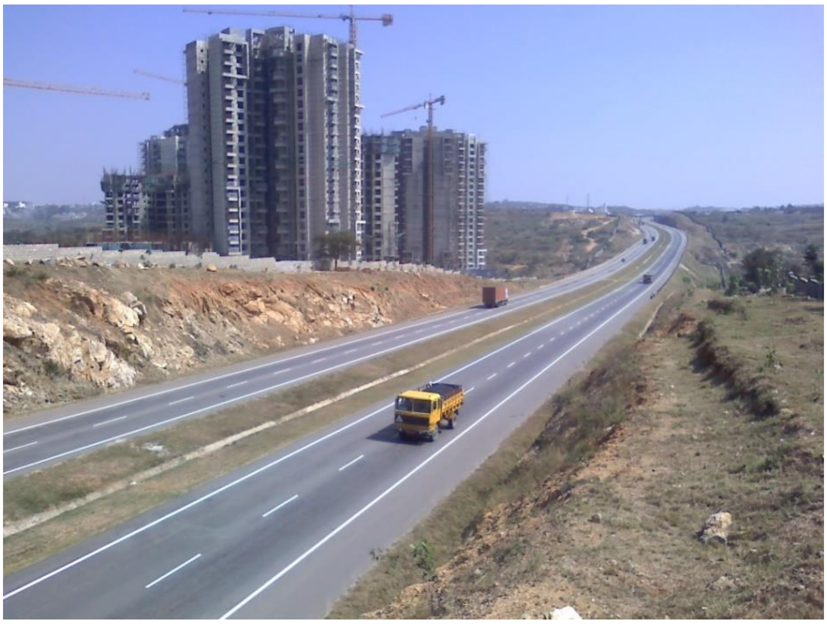

Figure 2: Recently constructed motorway in the state of Karnataka, India. A truck transporting construction material and buildings in construction in the background are visible. From Wikipedia.

C) Urban Heat island effect

Cities were shown to be warmer than adjacent rural areas: this consequence of land cover changes is a well-known phenomenon termed Urban Heat Island (UHI) effect (Forman 2014 p133-143). Urbanization modifies the physical characteristics of surfaces and atmospheric conditions, inducing warmer surface and air temperature in urban areas, particularly at night-time (Fig. 3, Dousset et al. 2011). Several factors are responsible for the intensity of urban heat islands: impervious surfaces have low albedo and high solar radiation storage capacity, they concentrate heat during the day and release it at night, significantly increasing nighttime air temperature (from 2°C to 10°C higher in urban areas than neighboring ones, Gaston 2010 p36) in cities cores (i.e. having the highest proportion of impervious cover). As a consequence, nocturnal UHI effects are generally more pronounced than daytime effects (Fig. 3, Forman 2014 p135).

Additionally, there are at least three types of UHI: air UHI, surface UHI (Fig. 3) and sub-surface UHI (Oke 2006a, Heisler and Brazel 2010). These three UHI types are likely to behave differently in response to land cover composition and structure variations along urban gradients, or simply between night and day (Fig. 3).

Soil moisture and vegetation through evapo-transpiration, play a crucial role in heat dissipation. Therefore, non-urban areas, which generally comprise a high proportion of vegetation cover, cool more efficiently than urban areas dominated by impervious surfaces. Vegetation, especially trees, also prevents solar radiation from reaching impervious surfaces. Within cities, “green” spaces are therefore key features of heat dissipation and even small patches of vegetated cover can significantly reduce local heat island (Fig. 4, Gaston 2010 p39).

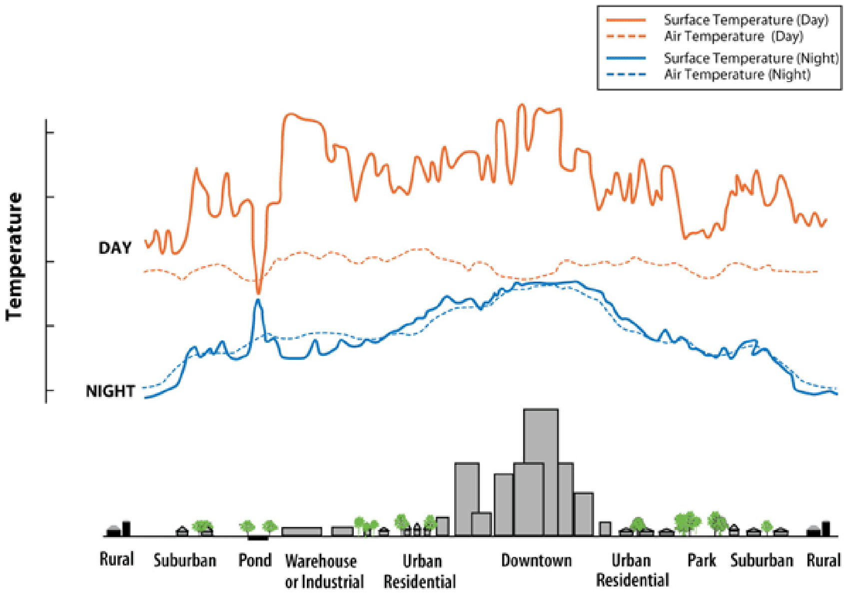

Figure 3: Air and surface temperature during the day and night along an urban gradient. From EPA (https://www.epa.gov), modified from Vogt 2000).

Stulpnagel et al. (1990) compared the air temperatures of 42 Berlin greenspaces of various sizes and the air temperature of their built-up surroundings. They found a strong positive correlation between size of the greenspace and difference of air temperature with its built-up surroundings (Fig. 4). It appears clearly that the larger the park, the more efficient the cooling.

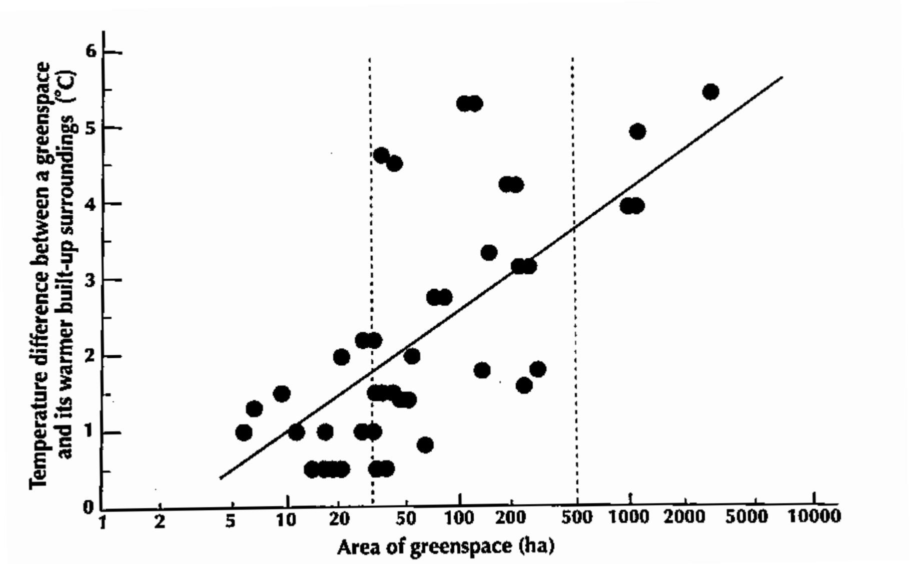

Figure 4: Relationship between air cooling and size of the urban greenspace in Berlin. From von Stulpnagel et al. (1990) in Forman 2014 (p137).

D) Pollution

Because of intensive human activities, urban areas are submitted to three principal classes of environmental stressors: noise, light and chemical pollution.

Noise - Roads, railways and airport are major sources of noise. While trains and aircraft induced noises are loud, brief and inconstant, road associated noises are extremely frequent and correlated to the amount of traffic. Road traffic is consequently the major source of acoustic pollution within cities. Given the intensity of human activities inside and around cities, urban areas are noisier than rural areas; with an acoustic pollution dominated by low frequency noises below 2000 Hz (Slabbekoorn and Peet 2003) (Fig. 5).

Figure 5: London, UK (Bayswater, Paddington) acoustic pollution map at day and night. From http://services.defra.gov.uk/wps/portal/noise

Light - The invention of electric lighting and its rapid proliferation have sharply increased Earth's surface artificial light emissions over the last century. Because electric lighting correlate with human populations and activities, cities are hotspots of nighttime light pollution (Fig. 6).

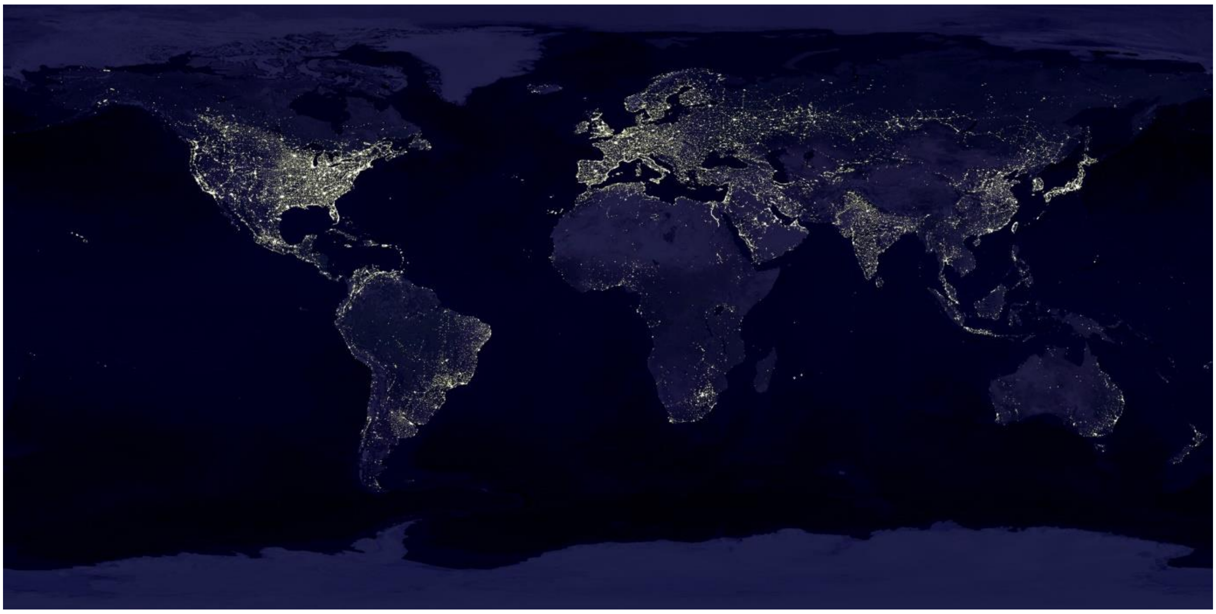

Figure 6: Satellite image of Earth at night. From NASA (http://visibleearth.nasa.gov).

Night light pollution has a large range of sources: lighted buildings, streetlights, security lights, terrestrial, maritime and aerial vehicles and flares on oil platforms (Longcore and Rich

2004); it can be used as an indicator of human population and activities density. Night light pollution patterns are likely to vary among countries because of economic and political features. For example, Belgium produces much more night light pollution per square kilometer than France because Belgian highways are lit and French ones not (Fig. 6, Bennie et al. 2014).

Chemical - Because of the long history of human activities (especially industrial activities and vehicles' fuel combustion) on spatially reduced surfaces, urban areas often experience large amounts of chemical pollution by accumulation of a broad diversity of pollutants. Pollutants are found in the atmosphere, soils, water and living organisms, including human beings (Forman 2014 p144).

Urban air pollutants comprise suspended particular matter of variable size and gases such as SO_x, NO_x and CO). Air pollutants mostly originate from the combustion processes associated with motorized vehicles, household heating or industrial activities.

Urban areas are generally associated with a decline of streams, rivers and ground water quality (Gaston 2010 p38). Water pollutants arise from several origins, such as stormwater run-off, wastewater, fertilizers and sewage discharges. Urbanization therefore often results in an increase of sediments, salinity, sodium, chloride, nutrients, heavy metals and organic compounds in the water (Steele et al. 2010).

Urban soils show the same tendency than air and water and, through atmospheric deposition, stormwater run-off, industrial activities and solid wastes burying, they accumulate high concentrations of diverse pollutants (Forman 2014 p113-119, Fig. 7). Typical pollutants of urban soils are polycyclic aromatic hydrocarbons (PAHs, Tang et al. 2005), radionuclides and heavy metals. Heavy metals, because of their high toxicity for organisms and their ability to remain and accumulate in soils for many years (Imperato et al. 2003, Wei and Yang 2010), have been particularly studied in urban contexts. Heavy metals concentrations have been found to increase between 1974 and 1999 in the urban area of Naples (from 17% to 878% depending on the metal and the site), presumably as a consequence of continuous inputs via vehicles emissions and tire and brake abrasion (Fig. 7, Imperato et al. 2003).

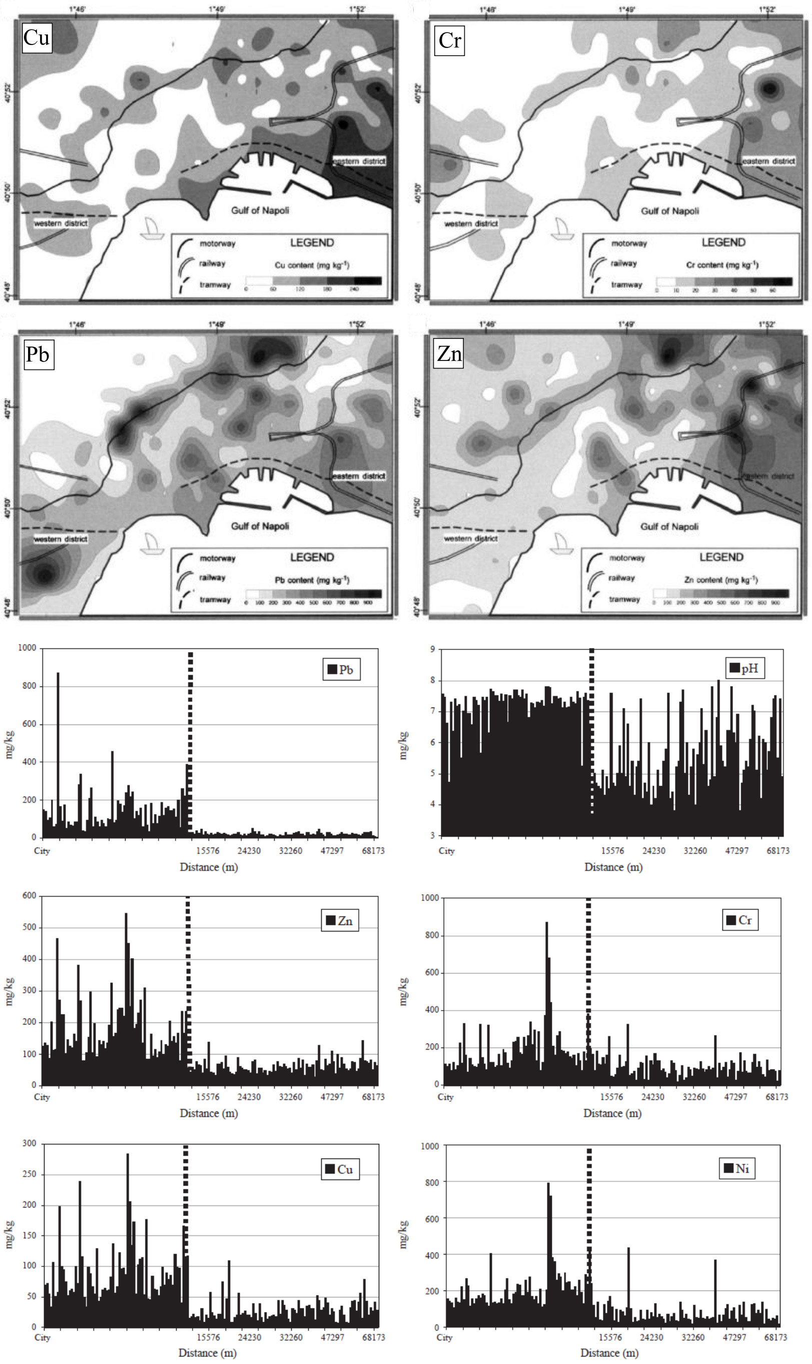

Figure 7: (above) Krigged maps of four heavy metals (Cu: Copper, Cr: Chromium, Pb: Lead and Zn: Zinc) content in surface soils of Naples, Italy, urban areas (modified from Imperato et al. 2003). (below) Histograms of five heavy metals (same four plus Ni: Nickel) content and the pH in surface soils inside and outside the city of Torino, Italy. The dashed line shows city administrative border (from Biasioli et al. 2006).

E) Urban soil properties

Higher temperatures and concentrations of chemical pollutants are not the only characteristic of urban soils, as the urbanization process has strong consequences on the physical properties of soils (Scharenbroch et al. 2005). Urban soils are subject to higher compaction through diverse human activities such as repeated walking or vehicles’ passing (Forman 2014 p92), reducing soils porosity and altering their structure, which result in the modification of soil drainage and aeration properties. This has significant consequences on stormwater infiltration by limiting water infiltration into urban soils and increasing run-off (Yang and Zhang 2010). Urban soil moisture is highly variable because of the multiplicity of factors likely to affect it, including soil type (e.g. sandy, clayey, organic), particles size, compaction or management type (e.g. irrigation, planted vegetation, gardening practices) (Volder p123, Forman 2014 p95). Urban soils also tend to be more alkaline than non-urban ones: elevated levels of calcium reaching urban soils (by water leaching calcium-rich surfaces such as concrete and building) raise urban soil pH (Fig. 7, Forman 2014 p94). Finally, urban soils demonstrate higher spatial heterogeneity, resulting in a finer mosaic, than non-urban soils. Soil patch types are very fragmented and bounded under the geometric rules of urban construction (Forman 2014 p92).

F) Nutrient flows

Urban nutrient fluxes of nitrogen (e.g. ammonium, nitrate), phosphorus and carbon have been widely studied in surface waters (Volder p297). Agricultural areas are known to increase N deposition by the use of fertilizers for crops production (Tilman et al. 2009). The produced food is partly consumed by urban dwellers, indirectly concentrating nitrous fertilizers at wastewater treatment facilities and increasing nitrogen loads in urban watersheds. Interestingly, pet food is likely to represent an important part of total N inputs because pet excrements are more likely to be deposited onto green spaces and thus to not enter wastewater stream with human wastes (Volder p301). Globally, food imported in urban areas was estimated to contribute between 13 and 90% of imported N in urban watersheds (Bernhardt et al. 2008). Other sources of nitrogen inputs in urban ecosystems exist including fertilizers used in urban lawns (Law et al. 2004) and fuel combustion (Bernhardt et al. 2008).

Surface waters from urban areas often exhibit higher phosphorus concentrations than surface water from rural areas (Volder p306). P sources in urban areas include wastewater, fertilizers and impervious surfaces leached by water run-off.

Despite its important role in biogeochemical cycles, organic carbon amounts, sources and fluxes in urban areas are poorly understood compared to more natural ecosystems (Volder p311). Both vegetation patches (essentially lawns and forest) and human activities are known to participate to natural organic carbon production in urban catchments. Moreover, human activities are equally responsible for novel organic carbon release in urban ecosystems. Novel organic carbons include hydrocarbons (e.g. fuel, oils), pesticides (e.g. insecticides, herbicides) and pharmaceuticals and personal care products (e.g. painkillers, antibiotics, narcotics) (Volder p316), also participating in previously discussed chemical pollution.

Despite the importance of biochemical fluxes for biodiversity, ecosystems functioning and urban population health, biochemical fluxes of global urban ecosystems are yet poorly known and models used to study them were first designed for natural ecosystems and need to be adjusted to urban areas (Forman 2014 p69).

III) Ecological and evolutionary consequences of urbanization on biodiversity

A) Biological Communities: Patterns of species richness and abundance

The relationship between urbanization and species diversity has been investigated at various spatial scales. At broad scales (national to continental), urbanization is usually described using human population density (HPD). A majority of studies using this indicator report a positive correlation between HPD and species richness, for a large range of taxonomic groups (Gaston 2010 p89). Given the negative impact of human activities on species persistence, these results are surprising. Meta-analyses showed that grain size (i.e. the spatial resolution or "pixel size") had a significant effect on the slope of the relationship. Indeed, while for large grain size, strong positive relationship between HPD and species richness is generally observed, for small grain sizes, the correlation can be inexistent or negative (Luck 2007a). This illustrate the crucial role played by the way urbanization is described and suggest that even broad scale analyses cannot escape a fine environmental description (small grain size) when studying urbanization, because environmental changes associated with it happen at local spatial scale and can lose their interpretability when described with too coarse variables. Positive relationship between HPD and species richness can however be explained by the fact that environmental conditions increasing species richness, such as rainfall and productivity, are also likely to increase human density (Gaston 2010 p91-95, Kuhn et al. 2004).

At a local scale, urbanization is usually described with multiple small grain sized environmental variables (e.g. impervious surface cover, road density, vegetated patches fragmentation). Usually, species richness is lower in urban compared to rural areas, but gradient sampling often showed that species richness was higher at intermediate level of urban development and was lower in urban cores and in rural and natural areas (Gaston 2010 p97), which fit the intermediate disturbance hypothesis (Connell 1978, Fig. 8).

McKinney (2008) reviewed 105 studies describing the effect of urbanization on species richness for non-avian vertebrates, invertebrates and plants. He found that most studies detected a peak of species richness at intermediate urbanization for plants and a constant decrease of vertebrates and invertebrates species richness from low to high levels of urbanization. Exceptions to these patterns exist, suggesting that responses of biological communities to urbanization are complex and likely to be affect by several factors, including climatic and societal context and the taxonomic group in focus. Finally, within dense urban areas, vegetation cover appears to promote faunal species richness (Gaston 2010 p100).

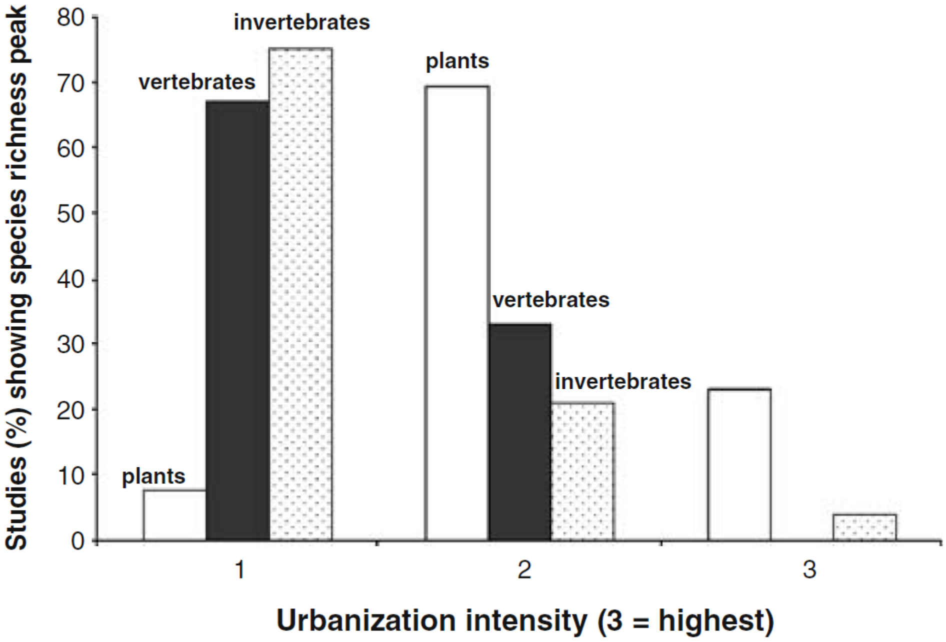

Figure 8: Relationship between urbanization level and species richness peak reviewed in 105 studies for three taxonomic groups. While vertebrates and invertebrates species richness strongly decrease with urbanization intensity, plants species richness is higher at intermediate intensity. From McKinney (2008).

Species abundance has also been shown to vary along the urbanization gradient. If the response of global abundance (the abundance of all species) to urbanization remains unclear (Saari et al. 2016), three distinct species response types have been identified. Urban avoiders, whose abundance is lowest in urban areas; urban exploiters, whose abundance peaks in intermediate areas; and urban adapters, whose abundance peaks in the most heavily urbanized areas (Blair 1996). McIntyre (2000) proposed a fourth response type: urban tolerant species, whose abundance does not vary along the urban gradient. Thereafter, for clarity purpose, I will consider urban adapters and exploiters as the same group: urban exploiters. We will thus consider only three categories: urban avoider, urban tolerant and urban exploiter.

Individual species responses to urbanization are the result of complex interactions between species biological traits (see Introduction part II) and multiple environmental changes caused by urbanization (Poff 1997, Verberk et al. 2013). Environmental conditions of urban areas are likely to filter species depending on their biological traits (Croci et al. 2008, Brown et al. 2009). Very few studies have yet investigated species-environment relationship based on biological traits in an urbanization context. Dispersal traits, such as the ability to fly have been found to be favored in urban carabid beetles assemblages (Niemela and Kotze 2009) but not in plant assemblages (Vallet et al. 2010). Behavioral traits have also been identified: Moller (2010) documented the relationship between urbanization and interspecific variations in fear in birds by analyzing flight initiation distances (FIDs) in 48 bird species when approached by a human. The author showed that urban species had lower FIDs than rural ones, which may enhance their foraging efficiency in densely populated areas. A consistent result among three taxa (plants, birds and butterflies) was that species demonstrating higher abundances in urban areas tended to be the most generalist (Vallet et al. 2010, Lizée et al. 2011). Relationships between urbanization and biological traits are attracting increasing attention (Croci et al. 2008, Vallet et al. 2010, Lizée et al. 2011) and recent methodological advances now produce predictive assessments of species responses to urbanization based on biological traits (Verberk et al. 2013).

B) Biological invasions

Another common pattern of biological community response to urbanization is the increase of exotic species richness in urban areas (Gaston 2010 p96). Indeed, urban landscapes often experience the simultaneous spread of several invasive species (Bertelsmeier & Courchamp 2014). The first cause of multiple invasions in urbanized landscapes is the dispersive characteristics of human activities and their concentration inside and around cities (Murphy et al. 2013, see Introduction part II-B). The dispersal of native or invasive species by human activities is called human-mediated dispersal (HMD, Auffret et al. 2014). Cities often host major transportation hubs of national and international human activities such as commercial exchanges and tourism. Such hubs, typically seas, rivers and airports, are recurrent introduction points for exotic species (Hulme 2009). With the globalization of human activities in the last decades, the number of exotic species introductions has sharply raised worldwide (Fig. 9, Hulme 2009).

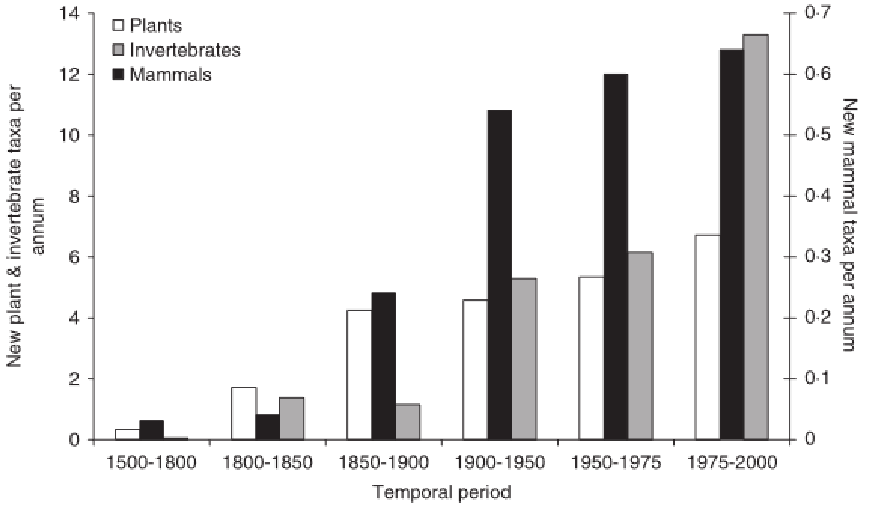

Figure 9: Annual rates of increase in the establishment of alien mammals, invertebrates and plants in Europe since 1500 AD. From Hulme 2009.

Once an exotic species is introduced, the intense human activities associated with urbanized landscapes are likely to enhance its secondary spread at regional and landscape scale. HMD involved in secondary spread of invasive species has been far less studied than introduction related HMD (Gippet et al. *submitted*). A more extensive discussion about HMD is available in Chapter 2.

Secondary spread HMD has been observed for both terrestrial and aquatic species. Crespo-Perez et al. (2011) modeled the HMD of potato moths (*Tecia solanivora*) in the Ecuadorian Andes using gravity models describing how the pest species had spread among villages stocks at landscape scale. Johnson et al. (2001) investigated the potential of recreational boating in the secondary spread of the invasive zebra mussels (*Dreissena polymorpha*) in North American Great Lakes. They showed that, despite a low probability of successful transport (0.12% maximum), 170 dispersal events to inland waters were likely to occur within one summer because of high levels of vector activity (96800 real transport events had the potential to transport zebra mussels).

While dispersive characteristics of urbanized landscape are key factors for starting, multiplying and intensifying biological invasions within and around cities, disturbances and stresses induced by urbanization increase habitat invasibility (i.e. the susceptibility of a community or habitat to the establishment and spread of new species), promoting invasive species establishment (Alpert et al. 2000). A recent study even shows that intermediate disturbance bolstered the abundance of invasive ants in California, USA (Vonshak et al. 2015). Alpert el al. (2000) suggested that habitats with a longer history of human disturbance (typically city cores) may have lower invasibility because their species have already been selected to perform well under disturbed conditions.

Another feature of invasion success is species invasiveness: the combination of biological traits enabling a species to invade a new habitat (Alpert et al. 2000). Because species introductions depend on the ability of a species to be transported and to survive during the transport, the first biological trait involved in invasiveness could be the propensity of being transported with human activities. Indeed, such trait is likely to filter species during the introduction process but also during the secondary spread process, especially when the species have low natural dispersal ability and depend on HMD to spread. Because urban areas are likely to promote species spreading via HMD, biological communities in urban areas are likely to concentrate native and invasive species (Pysek et al. 2009) with high propensity to HMD, which are therefore likely to cross their biogeographic barrier thanks to long distance human transportation activities (see Chapter 2).

A similar process, involving thermotolerance rather than HMD propensity, has recently been documented by Foucaud et al. (2013) who showed that thermotolerance adaptation to human-modified habitats occurred in the native range of the invasive ant *Wasmannia auropunctata* before long-distance dispersal. The authors demonstrated that thermal tolerance to heat stress occurred in urban populations in the native range (South America) of the ant and that, unlike native rural populations, invasive populations (in North America) demonstrated the same adaptation. This study illustrated the impact of Urban Heat Island effects on ecological and evolutionary processes (see Introduction part III-D). Thermophily is a recurrent trait found in invasive species in urban contexts (Gaston 2010 p123).

Because urban areas worldwide experience similar environmental changes, two distant city environments will tend to be more environmentally similar than to their adjacent rural areas. As a result, they should demonstrate similar environmental filters selecting species with similar biological traits and exchanging invasive species through transportation networks, leading to “biotic homogenization” (McKinney and Lockwood 1999, McKinney 2006).

C) Biotic interactions: intra- and interspecific interactions

By attracting and favoring invasive species, urbanization adds new interacting species into biological communities. Environmental factors associated with urbanization have also been shown to modify intra- and interspecific interaction including communication, reproduction, competition, predation and parasitism.

Acoustic pollution has been shown to reduce effectiveness of acoustic communication in many taxa including fish, frogs, birds and mammals (Gaston 2010 p59, Barton and Holmes 2007, Francis et al. 2009). While most studies investigating urbanization impacts on intra- and interspecific communication focused on acoustic pollution effects on bird vocalizations(Brumm 2006, Slabbekoorn and den Boer-Visser 2006), chemical and light pollution, and other factors (such as temperature or land cover), are likely to affect all sorts of information exchanges in a wide range of taxa. For example, Van Oudenhove et al. (2012) have shown that high surface temperature accelerated trail pheromone evaporation in the ant *Tapinoma nigerrimum*, altering recruitment intensity and thus, foraging efficiency. As a consequence, surface UHI is likely to affect ant intra colonial communication, decreasing foraging efficiency in urban compared to rural areas. Plant and birds phenology are often impacted by urbanization as UHI modified temperature and anthropogenic provisioning of food can result in early reproduction (Neil and Wu 2006, Gaston 2010 p66), which is likely to lead to reproductive isolation between urban and rural populations.

Urbanization has also been related to disruption of predator-prey interactions in bird assemblages (Friesen et al. 2013). Barton and Holmes (2007) showed that bird nesting success was higher and predation lower in loud (i.e. with high levels of acoustic pollution) nesting sites, which can be explained by the preference of the major nest predator (the western scrub jay, *Aphelocoma californica*) for quiet sites and possibly by the incapacity of the predator to use acoustic cues to find its prey in loud sites. At interspecific levels, urbanization increased the opportunity for encounters between two competitive wild felids (*Puma concolor* and *Lynx rufus*) by modifying the behavioral response of bobcats to puma depending on the landscape context (Lewis et al. 2015).

Urbanization impacts on host-parasite relationships and pathogens prevalence have been widely documented but general trends have yet to be established (Gaston 2010 p65). Urban blackbirds (*Turdus merula*) populations have lower abundance of *Ixodes* ticks and avian malaria than rural populations (Evans et al. 2009b) while house finches (*Haemorhous mexicanus*) populations demonstrated the opposite pattern for two viruses (*Isospora* sp and *Avipoxvirus*) along an urban-rural gradient (Giraudeau et al. 2014). Such opposite findings reveal the multiplicity of factors likely to drive host-parasite interactions along urban-rural gradients, including host densities (likely to affect pathogens transmission, Bradley and Altizer 2006), host and parasite response to urban pollutants (e.g. by immunosuppressive effects) and other abiotic conditions, species richness in intermediate hosts and species richness in parasites (often linked to invasive species introductions, Gaston 2010 p 64-65).

Mutualistic interactions (e.g. plant-pollinator or plant-seed disperser) are also impacted by urbanization. Geslin et al. (2013) showed that the number of flower visitors was lower in urban compared with suburban, agricultural and semi-natural areas. Among the most studiedtaxa, solitary bees and hover flies demonstrated the most important decrease in abundance in urban contexts while other taxa such as bumblebees were abundant in all contexts.

A recent study (Hase et al. 2013) even showed that human induced re-contact between two distinct toad subspecies (the native *Bufo japonicus formosus* and the exotic *Bufo japonicus japonicus*) lead to hybridization and introgression (Fig. 10). Such phenomenon was associated with increased fitness of introgressed *B. japonicas formosus* in urban context compared to non-introgressed ones.

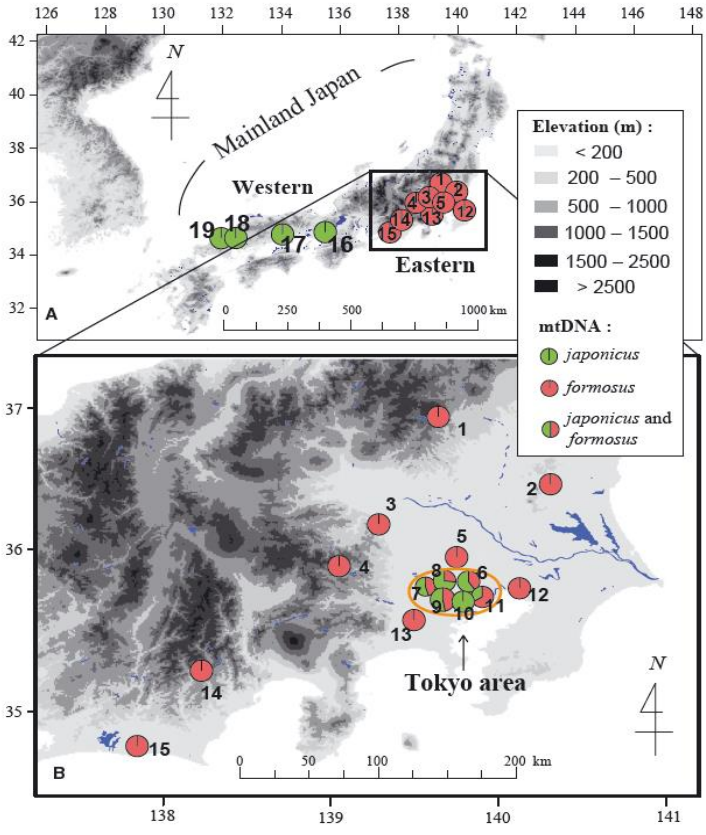

Figure 10: Sampled populations of two lineages of Japanese common toads: *Bufo japonicus formosus* and *Bufo japonicus japonicus*. (**A**) *B. japonicus japonicus* and *B. japonicus formosus* are naturally distributed in the western and eastern regions of mainland Japan, respectively. (**B**) Zoom on eastern Japan and Tokyo area. Populations of *B. japonicus formosus* located in the urban area of Tokyo are clearly introgressed with *B. japonicus japonicus* based on mtDNA and seven microsatellites loci.

By modifying environmental conditions and biotic interactions, urbanization has strong, although poorly understood, consequences on food webs and trophic dynamics (McIntyre et al. 2001, Faeth et al. 2005). An illustration of how urbanization alters trophic dynamics is given by Faeth et al. (2005) who demonstrated that, while the studied natural areas were controlled by bottom-up forces (i.e. rural arthropods abundance depended on resource availability), in urban areas, disruption of predation on birds increased their abundances and lead to strong top-down effects of birds on arthropods abundances. Globally, urban ecosystems are frequently correlated with simplifications of food webs and declines in the efficiency of ecological processes, with concerns for ecosystems functioning (Alberti 2005, Chadwick et al. 2006).

D) Intraspecific variation

As urbanization provokes short scale spatial differentiation of environmental conditions (see Introduction part II), organisms living along urban-rural gradients are subjected to these changes and are therefore likely to demonstrate intraspecific variations in morphology, physiology or behavior, either by phenotypic plasticity or local adaptation (Alberti 2015).

Morphological differences have been observed for a broad range of taxa and associated with a variety of biological traits. First, reduced body size in urban population is a recurrent finding for various taxa (including birds, reptiles and invertebrates, Magura et al. 2006, Gaston 2010 p57) but the opposite pattern (larger body size in urban populations) has also been reported (Sadler et al. 2006). Two major hypotheses may explain reduced body size in urban areas, both related to UHI induced warmer temperatures in cities: 1) For ectotherms, physiological constraints on cell division, replication and growth under warm temperature lead to reduced body size (Angilletta et al. 2005, Gaston 2010 p58), therefore urban ectotherms experiencing UHI should demonstrate such pattern. 2) For endotherms, Bergmann’s rule (Bergmann 1847, Teplitsky and Millien 2013) predicts that species experiencing warmer habitats will exhibit smaller body sizes than species experiencing colder habitats. Applied to conspecific populations along an urban gradient, this rule predicts that urban populations, because they experience warmer temperature induced by UHI, should have smaller body sizes than rural ones. A third set of hypotheses states that trade-offs between variable predation risks, reproductive rates and relative resources availability in urban and rural areas are likely to affect body size and could explain the larger body sizes sometimes observed in urban populations (Gaston 2010 p58, Dulisz et al. 2016).

Urban related intraspecific variation in body size has been widely investigated but some studies focused on particular morphological features, usually locomotor organs such as legs and wings because dispersal abilities have often been identified as a key factors of species response to urbanization (in response to fragmentation, Cheptou et al. 2008, San Martin y Gomez and Van Dyck 2012). The relationship between urbanization and differences in dispersal capabilities among urban and rural populations have occasionally been explored and opposite results have been found. While larger dispersal organs (and thus better potential dispersal abilities) have been found in urban populations of grasshoppers (*Chorthippus brunneus*, longer femurs and wings, San Martin y Gomez and Van Dyck 2012) and butterflies (*Pieris rapae*, larger wings for males, Schoville et al. 2013), urban populations of the weed *Crepis sancta* were found to produce higher proportions of non-dispersing seeds (and thus lower proportions of dispersing seeds) than non-urban populations (Cheptou et al. 2008). These opposite findings could reflect interactions between species biological traits, in this case, the differences in species mobility (mobile versus fixed organisms). Snell-Rood and Wick (2013) showed an increase in cranial capacity for urban populations of two species of rodents (among 10 studied, *Microtus pennsylvanicus* and *Peromyscus leucopus*) compared to rural populations and hypothesized that selection for behavioral plasticity in urban environments could be a driver of such differentiation.

Because urban environments present multiple stressors (e.g. temperature, heavy metals), they are predicted to negatively affect developmental stability and thus provoke fluctuating asymmetry (i.e. departure of bilateral structures from perfect symmetry) (Weller and Ganzhorn 2003). While some studies verified this prediction (for Lizards: Lazic et al. 2013, for bees: Nunes et al. 2015), other showed no differences (for bats: de Figueiredo et al. 2015) or only for urban avoider species (Weller and Ganzhorn 2003).

Physiological consequences of urbanization-induced multiple stressors have also been investigated. Given the warmer temperatures in urban areas, differences in thermal tolerance were tested between urban and rural populations. Greater tolerance to high temperatures have been found for urban populations of the ant *Atta sexdens* (Anguilletta et al. 2007) and for native urban and invasive populations of the ant *Wasmannia auropunctata* (Foucaud et al.

2013) compared to native rural populations, suggesting that urban environment may pre-adapt species to invasion.

Oxidative stress, which occurs when levels of pro-oxidants (ROS for reactive oxygen species) exceed the capacity of antioxidants defenses, induces damages to lipids, proteins and nucleic acids that can lead to cellular dysfunction and apoptosis and affect survival and senescence in organisms (Isaksson 2015). Most of studies linking urbanization to damages and defenses related to oxidative stress focused on birds and showed differences between urban and rural populations (Costantini et al. 2014, Herrera-Dueñas et al. 2014). Similar results were observed in plants (Oztetik 2015) and terrestrial snails (Radwan et al. 2010). These studies reported that urban populations usually had lower oxidative damages and sometimes higher antioxidants levels than rural conspecifics. However, responses may be unclear (Isaksson 2009) and more empirical studies with a broader taxonomic range are needed.

Our knowledge on the consequences of urbanization on intraspecific variation in immunity suffers from the same lack of empirical studies and biological model diversity. Among the few studies exploring the differences in immunity between urban and rural conspecifics, negative (Tian et al. unpublished (thesis) in *Rattus norvegicus*), positive (French et al. 2008 in *Urosaurus ornatus* lizards) or no (Youngsteadt et al. 2015, Appler et al. 2015 in *Apis melifera*) relationships were found.

Physiological differences in glucocorticoid steroid hormones levels between urban and rural populations of blackbirds (*Turdus merula*) have been reported (Partecke et al. 2006). These hormones are implicated in vertebrate behavioral responses to environmental stress. In common garden experiments, urban blackbirds had lower levels of corticosterone when manipulated (stressed) compared to rural birds. The same outcomes were reported in *Junco hyemalis* and related to exacerbated boldness behavior in urban population (Atwell et al. 2012).

Behavioral differences between urban and rural conspecifics have been observed in several bird species (*Turdus merula, Parus major, Passer domesticus, Junco hyemalis, Carpodacus mexicanus*) while some examples exist in other taxa (e.g. in mammals, Ditchkoff et al. 2006). Blackbirds and great tits (*Parus major*) are known to sing at higher pitch in cities (Nemeth and Brumm 2009, Slabbekoorn 2003). Such differences in communication parameters between urban and rural conspecifics can result from local adaptation to the urban acoustic conditions likely to hamper reception of low frequency songs but several alternative hypotheses exist and further investigation is thus needed (Nemeth and Brumm 2009). Other urban–rural intraspecific variations, such as increased sedentariness (Partecke and Gwinner 2007), differences in neophobia and neophilia (Bokony et al. 2012, Miranda et al. 2013), changes in timing of daily activity (in relation to night light pollution, Dominoni et al. 2013) and the use of cigarette butts reducing ectoparasite loads (Suarez-Rodriguez et al. 2013) have been identified as possible adaptations to city life (Garroway and Sheldon 2013). Recently, Mueller et al. (2013) confirmed behavioral adaptations during urbanization by identifying a significant association between urban life and a gene implicated in harm avoidance behavior (SERT) among 10 out of 12 pairs of urban-rural populations across the Western Palearctic. In the odorous house ant *Tapinoma sessile*, Buczkowski (2010) showed that urbanization strongly impact the social and spatial structure of colonies: while colonies found in natural environments were small, monogyne (single queen) and monodomous (single nest), colonies from urban environments exhibit high polygyny and polydomy, forming large colonies of interconnected nests. Another study (Menke te al. 2010) based on genetic analyses of *T. sessile* colonies from urban and natural habitat showed that *T. sessile* colonized urban habitats repeatedly and suggested that plasticity in life history strategies already present in natural populations is responsible for the social structure observed in colonies from urban environments.

E) Evolutionary dynamics in urban environments

As the number of empirical studies investigating intraspecific variations along urbanization gradients grows, new insights arise. Indeed, urbanization constitutes a spontaneous field of experiment, with thousands of potential replicates, for studying fundamental processes responsible for biological evolution (selection, drift, mutation and gene flow), at reduced spatial and time scales (Donihue and Lamnbert 2015). Urban environments, more than sinks for biodiversity, have the potential to grow whole biological communities of adapted populations in some decades or centuries, as rapid evolution has been shown to happen in few generations (Phillips et al. 2006).

The rapid and strong environmental changes imposed by urbanization can result in intense selection pressures likely to accelerate the establishment of local adaptation, leading to rapid contemporary evolution (Stockwell et al. 2003, Winchell et al. 2016). However, the strength of urban induced selection pressures is not the only parameter governing the establishment of local adaptation in urban areas. Indeed, the establishment of local adaptation is likely to depend on a precise multi-modal equilibrium point constituted of three major interacting parameters: strength of the selection pressure, effective size of urban populations (Ne) and amount of gene flow between urban and rural populations (Stockwell et al. 2003, Fig. 11). The establishment of local adaptations is likely to be promoted by strong selection pressures, high effective urban population sizes and limited gene flow between urban and rural populations. However local adaptation can emerge even in theoretically inappropriate conditions such as low Ne (McKay et al. 2001) or important gene flow (Tigano and Friesen 2016). Urban areas might constitute great opportunities to investigate the emergence of local adaptation as they are challenging environments generating potentially strong selection pressure and because they are likely to affect both gene flow (Jha and Kremen 2013) and populations effective size (e.g. by bottleneck effect, Yamamoto et al. 2013, Munshi-South et al. 2013).

In addition, potential urbanization induced changes in dispersal abilities, phenology, reproductive behavior or social behavior, either by local adaptation or phenotypic plasticity, would be likely to disrupt gene flow between urban and rural populations, which could, in turn, lead to rapid genetic differentiation of urban populations by adaptation (Orsini et al. 2013) or drift (Munshi-South and Kharchenko 2010, Vangestel et al. 2012) and ultimately, might conduct to speciation (Ushimaru et al. 2014, Parris 2016).

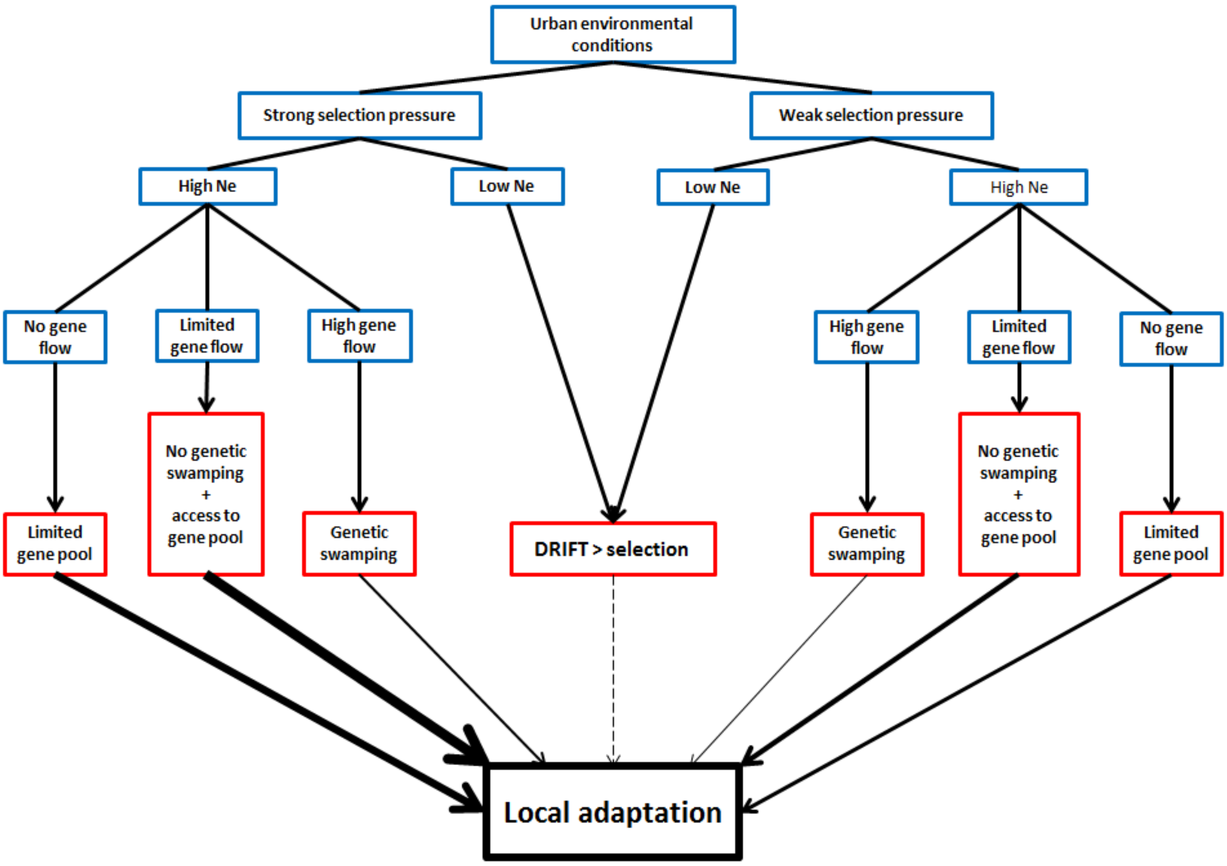

Figure 11: Theoretical framework of the establishment of local adaptation in urban areas. The establishment of local adaptation in urban populations depends on the combination of several conditions and parameters: the strength of the selection pressure, the effective size of the population (Ne) and the amount of gene flow between urban and non-urban adjacent populations. The width of the arrows pointing toward "Local adaptation" indicates the (theoretical) relative probabilities of observing local adaption under each scenario (larger arrows: high probability; finer arrows: low probability).

IV) Concluding remarks

Urban ecology still suffers from a lack of diversity in the biological models used to assess patterns and test hypotheses, as most of studies, for all levels of biological organization, are focused on birds (Gaston 2010 p54). As a consequence, a first step in future urban ecology studies would be to find novel relevant biological models enabling the study of urbanization at all organization levels. The optimal choice of taxa might be based on particular characteristics. First, at the biological community level, relevant "urban model taxa" should present several species with diverse biological traits, resulting in several species-urbanization relationships among the community (i.e. urban avoiders, urban exploiters, urban tolerant). Urban tolerant species, i.e. species abundant all along the gradient, allow the investigation of intraspecific variations and testing for local adaptation in urban environments, which means that the possibility to realize common garden and transplant experiments should be an essential criterion. Taxa containing species with broad distributions should be favored because they would enable replications across cities and multiple gradients studies. Furthermore, taxa fulfilling these conditions are likely to share diverse interactions with other species and to have a strong influence on ecosystems functioning in both natural and man-impacted environments. Another, more practical, criterion would be that time and cost-effective sampling organisms should be preferred in order to maximize the numbers of independent studies across countries globally (Gaston 2010 p54). As a consequence, taxa such as herbaceous plants, insects such as carabid beetles and ants, spiders, birds and rodents are organisms allowing effective and generalizable study of urbanization impacts on ecological and evolutionary dynamics (Alberti 2015).

For a long time, urban ecology has been mostly made of observational rather than experimental studies (Shochat et al. 2006) and it is only recently, that experimental approaches focusing on mechanisms appeared (see Introduction part III-D). Technological advances such as genomics already allow new insights in our understanding of urbanization consequences on eco-evolutionary processes (Munshi-South et al. 2016). Because urban ecology studies are often idiosyncratic case studies (Grimm et al 2008b), generalizing our comprehension of urbanization impacts on biodiversity and ecosystems functioning will have to integrate climatic and societal factors (Turrini et al. 2015, Diamond et al. 2015). By understanding how the effect of urbanization on biodiversity varies along climatic and societal gradients, it should be possible to develop a general framework and models allowing global understanding of urbanization consequences on eco-evolutionary dynamics, and consequently, begin the construction of predictive scenarios and management plans.

V) Aims of the thesis

This thesis was built to investigate the consequences of urbanization on biodiversity at different organization levels. We have chosen to study ants (Hymenoptera, Formicidae) which are a well suited model for studying urbanization as they fulfill almost all the "urban model taxa" criteria discussed earlier, except for the possibility to realize common garden experiments on several generations (most ants species do not reproduce in the laboratory). Ants occur on all landmasses worldwide (except for the poles and isolated islands) and feature more than known 12,000 species. Because they are eusocial organisms, ants have been extensively used as biological models for studying social organization, animal communication and kin selection (Holldobler and Wilson 1990). They also constitute a recurrent model for studying biotic interactions (Passera 2006 p229-276) and biological invasions (Suarez et al. 2000, Holway et al. 2002), as they interact in various ways (e.g. mutualism, parasitism, predation) with many organisms (including animals, plants, fungi and bacteria) and a number of species are major invaders worldwide causing billions of dollars of damages each year (e.g. *Solenopsis invicta*, Bradshaw et al. 2016). Finally, ants are able to settle in urban areas and to establish even in the densest urban cores (Pećarević et al. 2010).

This thesis is divided in four chapters, each focusing on a particular feature of urbanization consequences on ants. As I developed in previous chapters of this manuscript, urbanization is inseparable from human activities and then tightly related to biological invasions. Invasion ecology is consequently a recurrent theme of this thesis that I have deliberately kept intertwined with urban ecology.

This thesis contains four chapters:

Chapter 1: Urbanization and biological communities: How does urbanization affect the landscape distribution of ant species?

Chapter 2: Urbanization and human-mediated dispersal: Conceptualizing and modeling human-mediated dispersal by focusing on human activities and networks topology.

Chapter 3: Urbanization and biotic interactions: How does urbanization affect co-invasion patterns of a host-parasite invasive couple?

Chapter 4: Urbanization and intra-specific variations: Does urbanization induces phenotypic changes between urban and rural conspecifics of an urban tolerant ant?

Chapter One investigates how environmental changes associated with urbanization affect ant species distributions at the landscape scale. Because the ability of species to establish in urban areas depends on complex interactions between multiple environmental factors and species biological traits, responses to urbanization were predicted to be species-specific.

Urbanization and biological invasions have a long common history. The development of human population and activities sharply affected the dynamics of species invasions by multiplying introduction events, facilitating establishment and accelerating secondary spread. Chapter 2 investigates human mediated dispersal of invasive species in two parts. The first part proposes a new conceptual framework for the study of human-mediated dispersal and particularly points out the necessity to focus on human activities rather than directly on species. The second part proposes a model of human-mediated secondary spread by transport (MoRIS). This model aims to bring a novel and simpler way to simulate dispersal by transport, to estimate its parameters from simple ecological data and to predict its spatial and temporal dynamics.

Urban areas concentrate human activities. As a consequence, they often experience multiple simultaneous invasions. Chapter 3 investigates the effects of urbanization on the invasion pattern of two invasive species (ant and fungus) sharing a host-parasite relationship. Urbanization may increase the probability of invasive species to encounter each other, but also affect the outcomes of their encounter.

Finally, urbanized landscape often host urban tolerant species, i.e. species that are not affected by urbanization in terms of spatial distribution and abundances along this distribution. In such species, urban and rural populations experience very different environmental conditions. Chapter 4 proposes to test for the existence of phenotypic and potential evolutionary shifts between urban and rural populations of a widespread urban tolerant a species (*Lasius niger*). Morphological, physiological and behavioral changes are investigated in queens and workers originating from urban and rural populations.

CHAPTER 1

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I'm not like everybody else: urbanization factors shaping spatial distribution of native and invasive ants are species-specific

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Abstract

Urbanization is a major global change inducing complex and multiple modifications of landscapes and ecosystems. The spatial distributions of organisms experiencing these modifications will likely shift specifically, depending on each species' response to each environmental modification induced by urbanization. We sampled two ant genera (*Lasius* and *Tetramorium*) at 1248 locations along an urbanization gradient in Lyon, France and used high resolution spatial layers for 18 spatial (e.g., open habitat fragmentation, bioclimatic data and surface temperatures) and temporal (e.g., comparison of Normalized Difference Vegetation Index between 1986-2015) environmental variables associated with urbanization. Coupling two different analytical methods (Outlying Mean Index and Boosted Regression Trees), we showed that each species' distribution was influenced by its own combination of environmental factors. Two morphologically cryptic *Tetramorium* species (*T. spE* and *T. spU2*) were both highly abundant but with opposite responses to urbanization: while *T. spE* was favored by urbanized habitat, *T. spU2* avoided urbanized areas. Among *Lasius* species, we detected 63 occurrences of the invasive ant *Lasius neglectus,* the distribution of which was favored only by embankments along roads. We found that, even at this reduced spatial scale, climatic effects influenced most species and interacted with urbanization factors.

Keywords Urbanization, global change, species distribution, biological invasions, Formicidae, *Lasius neglectus*

Introduction

The pace of urbanization has dramatically accelerated over the past decades to become a major concern, severely impacting biodiversity dynamics, ecosystems functioning, as well as human well-being (Grimm et al. 2008a). Urbanization can be defined as the modification of an ecosystem to support dense human populations and their activities (Gaston 2010). In urban areas, original plant communities are partially or totally replaced by impervious surfaces (e.g., roads, concrete and building), exotic plant species and bare ground (Heterick et al. 2013). Outside of cities, the agricultural area necessary to feed urban populations is growing with the pace of urbanization, replacing diverse native plant communities by cultivated monospecific patches (Flinn & Vellend 2005). These land cover changes are responsible for habitat fragmentation (Jenerette & Potere 2010) and for an increase in open-vegetation patches (typically lawns) to the detriment of forests (James & Bound 2009, Smith et al. 2015). Land cover changes are also characterized by spatial and temporal dynamics as they follow the development of cities. Among the direct consequences of land use changes are the alteration of physical characteristics of surfaces, i.e. emissivity, albedo, and thermal conductivity. Furthermore, removing plant cover reduces evaporative cooling and modifies radiative fluxes in urban areas. These changes induce warmer surface and air temperature in urban areas, particularly at night-time, a phenomenon termed Urban Heat Island (UHI) effect (Dousset et al. 2011). Urban areas are also subjected to heavier air, soil and water pollution than surrounding rural areas (Grimm et al. 2008a).

In addition, urban areas are exposed to frequent introductions of exotic species through national and international commercial exchanges, as well as to the secondary spread of invasive species. Secondary spread is exacerbated by recurrent human-mediated transport facilitating dispersal (Hodkinson & Thompson 1997, King et al. 2009) and by increased habitat invasibility associated with disturbed landscapes, which facilitate establishment (Alpert et al. 2000, With 2002, Vonshak & Gordon 2015).

Changes associated with urbanization therefore constitute new environmental filters for biodiversity, modifying species' distribution patterns and affecting community composition along the urbanization gradient (Van Nuland et al. 2014). Several studies have already shown that assemblage parameters such as native and invasive species richness and abundance are affected by urbanization for a wide range of taxa (Hansen et al. 2005). While urban environments are often described as species-poor ecosystems (Niemelä & Kotze 2009, but see Pećarević et al. 2010), several studies have shown that suburban areas can display high species richness and diversity because of greater habitat heterogeneity, intermediate disturbance regime and invasive species enrichment (Angold et al. 2006, Vonshak & Gordon 2015, Menke et al. 2011). Further studies have shown that community responses vary depending on the taxonomic group considered (Croci et al. 2008, McKinney 2008, Vergnes et al. 2012). Since Blair's classification of species responses to urbanization (Blair 1996), several authors categorized species into urban exploiters, urban tolerant and urban avoiders (McIntyre 2000, Kark et al. 2007). However, this characterization has been criticized as over-simplistic and prone to masking nuanced or complex species responses to urbanized landscapes (Conole 2014). While urban-rural gradient approaches have been widely used to describe species and community responses to urbanization (McDonnell & Hahs 2008), precisely identifying which environmental factors are implicated has become a major issue of urban biodiversity studies (Philipott et al. 2014, Turrini & Knop 2015). As the environmental changes induced by urbanization depend on the interactions between multiple environmental factors and species biological traits (Poff 1997, Brown et al. 2009, Verberk et al. 2013), species responses to urbanization are likely to be specific and to depend on the local environmental background, which includes climate or social and economic activities (Grimm et al. 2008b, Oleson et al. 2015). Consequently, clearly identifying environmental factors associated with urbanization and understanding their impacts on ecological dynamics should constitute a first step towards evaluating and predicting the consequences of urbanization on biodiversity. To this end, Arthropods have recently been pointed out as highly relevant organisms, and among them, ants (Stringer et al. 2009, Menke et al. 2011, Bang & Faeth 2011, Ślipiński et al. 2012, Heterick et al. 2013), because they are among the few taxa able to establish stable populations in densely urbanized habitat such as big city cores (Pećarević et al. 2010, Guénard et al. 2015, Savage et al. 2015). Ants have also been identified as effective city scavengers (Youngsteadt et al. 2014) and several invasive ant species are known to be dominant in urban habitats (Ward 1987, Suarez et al. 1998, Buczowski & Bennet 2008). Community ecology studies have shown that ant species richness and community composition differ along urban to rural gradients (Ślipiński et al. 2012, Heterick et al. 2013) but most of them focused on forested habitat and urban parks (e.g., Yamaguchi 2005, Clarke et al. 2008, Carpintero & Reyes-Lopez 2013) while lawns and open-vegetation green spaces, the most common habitat type in urbanized landscapes, have been neglected (but see Uno et al. 2010). These habitats are, however, likely to host rich ant communities as many ant species are open and edge habitat specialists. Among these, the genera *Lasius* and *Tetramorium* are particularly well represented: they are known to be both highly species diverse and exhibit broad spatial distributions ranging over most of continental Europe. Both contain species that invasive in Europe, such as *Lasius neglectus* (Ugelvig et al. 2008), or in North America, such as *Lasius alienus*, *Tetramorium* spE and *Tetramorium tsushimae* (Steiner et al. 2008), as well as species highly tolerant to urban conditions such as *Lasius niger* (Vepsäläinen et al. 2008).

We conducted surveys of *Lasius* and *Tetramorium* ants in the landscape of the urban area of Lyon, which is the second largest in France. The aims of our study were (i) to describe *Lasius* and *Tetramorium* responses to environmental changes associated with urbanization and (ii) to identify landscape-scale factors shaping the spatial distributions of species. Our main hypothesis was to find species-specific response patterns to urbanization: as species' ecological niches are characterized by unique combinations of environmental optima, therefore each species should be affected by a particular set of environmental features and have its own response pattern along the urbanization gradient. Additionally, we predicted that the occurrence of invasive species should be associated with factors related to the intensity of human activities.

Methods

Study area and sampling

The study was conducted in the urban area of Lyon, France (Fig. 1), and along the two major sets of transportation infrastructures serving the city (A7 motorway, train and Rhône River to the South and West, A43 motorway to the East) which concentrate industrial and commercial activities and are consequently important features of urban gradients. In 2012, the Lyon urban area hosted 2,214,000 people and covered 6,017 km². Lyon is located at the confluence of the Rhône and Saône rivers and has a continental climate with Mediterranean influences. Mean temperatures are 3.2°C in the coldest month (January) and 21.9°C in the warmest month (July), with an annual mean temperature of 12.3°C. The annual average rainfall is 820 mm (Infoclimat.fr).

1248 locations (Fig. 1) were sampled within this area, during spring and summer 2011, 2012 and 2013, following the method of Roura-Pascual et al. (2009). Sampling locations were herbaceous patches, with or without trees and shrubs, and generally close to or along roadsides. They were randomly selected along road transects and were located at least 500 m apart when outside the city center and 200 m apart when inside the city center. Sampling was performed by teams of two to five persons for an added search time of 40 minutes (the actual sampling time depended on the number of samplers, e.g., four samplers took 10 minutes per site; two samplers, 20 minutes per site) within a radius of 15 m, and only when air temperatures were comprised between 16°C and 28°C (Seifert 2007). Sampling was a direct search of ants nest and trails on the ground, trees and shrubs, followed by hand collecting using an entomological aspirator. Ants from the genera *Lasius* and *Tetramorium* were collected (except for hypogeic *Lasius* species). Spatial coordinates of sampling locations were recorded and imported into ArcGIS v.10.1 (http://www.esri.com/software/arcgis).

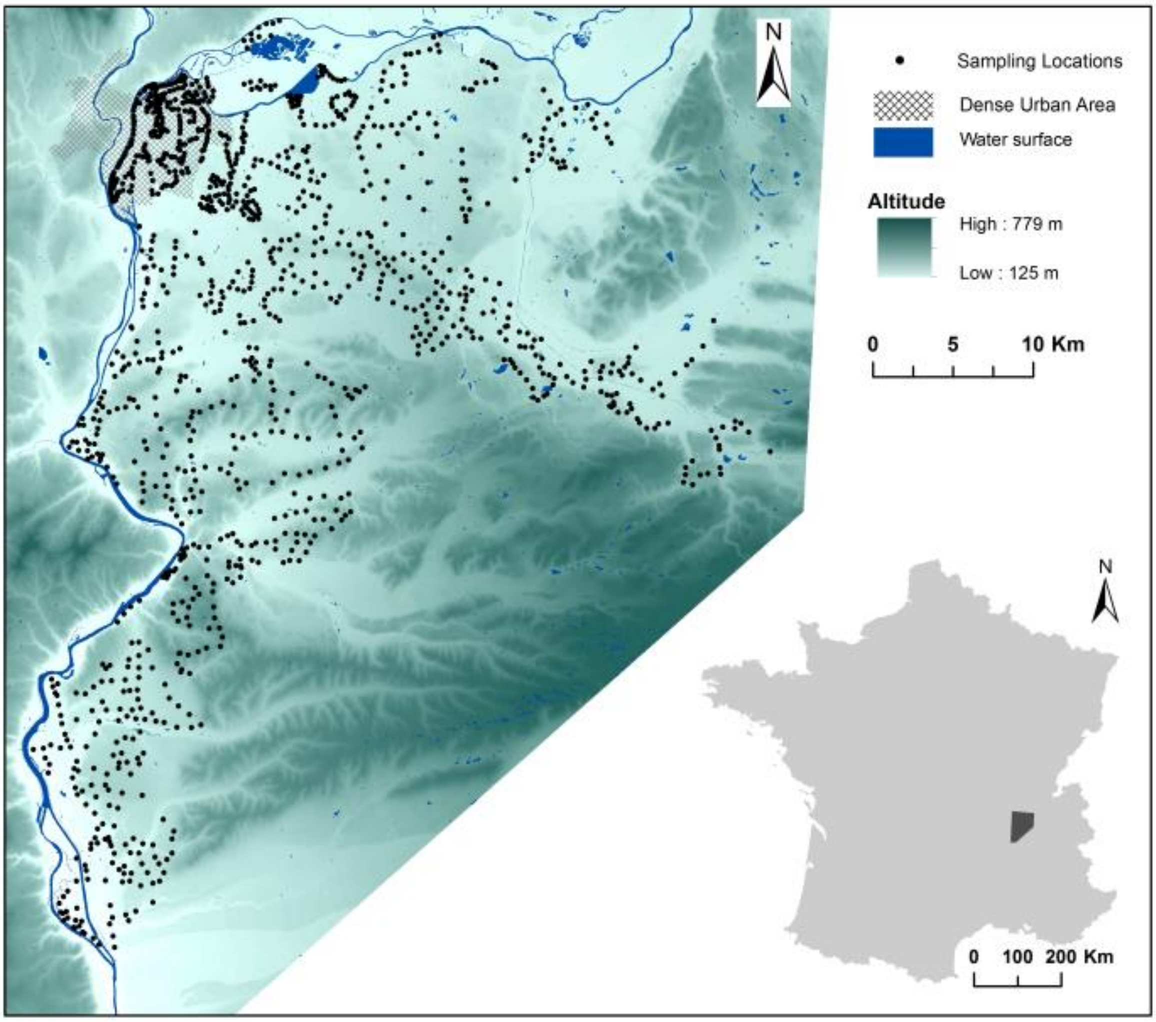


Figure 1: Sampling locations in the urban area of Lyon, France. Black dots indicate sampling locations, altitudes are represented by shades of green, with darker shades showing higher altitudes.

Morphological and molecular identification

All collected ants were preserved in 96° alcohol at -20°C and morphologically identified to genus or species whenever possible, following Seifert (2007). One individual per colony of

Tetramorium and taxonomically ambiguous *Lasius* species (i.e. with short or appressed scape setae) were systematically identified using Cytochrome Oxydase I (COI).

DNA was extracted from whole individuals for *Tetramorium*, from head and thorax only for *Lasius* to remove formic acid. *Lasius* ants were crushed and then mixed with 150 µl of hot (90°C) Chelex® 100 then cooled; *Tetramorium* ants were crushed and then mixed with 150 µl of room-temperature Chelex® 100. In both cases, 10 µL of proteinase K (15mg/mL) were added after crushing and cooling, and the solution was incubated at 55°C overnight (Casquet et al. 2012).

DNA was amplified by PCR using specific primers developed from complete COI sequences found in Genbank (COI_neg_F1, TTATTAATGAYGGAGTYGGAACAGGATG; COI_neg_R1, CTCGTCGTTATTCAGATTAYCCAGATA, derived from PAT L2-N-3014r (Simon et al. 1994). PCR Reactions were carried out in 35µL solutions with 0.17 µM/µl dNTPs, 0.1 µg/µl BSA (New England Biolabs, Ipswich, USA), 0.16 µM/µl of each primer, 0,04 U/µl Taq Polymerase (Biolabs), 1X PCR Buffer (Biolabs), and 4 µl of DNA. Cycling was conducted on a PTC-200 (MJ Research) thermal cycler with following parameters: (i) initial denaturation for 2 min at 94°C, (ii) 40 cycles with denaturation for 30 s at 94°C, annealing for 30 s at 48°C and extension for 30 s at 72°C; (iii) final extension for 1 min at 72°C. In Europe, the genus *Tetramorium* has numerous cryptic taxa (Schlick-Steiner et al. 2006), and COI sequencing is the only reliable means to identify them. DNA was amplified by PCR using specific primers developed from longer stretches of COI from the literature (Schlick-Steiner et al. 2006), with the following sequences (Tetra_F: TAGCATCTAATRTCTTTCAYAGAGG; Tetra_R: AGTATCAGGATA ATCTGAGTAYCGAC); PCR reagents and conditions were identical to *Lasius* PCRs. All PCR products were purified sequenced and ran on a 3730xl DNA Analyzer (Applied Biosystems) by a service provider (BIOFIDAL, Vaulx-en-Velin). All obtained sequences were compared to existing sequences present in Genbank using Blast-n.

Environmental factors associated with urbanization

18 environmental factors associated with urbanization and climate were extracted from various sources (see Table S1). Variables extraction was conducted using ArcGIS 10.1.

Land cover variables were extracted from an initial raster layer (1.5m spatial resolution) obtained from a three-step process. First, we extracted topographical vectorial data from national maps (BD TOPO® (2013) IGN) and Graphical Parcel Register (RPG 2012, www.data.gouv.fr). Second, unassigned surfaces (essentially impervious surfaces and open-vegetation surfaces) were processed with an image processing method using SPOT 6 imagery obtained by GEOSUD (http://geosud.teledetection.fr/) (1.5m spatial resolution) to assign each pixel to a "green" or a "grey" surface. Third, all layers were merged to produce a land cover map (1.5m resolution) with five different classes: water, forest, open-vegetation (i.e. open spaces with permanent grass cover such as lawns, meadows and pastures), agricultural and impervious. For each sampling location, areas of each land cover class (except water) were calculated within both a 50 and a 500m radius buffer.

Surface temperatures (ST) were calculated from remote sensing data (Diallo-Dudek et al. 2015) based on Landsat 8 imagery from the 21st of July, 2013. For each sampling location, mean surface temperatures were calculated within a 50m radius buffer.

A land cover change index (deltaLC), representing land cover changes over the last 31 years, was produced following a three-step process. First, we produced NDVI (Normalized Difference Vegetation Index) from Landsat images (30m resolution, using Red and Near-Infrared) from two dates: 25th of June, 1986, and 11th of July, 2015. Second, for each NDVI, we determined a threshold value that best discriminated urbanized from non-urbanized pixels. To choose the best threshold at each date, we produced binary urbanized versus non-urbanized raster layers for eleven different thresholds (from 0.15 to 0.25, incremented by 0.01; with 0: non-urbanized, 1: urbanized), and randomly selected 1000 pixels in each Landsat images (1986 and 2015). We then visually assigned each pixel to "urban" or "non-urban", ambiguous pixels being removed, for a final total of 354 and 400 correctly assigned pixels for 1986 and 2015 respectively. We finally used the Kappa-Cohen's coefficient (Cohen, 1960) to select the best NDVI threshold for each date, which is the threshold for which the best agreement score between our visually discriminated pixels and the threshold-induced classification was obtained. For the two dates, the best agreement score was 0.71, indicating that our classification was moderately accurate because of initial data quality and methodological limitations, which were likely to add stochastic variability to the final variable (i.e. deltaLC). Third, using the best thresholds (i.e. 0.22 and 0.21 for 1986 and 2015 respectively), we obtained a binary raster layer for each date and, by subtraction, a final third raster map containing three different values: -1 (changes from vegetated to urbanized between 1986 and 2015), 0 (no changes between 1986 and 2015) and 1 (changes from urbanized to vegetated between 1986 and 2015). We finally calculated the mean change index within a 100m radius buffer around each sampling location.

As a measure of open habitat fragmentation (OHFrag), we used the mean area of open-vegetation patches (e.g. lawns, meadows, semi-natural grasslands), calculated within a 500m radius buffer around each sampling location.

The intensity of human activities was assessed using three variables: distance from sampling locations to the closest main road (DMainRoad), distance from sampling locations to the closest embankment (Dbanks), and secondary road network density within a 100m radius (RoadsDensity). Main and secondary roads networks as well as embankments were extracted from BD TOPO® (2013) IGN. Roads and embankments were considered because the former are often related to invasive species occurrences (Prasad et al. 2010, Rouifed et al. 2014) while the latter are intrinsically associated with soil transportation at short to medium distances and are therefore likely to be implicated in the spatial spread of invasive species, especially ants.

Altitudinal and climatic variables were considered because our most southern sampling locations showed stronger Mediterranean climatic influences and because, even if sampling was designed to minimize altitudinal variation, sampling locations ranged from 132 to 405 meters above sea level. Altitudinal data were obtained from BD Alti® (2013, 25m spatial resolution) IGN and we used two Bioclim variables (Hijmans et al. 2005): mean annual temperature and mean annual precipitation (Table S1).

All 18 variables (Table S1) were classified as follows: land cover (LC, containing 8 variables), surface temperature (ST, 1 variable), temporal land cover change (deltaLC, 1 variable), open habitat fragmentation (OHFrag, 1 variable), human activities (HA, 3 variables) and altitudinal/climatic effects (CE, 3 variables).

In order to avoid variables redundancy and to facilitate interpretation, we reduced the number of variables using principal component analyses (PCA), performed in classes LC and CE as they contained highly collinear variables (Fig. S1). This operation enabled us to reduce the initial number of variables from 18 to 10 by extracting a set of synthetic orthogonal (uncorrelated) variables (Table 1).

Data analysis

All statistical analyses were performed in R (R Development Core Team 2014).

Data were analyzed using a multivariate ecological niche analysis (Outlying Mean Index, OMI; Doledec et al. 2000; R package *ade4*, Dray & Dufour 2007) and a machine learning method (Boosted Regression Trees, BRTs; Elith et al. 2008; R package *dismo*, Hijmans et al. 2011). These two methods investigate species-environment relationships in different ways. While OMI allows a simultaneous comparison of the response of all species to environmental factors, BRT investigate each species individually and allows a more specific assessment of species-environment relationship (as described below).

The Outlying Mean Index analysis maximizes the variance in species occurrences along ordination axes obtained from the input of environmental factors. This method assesses for each species the marginality or OMI (i.e. the difference between the average environmental conditions found for a species and the average environmental conditions for all locations), the tolerance (i.e. the breadth of the species' niche) and the residual tolerance (i.e. the variation in species occurrence not accounted for by the main gradient). To identify the main factors affecting species marginality, for each species and for each environmental factor, we measured the significance of the deviation of average factor values in the locations used by the species and the average factor values in all locations by running permutations tests (9999 permutations and using Bonferroni correction).

Boosted Regression Trees is a machine learning algorithm that uses a combination of decision trees and boosting methods to fit models. BRT has strong predictive performance, allows nonlinearities of predictor variables and detects interactions between them. It also enables predictor variables to be classified by their relative contributions (Elith et al. 2008). This method is relatively new in ecological studies and globally outperforms other more traditional methods (Elith et al. 2006). BRT models were fitted using a tree complexity of 5 and a learning rate of 0.001. Ten-fold cross validation was used by randomly selecting 50% of the sampling locations for model fitting and the remaining 50% for model validation. This enabled the calculation of two values for each model: the explained deviance and the discrimination as measured by the area under the receiver operator characteristic curve (AUC). Explained deviance indicates the goodness-of-fit between predicted and raw values, it was expressed as a percentage of the null deviance. AUC indicates the degree to which fitted values discriminate between observed presences and absences. AUC values range from 0.5 (discrimination no better than random) to 1 (perfect discrimination) (Leathwick et al. 2008, Radinger et al. 2015).

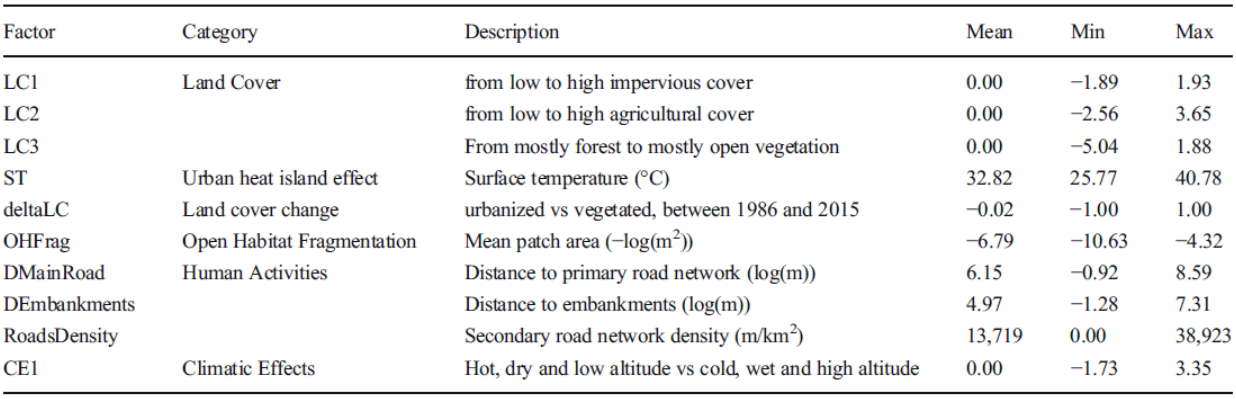
We identified the main factors affecting species distribution by coupling permutation tests and BRT relative contribution outputs. We only retained variables that showed significant effects according to permutation tests and that contributed to more than 10 % of total variance according to BRT models.

Results

From the 1248 sampled locations, we obtained a total of 2503 *Lasius* and *Tetramorium* occurrences with 7 and 4 species for each genus respectively. *Lasius niger* and *Tetramorium spE* were the most frequent species, respectively found in 74.9 and 49.1% of locations.

Lasius neglectus, known to be invasive in Europe, was found in 69 sites (5.5 % of the sampling locations), a large number of occurrences for this species considering our reduced spatial scale. For comparison, to date, 174 occurrences of *L. neglectus* were known for all Europe, of which 47 in France (Espadaler & Bernal 2015). The other common species were *T. spU2* (24.7 %), *L. alienus* (21.1 %), *L. emarginatus* (16.3 %), and *L. paralienus* (4.3 %) (Table 2).

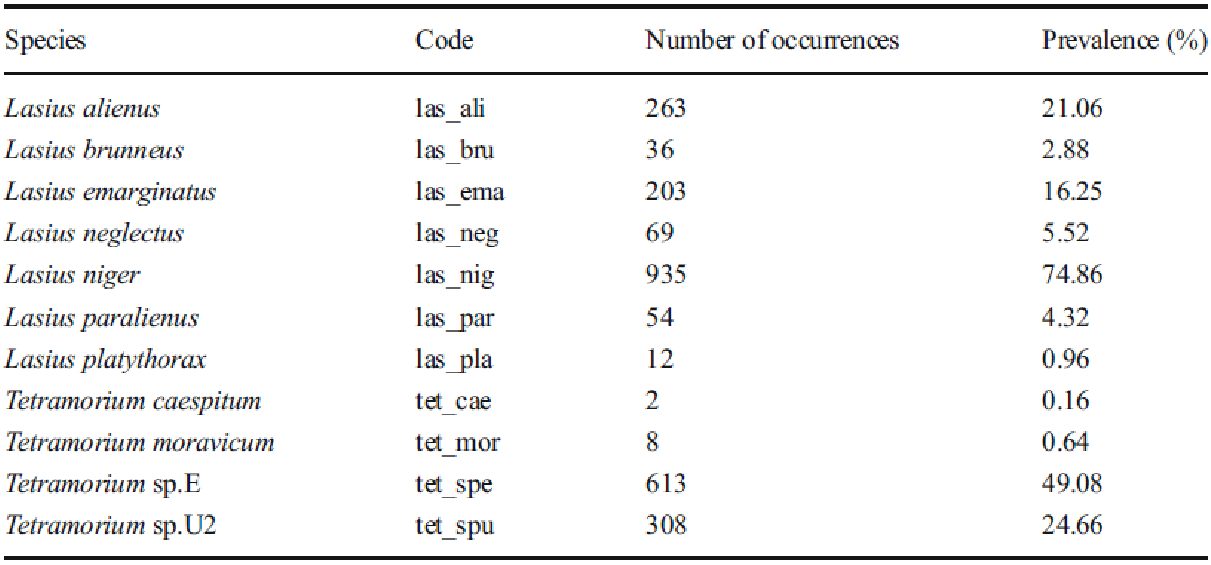
Table 1: Environmental factors used for Outlying Mean Index and Boosted Regression Trees analyses.



Factor	Category	Description	Mean	Min	Max
LC1	Land Cover	from low to high impervious cover	0.00	−1.89	1.93
LC2		from low to high agricultural cover	0.00	−2.56	3.65
LC3		From mostly forest to mostly open vegetation	0.00	−5.04	1.88
ST	Urban heat island effect	Surface temperature (°C)	32.82	25.77	40.78
deltaLC	Land cover change	urbanized vs vegetated, between 1986 and 2015	−0.02	−1.00	1.00
OHFrag	Open Habitat Fragmentation	Mean patch area (−log(m^2))	−6.79	−10.63	−4.32
DMainRoad	Human Activities	Distance to primary road network (log(m))	6.15	−0.92	8.59
DEmbankments		Distance to embankments (log(m))	4.97	−1.28	7.31
RoadsDensity		Secondary road network density (m/km^2)	13,719	0.00	38,923
CE1	Climatic Effects	Hot, dry and low altitude vs cold, wet and high altitude	0.00	−1.73	3.35

The first two axes of the OMI explained 88.9% of total inertia (axis 1: 79.16% and axis 2: 9.76%) (Fig. 2). The first axis was a mix of climatic and urbanization effects, opposing fragmented, impervious, hot and dry locations to non-fragmented, non-impervious, cold and wet locations. The second axis was mainly negatively correlated to two factors: ST (surface temperature) and LC3 (amount of forest versus open-vegetation). Because forests induce evaporative cooling, they are likely to be associated with low surface temperatures (Fig. 2, Table 3).

Table 2: List of sampled *Lasius* and *Tetramorium* ant species. Only bold-typed species were used in further analyses, the others being rare.



Species	Code	Number of occurrences	Prevalence (%)
Lasius alienus	las_ali	263	21.06
Lasius brunneus	las_bru	36	2.88
Lasius emarginatus	las_ema	203	16.25
Lasius neglectus	las_neg	69	5.52
Lasius niger	las_nig	935	74.86
Lasius paralienus	las_par	54	4.32
Lasius platythorax	las_pla	12	0.96
Tetramorium caespitum	tet_cae	2	0.16
Tetramorium moravicum	tet_mor	8	0.64
Tetramorium sp.E	tet_spe	613	49.08
Tetramorium sp.U2	tet_spu	308	24.66

The investigation of ecological niche parameters indicated that a large part of the total variance in species occurrences remained unexplained, with residual tolerance ranging from 63.1% to 89.4% (Table 4). However, species exhibited marginality scores ranging from 0.2 % (*L. niger*), indicating a very weak (although significant) difference from the environmental average conditions, to 13.3 % (*T. spU2*). Tolerance ranged from 9.9 % (*L. paralienus*) to 30.1 % (*L. emarginatus*) (Table 4).

Tetramorium species were mainly spread along the first axis and showed opposite positions (Fig. 2). *T. spE and* T. spU2 respectively showed positive and negative associations with fragmented open-vegetation patches, impervious areas and hot/dry climate (Fig. 2).

Lasius species were spread along the two axes. *Lasius niger* was the most central species, as indicated by its OMI score. *L. alienus*, *L. emarginatus* and *L. paralienus* occurrences were associated with the less impervious, less fragmented and cold/wet part of the environmental gradient. Finally, *L. emarginatus* and *L. neglectus* occurrences showed positive scores along the second axis, indicating that they were likely to be associated with low surface temperature or forested areas.

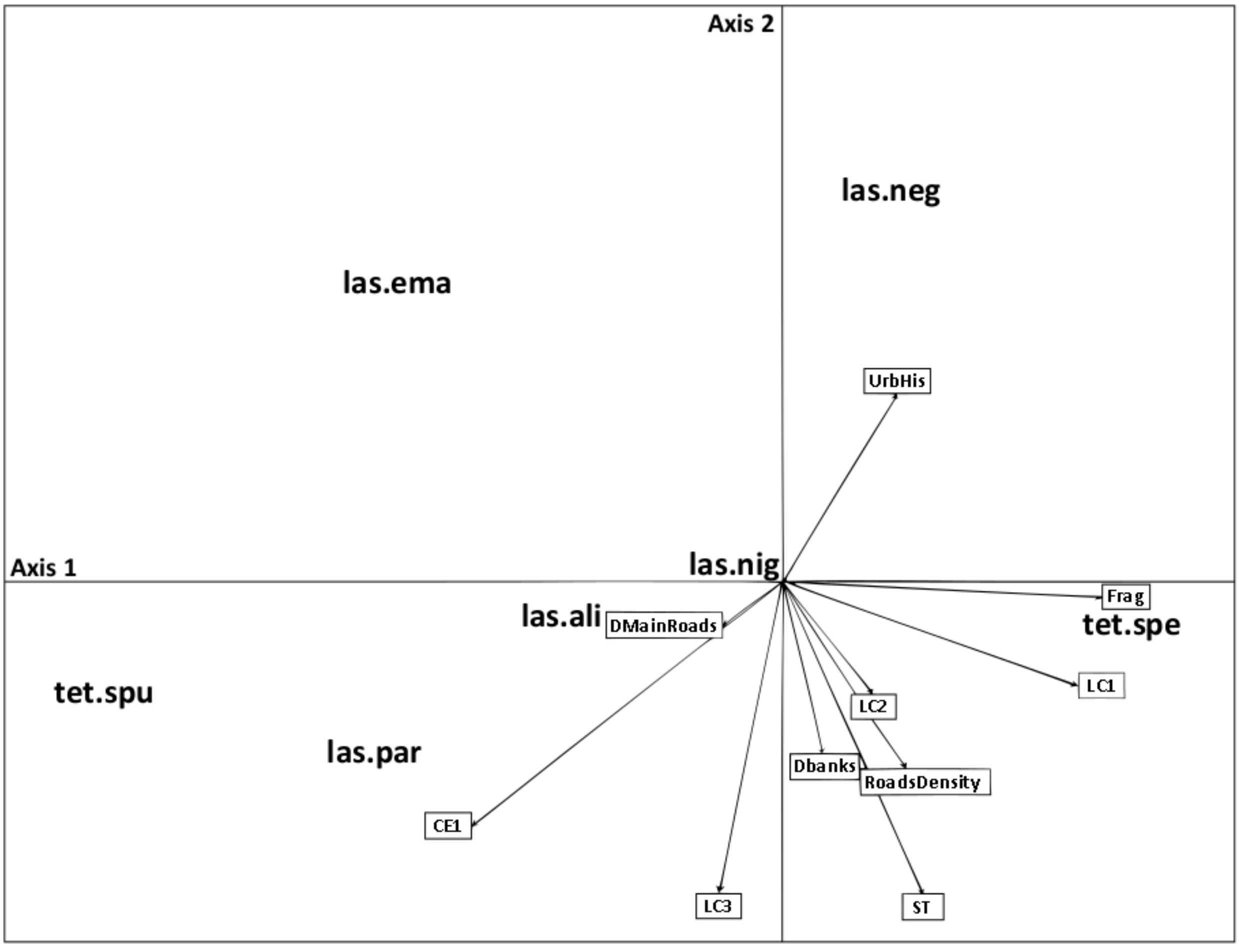


Figure 2: OMI analysis plot. The first axis is horizontal; the second axis is vertical. The origin of the plot represents the mean environmental conditions. Environmental factors (boxed) are represented as vectors; their relative size reflecting their influence (longer lines indicate greater influence) and their direction indicating their correlation to each axis. Species are indicated by their abbreviation code, their position on the factorial plane reveals the degree of difference of their ecological niche to the mean environmental conditions and their correlation to each axis.

BRT provided 'acceptable' (AUC = 0.7-0.8) to 'excellent' (AUC = 0.8-0.9) model performance for 4 out of 7 species, but weak model performance for *L. alienus*, *L. emarginatus* and *L. niger* (AUC = 0.6-0.7) (Leathwick et al. 2008). Levels of explained deviance were relatively low (ranging from 3.32 % (for *L. niger*) to 24.13 % (for *T. spU2*) but in accordance with OMI results (Fig. 3, Table 4, Table 5).

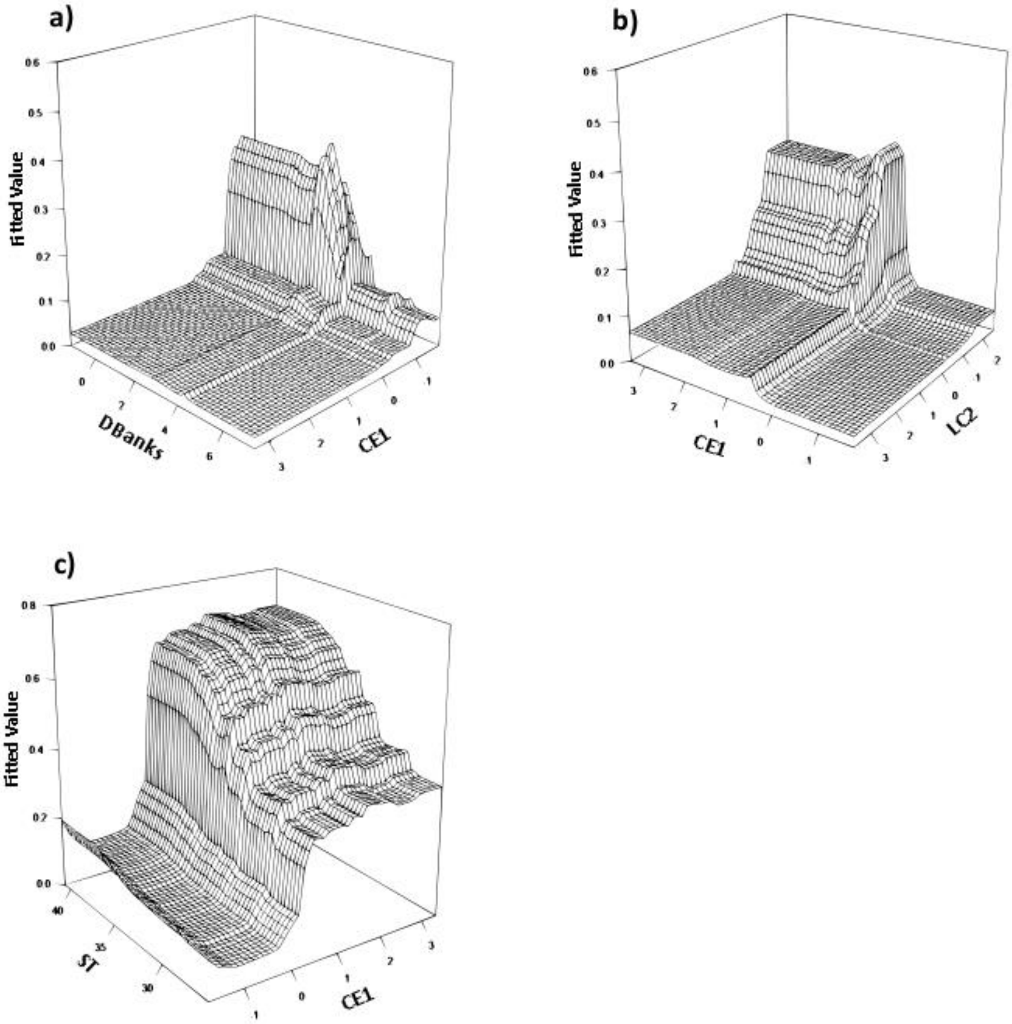
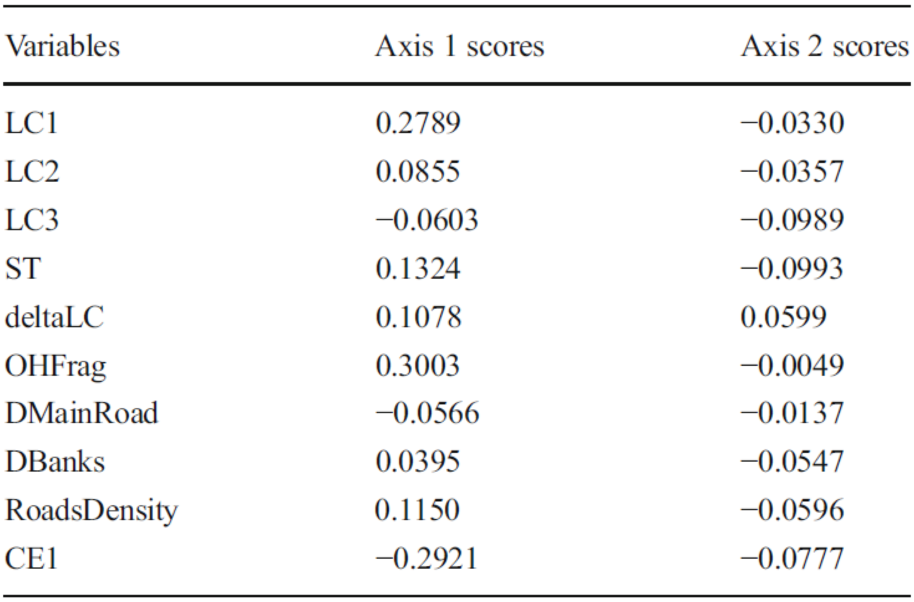


Figure 3: Plot of the most important pair-wise interactions detected by BRTs. a) Interaction between CE1 (Climatic Effects) and Dbanks (Distance to Embankments) for *L. neglectus*; b) Interaction between CE1 and LC2 (Land Cover factor associated with agricultural surfaces amount) for *L. paralienus*; c) Interaction between CE1 and ST (Surface Temperature) for *T. spU2*.

Species distributions were affected by at least 2 environmental factors (except *L. emarginatus* with 3 factors). Climatic effects were detected for 5 species out of 7, which confirmed field observations attesting to stronger Mediterranean climatic influences in the South of the study area. *L. neglectus* and *T. spE* distributions were negatively correlated with CE1, i.e favored by hot/dry climatic conditions, whereas *L. emarginatus*, *L. paralienus* and *T. spU2* showed the opposite pattern (Fig. 2, Table 6). Land cover factors affected *L. alienus* (LC1: negatively correlated with impervious surfaces), *L. emarginatus* (LC3: negatively correlated with open vegetation and positively with forested areas), *L. paralienus* (LC2: negatively correlated with agricultural areas) (Table 6). *L. emarginatus* occurrence was negatively correlated with surface temperatures. Land cover change was negatively correlated with *L. alienus*, i.e. it is favored by newly urbanized areas, and positively correlated with *L. niger* occurrences, which is favored by newly vegetated areas (Table 6). Land cover change was not correlated to any other species occurrences, however, despite acceptable overall kappa scores, some agricultural areas were misclassified as urban, due to absence of plant cover at the date of the imagery, thereby decreasing the accuracy of the measure in parts of the landscape. Only occurrences of *Tetramorium* species were directly correlated to fragmentation: while *T. spU2* was negatively affected by open habitat fragmentation, *T. spE* showed the opposite pattern (Table6).

Table 3: Correlation between each environmental factor and the first two axes of the OMI. The first axis explained 79.16% and the second axis 9.76% of variation.



Variables	Axis 1 scores	Axis 2 scores
LC1	0.2789	−0.0330
LC2	0.0855	−0.0357
LC3	−0.0603	−0.0989
ST	0.1324	−0.0993
deltaLC	0.1078	0.0599
OHFrag	0.3003	−0.0049
DMainRoad	−0.0566	−0.0137
DBanks	0.0395	−0.0547
RoadsDensity	0.1150	−0.0596
CE1	−0.2921	−0.0777

Table 4: Niche parameters of the seven species (in percentages of total variability). OMI represents the distance of the mean conditions of locations occupied by the species to the mean environmental conditions; Tolerance indicates niche breadth; rTol is the residual tolerance, i.e. the percentage of unexplained variability.

Species	Marginality (%)	Tolerance (%)	rTol (%)
L. niger	0.2	10.5	89.4
L. alienus	2.2	16.00	81.7
T. sp.E	3.7	28.50	67.9
L. emarginatus	6.8	30.10	63.1
L. paralienus	8.9	9.90	81.2
L. neglectus	10.3	11.80	77.9
T. sp.U2	13.3	12.70	74.00

Among factors related to human activities, only distance to embankments influenced ant distributions: the invasive ant *L. neglectus* occurred more often in locations close to embankments whereas *L. niger* showed the opposite pattern (Table 6) (See Table S2 for details). The interpretation of functions fitted by BRT models were made complicated by interaction effects, even for factors that were not previously found significant: BRT models detected 3 species for which there were strong interaction effects (i.e. interactions with the highest interaction scores): *L. neglectus*, *L. paralienus* and *T. spU2*. Among these interactions, the strongest implicated climatic factors (i.e. CE1), as well as distance to embankments (DBanks) for *L. neglectus* (Fig. 3a), the agriculture related land cover factor (LC2) for *L. paralienus* (Fig. 3b) and surface temperatures for *T. spU2* (Fig. 3c).

Table 5: Predictive performance of BRT models for each species.

Species	% Explained Deviance	AUC mean	AUC standard errors
L. alienus	5.649861	0.67081	0.02507422
L. emarginatus	6.385291	0.69566	0.01883043
L. neglectus	7.050766	0.70128	0.03554388
L. niger	3.320513	0.63409	0.01752321
L. paralienus	10.310401	0.74345	0.0366056
T. sp.E	10.529285	0.71924	0.01750342
T. sp.U2	24.129223	0.83013	0.01207758

Discussion

In the present study, ant species exhibited specific responses to environmental changes induced by urbanization. As urbanization generates a mixture of several environmental changes, the distribution of each species was affected by a subset of these changes and the factors composing these subsets varied greatly depending on the species considered. Rather unexpectedly, we showed that, even at a reduced spatial scale, climate had as strong explanatory contribution for species distribution, and that it often had interactive effects with urbanization factors. Furthermore, as expected, invasive species distributions (i.e. *L. neglectus*) were related to human activities, here the distance to road embankments; *L. neglectus* was also surprisingly prevalent in the landscape as our study did more than double the number of known occurrences (Fig. 4b) for this species in France, which is likely to be of concern as this kind of landscape invasional pattern is probably a widespread situation both in France and in the rest of Europe.

We found that ecological niche marginality and ecological niche breadth varied across ant species (OMI range from 0.2 to 13.3% and Tolerance from 9.9 to 30.1%). These results indicated that most ant species responded to changes induced by urbanization. Nevertheless, a large part of the variability in species-environment relationships remained unexplained (Fig. 2, Table 4), highlighting the need to take into account other habitat and microhabitat factors directly or indirectly associated with urbanization such as soil pollution (Eeva et al. 2004) or structural properties of soils (Boulton et al. 2005). This limit in explanative power could also be explained by the presence/absence nature of the dataset. Abundance data would improve the description of species-environment relationship, but such data require far more intensive sampling, which would limit the number of sampling locations and consequently hamper the ability to detect low-density species, such as *L. neglectus* in our landscape (Joseph et al. 2006).

Our study clearly showed a species-specific response to urbanization as each ant species was affected by a specific combination of factors (Table 6) and as only *Tetramorium* species were impacted, although in opposite directions, by the same factors (open habitat fragmentation and climate).

Among *Tetramorium* species detected in the sampling area, only two were common: *T. spE* and *T. spU2*. These species have not been formally described yet as they belong to a cryptic complex distributed all over Europe (Schlick-Steiner et al. 2006). *T. spE* is also known to be invasive in North-America since the early 19th century, and has been described as an urban specialist in its invasive range (King & Green 1995) but ecological preferences data in its native European range are scarce. Here, we showed that *T. spE* was indeed associated with urban environments, especially where open-vegetation patches were highly fragmented (Fig. 2, Table 6). OMI analysis also showed that *T. spE* had the largest niche breadth of all species (Table 4), which indicated its ability to tolerate a broad variety of environmental conditions. Globally, *T. spE* appeared the most urban exploiter species (Fig. 2, Fig. 4a), which raises questions about its status in Europe. Indeed, as pointed out by Valery et al. (2009), "invasive species can also be native" since species can, in response to environmental changes in their native range, show the same patterns of populations growth and spread than in their invasive ranges.

On the opposite, *T. spU2* favored the less fragmented areas and showed the typical distribution pattern of an urban avoider (Fig. 2, Fig. 4a). However, we cannot exclude the possibility that the absence of *T. spU2* in urban environments is the result of exclusive competition with *T. spE*. *T. spU2* distribution was also affected by the interaction between surface temperature and climate, suggesting that, although this species was generally absent from urban environments in our study area, it might be favored by urban heat islands at higher latitudes or altitudes.

Table 6: Factors influencing species occurrences. For each species, shaded cells indicate which factors were found to influence species occurrences in both permutations tests and BRT models (Fig. S1). Light grey shading indicates negative correlation between species occurrences and factor; dark grey shading indicates positive correlation between species occurrences and factor. Blank cells indicate factors identified by none or only one of the two analysis methods. "*" indicates main interactions between CE1 and the factor (two "*" indicate medium importance interaction, four "*" main importance interaction (Fig. 3)).

Factor	las_ali	las_ema	las_neg	las_nig	las_par	tet_spe	tet_spu
LC1	░				**		**
LC2					░ ****		
LC3		░					
ST		░	**				****
deltaLC	░			▓			
OHFrag					**	▓	░
DBanks			░ ****	▓			
DMainRoad							
RoadsDensity							
CE1		▓	░		▓	░	▓

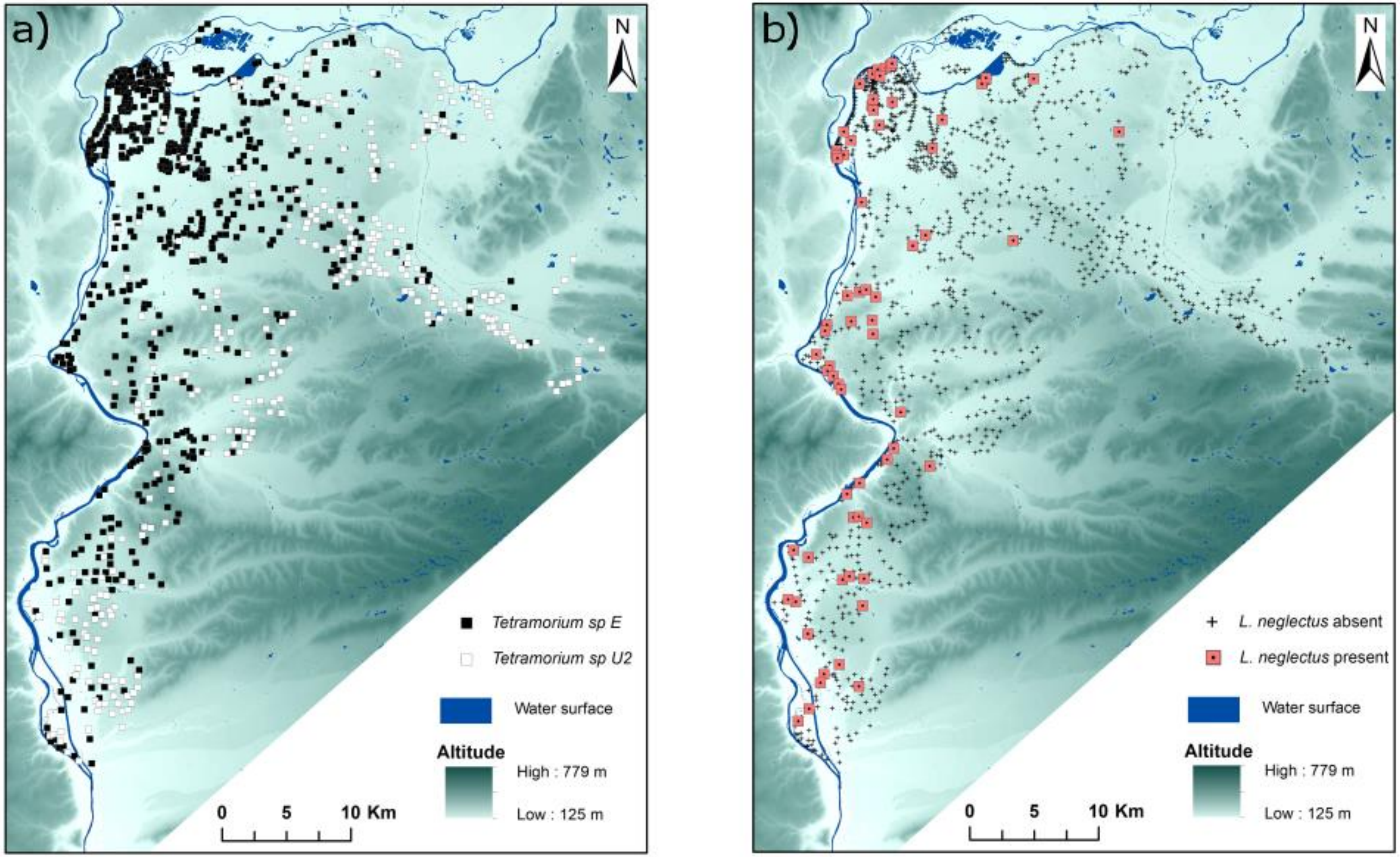


Figure 4: Spatial distribution of a) the two cryptic *Tetramorium* species and b) *Lasius neglectus*.

As expected, *Lasius niger* was the most generalist species, occupying almost 75% of all sampling locations and showing the lowest marginality. This species appeared to be significantly tied to newly vegetated areas (deltaLC), which was not surprising as most land cover changes from urban to vegetation occurred in dense urban areas: as a pioneering species inhabiting urban areas (Czechowski et al. 2013, Seifert 1991), *L. niger* is therefore likely to quickly recolonize these new environments. However, sites with *L. niger* tended to be more distant from embankments than expected by chance. This finding could reflect a competitive exclusion of *L. niger* by *L. neglectus* (the latter being on the contrary significantly tied to embankments) and highlights the possible negative impact of invasive species on biodiversity, *Lasius neglectus* having already been pointed out as a threat for native arthropods species, including other ants (Nagy et al. 2009).

The distributions of other native *Lasius* species (*L. alienus*, *L. emarginatus* and *L. paralienus*) were affected by very different sets of factors. Interestingly, all these species were absent from urban cores although they did not share any common explanative factor affecting their distribution, which illustrates the complexity of urbanization impacts on biodiversity. Additionally, *Lasius paralienus* appeared favored in cold climate agricultural landscapes (interaction between CE1 and LC2). As climate can influence agricultural practices and cultivated species phenology (Craufurd & Wheeler 2009), this relationship raises questions about ant responses to agricultural practices and crop species composition in agricultural landscapes (Peck et al. 1998).

As expected, *L. neglectus* distribution was driven by human activities factors. This is, to our knowledge, the first study indicating the importance of a factor such as the distance to embankments in explaining the spatial distribution of an invasive species. The invasive ant *L. neglectus* is characterized, like many other tramp ant species (e.g., *Linepithema humile*), by a high level of polygyny (several queens in one nest), supercolonial organization and very low self-dispersal abilities (Ugelvig et al. 2008). Unlike most ant species (including all the other species studied here), *L. neglectus* is incapable of dispersal by nuptial flight: colonies expand by budding, which consists in the increase of the area occupied by the colony over the years (Espadaler et al. 2007). This mechanism has been described as a short distance process (few meters to 89 m a year) and is thus very unlikely to explain the invasion pattern of *L. neglectus* throughout the landscape, as the median distance between the 69 invaded locations was 17.7 kilometers. The spatial distribution pattern of this species appears to be mostly due to human-mediated dispersal at reduced limited spatial scale (as 69 independent long distance introduction events are unlikely). Construction of embankments involves soil rearrangement and movement: ground dwelling arthropods, and particularly highly polygynous tramp ants are therefore likely to be displaced with queens, thus enabling colony survival and rapid growth in newly created embankments (King et al. 2009).

An alternative, although non-exclusive, mechanism could be responsible for the relationship between *L. neglectus* and embankments: as suggested by DeMers (1993), anthropogenic microhabitat structures, such as roads ditches or embankments, could act as corridors for range expansion of species experiencing climatic limitation as they reach their northern range limits. Embankments could make highly suitable environmental conditions because their structure is likely to modify abiotic conditions such as solar exposure (and therefore local temperature) or soil compaction and draining, as well as biotic factors such as plant community composition (Vasconcelos et al. 2014). Moreover, a recent study (Schaffers et al. 2012) showed that roadside verges, rather than acting as biodiversity sinks, appeared to be highly suitable reproduction and overwintering habitats for a wide range of arthropod taxa. *L. neglectus* showed higher probability of occurrence embankments located in hot/dry sites, than in cold/wet sites (interaction between Dbanks and CE, Fig. 3a). This observation strengthens the hypothesis that embankments act as human-mediated dispersal endpoints, rather than as warm shelters in a cold and rainy environment.

Because of the spatial scale of our study area, we did not expect strong and general effect of climate on ant species distributions. Remarkably, climatic effects (CE1) influenced the spatial distribution of 5 out of 7 species, highlighting that, even at reduced spatial scales, coarse environmental features can affect biodiversity patterns and consequently have to be taken into account. Furthermore, climatic effects often acted in interaction with factors associated with urbanization (Fig. 3). This suggested that, for a given species, the explanative combination of factors may depend on climatic context, which is likely to affect species responses to urbanization along latitudinal and altitudinal gradients. For example, a species could be defined as an urban avoider at lower latitudes (hot and dry) and an urban exploiter at higher latitudes (cold and wet); because urban heat island effects would turn urban environments into a more favorable habitat in the second case. This example was illustrated in our results by the interaction between climatic effects (CE1) and surface temperature for *Tetramorium spU2*.

Conclusion

Shochat et al. (2006) discussed the necessity for urban ecology to balance between descriptive and experimental (mechanistic) ecology in order to develop a better understanding of ecological and evolutionary processes operating in urbanized landscapes and ecosystems. Precisely identifying species-environment relationships in urbanized contexts is therefore a necessary logical step that should offer pertinent directions for future experimental studies (Stewart et al. 2015). Furthermore, it clearly appears that future urban ecology studies will have to deal with complex interactions between global changes (Radinger et al. 2015). Consequently, further studies focusing on the effects of urbanization on biodiversity should aim to compare urban areas, using multiple gradient (e.g., climate, human social and economic activities) sampling designs in order to better disentangle the many intricate factors affecting species distributions.

Electronic Supplementary Material

Table S1: Initial environmental variables

Table S2: Permutation tests and BRT results.

Figure S1: Principal component analyses used to reduce the number of variables.

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Author contributions

JMWG wrote the paper; NM and BK supervised the writing and bring significant improvements to the paper; LM, JMWG and BK conducted most of the field and ant identification work; JD produced the surface temperatures data; AB produced the land cover data; JMWG processed all other GIS data and all statistical analyses. AD realized most molecular identifications.

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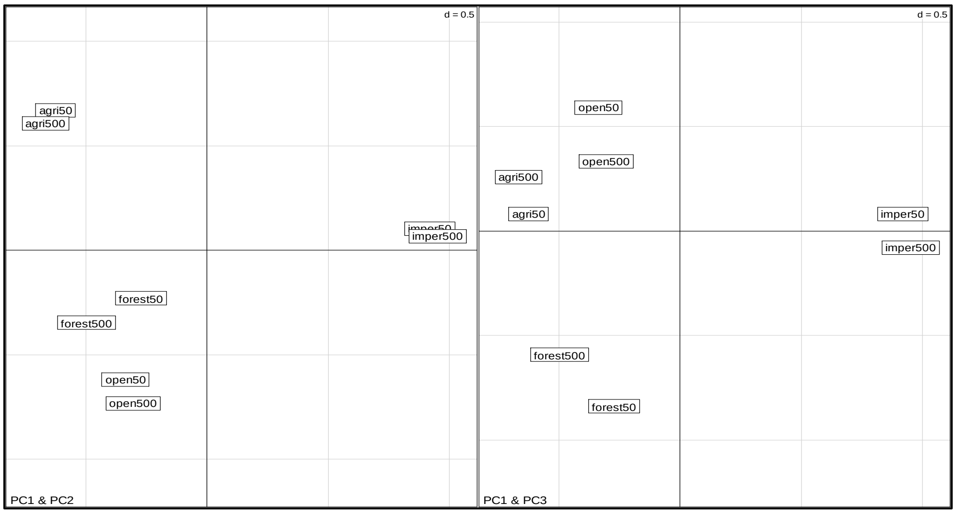
Electronic Supplementary Material

Figure S1: Principal component analysis used to create artificial variables

1) PCA on Land Cover (LC) variables set

Principal components (PCs) 1, 2 and 3 are selected:
LC1= PC1: 38.95 %
LC2= PC2: 24.1 %
LC3= PC3: 19.97 %
(PC4: 6.61 %)
Explained variability of the selected PCs: 83.02 %



2) PCA on Topography and climate (CE) variables set

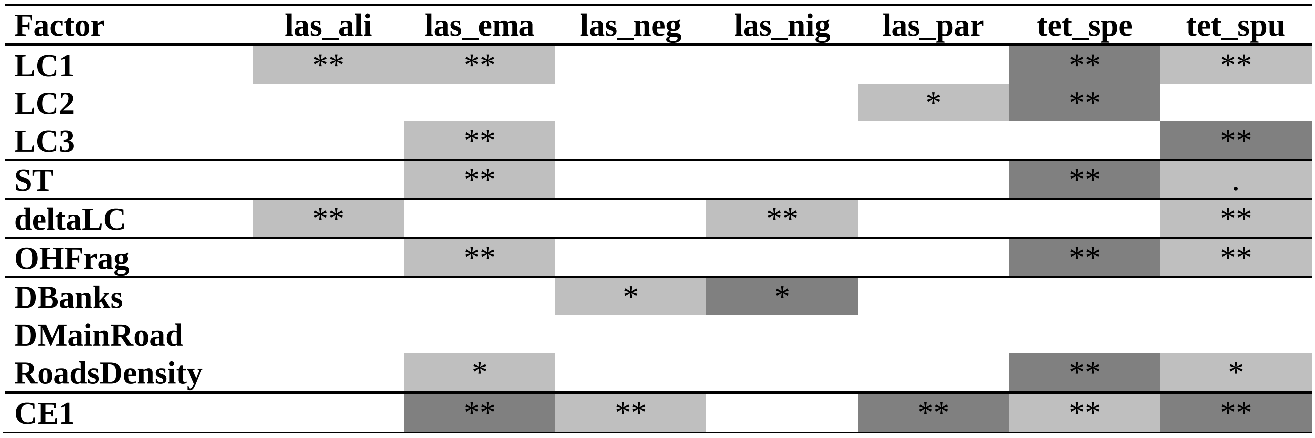
Principal component 1 is selected:
CE1= PC1: 86.03 %
(PC2: 9.85 %)
Explained variability of the selected PC: 86.03 %

Table S1: Initial environmental variables

Factors category	Factors	Source	Extraction type	Initial Resolution	Unit
Land Cover	impervious 50	BDTOPO® IGN + SPOT 6	50 m buffer	1.5m	m²
	impervious 500	BDTOPO® IGN + SPOT 6	500 m buffer	1.5m	m²
	agricultural 50	RPG12	50 m buffer	1.5m	m²
	agricultural 500	RPG13	500 m buffer	1.5m	m²
	open 50	BDTOPO® IGN + SPOT 6	50 m buffer	1.5m	m²
	open 500	BDTOPO® IGN + SPOT 6	500 m buffer	1.5m	m²
	forest 50	BDTOPO® IGN	50 m buffer	1.5m	m²
	forest 500	BDTOPO® IGN	500 m buffer	1.5m	m²
Urban Heat Island effect	Surface temperature	Landsat8	50 m buffer	30 m	°C
Land cover change	Delta NDVI	Landsat5 and Landsat8	100 m buffer	30 m	none
Open habitat fragmentation	Mean patch area	BDTOPO® IGN + SPOT 6	500m buffer	1.5m	-log (m²)
Human Activities	Distance to main road	BDTOPO® IGN	distance	vectorial	log(m)
	Secondary roads Density	BDTOPO® IGN	ponctual	vectorial	m/km²
	Distance to Talus	BDTOPO® IGN	distance	vectorial	log(m)
Altitude and climate	Altitude	BDALTI® IGN	ponctual	25 m	m
	Annual mean temperature	Bioclim	ponctual	750 m	°C
	Annual mean precipitation	Bioclim	ponctual	750 m	mm

Table S2: Permutation tests and BRT results.

A) Permutations tests factors identification. Light grey shading indicates negative correlation between species occurrences and factor; dark grey shading indicates positive correlation between species occurrences and factor.



Factor	las_ali	las_ema	las_neg	las_nig	las_par	tet_spe	tet_spu
LC1	**	**				**	**
LC2					*	**	
LC3		**					**
ST		**				**	.
deltaLC	**			**			**
OHFrag		**				**	**
DBanks			*	*			
DMainRoad							
RoadsDensity		*				**	*
CE1		**	**		**	**	**

B) BRT factors relative contribution for each species (%).

Factor	las_ali	las_ema	las_neg	las_nig	las_par	tet_spe	tet_spu
LC1	10.29	9.22	5.28	5.52	13.93	5.08	8.38
LC2	10.68	6.77	6.16	7.41	14.53	8.27	6.72
LC3	10.6	16.31	5.21	7.23	5.54	5.11	8.68
ST	6.17	15.17	10.18	12.7	8.1	6.12	8.74
deltaLC	14.2	5.99	10.07	13.86	3.65	6.86	4.3
OHFrag	11.08	8.43	7.23	5.14	12.71	27.03	15.96
DBanks	7.6	8.01	14.34	15.49	3.84	4.96	4.8
DMainRoad	7.74	7.64	11.24	7.55	5.53	9.37	5.6
RoadsDensity	8.58	9.07	8.86	9	7.93	5.05	3.92
CE1	13.04	13.38	21.42	16.09	24.23	22.15	32.9

CHAPTER 2

<u>PART 1:</u> Article submitted in **Diversity and Distributions**

Why ecologists should focus on the "human" in "Human-mediated dispersal"

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Abstract

The introduction of exotic species and their subsequent spread are rapidly increasing worldwide, owing to the intensification of human activities favoring species dispersal at multiple spatial scales. Although human-mediated dispersal plays a central role in both introduction and secondary spread processes, it is yet poorly understood and only few studies have attempted to conceptualize and model it. In this *Biodiversity Viewpoint*, we propose to address human-mediated dispersal by focusing on human activities rather than on dispersed species. The nature of human activities, as well as the vectors and the networks involved, are likely to affect the dispersal probability and parameters (e.g., its distance or its direction) for any given species. Our approach should contribute to a more general conceptual framework of human-mediated dispersal and promote the development of relevant models to describe and predict it. Multidisciplinarity is at the core of this approach, and will require interaction between ecologists, sociologists, economists, computer and transportation scientists, as well as applied scientists engaged in invasive species management and control.

Keywords Human activities, conceptual framework, transport, movement, biological invasions

Introduction

Biological invasions are characterized by three main processes in which human activities often play a crucial role: introduction, establishment and secondary spread, (Lockwood et al. 2013). While climate and land cover changes are likely to increase habitat invasibility (Alpert et al. 2000) by intensifying disturbance (Chown et al. 2015, Vonshak & Gordon 2015), dispersal is a *sine qua non* process of biological invasions. Human-mediated dispersal (HMD) is the cause of the majority of introduction events worldwide (Wilson et al. 2009), enabling species to cross biogeographic barriers and access new environments (Davis 2009, Lockwood et al. 2013), as well as leading to secondary introductions from previously invaded areas (Ascunce et al. 2011). Global scale HMD, generally referred as Long Distance Dispersal (LDD), generates species introductions through global scale transportation by national and international commercial exchanges, tourism, material transportation or trade in living organisms (Lockwood et al. 2013, Hulme 2015). Recently, Auffret et al. (2014) underlined the fact that HMD acts at multiple spatial scales and is thus implicated in both dispersal stages of biological invasions, i.e., introduction and secondary spread (Blackburn et al. 2011). There is indeed growing evidence that local commercial exchanges or small scale recreational activities are efficient vectors of secondary spread (Johnson et al. 2001, Crespo-Perez 2011, Clarke Murray et al. 2011, Bergey et al. 2014, Egizi et al. 2016). However, to our knowledge, the implication of HMD in secondary spread (local and regional propagation) has been neglected to date, in comparison to HMD involved in introduction (LDD, e.g., Robinet et al. 2011, but see Crespo-Perez et al. 2011), especially in theoretical and modeling studies (Lockwood et al. 2013, but see Pitt et al. 2009 and Savage & Renton 2014). Because most modeling studies are idiosyncratic (designed for a particular situation and species), the main concern in HMD modeling seems to be the lack of a strong theoretical framework establishing foundations and proposing research directions for future conceptual and modeling approaches.

In this *Biodiversity Viewpoint*, we argue that HMD studies should, contrary to current trends in the field, primarily focus on human activities rather than directly on organisms themselves. We also explore the interactions between the characteristics of human activities and species biological traits (Banks et al. 2015). This approach constitutes a first step towards elaborating a general framework for studying human-mediated dispersal, enabling the construction of general and relevant predictive models, as well as monitoring plans, at multiple spatial scales.

Anyway, anyhow, anywhere? Human activities and vectors control dispersal parameters

Humans are likely to displace organisms in several ways (Wilson et al. 2009). A broad variety of examples exist in the literature and two major dispersal modes have been described: (i) *dispersal by movement* and (ii) *dispersal by transport* (Auffret et al. 2014).

(i) Dispersal by movement is a well-known process in plant invasions (Zwaenopoel et al. 2006) where seeds are dispersed by attachment/detachment to persons, private and professional vehicles, pets, livestock (Auffret et al. 2014), and by the airflow of vehicles ("dispersal by traffic": von der Lippe and Kowarik 2008, von der Lippe et al. 2013). In dispersal by movement, dispersal parameters depend on attachment/detachment probabilities or on movements induced by air or water flows, resulting from complex interactions between vector characteristics and species biological traits (Wrange et al. 2016, von der Lippe et al. 2012).

(ii) Dispersal by transport consists in the displacement of organisms by human transportation activities, which move all kinds of goods and organisms (e.g., water, soil, construction material, plants, fruits, vegetables, grains, logs, livestock and human beings) from a point of departure to a point of arrival (Crespo-Perez et al. 2011). As a consequence, the dispersal parameters of a transported species are highly dependent on transport characteristics (e.g., distance between departure and arrival, frequency of transport events). Dispersal by transport is most likely to be involved in LDD events (i.e. global scale HMD), but also in HMD at regional and landscape scales (Johnson et al. 2001).

Table 1: How the characteristics of human activities affect the parameters of HMD.

HMD mode	Human activity characteristics	HMD parameter affected
Transport	Distance between departure and arrival	Dispersal distance distribution
	Frequency of movement	Propagule pressure
	Freight/passenger types	Species identity
	Amounts transported	Survival during and after transport
Movement	Traffic density	Dispersal frequency
	Attachment probability	Propagule pressure
	Detachment probability	Dispersal distance distribution
	Vehicle speed (generated flow)	Dispersal distance distribution

These two HMD modes may have very different outcomes on the dynamics of invasive species dispersal. Therefore, it is essential for ecologists to measure the relative importance of both processes in any biological invasion. For example, the dispersal parameters (e.g., transportation distance, see Fig. 1) of a seed strongly differ if it is attached to a car by mud or if it is transported with soil in a car trunk. Species biological traits may also affect the probability to be dispersed by movement or by transport (e.g., producing winged seeds facilitates dispersal by vehicles' airflow, von der Lippe et al. 2013). In addition, the probability to be dispersed by movement or transport depends on spatial features (e.g., distance to road side, von der Lippe and Kowarik 2008) and temporal or phenological features (e.g., dispersal by movement mostly affects seeds of a terrestrial plant, while dispersal by transport also affects rhizomes or adult individuals, Johnson et al. 2001).

Although poorly considered in most modeling studies, the characteristics of human activities, such as the nature and amount of material transported or the speed of vectors, are likely to strongly affect HMD parameters such as dispersal distance or propagule pressure (Table 1). The identity of human activities and the vector used may consequently have a major influence on the parameters of HMD (Perkins et al. 2014) (Table 1). Indeed, the movements of people and goods tend to be optimized following geographical, economical, time, political and environmental constraints (Bhat 1996, Beliën and De Boeck 2012, Wang et al. 2016, Karlson et al. 2016). For instance, private cars are mostly used in local movements, therefore participating in landscape secondary spread, whereas planes are used for global movements generating primary or secondary introduction events (Fig. 2).

A first step in disentangling the potential consequences of human activities on HMD should be to review the travel parameters of all human activities potentially involved in transport or movement dispersal. Such data is likely to be found in socio-economics and transportation studies and surveys (e.g., Great Britain road use statistics – www.gov.uk.com, New Zealand household travel survey – www.transport.govt.nz), but will necessitate significant efforts to be collected and homogenized. A promising opportunity to collect important amount of high quality data may lie in the combination of mobile phone carriers (e.g., Song et al. 2010) and interviews (Perkins et al. 2014), which could help to estimate several travel parameters for a broad range of human activities.

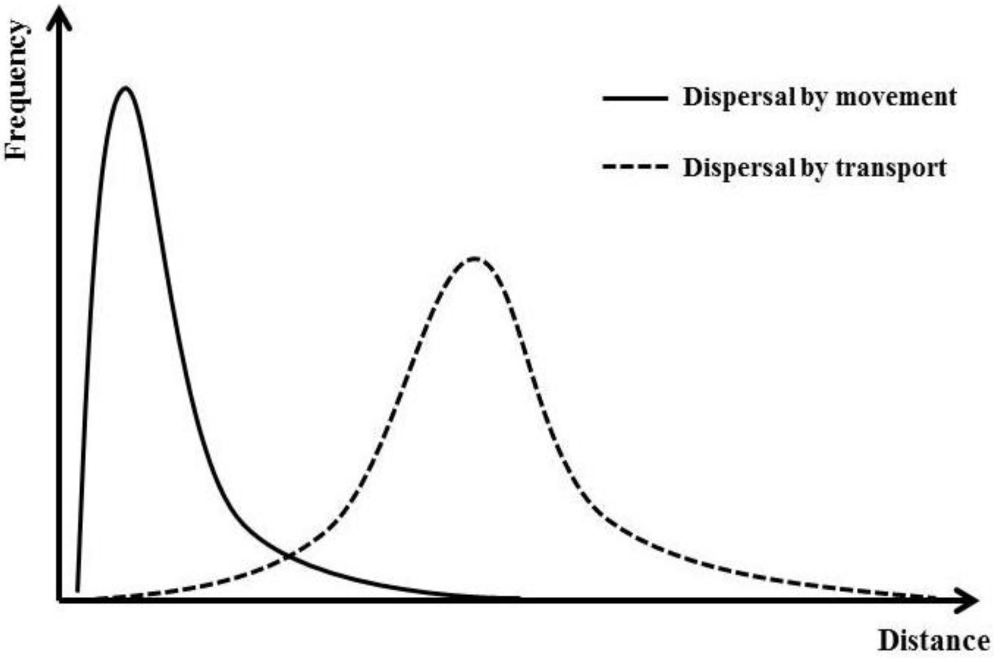

Figure 1: Comparison of expected dispersal distance distributions of a same species with the same vector (e.g., a car), depending on the HMD mode. Dispersal by movement is in solid line, dispersal by transport in dashed line. These theoretical distributions are based on von der Lippe et al. (2013) for dispersal by movement and on Great Britain road use statistics 2016 (www.gov.uk.com) and European driving patterns 2012 (http://ec.europa.eu/index_en.htm) for dispersal by transport.

And networks also matter: the geography of human-mediated dispersal

Transportation networks play a crucial role in invasion dynamics because they spatially connect geographical areas and make it possible for vectors to access locations throughout landscapes (Banks et al. 2015). Human activity networks (e.g., pedestrian and bicycle paths, roads, railways, fluvial, maritime and aerial routes) have specific topologies and a specific set of vectors using them (e.g., cars, trucks, freight trains, recreational boats and planes). Network types and topologies therefore have major impacts on the spatial and temporal outcomes of HMD (Banks et al. 2015, Fig. 3). Consequently, while maritime and aerial routes are strongly implicated in global HMD leading to introduction events, fluvial and road networks may be the principal sources of regional and local HMD enhancing secondary spread (Fig. 2).

In addition, HMD direction is biased by the topology of networks (e.g., HMD generated by freight trains follows railways linking major traffic hubs). This directional bias in the dispersal of invasive species, although generally neglected, determines connectivity at all spatial scales, from landscapes (e.g., via road networks, Ansong et al. 2013) to continents (e.g., via aerial routes, Hulme 2009). Taking into account the topology of transportation networks to estimate the direction of HMD may thus greatly improve modeling studies, where parameters controlling the direction of dispersal are often inexistent (e.g., Robinet et al. 2011) or isotropic (e.g., Pitt et al. 2009). Moreover, socioeconomic, political and historical features are likely to affect the structure and properties of transportation networks at all spatial and temporal scales. For example, speed limitations, management of road verges and materials importation policies, which vary between countries, counties and even neighborhoods as well as through time, are likely to affect HMD by modifying the parameters of dispersal by movement and transport (Table 1).

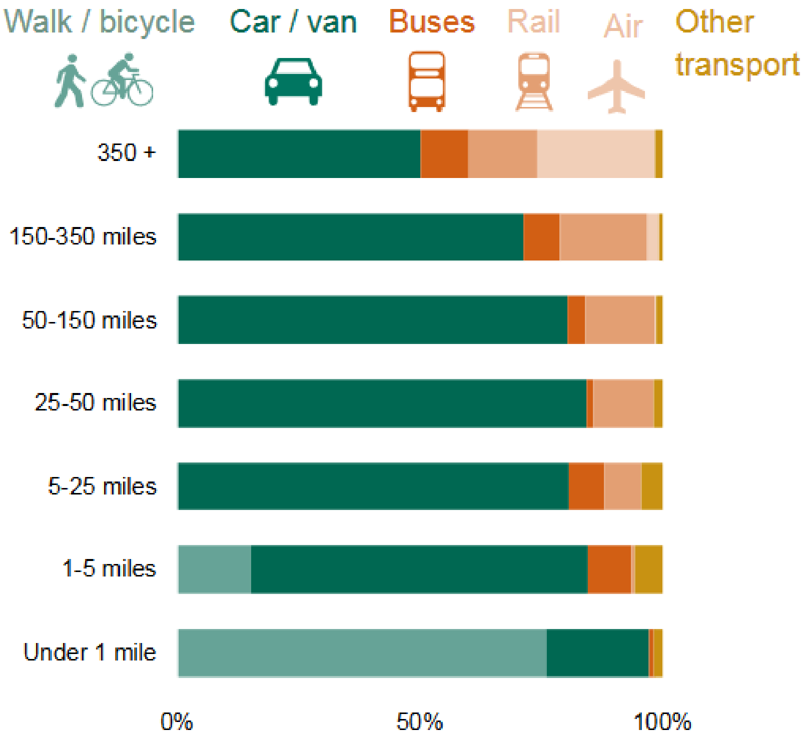

Figure 2: Average proportion of trips by main modes and distance in England in 2014. Trips over 50 miles represent 2% of all trips. These statistics include household activities such as commuting, business, education, shopping or recreation. From “Road use statistics Great Britain 2016” (NTS0308), National Travel Survey, Department of Transport, Gov.uk.com

Beyond the importance of network topology, it could be argued that networks and vectors are so intertwined that taking both into account would be redundant. However, a single network may be used by several types of vectors (example in Fig. 3), and networks can comprise several sub-networks with very different properties (gov.uk.com). For example in road networks, highways and local roads are unlikely to have the same impact on HMD because they differ in traffic density, vehicle identities (e.g., private car, van, truck) and speeds, human activities involved as well as spatial and temporal scale of movements.

In addition, networks and vectors involved in dispersal events provide indications on dispersal characteristics, and constitute valuable information for investigating human-mediated invasion dynamics at multiple spatial scales (Fig. 3).

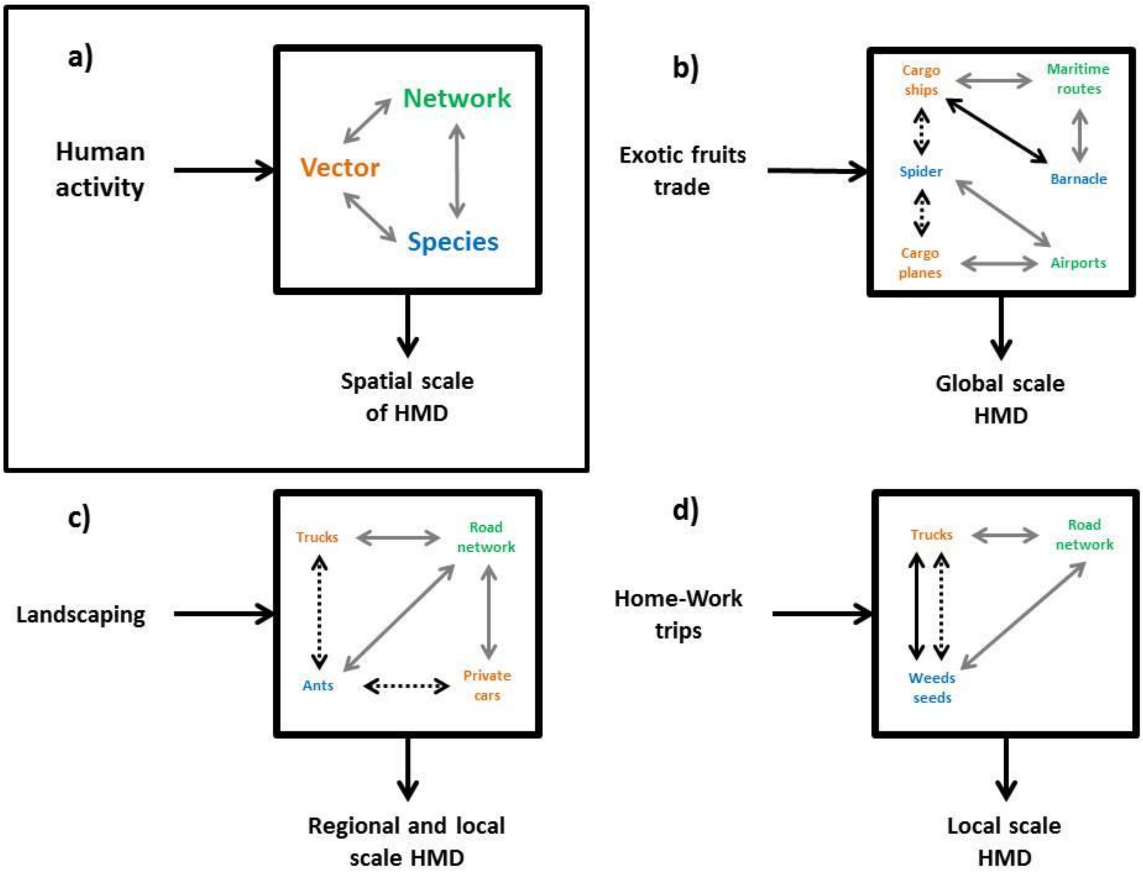

Figure 3: Human-mediated dispersal (HMD) conceptual framework. a) A general HMD framework involves a human activity, interacting with vectors, networks and species (double arrows) to shape invasion dynamics and particularly the spatial scale of HMD. **b) c) d)** Theoretical examples of HMD involving different human activities (with different consequences on vectors, species and networks) resulting in different spatial scales of HMD. Double arrows indicate possible interactions between vectors, species and networks. Black arrows indicate either dispersal by transport (dashed) and movement (solid). These examples are based on empirical studies: **b)** Nentwig 2015 for spiders and Wrange et al. 2016 for barnacles, **c)** King et al. 2009 for ants, **d)** van der Lippe et al. 2013 for seeds.

How many roads must an alien species walk down? Introduced species as hitchhikers

Local spatial scale HMD is often underestimated or even neglected because most invasive species can disperse on their own throughout landscapes (e.g., Robinet et al. 2011). Nevertheless, unintentional introductions of alien species result from HMD, and species transported across biogeographical barriers are likely to be those with a propensity to be transported, i.e. *hitchhiker species* likely to be picked up, to survive transport and to establish viable populations after transport (Rabitch 2011, Ansong et al. 2013, Wrange et al. 2016). Consequently, introduced species, even if they have natural dispersal abilities, should have higher probabilities of experiencing post-introduction HMD than any random native species. Depending on the natural dispersal capabilities of alien species, post-introduction HMD should contribute differently to spread depending on the spatial scale (Egizi et al. 2016). Alien species for which HMD is the major contributor to secondary spread and landscape infill, i.e. efficient hitchhiker species, should also have a higher propensity for secondary introduction from invaded landscapes. Such processes may generate strong selective pressure on the propensity of species to exploit HMD. In consequence, hitchhiking propensity could be considered as a biological trait under selection, enhancing the fitness of individuals better at hitchhiking. Maintaining selective pressures on hitchhiking propensity could even lead to the emergence of a “hitchhiking syndrome”, with tremendous consequences on invasion dynamics at multiple scales.

Conclusion

Human-mediated dispersal is a major mechanism of biodiversity displacement and is predicted to amplify with the increase of human populations, the urban revolution and the globalization of commercial exchanges (Hulme 2009). Given the negative consequences of species invasions on biological diversity, ecosystem functioning, as well as human economy and health (Davis 2009), management strategies are needed to “save what is left”, by preventing or at least limiting further invasions (Alpert et al. 2000). Identifying the key parameters of invasion dynamics and producing accurate invasion scenarios in order to adopt efficient control strategies should be the most efficient first step in a successful strategy, which in turn, may hinge on precise and generalizable modeling approaches, based on strong conceptual frameworks (Savage & Renton 2014, Banks et al. 2015). However, while natural dispersal is a well-known and well-modeled process (Lockwood et al. 2013), human-mediated dispersal is yet underrepresented in modeling studies, especially at reduced spatial scales (but see Crespo-Perez et al. 2011 and Pitt et al. 2009). Studying HMD through a multidisciplinary approach combining socio-economic, transportation and ecological sciences, and focusing on human activities rather than directly on species, may contribute to unlocking conceptual barriers which have prevented new insights and advances in modeling so far. The coming decades promise new cooperation between ecologists, sociologists, economists, transportation and computer scientists to lift science to the challenge of predicting how human beings disperse other species over space and time.

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Author contributions: JMWG and CR developed the idea; JMWG wrote the paper; CR, NM and BK brought significant improvements to the paper.

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CHAPTER 2

PART 2: Article in preparation

MoRIS: Modeling, Estimating and Predicting Human-Mediated Secondary Spread of Invasive Species

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Abstract

1. Human-mediated dispersal acts as a vector for many exotic species, both at the introduction and secondary spread stages. Primary and secondary introductions arise from human-mediated long distance dispersal happening at global scales. Secondary spread occurs at smaller spatial and time scales (e.g. landscape) and results from either natural or human-mediated dispersal. Despite the importance of materials transportation (e.g. for landscaping, construction) for the spread of invasive species, few studies have investigated short distance human-mediated dispersal and even less have tried to model it.

2. We developed MoRIS (*Model of Routes of Invasive Spread*), a spatially explicit spread model designed to simulate invasive species dispersal by transport at local to regional spatial scales. MoRIS is an innovative, yet simple model, taking into account the road network topology to influence the direction of dispersal events. MoRIS is designed to minimize *a priori* making (e.g., expert knowledge), to enable the estimation of human-mediated dispersal parameters based on a simple presence/absence locations dataset and to produce predictive maps of spread.

3. We applied MoRIS to the invasive garden ant *Lasius neglectus*, a particularly relevant species to validate the model as it has no natural dispersal abilities and is thus entirely dispersed by transportation activities. Based on a published presence/absence dataset of *L. neglectus* in an urbanized landscape (Lyon, France), we estimated the introduction area, the average transportation distance and the frequency of transportation events involved in the invasive ant landscape spread. Using indirect validations, we showed that our estimation were realistic and even accurate.

4. By focusing on human transportation activities and network topology rather than directly on species, MoRIS provides a flexible and objective approach widely generalizable to any taxa dispersed by human activities using road network. Besides constituting a useful tool for managers, MoRIS aims to fill a gap in the modeling of human-mediated dispersal and to bring new practical and conceptual insights in its study.

Keywords Human activities, network topology, spread model, secondary spread, dispersal by transport, road network

Introduction

Human activities are responsible for biological invasions worldwide by introducing species out of their native range, facilitating establishment, and enhancing secondary spread (Wilson et al. 2009, Davis 2010). Species distribution models (SDMs, Guisan and Zimmermann 2000) have been widely used over the past decades, but they mostly focused on establishment probabilities and neglected species dispersal (Guisan and Thuiller 2005). Despite their crucial role in invasion success, the stages of introduction and secondary spread are poorly represented in theoretical and modeling works (Puth and Post 2005). In addition, most of existing models focused on natural dispersal (Robinet et al. 2011, Hooten and Wikle 2008, Higgins et al. 1996, Adams et al. 2015), while the modeling of human-mediated dispersal (HMD) is globally underrepresented (Puth and Post 2005, Gippet et al. *submitted Viewpoint*). The few existing attempts to model HMD were generally case specific and focused on long distance dispersal (LDD) leading to both primary and secondary introductions (Robinet et al. 2011), but they ignored landscape-scale secondary spread (but see Crespo-Perez et al 2011,Pitt et al 2009 and Chkrebtii et al 2015). However, growing evidence suggests that HMD is not only a key process of invasion success at the introduction stage, but also during the secondary spread stage (Minchin et al. 2006, Rouifed et al. 2014, Egizi et al. 2016). Examples may be found in North American Great Lakes, Mediterranean Sea and Pacific Ocean coasts, where aquatic invasive species have been shown to be locally transported by recreational boating activities (Kelly et al. 2013, Clarke-Murray et al. 2011, Dan Minchin et al. 2006). In terrestrial landscapes, HMD involving transportation of materials such as soil, plant residues, horticultural plants or food for humans and livestock have been pointed out as potential vectors of invasive species' spread through landscapes, for a wide variety of organisms, including plants, crustaceans, insects (e.g., ants, mosquitoes), mollusks, earthworms and nematodes (Hodkinson and Thompson 1997, King et al. 2009, Bigsby et al. 2011, Rouifed et al. 2014, Crespo-Perez et al. 2011, Chkrebtii et al 2015, Gertzen and Leung 2011, Chytry et al. 2012, Aubry et al. 2006, Johnson et al . 2006, Robinet et al. 2009, Egizi et al. 2016).

Models of human-mediated secondary spread are therefore clearly needed and would constitute important predictive tools for invasion managers and conservation biologists. Taking into account HMD in secondary spread may produce more accurate predictions for a large range of invasive species, especially for species which mostly or exclusively disperse by HMD (Crespo-Perez et al. 2011, Egizi et al. 2016).

The methodological gap in HMD modeling probably results from the complex nature of human activities. Human movements constitute a whole topic of research (Gonzales et al. 2008), and ecologists cannot hope to properly incorporate it into HMD models with only basic stochastic long distance jump events (e.g., Pitt et al. 2009, Robinet et al. 2016). Spatially explicit mechanistic approaches using parameters with strong socioeconomic, geographic and ecological meaning are essential to construct relevant models, to clearly identify key parameters of HMD and to apply efficient management strategies (Auffret et al. 2014, Savage and Renton 2014, Adams et al. 2015). Using models specifically designed for HMD and thus focusing on human activities rather than directly on species (Gippet et al. *submitted Viewpoint*) should be central to understanding and predicting invasion dynamics.

We developed MoRIS (*Model of Routes of Invasive Spread*), a spatially explicit landscape spread simulation model designed to simulate invasive species dispersal by transport at local to regional spatial scales (Auffret et al. 2014, Gippet et al. *submitted Viewpoint*). Human activities involving materials transportation (e.g., road building and repair, private or industrial landscaping, firewood transport or recreational boating) are spatially constrained by the transportation network they use. As a consequence, network topology has a major influence on dispersal by transport, by inducing directionally biased spread paths through the landscape (Banks et al. 2015). Consequently, in MoRIS, the direction of transportation events depends on the topology of the network used by human vectors. In addition, rather than calibrating the model with expert knowledge, MoRIS enables the estimation of multiple parameters of human transportation activities (e.g., distance, frequency, introduction area), based on a simple species presence/absence dataset. MoRIS can be applied to any terrestrial landscape with linear transportation networks, and to any species transported by human activities.

In this article, we first present our modeling approach, as well as the different outputs of MoRIS. Second, we apply MoRIS to an empirical case: the invasive ant *Lasius neglectus*. This species is a well suited biological model for studying HMD and testing our modeling approach, because its spread is exclusively mediated by human activities involving soil transportation (e.g. landscaping or road building).

Materials and methods

MoRIS is a spatially explicit simulation model which aims to estimate and predict HMD with as little *a priori* expert knowledge as possible.

The minimal requirements of MoRIS are a map of presence/absence of the studied species and a description of the road network implicated in HMD. Four simple input files (text files) are necessary to run MoRIS and estimate human transportation parameters. Three of these files are obtained with basic spatial operations using geographic information system (GIS) software (see the construction of input files below, *Step 3*).

The model is based on an undirected weighted graph G representing the road transportation network of interest. This graph is generated using GIS methods. Basically, the landscape of interest is discretized in a regular tesselation of cells. Every road intersecting a common border between two cells constitute a link between them, thereby generating a simplified representation of the road network. The graph is thereafter modified by creating one node per cell, the weight w between each neighboring cell being the sum of individual roads connecting them, weighted by the type of road under concern (*e.g.*, heavier weights can be assigned to highways than to secondary roads). Hence, two nodes not sharing any road have a link of weight $w=0$. Each node i is given a presence/absence status $S_i \in [0, 1]$. HMD dispersal events are simulated by self-avoiding random walks on the graph, determined by five parameters: **(i)** mean transportation distance μ (in number of jumped cells), **(ii)** mean number of materials transportation events per year λ, **(iii)** location of the initial introduction ι, **(iv)** time in years since the initial introduction t, and **(v)** pick-up probability p (see Table 1 for a full description of MoRIS parameters). These parameters correspond to transportation parameters and are thus mostly extrinsic to the species being dispersed, except for p (discussed below). Consequently, they are applicable to any species likely to be dispersed by the same modalities of human transportation. MoRIS uses the topology of the transportation network to determine the direction of each jump. Three main equations determine the dynamics of the simulated dispersal.

(i) At each time-step t and for each node i, the number of jumps per year J_i is computed as following: the total number of transportation events T_i is drawn from a Poisson law $\mathcal{P}(\lambda)$.

Then, J_i is drawn from a binomial law $\mathcal{B}(n=S_i*T_i, p=p_i)$. $S_i=1$ if the cell is invaded (presence of the species), else $S_i=0$ (absence of the species).

(ii) Then, for each cell i with $S_i = 1$, a random walk is computed by performing μ cell-to-cell jumps. Because the random walk is self-avoiding, each cell i has a temporary status V_i indicating if the cell has been visited during the walk ($V_i = 1$) or not ($V_i = 0$). The probability to jump from cell i to cell j follows equation 1 rules:

$$\mathrm{P}(i \rightarrow j) = \frac{w_{ij} * (1-v_i)}{\sum_{k=1}^{n}(w_{ik} * (1-v_k))} \qquad \text{(eqn 1)}$$

With n the number of adjacent nodes connected to i in the graph G.

The presence/absence status S of the last visited cell of the walk is then set to 1. During a random walk, if all neighbors of the current cell i are visited (i.e. $p(i \rightarrow j) = 0$), the walk stops at i. If a jump leads to a map border, the transportation event is considered null. At each new random walk, the V status is reinitialized to 0 for all cells, and the V status of the introduction cell is set to 1. Using this methodology, the direction of dispersal events only depends on the structure of the road network, and can be highly anisotropic. At the end of each simulation, MoRIS provides a binary list of cell states (cells are invaded or not). It is thus necessary to run many simulations with the same parameters set (but with a different seed), in order to estimate the probability distribution of the invasion scenario.

(iii) Finally, a map of presence probabilities is computed. To this aim, for each cell i the proportion f_i of simulations that led to an invasion ($S_i = 1$) is computed, so that:

$$f_i = \frac{\sum_{i=1}^{l} s_i}{l} \qquad \text{(eqn 2)}$$

With l the number of simulations.

The map of simulated presence probabilities is then compared to the empirical map by computing a purpose-made distance score: the *Hypergeometric Score* (HS). This score takes into account the sampling effort in the empirical presence/absence map by considering the total number of samples per cell. To this aim, instead of simply using the observed frequency of the studied species among all the samples within a cell, we use the probability of observing this frequency knowing the sampling effort in this cell (i.e. the likelihood of the observation). This likelihood can be computed using several methods. We chose the hypergeometric law, which is used in the exact Fisher test for low sample sizes. For reasons of optimization efficiency (see below), the score is normalized and squared. The *HS* score is then computed as following:

$$HS = \sum_{i=1}^{n} \left(1 - \frac{\text{Hypergeometric}(y_i^{obs}, n_i^{obs}, y_i^{sim}, l)}{\text{Hypergeometric}(y_i^{sim}, l, y_i^{sim}, l)} \right)^2$$

*(*eqn 3*)*

With *n* the total number of cells. N_i^{obs} is the total number of empirical samples in cell I, y_i^{obs} being the number of samples where the invasive species is present.

The complete simulation algorithm of MoRIS is represented in a simplified form in Fig. 1.

Table 1: Parameters included in MoRIS.

Parameter	Variable	Description	Comments	Estimation
Age	*t*	Number of years since the introduction	The parameter is fixed but can be estimated by running MoRIS with different numbers of cycles	Indirect
Introduction	ι	Cell of introduction	Two sub-parameters: the coordinates (x,y) of the cell.	Direct: CMA-ES
Pick-up probability	*p*	Probability of picking up the species during a transportation event	see eqn 2. One independent value per cell.	None. Parameter fixed by the user or determined by a submodel.
Frequency	λ	Number of transportation events per cell and per year	Mean of a Poisson law. Value common to all cells.	Direct: CMA-ES
Distance	μ	Length of a self-avoiding random walk during one dispersal event	The probability distribution of dispersal events length is a Dirac law of parameter μ (other laws are possible)	Direct: CMA-ES

To estimate parameters, we coupled MoRIS to an optimization algorithm: CMA-ES (Covariance Matrix Adaptation Evolution Strategy), an evolutionary algorithm enabling time-efficient estimation of several parameters simultaneously (Hansen et al. 2003). CMA-ES generates sets of parameters used to run MoRIS, and subsequently produces invasion scenarios (maps of presence probabilities). Each scenario is evaluated by computing the HS score (eqn 4). By evaluating the quality of each set of parameters, CMA-ES produces better sets until it converges to an optimal solution (Fig. 2). The HS landscape being multidimensional and noisy (i.e. containing a lot of random local optima), it is necessary to perform thousands of minimizations with CMA-ES to increase the probability to find the best solution (see validation)

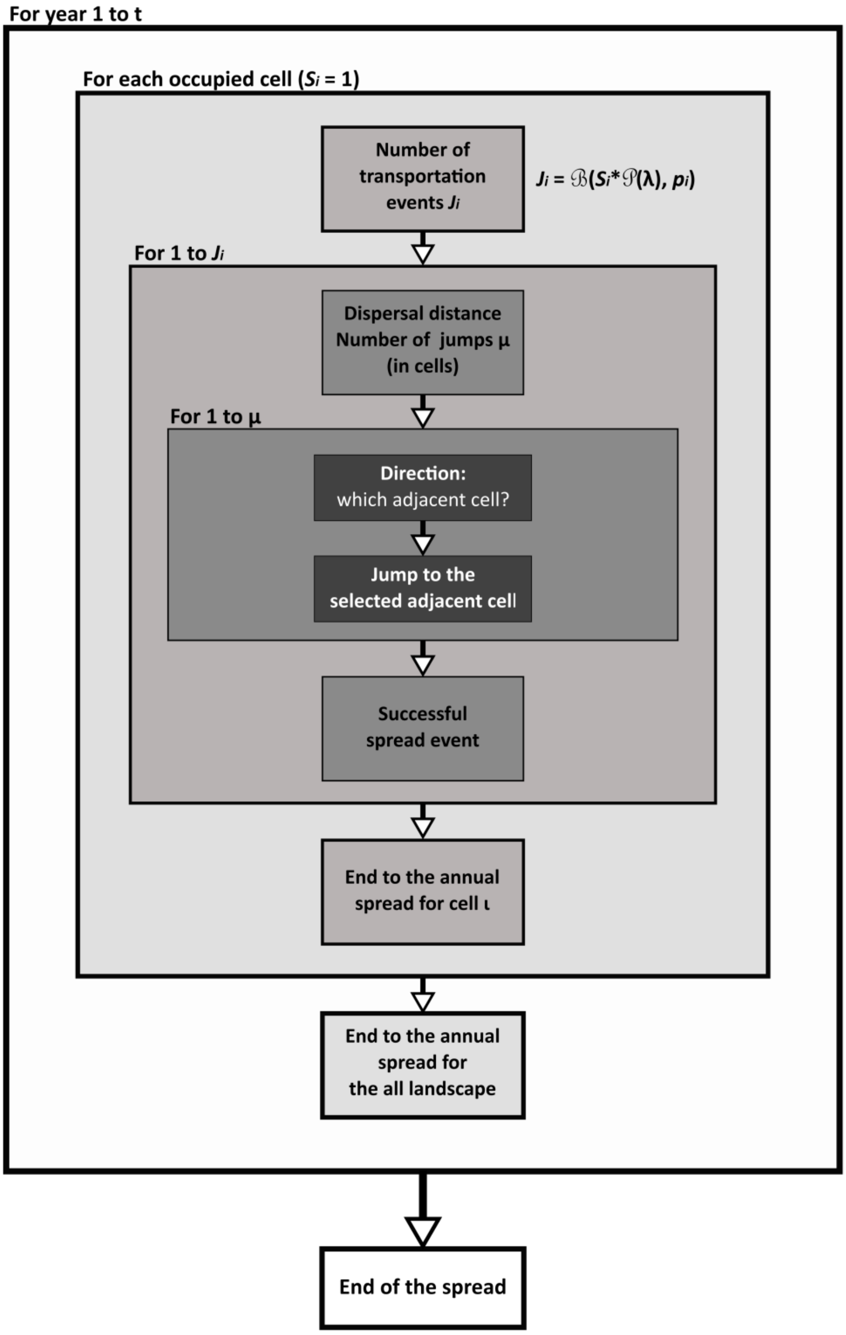

Figure 1: The MoRIS algorithm.

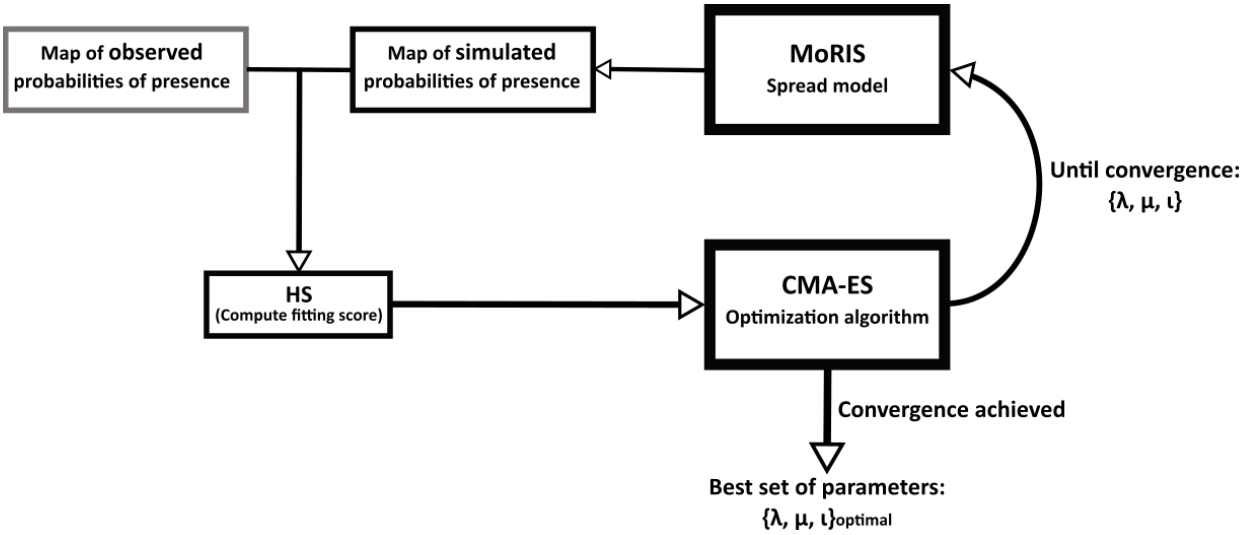

Figure 2: Parameter estimation coupling MoRIS and CMA-ES. The diagram represents one optimization run. Optimization is initiated with several random set of parameters, these are run by MoRIS to produce maps of simulated probabilities of presence, each map is evaluated by comparing it to the observed probabilities of presence using the Hypergeometric score (HS), this score is then given to CMA-ES which regenerate sets of parameters depending on HS. At each cycle, CMA-ES produces better set of parameters until it converges to an optimal solution, where it stops.

MODEL FRAMEWORK

The utilization of MoRIS requires three steps to estimate the optimal set of parameters. Once these parameters estimated, two additional steps are necessary to calculate the dispersal parameters of the invasive species of interest (SD parameters), and to make predictions about future spread dynamics.

Step 1: Construction of MoRIS input files

First, a regular tesselation of squares or hexagons is constructed within a GIS. This tesselation comprises the entire spatial extent of the study area, plus broad edges (at least half of the length of the study area extent). Edges limit simulation biases due to dispersal events jumping out of the study area. Cell size is determined by the user considering the characteristics of the system under focus (*e.g.,* structure of the transportation network, spatial extent of the study area). A first input file (the *map file*) is created containing the unique identifier value and coordinates (usually, the centroid) of each cell of the tesselation.

Second, a map of the transportation network, generally a polylines vectorial layer, must be imported into the GIS and intersected with the tessellation, to assess the number of roads connecting each adjacent cell. A second input file (the *network file*) is created containing the adjacency list of the graph *G*, each line indicating a pair of adjacent cells, and the number of roads between them.

The third input file (the *sample file*) contains the observed frequencies of the species under focus. This input file must contain two values for each cell: the total number of sampling points *n*, and the number of positive sampling points *y*, where the species is observed. These two values allow for the computation of the observed frequency, the probability of presence and the sampling effort, all necessary to compute the fitting score HS (eq. 3).

The fourth input file necessary to run MoRIS is the model instructions (the *parameters file*). This file contains parameter values, exploration ranges, and the conditions of parameters optimization.

Step 2: Validation of the parameters estimation method

Because several factors (including global sampling effort, network structure and possible interactions between parameters) are likely to affect the reliability of our estimation method, a validation step is necessary. To evaluate the ability of MoRIS/CMA-ES to correctly estimate human transportation parameters, *artificial datasets* are created by running MoRIS with known parameters sets. For each set, we simulated an invasion scenario and we sampled an artificial dataset of observed frequencies. The sampling effort can be adjusted to mimic the real observed frequencies data (*field data*). These *artificial datasets* correspond to the *sample file* described above, and are used to feed the MoRIS/CMA-ES estimation method (Fig. 2). Comparing the optimal parameters set to the known original parameters used to generate the artificial data allowed us to evaluate the accuracy of MoRIS.

Step 3: Parameters estimation on empirical data

MoRIS/CMA-ES minimizations are run with empirical data (the *sample file* described above). A large number (at least a thousand) of minimizations should be realized to maximize the probability of finding the best solution and discriminate local optima. Each optimization result is written in an output file, associating the estimated parameters with the best fitting score.

We highly recommend to run complementary analyzes to test the robustness of estimated parameters, such as cross-validation tests (see below).

Step 4: Computation of spread characteristics and history

Once the human transportation parameters are estimated, MoRIS can be re-run with the best set of parameters in order to extract specific information about spread. Dispersal paths generated by MoRIS follow road network topology and are therefore rarely straight. Consequently, the Euclidean distance of each transportation event may not necessarily correspond to the distance travelled along the graph. Secondly, the probability *p* for the invasive species to be picked-up during a transportation event should affect the number of effective spread events (i.e. transportation events that actually dispersed the species). Consequently, the way *p* is computed should affect the global pattern of the spread. This could be used, for example, to compare the spread dynamics of two different invasive species dispersed with the same human activities in the same landscape but with different pick-up probabilities. In MoRIS, several options allow the user to save the history of the spread, by saving every jump events in output files. It is then possible to compute important properties of the invasive scenario, e.g. the distribution of jump distances (geodesic distance on the graph, or Euclidean distance on the landscape). It is also possible to build the genealogic tree of the dispersal events, and compute, for example, the distance (in number of jumps, or in number of years) between two occupied cells.

Step 5: Prediction of future spread and spread scenarios

MoRIS can be run to produce spatially explicit spread predictions (maps of simulated frequencies of presence), by using the best set of parameters previously estimated, or different set of parameters in order to provide scenarios. As MoRIS is a stochastic model, several simulations have to be computed to produce a consensus map for a given age of invasion.

Case study: the invasive garden ant *Lasius neglectus* in South-East France

We applied MoRIS to an invasion occurring in a terrestrial landscape surrounding the second most populated urban area of France, the Lyon metropolis. In 2012, the Lyon urban area hosted 2,214,000 people and covered 6,017 km². Lyon is located at the confluence of the Rhône and Saône rivers and has a continental climate with Mediterranean influences. The city is served by two major sets of transportation infrastructures (A7 motorway, train and Rhône River to the South and West, A43 motorway to the East) and a well-developed road network that is constrained by hills and mountain ranges. This complex landscape should be especially vulnerable to spread anisotropy and a relevant testing ground for MoRIS (Fig. 3).

We applied MoRIS to understand the landscape secondary spread of the invasive ant *Lasius neglectus*. This species has several characteristics making it particularly relevant for the investigation of human-mediated secondary spread in terrestrial landscapes. First, *L. neglectus* is a recently introduced species in Western Europe and has thus not completely filled in any European landscape. Secondly, *L. neglectus* has no natural long range dispersal capability. It forms colonies of interconnected nests without intraspecific aggression, and its winged reproductive females have never been recorded to fly and disperse, which means that secondary spread in this ant is solely by human-mediated dispersal through materials transportation (Van Loon et al. 1990, Schultz and Seifert 2005). Finally, *L. neglectus* has no strong climatic limitation in our study area and is even predicted to be one of the less climatically limited invasive ants in France (Bertelsmeier & Courchamp 2014). Recently, Gippet et al. (2016) showed that the distribution of *L. neglectus* in the studied landscape was poorly explained by environmental features related to urbanization, except for the distance to embankments, which corresponds to the hypothesis that HMD has strong impacts on the spatial distribution of *L. neglectus*. The combination of these characteristics make *L. neglectus* a highly relevant biological model for studying HMD at landscape spatial scales, as its spread is exclusively the consequence of HMD, which is likely to facilitate the estimation of Human transportation parameters by reducing the noise due to other factors involved in observed distribution patterns. The first known occurrence of *L. neglectus* outside of its native range (Central Turkey) was recorded for 1973 in Hungary and recent genetic analyses suggested an unique introduction for all French colonies (Espadaler et al. 2007, Ugelvig et al. 2008). To build our model, we considered that a single introduction occurred in our landscape 40 years ago.

INITIAL DATASETS AND MODEL INPUTS

Field sampling

We used a recently published dataset on *L. neglectus* landscape distribution (see Gippet et al. 2016 for details). 1248 locations were sampled within the studied landscape, during spring and summer 2011, 2012 and 2013, following the method of Roura-Pascual et al. (2009). Spatial coordinates of sampling locations were recorded and imported into ArcGIS v.10.1 (http://www.esri.com/software/arcgis). 67 *L. neglectus* colonies were detected, representing 5.5% of all sampling locations.

Construction of the graph G

The graph *G* of road network connections between adjacent cells was constructed using a three steps process. First, the landscape was discretized in a regular hexagonal grid (hexagons inscribed in a 1000m radius circle) composed of 5586 cells (Fig. 3). We chose this cell size because, given the spatial extent of our focus area, it was a good trade-off between simplification and precision. Second, the road network was extracted from national GIS databases (vectorial layer from BD TOPO® (2013) IGN, Fig. 3) and spatial intersections between roads and cells boundaries were computed using basic operations in ArcGIS 10.1®. Finally, the number of roads crossing each boundary was calculated (i.e. the *network file*).

Presence/absence dataset computation

The 1248 sampling locations were then spatially intersected with the hexagonal grid. For each cell, the total number of samples n^{obs}_i and the number of positive samples y^{obs}_i were computed.. A total of 272 cells contained at least one sampling location (in average 4.5 sampling location per cell) and 40 cells (15% of the sampled cells) contained at least one invaded location (Fig. 4).

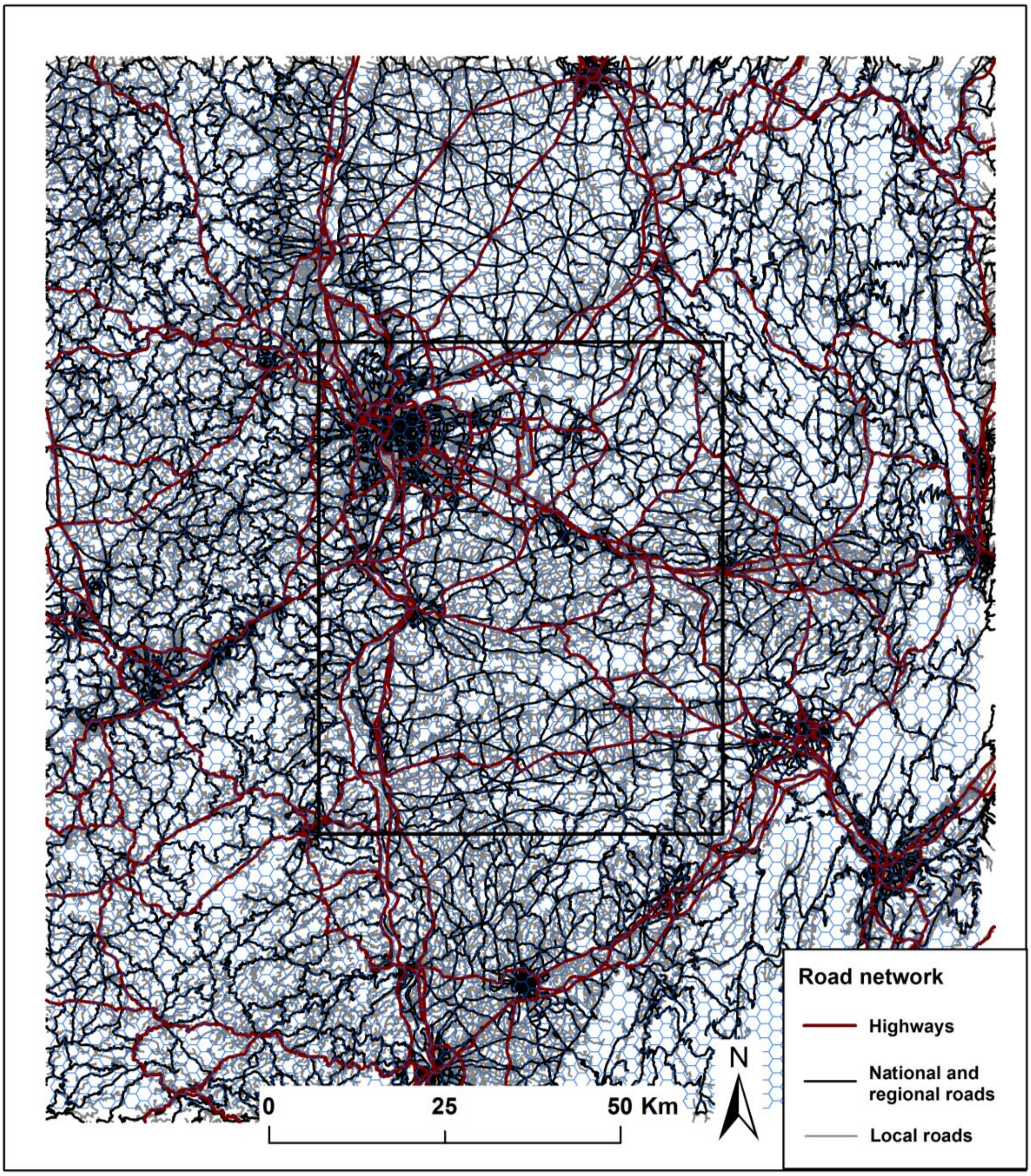

Figure 3: Map of the tesselation used for the *L. neglectus* dataset. The focus area (black frame) constitutes the spatial area in which the introduction cell is estimated. A large buffer area is needed to avoid edge bias. The road network is presented. Adjacent cells are connected by 0 to 28 roads (mean ± SD: 2.38 ± 2.69), cells limits are 1155 m long).

Model instructions

MoRIS was then parameterized to estimate the introduction cell, the frequency of transportation events and the mean dispersal distance. (Table 2).

Table 2: List of estimated and fixed parameters in the case study

Parameter	Range	Estimated or fixed
ι (introduction cell)	Focus area (Fig.3)	Estimated using CMA-ES
λ (transport frequency)	[0,3]	Estimated using CMA-ES
μ (movement)	[0,20]	Estimated using CMA-ES
t (invasion age)	40	fixed
d (direction)	[0,1] in each direction	fixed (*G* graph)
p (pick-up probability)	[0,1]	Fixed

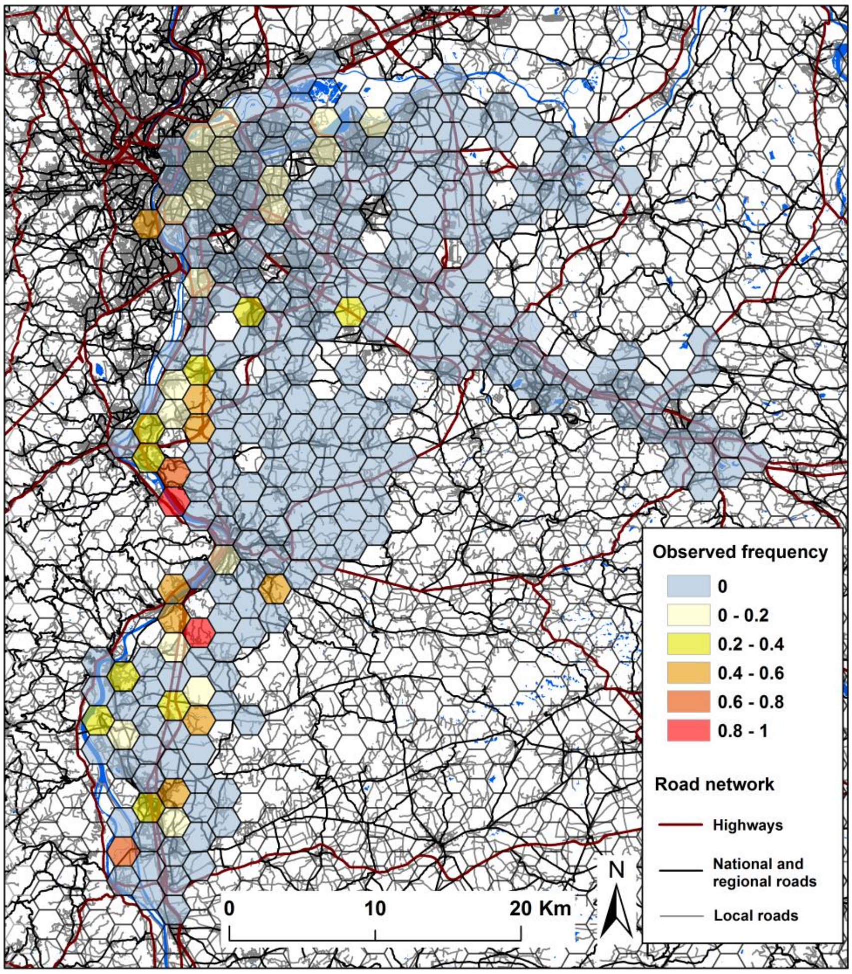

Figure 4: Map representing the focus area of the hexagonal tesselation (see Fig. 3), the road network, the sampled cells (grey and colored cells) and the observed frequency of *L. neglectus* among sampled cells. Open water is represented in blue.

VALIDATION OF THE ESTIMATION METHOD

Before estimating transport parameters of the *L. neglectus* invasion, we validated our parameters optimization method using artificial datasets and testing for sampling effort consequences on our ability to estimate the best fitting parameters set.

Method

We generated 54 sets of parameters (µ, λ and ι) with 54 different combinations of values: three for µ (5, 10, and 15), three for λ (0.5, 1 and 1.5) and six for ι (6 different cells). For each set of parameters, we ran MoRIS 10,000 times and produced one *artificial dataset* corresponding to a map of simulated probability of presence by cell. We then randomly sampled each map (5 samples per cell in average). Each sample results in "invaded" or "not invaded", depending on the simulated probability of presence in the cell. 54 such *artificial datasets* (i.e. *sample files*) were produced. Finally, we ran 100 optimizations (parameters estimation method using MoRIS/CMA-ES) for each *artificial dataset*.

Results

We assessed the capability of MoRIS to find the true parameters set for each *artificial dataset*. We found that the fitting score (HS) did not allow us to discriminate the original set of parameters among the optimal sets provided by CMA-ES. Because of the stochastic nature of MoRIS, the fitness landscape is noisy, and many random local optima exist near the best solution. Despite this fact, performing repetitions allowed us to show that MoRIS statistically converges to the optimal set, by smoothing the noise due to random local optima. In this view, a majority of optimization runs should converge to the real optimum.

For each *artificial scenario*, we analyzed the distribution of the estimated values for each parameter (µ, λ and ι) and compared the true values to **(i)** the mean, **(ii)** the median and **(iii)** the mode of the distributions of estimated values. We found that the mode of the distribution of estimated values was the best index to estimate the true value (Fig. 5). This result confirms our hypothesis about the noisy HS landscape, since the mode indicates the most frequent value of a distribution.

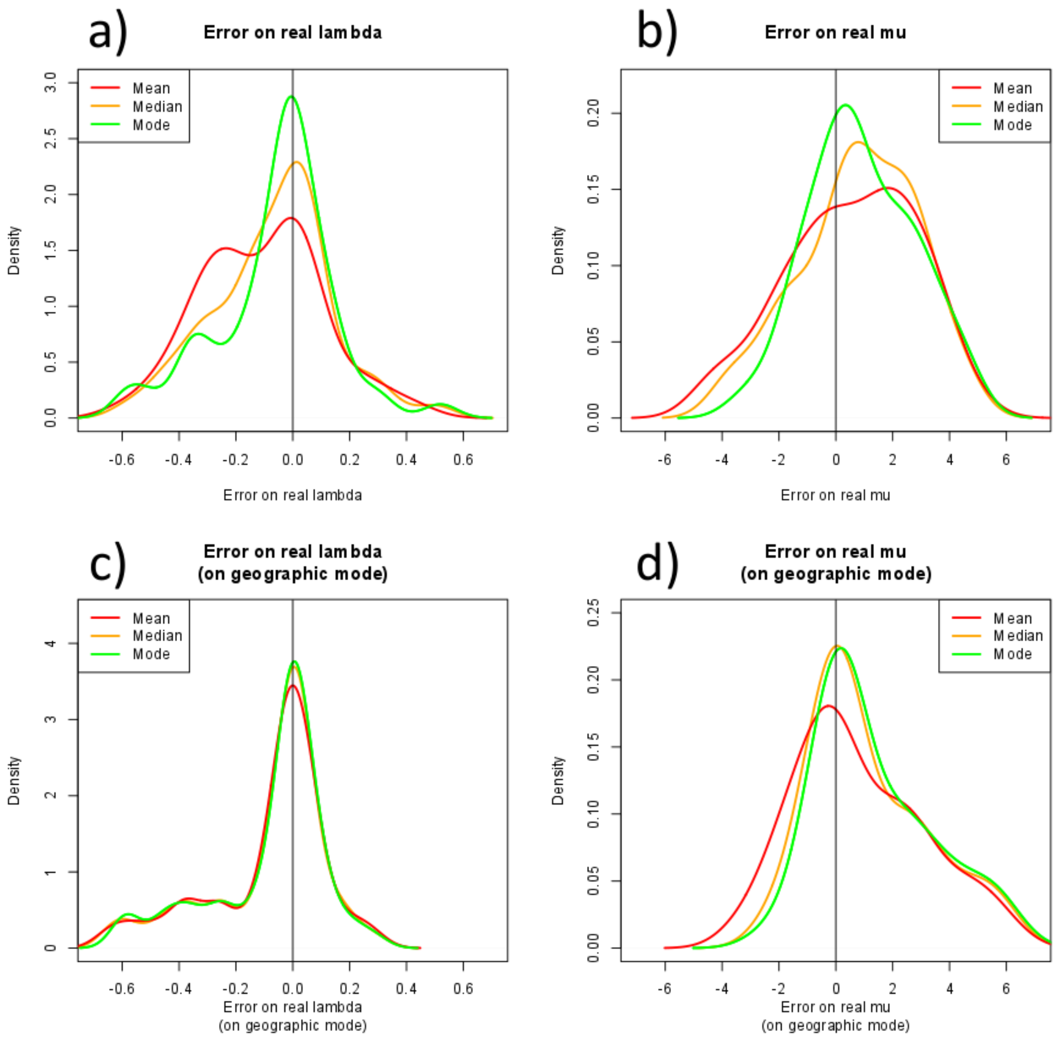

Figure 5: Validation of the parameter estimation method. Determination of the best index to estimate the true parameter values of the *artificial datasets*. Shown here are the distributions of the differences between true and estimated values (for λ and μ) when the estimated value is determined by the mean, the median or the mode of the distribution of estimated values for each *artificial dataset*. **a)** and **b)** represent, for each index, the distribution of the distance to the true values for λ and μ before determination of the best estimated introduction cell (all convergences are included). **c)** and **d)** represent the same distributions but without the convergences that had not identified the best cell of introduction.

The values determined by the modes of the distributions were the closest to the true parameter values as their distance to the true value (the “error” in fig. 5) was closer to 0. However, when the estimations were refined by selecting only those that estimated the most probable cell of introduction (the cell that was the most often estimated in convergences), the mode and median gave approximately the same results for μ and λ estimation.

ESTIMATION OF HUMAN TRANSPORTATION PARAMETERS

As we showed in the validation step, 100 optimizations were generally good enough to identify the best set of parameters. We realized 10,000 optimizations to estimate the best set of parameters for *Lasius neglectus*. We identified the best set of parameters as: $\mu = 10$, $\lambda = 0.81$ and ι is cell 336 (Fig. 6).

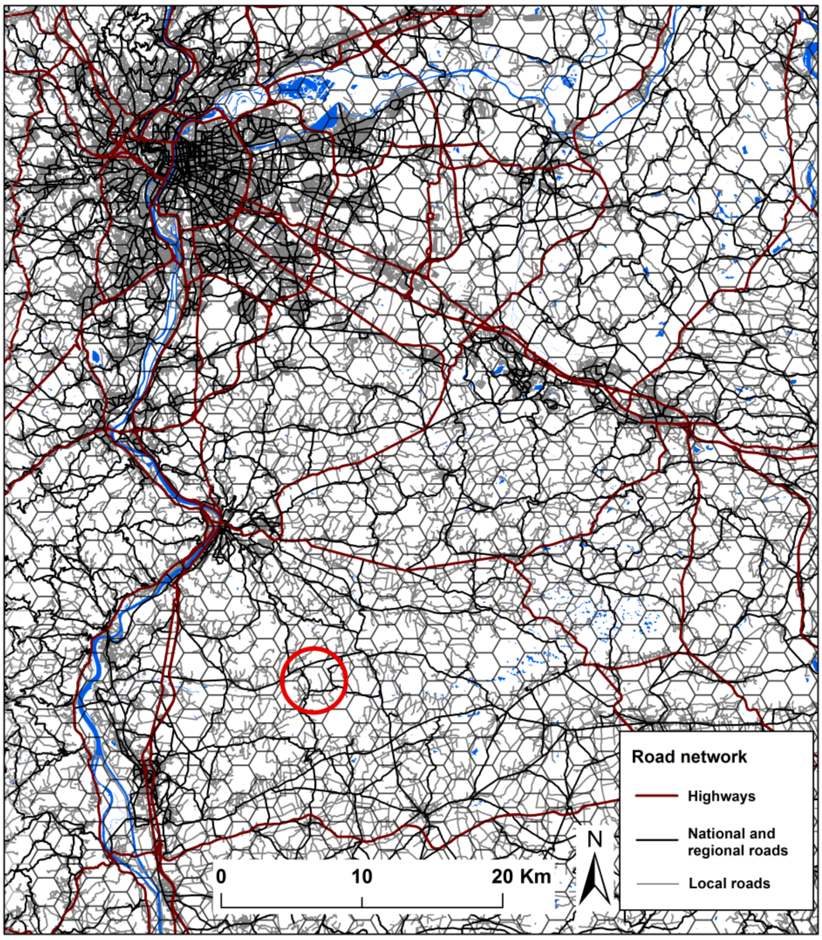

Figure 6: Localization of the estimated cell of introduction (red circle) in the focus area. Open water is represented in blue.

Using this best set of parameters, we computed 1000 simulations and assessed the difference between empirical observation and simulation results for each sampled cell (Fig. 7). We found that on 42 invaded cells in our empirical dataset, only two (5%) were not predicted by the simulations. In invaded cells, the simulations globally underestimated the presence of *L. neglectus* by 0.15 (median value). Among 223 non-invaded cells, simulations predicted very low probabilities of presence of *L. neglectus* globally (median = 0.03, maximum overestimation of 0.12) (Fig. 7).

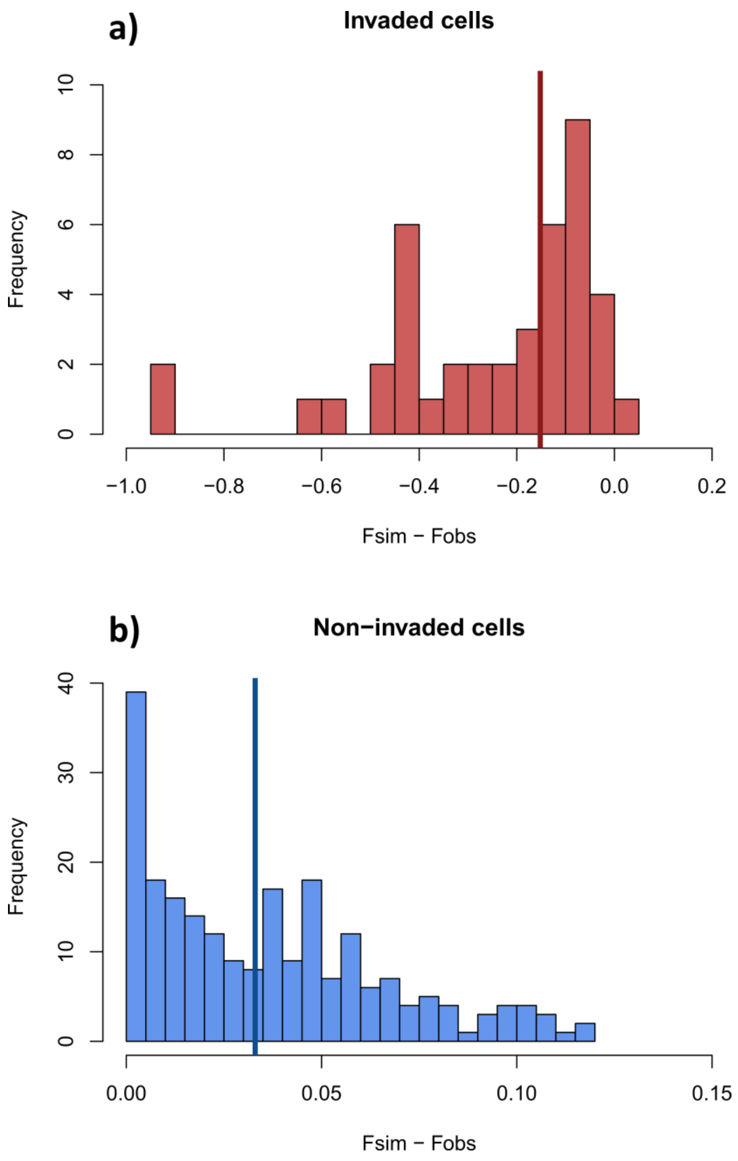

Figure 7: Differences between simulated and observed probabilities of presence of *L. neglectus* (respectively Fsim and Fobs) for **a)** cells for which *L. neglectus* was detected (invaded cells) and **b)** cells for which *L. neglectus* was not detected (non-invaded cells), in the empirical dataset. A negative value indicates that the empirical (observed) probability of presence is underestimated by simulations while a positive value indicate that the empirical (observed) probability of presence is overestimated. A value of 0 indicates perfect match. Vertical lines indicate median values (-0.15 for a) and 0.03 for b)).

Computation of spread characteristics and history: indirect validation of the estimation

We run 1000 simulation using MoRIS with fixed values for each parameter ($\mu = 10$, $\lambda = 0.81$ and ι is cell 336, see fig. 6), according to the best set of parameters. For each effective dispersal event occurring during a simulation, we recorded the cell of origin and the cell of arrival. We then calculated the number of effective dispersal events, the Euclidean distance of each dispersal event, the distance between each invaded cell and finally constructed a genealogical tree representing the history of invasion.

The empirical and simulated distributions of distance between colonies were very similar (Fig. 8) with an average distance between invaded cells of 20 km.

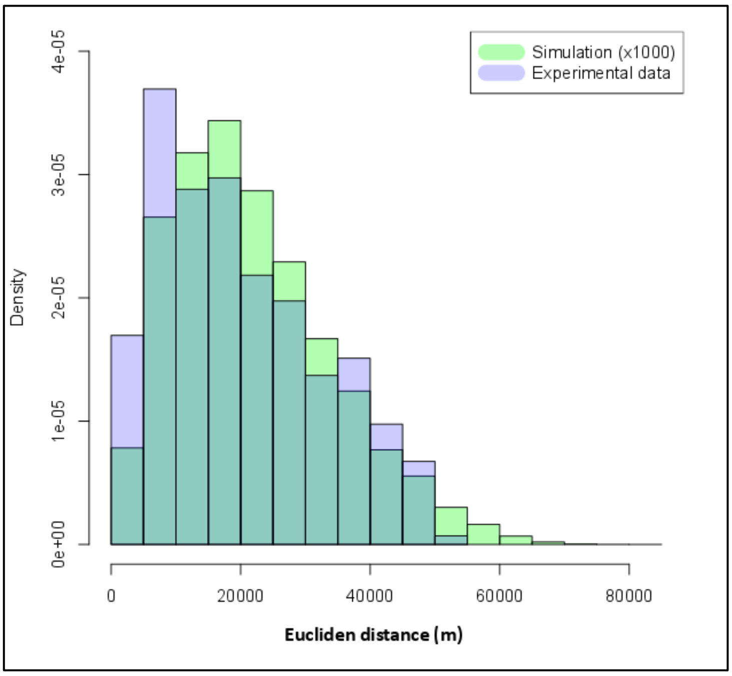

Figure 8: Distribution of the Euclidean distance between colonies for empiric and simulated data.

We used a genetic dataset of 34 *L. neglectus* colonies present in the studied landscape (Gippet et al. 2016) (Gippet et al. *submitted*) allowing us to build genetic and Euclidean distances matrices between these 34 colonies. From these matrices, we produced a Mantel correlogram to assess the genetic spatial autocorrelation of the colonies of *L. neglectus* in our studied landscape.

Using the genealogical trees produced by simulations, we computed a matrix of *simulated genetic distance* between simulated colonies. The *simulated genetic distance* between two simulated colonies was calculated as the length of the branches between the colonies in the genealogical trees. Using Euclidean distances matrices between simulated colonies, we produced simulated Mantel Correlograms. We compared empiric and simulated Mantel correlograms (Fig. 9). The main result of the empirical Mantel correlogram was that colonies distant from 5 to 10 kilometers were (significantly, $p<0.05$) genetically closer than colonies distant from other distance classes. This result was interpreted as a signal of the strong role of HMD on the spread of *L. neglectus*. The simulated Mantel correlogram described the same phenomenon (Fig. 9) and globally fitted the empirical results for all classes of distances, adding credit to the results of our estimations and more globally to our model.

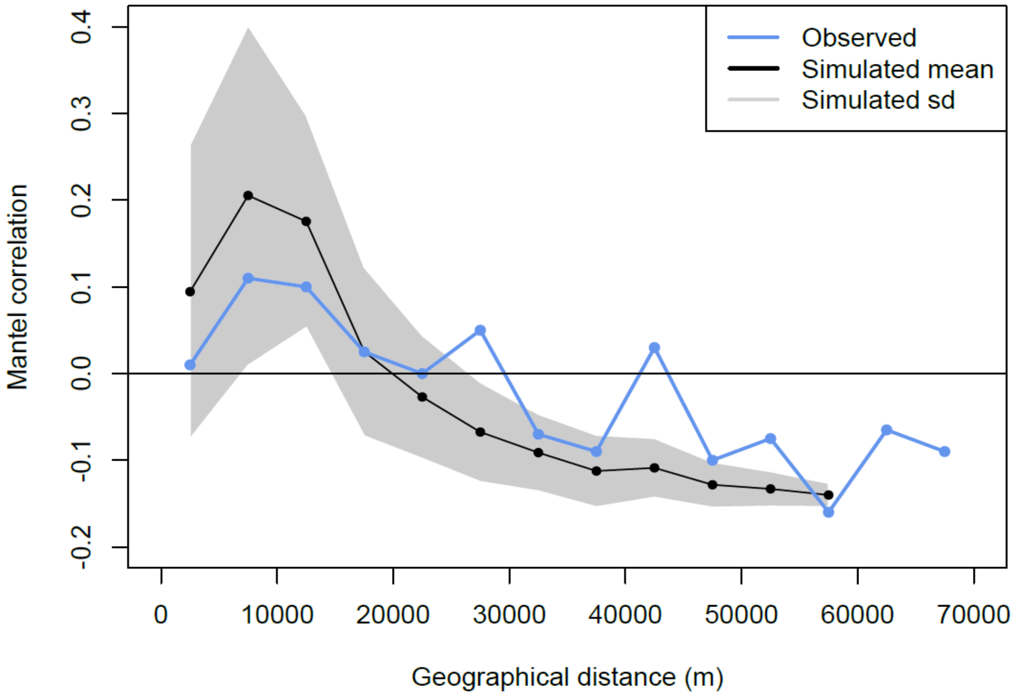

Figure 9: Empiric (observed) and simulated genetic spatial autocorrelation using Mantel Correlograms.

Predictions of spread

We computed predictions of *L. neglectus* probabilities of presence for the years 2025 and 2050 by running 1000 simulations with the best set of parameters (μ equals 10, λ equals 0.81 and ι is cell 336) (Fig. 10). *L. neglectus'* spread clearly followed a North-South orientation. In 2025, our simulations predict that the spread should push into a centre-west valley which leads to another urban area, that of Saint-Etienne. The patterns of spread appeared more diffusive in the East as the area is much more urbanized, connected to Lyon and without the mountains ranges of the West that stongly reduce road densities.

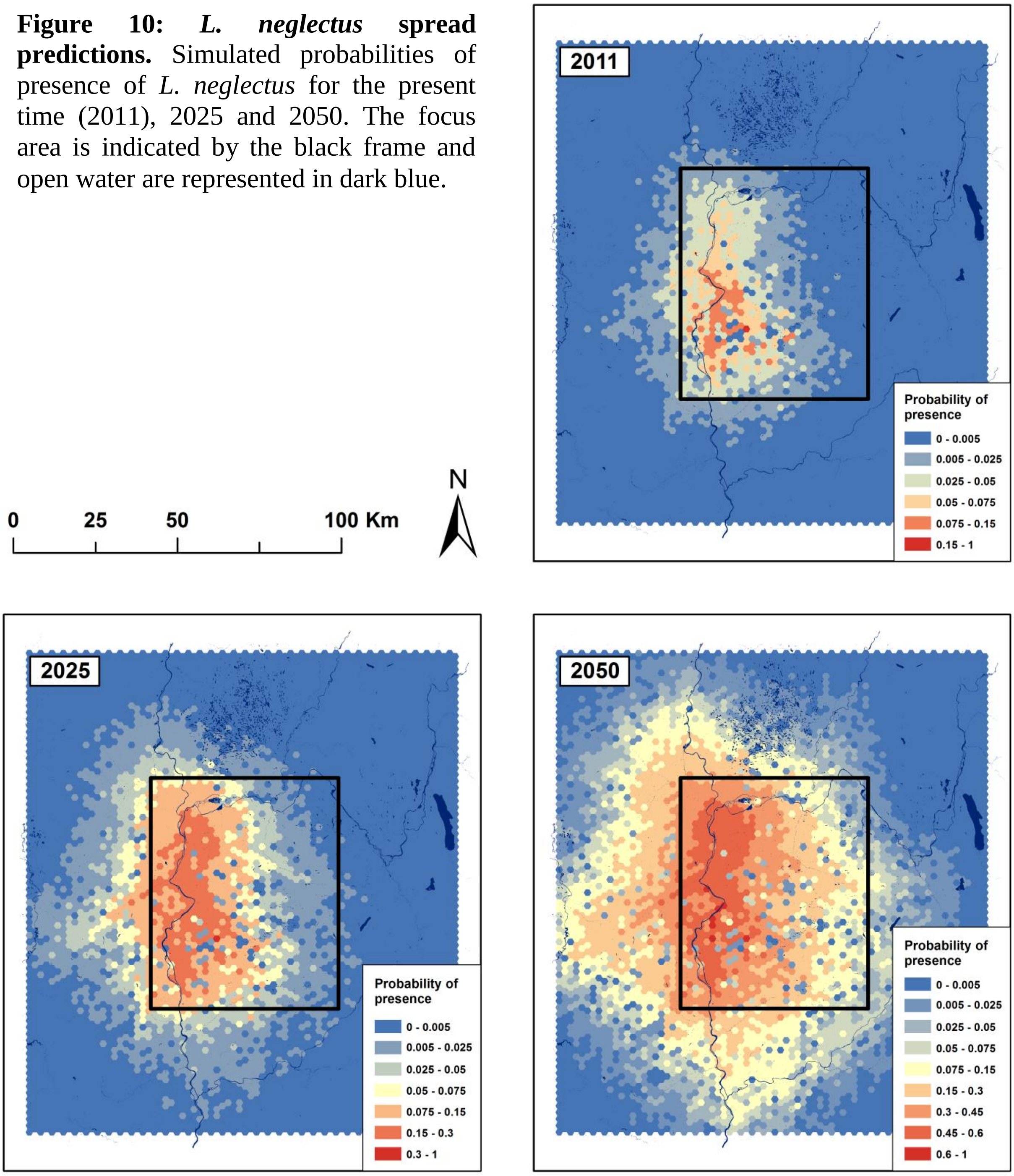

Figure 10: *L. neglectus* spread predictions. Simulated probabilities of presence of *L. neglectus* for the present time (2011), 2025 and 2050. The focus area is indicated by the black frame and open water are represented in dark blue.

Discussion

We developed and applied MoRIS, a spatially explicit stochastic simulation model of human-mediated secondary spread. MoRIS simulates transport activities to model species spread and controls the direction of dispersal events by taking into account the topology of the transportation network. MoRIS uses five parameters (time since introduction, cell of introduction, dispersal distance, frequency of transportation events, and pick-up probability) which can be estimated directly or indirectly using a fitness score (HS) and an optimization algorithm (CMA-ES). Using as case study the spread of the invasive ant *Lasius neglectus*, we showed that our model was able to estimate parameters maximizing the explanation of HMD in *L. neglectus*. In addition, using two indirect indicators, distance between invaded cells and genetic spatial autocorrelation, we demonstrated that our model produced realistic and even accurate scenarios.

Main achievements

Besides the fact that it requires simple datasets and little expert knowledge (minimizing *a priori* on spread parameters), MoRIS proposes an original way to model human-mediated dispersal, more specifically dispersal by transport, by focusing on human activities rather than directly on species (Gippet et al. *submitted Viewpoint*).

MoRIS computes the direction of dispersal by using the topology of the transportation network involved in HMD. For this reason, the directional bias induced by the network topology is included during the construction of the initial datasets required to run MoRIS. This generates anisotropic dispersal direction probabilities. To our knowledge, this is the first HMD model that manages direction of dispersal in this way and, as such, it might offer promising insights for the improvement of future HMD modeling.

Among the five parameters used in MoRIS, four are human transportation parameters and are mostly independent of the species being transported. The fifth parameter, i.e. pick-up probability (p), reflects the interaction between characteristics of human activities and species traits, which affects the number of realized dispersal events (i.e. the number of transportation events for which the material transported actually contains living individuals of the studied species). The pick-up probability should mostly depend on the proportion of occupancy of the area (the cell) undergoing a materials transportation event. The pick-up probability (p) is actually a very complex parameter which could be affected by several other parameters such as species reproductive rates, or natural dispersal abilities. This offers stimulating insights for future uses of MoRIS to investigate the intricate outcomes of species intrinsic characteristics on spread dynamics. In our case study, we fixed *p* with a human transportation index (the number of roads penetrating the cell) in order to simplify our model but the use of a species-specific demographic sub-model would be more appropriate.

Insights from the Lasius neglectus case study

Our case study was based upon a very simple dataset of presence/absence locations of the invasive ant *Lasius neglectus* and the road network of the urbanized landscape around Lyon.

We evaluated the ability of MoRIS to correctly estimate Human transportation parameters based on artificial datasets from which parameter values were known. We showed that, for our case study situation, our estimation method coupling MoRIS and CMA-ES was globally accurate, even with few optimization tries (validation step: 100 optimizations per scenarios). These first results showed that our estimation method, even when it must estimate several parameters simultaneously (in this case three), was effective.

We also estimated the set of parameters that best explains the spatial distribution of *L. neglectus* in the studied landscape. We found that the introduction cell was located in the south of our focus area. Our study area is characterized by the presence of the Rhône River (Fig. 6), which concentrates human activities and transportation networks (highways, national roads) in a North-South orientation. In addition, *L. neglectus* is particularly abundant in the South of the Rhône valley (see www.creaf.uab.es/xeg/lasius/index.htm) which supports the scenario of a spread following the valley from South to North.

We estimated μ as 10 cells jumped at each transportation events. If all dispersal pathways were straight, this would correspond to approximately 22 km as cells' maximal radius is 2 km. Because dispersal events are directionally biased by the topology of the road network at each jump between cells, dispersal pathways are rarely straight and euclidean distance between the origin and the arrival of transportation events are shorter than 22 km. We found that transportation events correspond to an average distance of 9.285 km ranging from 2 km to 22 km (Fig. 11).

We computed a Mantel correlogram with artificial genetic distances between simulated invaded cells. We compared it to the empirical genetic spatial autocorrelation of *L. neglectus* colonies present in our landscape. The same pattern emerged from the two correlograms: colonies distant from 5 to 15 kilometers are genetically closer than colonies distant from other classes of distance (even closer colonies). This result correspond to what could be expected from the simulated transportation distances (Fig. 11) and brings credit to our estimations and more broadly, to the relevance of MoRIS for modeling HMD.

We produced predictive maps of probability of presence of *L. neglectus* for 2025 and 2050. These showed that the Rhône Valley by concentrating activities and thus road network in a North-South orientation had a disproportionate impact on spread dynamic (Fig. 10).

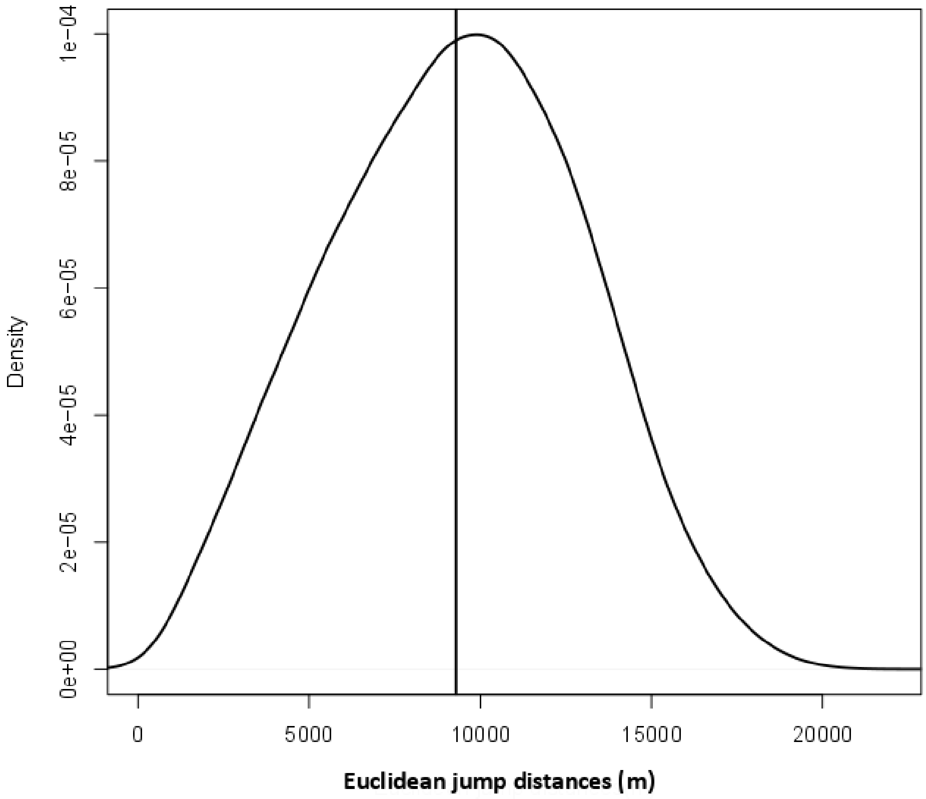

Figure 11: Distribution of the Euclidean distance between the origin and the arrival of a transportation event. The vertical line is the mean (9285 m).

The uses of MoRIS

MoRIS constitutes a useful tool for many applications such as invasive species management strategies where it could be used to identify key parameters of secondary spread, to choose the more relevant management strategy, to locate risk areas in landscapes or to determine efficient sampling surveys schemes in order to apply control strategies at the earliest stages of invasions. It could also be useful to predict the possible consequences of networks development and urban sprawl on landscape infill by invasive species (Crespo-Perez et al.

2011), to improve biodiversity scenarios (Titeux et al. 2016, Bellard et al. 2016) or to test ecological theories (Guisan and Thuiller 2005).

Future improvements

MoRIS was designed to be generalizable, flexible and modular. Several possible improvements are listed hereafter.

Demographic sub-model - As discussed above, while most parameters of MoRIS are human transportation parameters, the pick-up probability (*p*) is a parameter taking into account species characteristics in HMD. *p* is likely to vary depending on the proportion of infill of a cell. In other words, the more an invasive species fills a habitat, the more likely it is to be picked-up when a transportation event happens in this habitat. We thus propose to nest a demographic sub-model simulating habitat infill into each cell over time. At the end of each year, *p* can be computed for each cell as the proportion of habitat occupied by the species (i.e. when the species has completely filled the cell, *p* equals 1). Such sub-model would take into account natural dispersal abilities of invasive species, landscape heterogeneity in the availability of habitat and to implicitly model the lag time observed in many invasions (Adams et al. 2015).

Environmental limitations - Because invasive species often undergo environmental limitations during their spread, MoRIS needs to implement environmental constraints on species spread. This can be done by modifying the probability of establishment in arrival cells depending on one or multiple environmental layers. Such environmental limitations could also be added to the demographic sub-model described earlier by affecting reproductive and growth rates or other demographic parameters.

Network history - Human activities have intensified over the past centuries (Grimm et al. 2008) and using today’s transportation networks for invasions which began decades ago could produce misleading results, especially in newly developed areas which are prone to biological invasions. Known historical topologies of the network could be used to update network maps during MoRIS simulations by replacing the graph of road connection (*G*) during simulations. This would also be useful for testing the impact of potential future networks extensions on landscape invasibility (Alpert et al. 2000).

Conclusion

MoRIS is an innovative, yet simple, approach for modeling HMD by transport, as it explicitly simulates human activities movements, manages dispersal direction using network topology, estimates multiple parameters simultaneously with very little *a priori* and leads to improved knowledge of invasive species spread characteristics and history. We think that MoRIS constitutes a promising methodology to fill in the gap in human-mediated dispersal modeling and bring new insights to future research. As shown by our case study, MoRIS has the potential to provide important indications to natural resource managers but also significant refinements to biodiversity scenarios which often ignore or underestimate HMD, especially at landscape scale.

Acknowledgements

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Author contributions: JMWG and CR developed the idea and created the model; CR wrote the program code; JMWG and CR wrote the paper; SF run the simulations; BK performed the field and genetics study; BK and SF brought significant improvements to the paper.

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CHAPTER 3

Article submitted to **Biological Invasions**

Come together: host-parasite co-invasion is affected by urbanization

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Abstract

Co-invasion events remain understudied although they are likely to affect our ability to understand and predict invasion dynamics. Here we investigated a co-invasion process involving the eastern Mediterranean ant *Lasius neglectus* and the American ectoparasite fungus *Laboulbenia formicarum* in the urban area of Lyon (France). An extensive field survey was conducted to characterize the host-parasite relationship in an urbanized landscape. 80 *L. neglectus* colonies were detected, of which 58% were infected by *L. formicarum*. The fungus was, for the first time *in natura*, detected in colonies of the native ant *Lasius niger*, but not in any of five other *Lasius* species examined. Microsatellite marker data in *L. neglectus* reflected short distance, human-mediated dispersal of ants. Infection status was related to genetic proximity among colonies, attesting a frequent 'mother' to 'daughter' colony transmission of the parasite, without completely excluding other transmission pathways (e.g. by *L. neglectus* males' swarms). The prevalence of *L. formicarium* was highly variable at both inter- and intra-colonial level. Among infected colonies, fungus prevalence was positively correlated to urbanization, which was measured as the proportion of impervious land-cover in 50m buffers around colonies. Size of *L. neglectus* colonies was negatively correlated to urbanization and fungus prevalence, indicating that the parasitic fungus has the potential to disrupt the spread of invasive *L. neglectus* populations, especially in urban environments. This could be of value for biological control, but the potential negative impacts of the fungus on native ant species would require further investigation.

Keywords Co-invasion, Urbanization, secondary spread, parasite, *Lasius neglectus*, *Laboulbenia formicarum*

Introduction

With the globalization of human activities, the number of transported species has sharply risen, resulting in landscapes worldwide experiencing the simultaneous spread of numerous invasive species (Vitousek et al. 1997, Bertelsmeier & Courchamp 2014). The impacts of co-occurring invasive species are difficult to predict because such species are likely to interact with each other, which can intensify (e.g. by facilitation or mutualism, Ness et al. 2012, McNeil & Dick 2014) or mitigate (e.g. by competition, predation or parasitism, Jackson 2015) invasion dynamics. Among co-invasion processes, host-parasite relationships have raised considerable attention because parasites are susceptible to negatively impact invasive species fitness, possibly leading to the crash of populations, which can make them good candidates for biological control (Porter 2000). However, invasive species have also been pointed out as reservoirs of parasite species, threatening phylogenetically close native species (e.g. parapoxvirus and invasive grey squirrels threatening red squirrels in Great Britain, Sainsbury et al. 2000).Therefore, understanding co-invasion processes appears essential for improving predictions of invasion dynamics and impacts in order to make appropriate management decisions (Kuebbing et al. 2013).

Human activities involved in the introduction of invasive species are also linked to urbanized landscapes, which concentrate people, commercial exchanges and transportation networks (Vitousek et al. 1997, Rouifed et al. 2014). These landscapes are thus likely to be subjected to co-invasion processes but, because of their constraining conditions (e.g. fragmentation, urban heat island effect, pollution, Grimm 2008), they represent challenging environments for invasive species, which is in turn likely to affect co-invasion dynamics. Consequently, urbanized landscapes should be monitored for co-invasion events, especially when they involve host-parasite relationships likely to affect native biodiversity.

Such an invasive host-parasite couple has been recently reported in Europe: the invasive tramp ant *Lasius neglectus* and the ectoparasite fungus *Laboulbenia formicarum* (Ascomycota, Laboulbeniales) (Espadaler et al. 2011). *Lasius neglectus*, originating from Asia Minor, is a widespread invasive ant forming colonies of interconnected nests with numerous queens and low intraspecific aggression. Nuptial flights are absent, which means that natural colony expansion only occurs by budding over limited distances (a few to a hundred meters per year, Espadaler et al. 2007). Secondary spread in this ant occurs therefore almost solely by human-mediated dispersal through road-building or the transportation of landscaping materials (Van Loon et al. 1990, Schultz & Seifert 2005). In Europe, 174 colonies of this species have been detected to date, of which four were infected by *Laboulbenia formicarum*, an ectoparasitic fungus originating from North-America and invasive in Europe (Espadaler & Santamaria 2003). It is transmitted by spore attachment to ant cuticles during contact with infected conspecific or heterospecific ants (De Kesel 1995, Konrad et al. 2015, Tragust et al. 2015). Colonies of *L. neglectus* are ideal targets for such parasites, because they comprise millions of constantly interacting workers and because the two species never evolved together, with the consequence that the host species has no efficient antiparasite adaptations (Tragust et al. 2015). A recent study has shown that *L. formicarum* can be highly prevalent in *L. neglectus,* reaching 100% of the workers within colonies and is likely to have direct or indirect effects on colonies fitness (Konrad et al. 2015). It was also shown that survival of *L. neglectus* workers was negatively correlated with *L. formicarum* infection levels, but that infection by the fungus could provide a prophylactic protection upon later exposure to pathogens (Konrad et al. 2015). *L. formicarum* has equally been reported infecting one native European ant species (*Lasius grandis*, Portugal: Madeira archipelago, Espadaler & Santamaria 2012), and infection experiments have shown its ability to infect *Lasius niger* (Tragust et al. 2015), indicating that it is likely to have a wider range of potential hosts among native *Lasius* species.

In the present study, we explored the co-invasion patterns of *L. neglectus* and *L. formicarum* at landscape scale in the urban area of Lyon (France). To our knowledge, there is no other area in Europe this heavily invaded by *L. neglectus*, with 80 *L. neglectus* colonies detected over a ~2000 km² area (Gippet et al. submitted). The detection of *L. formicarum* in these populations gave us the opportunity to investigate the ant-fungus co-invasion. We combined several approaches to clarify how the ant is dispersed across the landscape, how the fungus is dispersed among ant populations, and how the environment can influence the expansion of both species. First, we investigated the spatial and local phylogeographical patterns of spread in *L. neglectus* by conducting an extensive field survey followed by genotyping 6 microsatellite loci. Ugelvig et al. (2008) showed that, at the continental scale, *L. neglectus* colonies were likely to arise from very few independent introductions. Therefore, we expected that, at landscape scale, all *L. neglectus* colonies originated from a unique introduction event and that their spatial distribution pattern would result from secondary spread, itself the consequence of short distance human-mediated dispersal. Second, we investigated *Laboulbenia formicarum* invasion patterns by addressing several questions: (i) What is the fungus host range? We screened six native *Lasius* species and *L. neglectus* for the presence of *L. formicarum*. (ii) How is the fungus transmitted in *L. neglectus*? As *L. neglectus* colonies are founded when individuals from a 'mother' colony are displaced through the landscape, it is likely that its ‘daughter’ colonies will inherit the infection when the ‘mother’ colony hosts the fungus – a process hereafter referred to as ‘vertical transmission’, while ‘horizontal transmission’ will be used to designate post-dispersal infection of initially fungus-free colonies. As the fungus is transmitted via direct contact, the most likely way for a colony to be infected is by vertical transmission. Consequently, we expected to observe a genealogical signal in fungus infection of colonies. (iii) Are some colonies more vulnerable than others? Previous studies have reported that *L. formicarum* prevalence in *L. neglectus* varied between colonies (Tragust et al. 2015). Because low genetic diversity can decrease immune defenses, therefore increasing parasite prevalence (Whitehorn et al. 2011), we tested the relationship between genetic diversity and fungus prevalence in infected colonies. (iv) How is the infection distributed within colonies? Intra-colonial *L. formicarum* prevalence patterns were surveyed in order to assess spatial heterogeneity in fungus prevalence across the extent of colonies.

Third, we investigated the impact of urbanization on co-invasion patterns. Because urban areas are stressful environments for biodiversity (Shochat et al. 2006), urban *L. neglectus* colonies are likely to have weakened immune defenses (Sorvari et al. 2006, Imperato et al. 2003). We therefore tested if parasite prevalence was higher in urban colonies than in rural ones. Finally, as both urbanization and *L. formicarum* prevalence are likely to affect *L. neglectus* colonies fitness, we tested the relationship between these two factors and the area size of colonies, used here as an indicator of fitness.

Methods

Study area

The study was conducted in the urban area of Lyon, France, and along the two major sets of transportation infrastructures serving the city (A7 motorway, train and Rhône River to the South and West, A43 motorway to the East). In 2012, the Lyon urban area hosted 2,214,000 people and covered 6,017 km². Lyon is located at the confluence of the Rhône and Saône rivers and has a continental climate with Mediterranean influences. Mean temperatures are 3.2°C in the coldest month (January) and 21.9°C in the warmest month (July), with an annual mean temperature of 12.3°C. The annual average rainfall is 820 mm (Infoclimat.fr).

Sampling

1248 locations were sampled following the method of Roura-Pascual et al. (2009). This was done during spring and summer 2011, 2012 and 2013, only when air temperatures were comprised between 16°C and 28°C (Seifert 2007). Sampling locations were herbaceous patches, with or without trees and shrubs, and generally close to or along roadsides. They were randomly selected along road transects and were located at least 500 m apart when outside the city center and 200 m apart when inside the city center. Sampling was performed by teams of two to five persons for a cumulative search time of 40 minutes (the actual sampling time depended on the number of sampler, e.g. four samplers took 10 minutes per site; two samplers, 20 minutes per site) within a radius of 15 m. Sampling was a direct search of ants nest and trails on the ground, trees and shrubs, followed by hand collecting using an entomological aspirator. Spatial coordinates of sampling locations were recorded and imported into ArcGIS v.10.1 (http://www.esri.com/software/arcgis).

We detected 69 *L. neglectus* colonies with this sampling protocol. Eleven other colonies used in the present study came from accidental detection during other surveys or were pointed out by inhabitants of invaded areas.

Morphological and molecular identification

All collected ants were preserved in 96° alcohol at -20°C and morphologically identified to genus or species whenever possible, following Seifert (2007). One individual per colony of ambiguous *Lasius* species (i.e. with short or appressed scape setae) was systematically identified using Cytochrome Oxydase I (COI) because of complex, error-prone morphological identifications.

DNA was extracted from head and thorax only (to remove formic acid). Ants were crushed and then mixed with 150 µl of hot (90°C) Chelex® 100 then cooled;10 µL of proteinase K (15mg/mL) were added and the solution was incubated at 55°C overnight (Casquet et al. 2012). DNA was amplified by PCR using specific primers developed from complete COI sequences found in Genbank (COI_neg_F1, TTATTAATGAYGGAGTYGGAACAGGATG; COI_neg_R1, CTCGTCGTTATTCAGATTAYCCAGATA, derived from PAT L2-N-3014r (Simon et al. 1994). PCR Reactions were carried out in 35µL solutions with 0.17 µM/µl dNTPs, 0.1 µg/µl BSA (New England Biolabs, Ipswich, USA), 0.16 µM/µl of each primer, 0,04 U/µl Taq Polymerase (Biolabs), 1X PCR Buffer (Biolabs), and 4 µl of DNA. Cycling was conducted on a PTC-200 (MJ Research) thermal cycler with following parameters: (i) initial denaturation for 2 min at 94°C, (ii) 40 cycles with denaturation for 30 s at 94°C, annealing for 30 s at 48°C and extension for 30 s at 72°C; (iii) final extension for 1 min at 72°C. All PCR products were purified, sequenced and ran on a 3730xl DNA Analyzer (Applied Biosystems) by a service provider (BIOFIDAL, Vaulx-en-Velin). All obtained sequences were compared to existing sequences present in Genbank using Blast-n.

Genotyping

In order to assess for the genetic structure of *L. neglectus* colonies, 34 colonies of *L. neglectus* were genotyped. Six microsatellites markers from the literature (Table S1) were organized into 2 multiplex and 2 simplex PCRs, all combined into one genotyping mix ran on a 3730xl DNA Analyzer (Applied Biosystems) by a service provider (BIOFIDAL, Vaulx-en-Velin). PCR mix composition were as follows : 1) mix 1, total volume of 20 µl, 0.4 µM of each primer (La32f, La33b), plus 0.8 µM of primer Lng-3, 1X Buffer, 0.5 U of TaqOzyme HS (ref OZYA002-250 ; Ozyme), and 2µl extracted DNA; 2) mix 2, total volume of 15µl, 0.4 µM of each primer (La36d, Lng-1), 1X Buffer , 2U of TaqOzyme HS, and 2µl extracted DNA; 3) mix 3 for simplex reaction, total volume 15µl, 0,4 µM of each primer (L1-5, L10-174), 1X Buffer, 0,5 U of TaqOzyme HS and 2µl extracted DNA. All PCRs were run with the same cycling program : 1 min. annealing at 94°C, 40 cycles with denaturation (15 s) at 94°C, annealing (15 s) at 48°C, extension (30 s) at 72°C, and a final 5 min extension at 72°C. Electrophoregrams were read and interpreted with Genemarker 1.95 (Softgenetics).

Colonies area measurement

33 colonies of *L. neglectus* were measured during spring and summer 2012 and 2013. Colonies were measured by teams of two to five persons by direct search of ant nests and trails on the ground, trees and shrubs in every direction from the initial point of detection. Ants were sampled at regular intervals, and samples were spatially referenced on a precise map. On average 12 such samples were collected per colony, ranging from two to 58 depending on the extent of the colony. Colonies boundaries were determined and search stopped when, in a given direction, no more *L. neglectus* or individuals of morphologically close *Lasius* species were found for more than 50 meters from the last location where *L. neglectus* was detected. Colony measurement was considered finished when bounded in all directions, closing the colony polygon. All mapped *L. neglectus* occurrences (sampling locations and field observations without sampling) were then imported into ArcGIS 10.1 and colonies surface area was calculated as the area of the polygon obtained from mapped locations by minimum bounding geometry using ArcGIS 10.1.

Fungus detection and prevalence

Five *Lasius* species were screened for the presence of *L. formicarum*: *L. neglectus* (66 colonies, 142 ± 225 workers per colony were examined), *L. niger* (230 colonies, 19 ± 6 workers per colony), *L. alienus* (118 colonies, on average 18 ± 6 workers per colony), *L. paralienus* (39 colonies, on average 14 ± 4 workers per colony) and *L. emarginatus* (26 colonies, on average 16 ± 8 workers per colony). To guarantee an acceptable detection probability of *L. formicarum*, we only considered samples with at least 10 workers. Consequently, 14 out of the 80 initial *L. neglectus* colonies were removed from the dataset. We used a stereoscopic microscope at 50x magnification to examine ants for fungus detection. An individual was considered infected if at least one *L. formicarum* thallus was observed on its cuticle. All *L. neglectus* samples obtained from colony measurements were examined for *L. formicarum* presence to investigate intra-colonial prevalence patterns. Fungus prevalence was computed as the proportion of infected individuals over all examined ants.

Since *L. formicarum* is the only species of its genus parasitizing *Lasius* species in Europe (Espadaler et al. 2011), we considered all thalli detected as *L. formicarum*.

Urbanization index

To assess the impacts of urbanization on co-invasion patterns, an urbanization index was defined as the proportion of impervious land cover in 50 meters radius buffers around all locations invaded by *L. neglectus*. The impervious land cover map (1 m spatial resolution) was obtained from BD TOPO® (IGN 2013) and SPOT 6 images (http://geosud.teledetection.fr/).

Statistical analyses

Statistical analyses were carried out using R (R Core Team 2014). In order to test for spatial genetic autocorrelation in *L. neglectus* colonies, we used a Mantel test with 99999 permutations (package *ade4*, Dray & Dufour 2007). This analysis tested for the correlation between geographical and genetic distances between colonies. The geographic distance matrix was calculated using the package *fossil* (Vavrek 2011), and we used *Fst/(1-Fst)* (obtained using Genepop 4.2, Rousset 2008) to measure genetic distance . We used a Mantel correlogram to visualize the shape of the spatial genetic autocorrelation among geographic distance classes (5 km classes were used).

To test for the vertical transmission hypothesis, we used the D statistic with 99999 permutations (package *caper*, Orme et al. 2013). The D statistic was originally designed for measuring phylogenetic signal strength in binary traits (Fritz et al. 2010). It necessitated the construction of a genetic tree (Reynolds genetic distance matrix: package *poppr*, Kamvar et al. 2014; *nj* algorithm: package *ape*, Paradis et al. 2004) rooted by mid-point method (package phytools, Revell 2012). If a binary trait is randomly dispersed over the genetic tree, D will tend to 1; however, if a binary trait is clumped (vertical transmission under a brownian scenario), D will be tend to 0 (an extremely clumped trait will have negative values of D) (Fritz et al. 2010).

The relationship between genetic diversity (rarefied allelic richness, obtained with HP-Rare, Kalinowski 2005, Table S2) and fungus prevalence was tested using linear regression. We investigated the intra-colonial heterogeneity of *L. formicarum* prevalence by calculating a heterogeneity index that is the variance of samples prevalence divided by the maximum variance possible. This index ranges from 0 (homogeneously infected) to 1 (very heterogeneously infected). In order to assess for intra-colonial spatial patterns of *L. formicarum* prevalence, we interpolated fungus prevalence along colonies' extents from point samples using the inverse distance weight interpolation tool (IDW, ArcGIS Spatial Analyst).

Student's t-tests and linear regressions were used to test the effect of urbanization on fungus presence and prevalence. To test the relationship between urbanization, fungus prevalence and colony size, multiple regressions were used, and, because of colinearity between predictors (i.e. urbanization index and fungus prevalence), we used commonality analysis to disentangle predictors effects (package yhat, Nimon et al. 2013). All linear regressions were calculated with appropriate data transformation (log or logit, scaling and centering).

Results

1) *L. neglectus* invasion patterns

A clear isolation by distance pattern among *L. neglectus* colonies was detected (Mantel test, n=29, p= 0.0003). The Mantel correlogram (Fig. 3) showed significant positive genetic relatedness between colonies for the second geographic distance class (i.e. 5 to 10 km), but not for the first geographic distance class (0 to 5 km).

2) *L. formicarum* invasion pattern

Host identity

Among native species, all *L. alienus*, *L. paralienus* and *L. emarginatus* colonies were free of *L. formicarum*. However, the fungus was found in 5% of *L. niger* colonies and 58% of *L. neglectus* colonies (Table 1, Fig. 1). In infected *L. neglectus* colonies, *L. formicarum* prevalence (i.e. proportion of infected workers) varied from 1.6% to 100% (Fig. 2).The eleven infected *L. nige*r colonies were found in only six urban sites, four of which were invaded by fungus-infected *L. neglectus*.

Table 1: List of the five species monitored for *Laboulbenia formicarium* infection. N: number of worker individuals screened for infection by *L. formicarium*, N_{inf} number of individuals observed to be infected and the associated prevalence.

Species	**N**	$\mathbf{N_{inf}}$	**Prevalence**
L. neglectus	66	38	0.58
L. niger	230	11	0.05
L. alienus	118	0	0
L. paralienus	39	0	0
L. emarginatus	26	0	0

Inter- and intra-colonial variation in fungus prevalence

D-statistic analysis indicated that infection occurrence across the genetic tree was more likely to result from a Brownian than from a random scenario (D= -1.08, p= 0.76 and 0.09 respectively), indicating that the presence of *L. formicarum* in *L. neglectus* colonies probably resulted mainly from vertical transmission (Fig. 4). We tested the relationship between genetic diversity and *L. formicarum* prevalence in infected colonies and found no significant relationship (linear regression: n=17, p=0.76). Intra-colonial prevalence was, as predicted, highly variable (as illustrated by interpolation mapping, Fig. 5). Indeed, only 7 out of 23 infected colonies were homogeneously infected (Table 2).

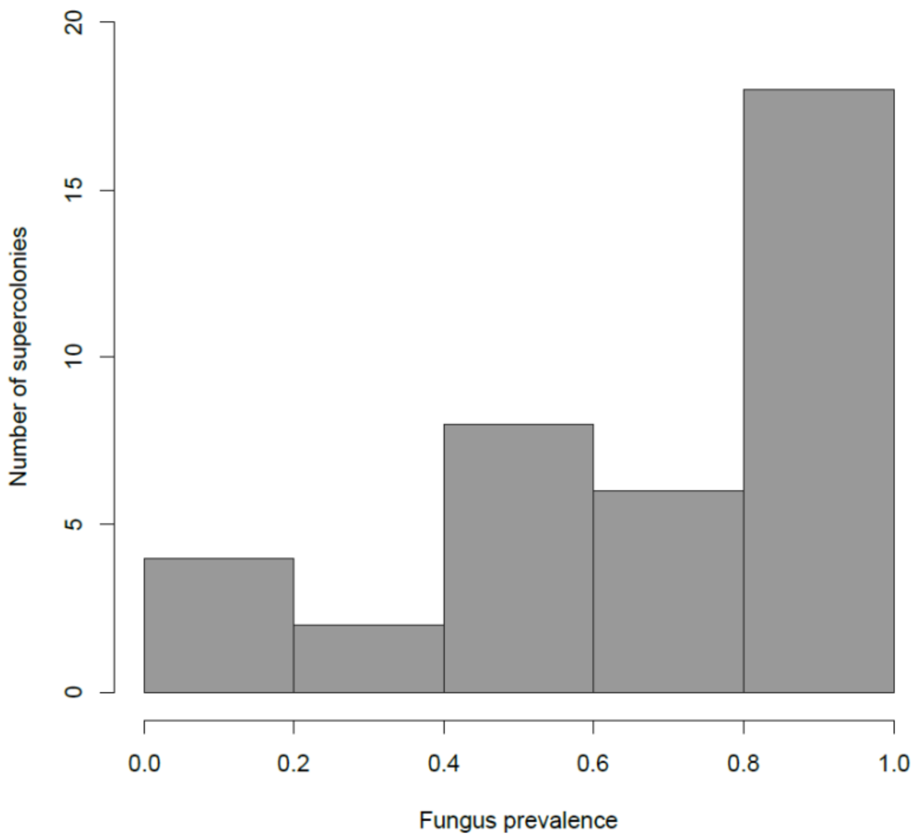

Figure 1: Distribution of the prevalence of *L. formicarum* (for infected *L. neglectus* colonies, n=38)

3) Impacts of urbanization on co-invasion patterns

Urbanization and fungus prevalence

Infected colonies were not located in more urbanized habitats than uninfected ones (t test: n=66, p= 0.88). However, we found a positive relationship between the urbanization index and *L. formicarum* prevalence in infected colonies (linear regression: n=38, R^2= 0.353, p=0.00005), indicating higher fungus prevalence in more urbanized areas (Table S3).

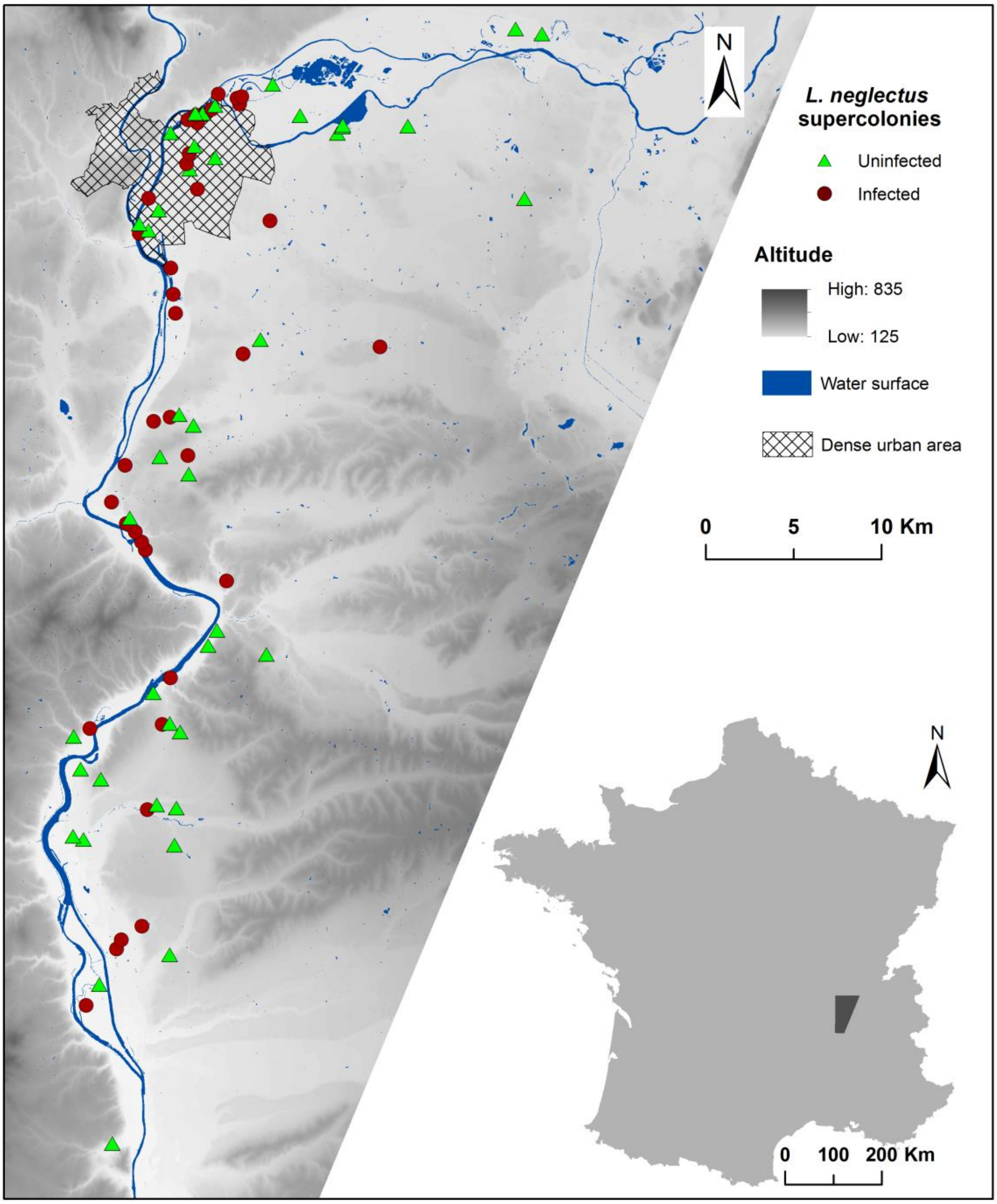

Figure 2: Spatial distribution of *L. neglectus* colonies and the fungal parasite *L. formicarum*.

Table 2: List of the 33 measured *Lasius neglectus* colonies with information on colony name, location (latitude, longitude), area, *L. formicarum* fungus prevalence, number of nests sampled within colonies and prevalence heterogeneity index.

Colony name	Latitude (WGS84)	Longitude (WGS84)	Colony area (m²)	*L. formicarum* prevalence	Nests sampled	Prevalence heterogeneity
NC06_07	45.6059	4.8068	243197	0.78	58	0.5
StDesirat	45.2583	4.7863	240037	0	57	0
BalanElec	45.8248	5.1004	49842	0	16	0
NC14	45.3694	4.8114	48030	0.85	21	0.25
DouaBohr	45.7849	4.8710	22096	0.29	18	0.36
NC15	45.3626	4.7961	20395	0.57	13	0.68
NC16	45.3580	4.7926	20266	0.49	12	0.79
CCbassin	45.7928	4.8950	19961	0.52	15	0.74
NC18	45.3400	4.7797	16711	0	15	0
NP12	45.7875	4.8762	12376	0.06	19	0.21
NC04	45.6256	4.8575	11316	0	12	0
CCtas	45.7935	4.8985	9591	0.01	7	0.01
NC10	45.5761	4.8069	9546	0.93	11	0.04
NP11	45.7246	4.8212	9407	0.18	8	0.54
NC19	45.3294	4.7695	8010	0.47	8	0.38
NC08	45.5872	4.7964	7802	0.22	14	0.62
NP13	45.7855	4.8695	6218	0	8	0
DouaDomus	45.7807	4.8654	5355	0.8	10	0.35
NC01	45.6281	4.8283	4024	0.55	15	0.48
NC03	45.6314	4.8472	3712	0	8	0
CCportail	45.7952	4.8813	1477	0.49	5	0.48
NP01	45.7263	4.8281	622	0	5	0
NC05	45.6099	4.8324	325	0	4	0
NC02	45.6300	4.8405	311	0.98	7	0.01
NP10	45.7625	4.8779	207	0	6	0
CCconfluent	45.7898	4.8967	163	0.3	4	0.91
NC13	45.5623	4.8203	91	1	3	0
NC12	45.5664	4.8176	62	0.75	4	0.14
NP07	45.7759	4.8459	56	0	5	0
NP14	45.7848	4.8647	54	0.47	2	0.53
NP05	45.7599	4.8571	52	0.97	2	0.01
NP06	45.7650	4.8590	48	1	3	0
NP03	45.7467	4.8645	43	0.93	2	0.01

L. neglectus colony size, fungus prevalence and urbanization

L. formicarum prevalence was significantly negatively correlated to infected colony size (p=0.029) while the proportion of impervious surface was not (p=0.95) (multiple linear regression: n=21, R²=0.30, p=0.016). Commonality analysis confirmed this result: 53.2% of explained variability was unique to fungus prevalence, 0.04 % was unique to the proportion of impervious surfaces and 46.8% was common to the two predictors.

On the contrary, a significant negative effect of urbanization on rcolony size (linear regression: n=8, R²=0.83, p=0.008) was found in uninfected colonies.

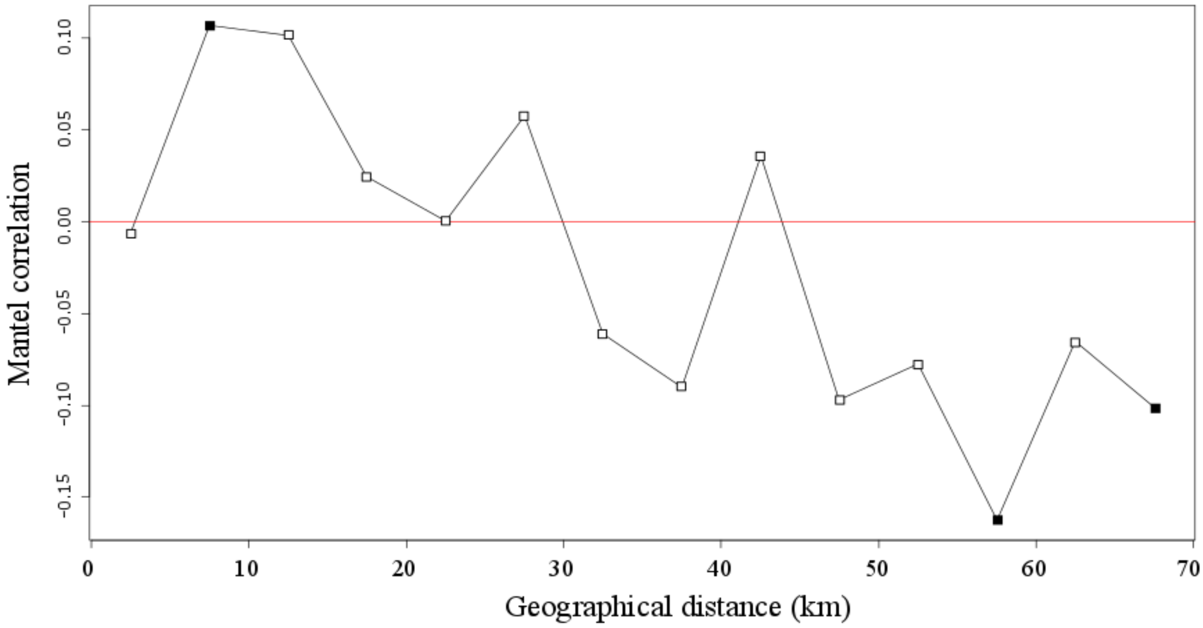

Figure 3: Mantel correlogram (distance class size : 5 km) performed on [Fst/(1-Fst)] genetic distances between ant colonies. Black doted classes indicate significant Mantel correlation (p<0.05).

Discussion

The present study made use of an uncommon ecological context involving a co-invading, but not co-introduced, host-parasite couple spreading through an urbanized landscape. We have demonstrated that at landscape scale both *L. neglectus* and *L. formicarum* were much more frequent than expected from previous studies. However, our study is the first systematical survey for *L. neglectus* at landscape scale, with 1248 sites investigated, and 66 *L. neglectus* colonies examined for the presence of the parasitic fungus; it is therefore highly likely that other areas in Europe are as strongly invaded than the urban area of Lyon. *Lasius neglectus* has been defined as an urban exploiter species until now (Ugelvig et al. 2008), however, colonies were detected all along the urbanization gradient, with the largest two (> 20 ha) found in a residential peri-urban area and an isolated village. Because they have low direct impacts (but see Rey & Espadaler 2005), invasions such as *L. neglectus* in Europe could easily go unnoticed until landscapes are filled and indirect impacts occur. From an invasion ecology perspective, the parasitic fungus could have contrasting effects on ant communities. On the one hand, it might limit the expansion of invasive *L. neglectus* through negative impacts on its fitness, especially in urban ecosystems. However, it might also infect and strongly affect native species, which would facilitate the progression of the invasive ant.

Lasius neglectus invasion patterns

We detected genetic isolation by distance among *L. neglectus* colonies and determined that colonies distant from 5 to 10km were genetically closer than colonies in other distance classes. Contrary to usual isolation by distance, which is characterized by a gradual decrease of genetic relatedness with geographic distance, geographically closest colonies were not the genetically closest ones. This pattern could be explained by the human-mediated dispersal scenario we expected for *L. neglectus*. This is in line with the idea that short (and not long) distance human-mediated dispersal plays a more important role in the secondary spread of invasive species than usually assumed (King et al. 2009).

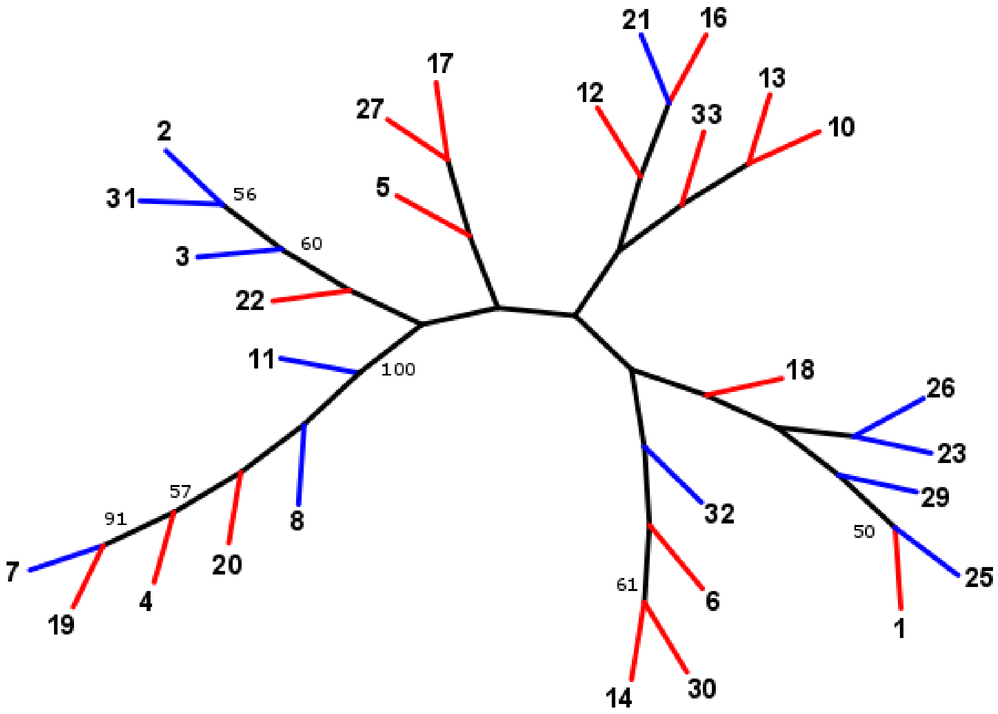

Figure 4: *L. neglectus* colonies genetic neighbor joining tree (unrooted) based on Reynolds genetic distance (bootstrap over 50% are indicated). colony infection status is indicated by color of terminal branches (red=infected; blue=uninfected).

Laboulbenia formicarum invasion patterns

58% of 66 *L. neglectus* colonies screened for *L. formicarum* were infected (38 colonies), which is indicative of a long co-invasion history between the ant host and the parasitic fungus. Previous studies suggested that the two species came into contact in Europe less than 20 years ago (Espadaler et al. 2011, Tragust et al. 2015). Our results suggest a longer association and a re-evaluation of the age of introduction of *L. formicarum* in Europe. We also recorded the first natural infection of *L. niger* by *L. formicarum*, which suggests the existence of cross-infections as was evidenced by Tragust et al. (2015) in laboratory experiments. Indeed, most of the infected *L. niger* colonies were sampled in locations occupied by infected *L. neglectus* colonies. In contrast, *L. formicarum* was not found on *L. emarginatus*, *L. alienus* or *L. paralienus*, but fewer colonies of these species were sampled and examined. Given the low occurrence of *L. formicarum* in *L. niger* colonies (5% of the colonies infected), we cannot exclude fungus infection in these species at similar or lower rates than in *L. niger*.

Our results confirmed a vertical transmission scenario for *L. formicarum*, however, the presence of the fungus over the terminal branches of *L. neglectus* genetic tree was not as clumped as what could have been expected under a hypothesis of strict vertical transmission that assumes obligate fungus transmission when the colony of origin is infected (Fig. 4). An explanation might be provided by intra-colonial infection patterns (Fig. 5, Table 2), because, as we showed, inside infected colonies fungus prevalence was spatially heterogeneous. Displacement of soil originating from an infected colony might therefore contain fungus-free ant workers only, depending on where the soil was extracted (Fig. 5), leading to uninfected 'daughter' colonies.

Another, non-exclusive, hypothesis could be that *L. formicarum* might be transmitted horizontally. Some *L. neglectus* colonies were spatially close, which might provide opportunities for the fungus to be horizontally transmitted, even without direct contact between *L. neglectus* workers. *L. neglectus* colonies are known to shelter several myrmecophilous species (e.g. Nitidulidae and Clytrinae beetles, myrmecophilous crickets, Nagy et al. 2009) which are likely to act as vectors when dispersing from one colony to another (Kronauer and Pierce 2011). Despite the lack of reported observations of swarming in *L. neglectus*, males could be another possible vector for the transmission of *L. formicaru*m: we did observe the take-off and active flight of *L. neglectus* males from infected colonies (Fig.6). To our knowledge, it is the first time that swarming behavior is documented for this species. As no reproductive females were observed leaving *L. neglectus* nests, male exchanges may occur between *L. neglectus* colonies, as observed in Argentine ants (Passera & Keller, 1994). Not only could males act as vectors for the fungus, but their ability to swarm might also strongly impact *L. neglectus* gene flow and genetic diversity wherever colonies are located at short distances from one another. This could have potential positive indirect effects on colonies fitness and immunity (Whitehorn et al. 2011), despite the fact that we found no relationship between genetic diversity and *L. formicarum* prevalence among *L. neglectus* colonies.

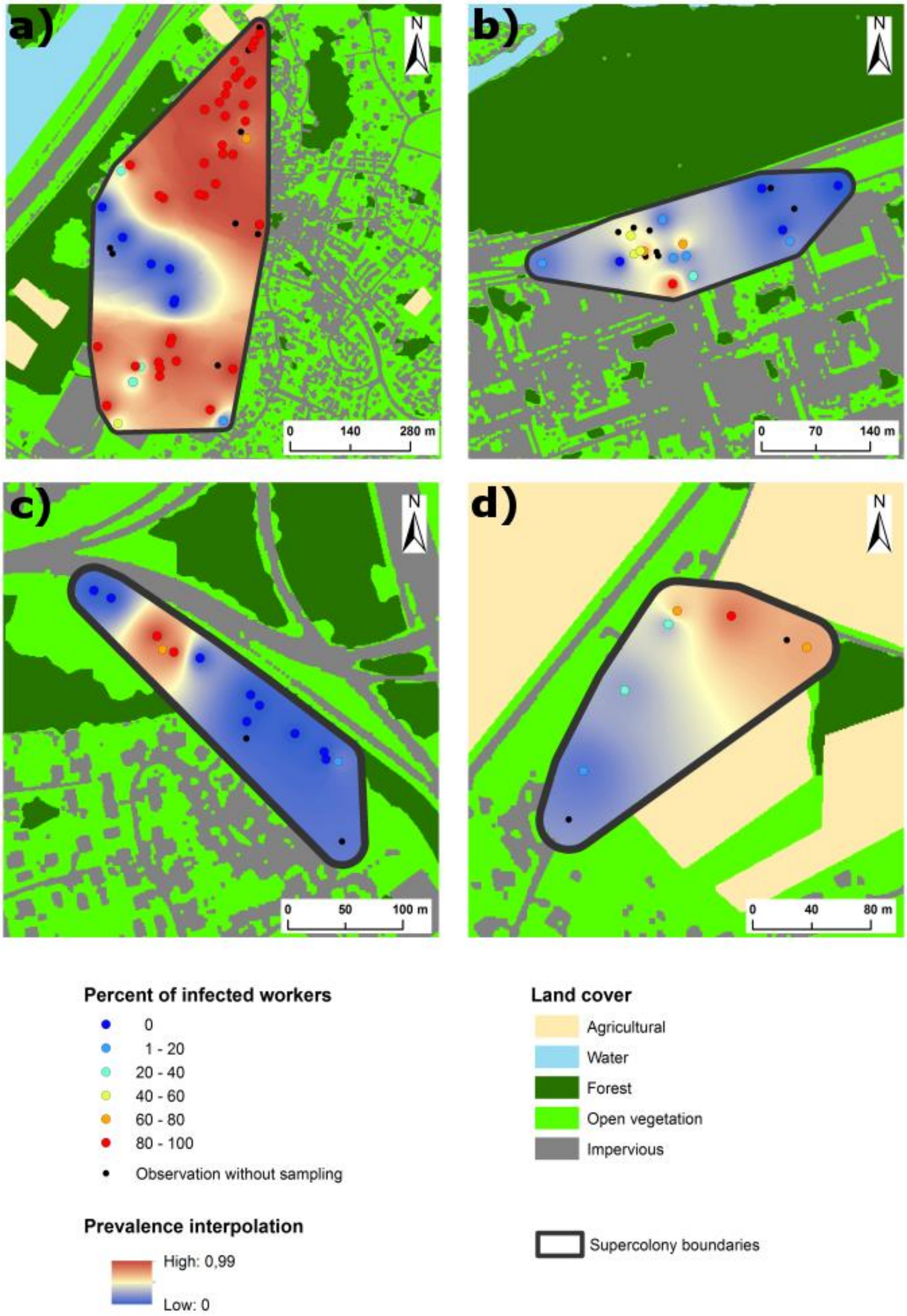

Figure 5: Intra-colonial prevalence of *L. formicarum* for four *L. neglectus* colonies. Shades of blue indicate uninfected status, red indicates infection. Colonies represented are: a) NC06_07, b) DouaBohr, c) NC08 and d) NC19 (Table 2).

Figure 6: Photographs showing *L. neglectus* males taking off from the nest, attended by a worker infected by *L. formicarum* (Photographs by T. Colin).

Impacts of urbanization on co-invasion patterns

Despite the importance of dispersal processes in co-invasion dynamics, environmental conditions are also likely to affect co-invasion patterns. In our study area, *L. neglectus* colonies were found all along the urban gradient, from semi-natural grasslands to small green spaces within the urban core, contrary to previous observations (Cremer et al. 2008, Ugelvig et al. 2008). We showed that the urbanization index was not correlated with the occurrence of *L. formicarum*, but positively correlated with its prevalence in infected colonies. These results confirmed our prediction that *L. neglectus* might be more vulnerable to parasites in an urban context, suggesting that environmental conditions associated with urbanization affect the host-parasite relationship. In line with this observation, urbanization has been associated to short-range variation in the prevalence of another *Laboulbeniales* species parasitizing a coccinellid beetle in the UK (Welch et al. 2001). In addition, all eleven infected *L. niger* colonies detected in this study were located in an urban context, hinting to the impact of urbanization on the vulnerability of native species to fungal infection. Furthermore, we showed that the size of infected colonies was negatively related to *L. formicarum* prevalence but not to the urbanization index. Because these two predictors were related (and thus co-linear), we tested the relationship between urbanization and colony size for uninfected colonies and found a significant negative relationship. Accordingly, we can conclude that *L. neglectus* colonies size is negatively related to both *L. formicarum* prevalence and urbanization.

Conclusion

The last decade has witnessed several population collapses of invasive ant species (Cooling et al. 2012, Kaspari& Weiser 2014, Cooling & Hoffmann 2015). Factors responsible for these crashes were unclear in most cases, but antagonistic interactions between independently introduced species have recently been demonstrated (LeBrun et al. 2014). Our results show that both the fungus *L. formicarum* and urbanization seemed to limit colony size and could lead to collapses of *L. neglectus* colonies in the future. However, as a control agent, this fungal parasite might turn into a double-edged blade that is likely to threaten native ant species. Finally, our findings highlight the fact that urbanization might affect co-invasions dynamics in multiple, antagonistic ways: on one hand urbanization increased human-mediated secondary spread of ants across the landscape, on the other hand, it hampered the spatial expansion of colonies and induced higher parasite prevalence.

Electronic Supplementary Material

Table S1: Microsatellite markers used.
Table S2: Basic multilocus population genetics statistics.
Table S3: Detailed information on *L. neglectus* colonies

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Author contributions

JMWG wrote the paper; NM, BK and JG supervised the writing and bring significant improvements to the paper; TC, MH, JMWG and BK conducted most of the field and ant identification work; FW, TC, MH, JMWG performed the ant screening for fungal infection; AB produced the land cover data; JMWG processed all other GIS data and all statistical analyses. AD realized most molecular identifications.

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Electronic Supplementary Material

Table S1: Microsatellite markers used: N alleles is the total number of alleles detected in this study.

Microsatellite name	Reference	Fluorochrome	PCR Mix	Size range (in bp)	N alleles
Lng-1	Ugelvig et al. 2008	HEX (Sigma)	2	200-216	6
L1-5	Fjerdingstad et al. 2003	6-FAM (Sigma)	3	276-296	9
L10-174	Fjerdingstad et al. 2003	PET (AppliedBiosystems)	4	220-286	15
La32f	Steiner et al. 2007	HEX (Sigma)	1	337-353	4
La33b	Steiner et al. 2007	NED (AppliedBiosystems)	1	213-221	3
La36d	Steiner et al. 2007	NED (AppliedBiosystems)	2	138-140	2

Table S2: Basic multilocus population genetics statistics for all colonies. N = number of workers genotyped, He = expected heterozygosity (unbiased), Ho = observed heterozygosity, A = mean allelic richness over all 6 loci, Ar = rarefied mean allelic richness over all loci, Fis and associated p-value for deviation from Hardy-Weinberg genotype frequencies over all loci.

Colony ID	N	He	Ho	A	Ar	Fis	p-value
1	36	0.3698	0.3687	2.33	2.11	0.0032	0.6502
2	24	0.4315	0.3978	2.83	2.70	0.0798	0.2033
3	24	0.4237	0.3711	2.83	2.55	0.1267	0.3607
4	24	0.3968	0.3641	2.17	2.08	0.0840	0.3914
5	24	0.4065	0.4028	2.17	2.13	0.0093	0.5302
6	24	0.5032	0.4994	3.00	2.74	0.0078	0.5614
7	36	0.4084	0.3981	2.67	2.44	0.0254	0.9277
8	24	0.4363	0.4127	2.50	2.31	0.0553	0.9947
9	12	0.4849	0.5000	3.33	3.33	-0.0326	1.0000
10	24	0.4240	0.4117	2.67	2.48	0.0296	0.4940
11	24	0.6097	0.5928	4.00	3.75	0.0283	0.3883
12	24	0.5925	0.5746	3.33	3.30	0.0309	0.9749
13	24	0.2926	0.2639	1.83	1.75	0.0999	0.7388
14	22	0.5394	0.5975	3.67	3.24	-0.1106	0.6159
15	33	0.5930	0.5258	4.33	3.65	0.1149	0.3562
16	24	0.4618	0.4786	2.83	2.70	-0.0381	0.9476
17	22	0.3201	0.2976	2.17	2.02	0.0724	0.4340
18	24	0.5282	0.4861	3.67	3.35	0.0813	0.6204
19	24	0.4441	0.3808	2.50	2.26	0.1453	0.1767
20	24	0.4082	0.4097	2.17	2.08	-0.0037	0.9980
21	24	0.3104	0.3215	2.17	2.03	-0.0370	0.3556
22	24	0.5262	0.5106	4.17	3.68	0.0304	0.7703
23	24	0.3733	0.3116	2.50	2.30	0.1683	0.2076
24	24	0.5923	0.5799	4.00	3.60	0.0216	0.0900
25	23	0.4288	0.4496	2.33	2.22	-0.0497	0.1554
26	24	0.2985	0.2708	1.83	1.75	0.0944	0.5387
27	24	0.4969	0.5236	2.67	2.46	-0.0547	0.3332
28	36	0.3528	0.3534	2.00	1.93	-0.0018	0.1565
29	24	0.4235	0.4792	2.50	2.45	-0.1348	0.0327
30	24	0.4727	0.4232	2.83	2.56	0.1070	0.2175
31	24	0.3905	0.3347	2.33	2.17	0.1457	0.6320
32	24	0.4740	0.4167	3.17	2.97	0.1233	0.1108
33	24	0.4829	0.4514	3.17	2.95	0.0665	0.4798
34	16	0.4228	0.4021	2.00	2.00	0.0505	0.2337

Table S3: Detailed informations on the 80 *L. neglectus* colonies of the study

Colony name	Colony area (m²)	Number of workers screened	Number of workers infected	*L. formicarum* prevalence	Colony IDs for Fig. 4	Proportion of impervious surfaces
Arbuel	na	9	0	na	na	0.12
BalanElec	54437.26	335	0	0	2	0.1
BalanMili	na	30	0	0	3	0.05
Bellecombe	na	20	0	0	na	0.78
C03_14	na	153	107	0.7	33	0.32
C10_01	na	20	16	0.8	na	0.38
C10_02	na	0	0	na	na	0.12
C11_04	na	120	63	0.53	1	0.22
C12_09	na	9	0	na	na	0.20
C12_11	na	10	0	0	na	0.42
C13_13	na	9	0	na	na	0.04
C14_13	na	98	0	0	7	0.04
CCbassin	40650.43	285	139	0.49	na	0.23
CCconfluent	899.99	60	18	0.3	4	0.04
CCpointe	na	10	0	0	na	0.02
CCportail	2466.31	142	61	0.43	10	0
CCtas	13833.69	125	2	0.02	na	0
DouaBohr	26291.93	174	46	0.26	13	0.49
DouaDomus	7138.55	146	113	0.77	12	0.70
IleBeurre	na	85	82	0.96	na	0.27
NC01	5427.91	435	273	0.63	22	0.32
NC02	1536.45	174	149	0.86	na	0.14
NC03	5115.11	259	0	0	29	0.66
NC04	14018.74	489	0	0	23	0.06
NC05	870.94	95	0	0	8	0.32
NC06_07	254311.09	1319	1036	0.79	30	0.38
NC08	11032.28	281	53	0.19	5	0.05
NC10	11852.88	245	219	0.89	6	0.61
NC11	na	43	39	0.91	na	0.28
NC12	1011.81	79	47	0.59	na	0.57
NC13	979.061	76	74	0.97	na	0.36
NC14	52999.93	376	322	0.86	20	0.43
NC15	24949.02	242	140	0.58	na	0.54
NC16	23881.88	247	113	0.46	na	0.25
NC18	20027.39	292	0	0	21	0.01
NC19	10328.44	122	57	0.47	na	0.21
NP01	1300.54	63	0	0	na	0.49
NP03	361.45	67	54	0.81	na	0.89
NP05	394.84	42	40	0.95	17	0.81
NP06	373.93	74	74	1	na	0.81
NP07	1049.11	79	0	0	na	0.78
NP09	na	25	19	0.76	na	0.32
NP10	616.38	80	0	0	31	0.82
NP11	16025.99	161	21	0.13	16	0.14
NP12	14970.84	304	20	0.07	14	0.07
NP13	8833.19	101	0	0	na	0.1
NP14	425.84	50	22	0.44	na	0.63
P02_17	na	15	15	1	na	0.31
P03_16	na	20	0	0	na	0.35
P03_27	na	18	0	0	na	0.07
P04_17	na	8	0	na	na	0.43
P09_18	na	20	20	1	na	0.85
P12_02L	na	30	0	0	32	0.54
StDesirat	250347.48	1153	0	0	26	0.15
T05_09	na	8	0	na	24	0.23
T05_10	na	58	0	0	25	0.29
T13_01	na	11	11	1	na	0.74
T14_11	na	12	0	0	na	0.10
T16_15	na	8	0	na	na	0.14
T19_04	na	14	14	1	27	0.22
T27_09	na	6	0	na	na	0.27
T32_13	na	23	23	1	na	0.69
T39_01	na	51	50	0.98	na	0.22
T39_03	na	54	0	0	na	0.52
T39_05	na	10	0	0	na	0.65
T39_06	na	3	0	na	28	0.21
T39_15	na	19	18	0.95	19	0.30
T39_16	na	0	0	na	na	0.55
T39_17	na	56	0	0	na	0.16
T40_14	na	0	0	na	9	0.63
T41_01	na	18	18	1	na	0.92
T41_02	na	30	30	1	18	0.38
V01_03	na	17	0	0	na	0.85
V10_08	na	9	0	na	15	0.64
V11_02	na	7	0	na	34	0.64
V11_10	na	13	0	0	na	0.67
V14_06	na	6	0	na	na	0.97
V21_02	na	12	0	0	na	0.44
V21_07	na	39	0	0	na	0.25
V21_13	na	48	0	0	11	0.32

CHAPTER 4

In preparation: This chapter combines many results from a common garden experiment realized in 2014. These results should be divided in two articles.

The town ant and the country ant: Multiple phenotypic shifts without genetic differentiation among urban and rural conspecifics

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Abstract

Urbanization induces contrasted environmental conditions over reduced spatial scales. These environmental changes can modify phenotypes of urban dwelling organisms, either by plasticity or local adaptation. Morphological, physiological and behavioral differences between urban and rural populations have been demonstrated for some species but no studies have investigated the three groups of traits simultaneously. However, changes in one trait are likely to have consequences on other traits (e.g., size and aggressiveness), and may even affect selective pressures in complex ways. In this study, we investigated simultaneously morphological, physiological and behavioral traits changes between urban and rural populations of the black garden ant *Lasius niger*. We showed that urban young queens were smaller and lighter, that they were less physiologically stressed by heat (lower expression of Hsp60) and that they performed multiple mating more often than rural queens. We also showed slight differences in colony founding success between urban and rural queens (urban queens had less pupae). Finally, workers produced under common garden experiments demonstrated higher inter- and intra-colony variability in size for urban colonies but no differences in HSPs expression between urban and rural colonies. Despite the fact that some of our results highly suggested local adaptation in urban populations of *L. niger*, we detected no genetic differentiation between urban and rural populations. These findings constitute the first evidence of simultaneous morphological, physiological and behavioral changes associated with urbanization. They raise several questions about eco-evolutionary dynamics happening in urban environments and their consequences on the evolutionary trajectories of urban dwelling organisms.

Keywords: Urbanization, morphology, physiology, behavior, common garden, HSP expression, *Lasius niger*

Introduction

Eco-evolution in an urbanized world

The spatial and temporal heterogeneity of environmental conditions can cause rapid evolutionary changes and population divergence, potentially leading to adaptive radiation (Gavrilets and Losos 2009, Price et al. 2003). This fundamental eco-evolutionary process of biological diversification has far-reaching consequences for the future of biodiversity in a rapidly changing world. Among global changes, the urban revolution associated with the development of human populations and activities is a major threat for biodiversity (Grimm et al. 2008, Maxwell et al. 2016), but is also leading to rapid environmental changes which constitute opportunities for eco-evolutionnary dynamics and biological diversification (Palkovacs and Hendry 2010, Alberti 2015). Moreover, urbanization induces localized and rapid changes in several environmental conditions simultaneously, as urban areas differ from adjacent rural or natural areas in land use and land cover, with increased habitat fragmentation, in air and ground temperatures (e.g., urban heat island effects where urban temperatures can rise several degrees above neighboring rural temperatures), in chemical, light and acoustic pollution and in modifications of nutrient flows (Gaston 2010, Forman 2014). These modifications have been associated with changes in the composition of biological communities, including the introduction and spread of alien species, modifications of biotic interactions and phenotypic variation among urban and rural conspecifics (Gaston 2010). Urban tolerant species, defined as taxa present in both rural and urban settings with no particular affinity for either (McIntyre 2000), are of particular interest for the study of biological diversification associated with urbanization. In such species, urban and rural conspecifics experience very different environmental conditions and could thus be predicted to undergo phenotypic shifts. Such differences might lead to contemporary adaptation (rapid evolution, Phillips and Shine 2004), population divergence and finally speciation (Slabbekoorn and Ripmeester 2008).

Phenotypic differences between urban and rural populations have been documented for morphology (body size: Magura et al. 2006 (carabids), San Martin y Gomez and Van Dyck 2012 (grasshoppers); locomotor organs: Schoville et al. 2013 (butterfly), Winchell et al. 2016 (lizards); cranial size: Snell-Rood and Wick 2013 (mammals), Tomassini et al. 2014 (bats); seeds and reproductive organs: Cheptou et al. 2008 (weed), Ushimaru et al. 2014 (weed)), physiology (heat tolerance; . Angilletta et al. 2007 (ant), Foucaud et al. 2013 (ant); oxidative stress: Costantini et al. 2014 (European Blackbird), Herrera-Dueñas et al. 2014 (House

Sparrow); hormones levels: Partecke et al. 2006 (European Blackbird)) and behavior (communication: Nemeth and Brumm 2009 (European Blackbird); circadian activity: Dominoni et al. 2013 (European Blackbird); sedentariness: Partecke and Gwinner 2007 (European Blackbird); social structure: Buczkowski 2010 (ants)). However, the direction of the changes is often case specific and the topic suffers from a lack of new biological models as most of the existing studies concern birds.

Ants as biological models for eco-evolution in an urbanized world

Ants (Hymenoptera, Formicidae) constitute good candidate models as many species are able to colonize urban areas and establish stable populations (Pećarević et al. 2010). Among such urban tolerant species, several are alien species considered major pests and have consequently been extensively studied over the past decades (e.g., *Solenopsis invicta*, *Linepithema humile*, Holway et al. 2002). Recent studies illustrated the potential of ants as a relevant biological model for studying phenotypic and evolutionary changes associated with urbanization, for instance, Angilletta et al. (2007) found that urban populations of the leafcutter ant *Atta sexdens* were more tolerant to heat stress than rural populations. Foucaud et al. (2013) corroborated this trend in the invasive ant *Wasmannia auropunctata* in its native range (South America) and additionally showed that invasive populations (in North America) demonstrated the same phenotype than native urban ants, pointing urban areas as potential pre-adaptation environments facilitating further invasions.

The black garden ant, *Lasius niger*, is a widespread Holarctic ant species characterized by its high tolerance to urban environments (Vepsäläinen et al. 2008, Ślipiński et al. 2012, Konorov and Nikitin 2015, Gippet et al. 2016). It is a generalist species foraging both on the ground and in trees, predating and scavenging on arthropods, but most of its energy requirements are met by carbohydrates-rich food such as extrafloral nectar and honeydew from aphids (Völkl et al. 1999). In early summer, alate males and gynes (unmated winged females) of *L. niger* leave their birth colony to take part in large mating flights involving many colonies (Aron et al. 2009). After mating with one to four males (Corley and Fjerdingstad 2011), queens land on the ground and dig a burrow in which they start laying eggs. *L. niger* is an independent founding species: new queens raise their brood by relying solely on the metabolic reserves they accumulated before nuptial flight (mainly fats stored in adipose tissue and proteins from the histolysis of their wing muscles). The first workers of incipient colonies emerge during late summer and forage for food until winter. Colonies then hibernate deep underground, and restart their activity in early spring (egg-laying for the queen; nest excavation, foraging and brood care for the workers). Once established, colonies can produce hundreds to thousands of alate males and gynes each year (Fjerdingstad et al. 2002) until the death of the queen, which can live up to 28 years (in captivity, Kutter and Stumper 1969). Nuptial flight and colony founding are consequently a crucial period in the life history of incipient colonies and their success determines colonies fitness (Fjerdingstad and Keller 2004). Parameters of survival and founding success of young queens are therefore essential for investigating the potential for urbanization to drive phenotypic and evolutionary changes.

Hypotheses and experiments

In this study, we investigated phenotypic and evolutionary shifts between rural and urban *L. niger* founding queens and the workers they produced under common garden experiments, as well as colony founding success and genetic differentiation between urban and rural populations.

1- Queens phenotypes

i) Morphological changes

We first measured morphological (weight and size), physiological (response to heat shock) and behavioral (number of matings) differences between urban and rural queens. Urban and rural conspecifics often demonstrate differences in body size (Gaston 2010). Size differences between urban and rural individuals can arise from phenotypic plasticity linked to developmental conditions (e.g., temperature, resource limitations; Angilletta et al. 2004, Chown and Gaston 2010) as well as from adaptive responses to fragmentation or other selective pressure (e.g., Cheptou et al. 2008). According to general trends in urban insects (Gaston 2010), we expected urban queens to be smaller and lighter than rural ones.

ii) Physiological changes

As previously demonstrated in two other ant species in response to urban heat island effects (Angilletta et al. 2007, Foucaud et al. 2013), we expected urban queens to be more tolerant to heat stress than rural queens. Cellular defense mechanisms can occur in order to deal with thermal stress using chaperone proteins. These molecules prevent the aggregation of polypeptides and assist in the refolding of proteins damaged by thermal stress but also play an essential role in unstressed cells by assisting in the initial folding processes. Heat Shock Proteins (HSP) are chaperone proteins commonly involved in thermal stress (Sorensen 2010). In a recent study, Ślipiński et al. (2015) showed that the ant *Formica cinerea* demonstrated up-regulation of Hsp60, Hsp75 and Hsp90 expression when experimentally exposed to 40°C and 45°C. We consequently chose to use the same HSP genes to investigate *L. niger* heat tolerance. According to Sorensen (2010), organisms acclimated or adapted to warm environments generally express lower levels of Heat Shock Protein coding genes (HSPs) when exposed to heat stress, compared to conspecifics of colder environments. Our hypothesis was that, because urban areas are warmer than adjacent rural areas, acclimation or adaptation to these thermal conditions should result in lower levels of HSP expression under heat stress in urban queens compared to rural queens. To test this hypothesis, we measured the expression levels of three HSPs (Hsp60, Hsp75 and Hsp90) under mild conditions (25°C, 1h) and heat stress (45°C, 1h).

iii) Behavioral changes

Simulation models (Rueppell et al. 2008 and Gove et al. 2009) predicted that mild or complex environments should favor multiple mating (because complexity favors a broader range of responses) while harsher and simpler environments should favor single mating (because combined, they favor high performance for a specific response). Corley and Fjerdingstad (2011) tested this prediction on a latitudinal gradient (from Uppsala, Sweden, to Cannes, south of France), assuming Northern Europe constituted harsher (because of low summer temperature constraining colony growth) and simpler (because of lower biotic diversity) environments while Southern Europe constituted milder (because of reduced summer temperature constraints on colony growth) and more complex (because of greater biotic diversity) environments. The authors found that mating frequencies were higher in lower latitudes, thus verifying their hypothesis. Transposed to our study context, urban areas correspond to milder (because of urban heat island effects) and more complex (because of environmental novelty and greater heterogeneity) environments while rural areas correspond to harsher and simpler environments. We thus expected to find more multiply mated queens in urban compared to rural areas. To measure mating frequencies, we dissected rural and urban foundress queens, isolated their spermatheca, and extracted its sperm contents. We genotyped sperm contents using 20 microsatellite markers (including 6 markers from the literature and 14 novel markers) to determine the number of haploid males mates.

2- Incipient colonies success

Second, we compared incipient colonies success by measuring the mortality and mass loss of queens, the time to emergence of the first worker, as well as brood development during foundation (number of eggs, larvae and pupae). *L. niger* ants have been found to exhibit high accumulation of pollutants such as heavy metals and phthalates (Grześ 2009, Grześ 2010a, Lenoir et al. 2014) but with no, or little, consequences on worker mortality or queens fitness (Grześ and Okrunitniak 2016a, Cuvillier-Hot et al. 2014). We thus expected no or little difference in colony founding success between urban and rural queens.

3- Workers phenotype

Third, we assessed morphological (size) and physiological (heat tolerance) differences between workers produced by urban and rural queens in a common-garden rearing experiment. Our objective was here to test for contemporary adaptation acting on the worker caste. As worker size has been related to alimentary range in some social hymenopteran species (Persson and Smith 2011, Quezada-Euán et al. 2014), we expected urban and rural workers to demonstrate contrasted mean or variance in size as urban and rural areas are likely to propose different types of resources and consequently to favor different worker size distribution in urban environments compared to rural ones (Penick et al. 2015). When foraging ant workers experience ground and air temperature outside the colony; as urban areas are warmer, we expected workers from urban colonies to demonstrate higher tolerance to heat stress and thus to exhibit lower levels of HSPs expression when exposed to heat stress (as hypothesized for queens, Sorensen 2010).

4- Genetic differentiation

Finally we assessed the genetic context of divergence by genotyping urban and rural queens at 20 microsatellite markers, as was done for their spermathecal contents. Boomsma and Van der Have (1998), using allozyme markers found no genetic differentiation between *L. niger* populations distant hundreds of kilometers and separated by the North Sea (e.g., England and Denmark). As a consequence, we expected no differentiation between the urban and rural populations of our study, which are only 25 kilometers distant from each other.

Material and methods

Field sampling

Freshly mated and dealated *L. niger* queens were collected on the ground, between June 27th and July 15th 2014, during or right after large nuptial flights. Queens were collected in two areas: in the urban core of Lyon and in a rural area 20 km away from the urban core. In the two areas, queens were sampled at different locations and days in order to obtain enough individuals. All sampled ant queens (78 urban queens and 145 rural queens) were weighed (±0.1 mg) immediately after nuptial flight (fresh mass) and then put in glass tube nests with a water reserve and closed with a cotton plug without food (Aron et al. 2009)

Common garden experiment

Incipient colonies were kept at 25°C in the dark for approximately 45 days. They were examined every 5 days to check for queen mortality and count eggs, larvae and pupae (with a stereomicroscope, magnification x40). Eggs were sometimes aggregated, making any exhaustive counting impossible; in such cases, only visible eggs were counted. After 35 days of colony founding, incipient colonies were checked every day to check for the time to emergence of the first worker.

After 44 days (on average, ranging from 41 to 48 days) of colony founding, surviving queens were re-weighed (except for the queens used in gene expression experiments) in order to assess their loss of mass (fresh mass). A total of 107 queens, 16 urban and 91 rural, were weighed. The mass loss was calculated as the difference between the initial mass and the final mass (we used the same precision balance as for initial weighting). Thirty urban queens were lost during rearing because of manipulation errors; they were consequently not taken into account for queen mortality, brood counting and weight loss estimations. At the end of the experiment, incipient colonies (queen, workers and brood) were killed and preserved in 96° alcohol at -80°C.

Morphometrics

We used inter-ocular distance (subsequently termed head width) as an estimate of queen and worker size (Aron et al. 2009). Queen head width was measured after the end of the colony founding survey, for a subset of the initially sampled queens (55 urban queens and 56 rural queens). Heads were mounted by pasting on a gridded labelled cardboard card in order to minimize angle differences for measurements (Tschinkel 2013). They were then photographed at x40 magnification using an Olympus DP25 camera mounted on an Olympus SZX16 stereomicroscope. Measures were realized with the cellD® software. The repeatability of measures was assessed by measuring twice 20 randomly chosen queens. Repeated measures differed on average by 0.006 mm and were highly correlated (Pearson correlation: $r = 0.97$, $P < 0.001$).

Four or five workers from 15 urban colonies and 19 rural colonies were used to measure head width and assess intra-colony, inter-colony and inter-origin (urban and rural) variation in size. Head width was photographed at a magnification of x100 and measured using the same equipment and protocol than for queens. The repeatability of measures was assessed by measuring twice 20 randomly chosen workers. Repeated measures differed on average by 0.014 mm and were highly correlated (Pearson correlation: $r = 0.92$, $P < 0.001$).

Heat shock proteins gene expression

Twenty incipient colonies (10 urban and 10 rural) were used to assess HSP expression in queens and workers when exposed to heat shock. Heat shock experiments were realized 40 to 47 days after the beginning of colony founding. Only incipient colonies with at least 6 workers were used. For five urban and five rural incipient coloniess, we split the colony in two groups: a first group containing the queen and three workers and the second group containing the other three workers. The two groups were kept in opened petri dishes (2.5 cm radius) with moist cotton placed in the center to avoid dehydration. The group containing the queen was exposed to 45°C for one hour while the group with only workers was kept at 25°C for one hour. To obtain controls for queens, five urban and five rural queens were kept at 25°C for one hour. All individuals were preserved at -80°C immediately after the one hour experiment.

We analyzed the expression of three Heat Shock Protein coding genes (Hsp60, Hsp75 and Hsp90) and two reference genes (α-tubulin and EF1) for each individuals (20 queens and 30 workers), by real-time qPCR. Normalized relative quantities (NRQs) were calculated from Cq (quantification cycles, Bustin et al. 2009; R package: EasyqpcR, Le Pape 2012) using both reference genes (α-tubulin and EF1) and calibration for inter-vial variation (only for workers, as queens were all in one vial). Primer design, sample preparation and calculation of normalized relative quantities are detailed in Supplementary Material I (TableS1).

Genetic analyses

Eighty queens (41 urban and 39 rural) were dissected: their spermatheca was extracted and the sperm was isolated and conserved separately. The sperm was genotyped using 20 microsatellites markers (see DNA extraction, genotyping details and characteristics of microsatellite loci in Supplementary Material II, Table S2). Because ant males are haploid, the number of mating per queen was assessed by identifying the number of alleles in genotyped sperm contents.

Using the same microsatellites markers, we genotyped the queens previously described to check for genetic differentiation between urban and rural populations.

Statistical analyses

We used linear models to assess the relationship between initial mass, size and origin (urban or rural) of sampled queens. We tested head width differences between urban and rural workers using linear mixed models (origin as fixed effect and colony identity as random effect). Urban-rural difference in the variance of average colony head width and urban-rural difference in colony coefficient of variation in head width were tested using Student t-test.

For queens, we used linear models to test the effect of temperature (25°C or 45°C), queen origin (urban or rural) and their interaction on the expression rate of each HSP gene (log transformed to approach normality). For workers, we used linear mixed models to test the effect of temperature (25°C or 45°C), origin (urban or rural) and their interaction (fixed effects) on the expression of each HSP gene (log transformed to approach normality) with colony identity as random effect.

We tested urban-rural difference in the number of mating per queen using a permutation test (10,000 permutations). Genetic differentiation between urban and rural populations was tested using Genepop 4.0 (Rousset 2008); STRUCTURE software (Pritchard et al. 2000) was used to detect genetic clustering between and within the two sets of queens (with admixture option, with a burn-in period of 100000 and 1000000 subsequent MCMC repetitions, with 10 runs at each K value from 1 to 5). Genetic methods and markers are detailed in Supplementary Material.

We used logistic regressions to test the effect of initial mass, origin and their interaction on the probability of queen death during colony founding.

Because of the small number of urban queens compared to rural ones ($n_{urban} = 16$ and $n_{rural} = 91$), we tested the difference in mass loss between urban and rural queens by randomly re-sampling 16 out of 107 individuals 10,000 times. For each re-sampling, we calculated a

Wilcoxon statistic (W). We calculated the Wilcoxon statistic of our observed dataset and calculated the probability of observing a higher value under the normal distribution of previously computed 10,000 W values from re-sampling.

Differences in brood development of urban and rural incipient colonies (respectively n = 40 and n = 128) were assessed by comparing the number of eggs, larvae and pupae at each surveyed date (5, 10, 15, 20, 25, 30, 35 and 40 days) using 95% confidence interval around the mean.

Results

Queens phenotypes

Size and weight – Rural queens were heavier than urban ones (n_{urban} = 78, n_{rural} = 145, 28.29 mg and 26.4 mg respectively, t = 4.9, $P < 0.0001$). The best model to describe the relationship between initial mass, size and origin of queens was the additive model (initial mass ~ size + origin). Both head width and origin had significant effect on initial mass (n_{urban} = 55, n_{rural} = 56, P = 0.0001 and P = 0.017 respectively; $R^2 = 0.2$, $P < 0.0001$).

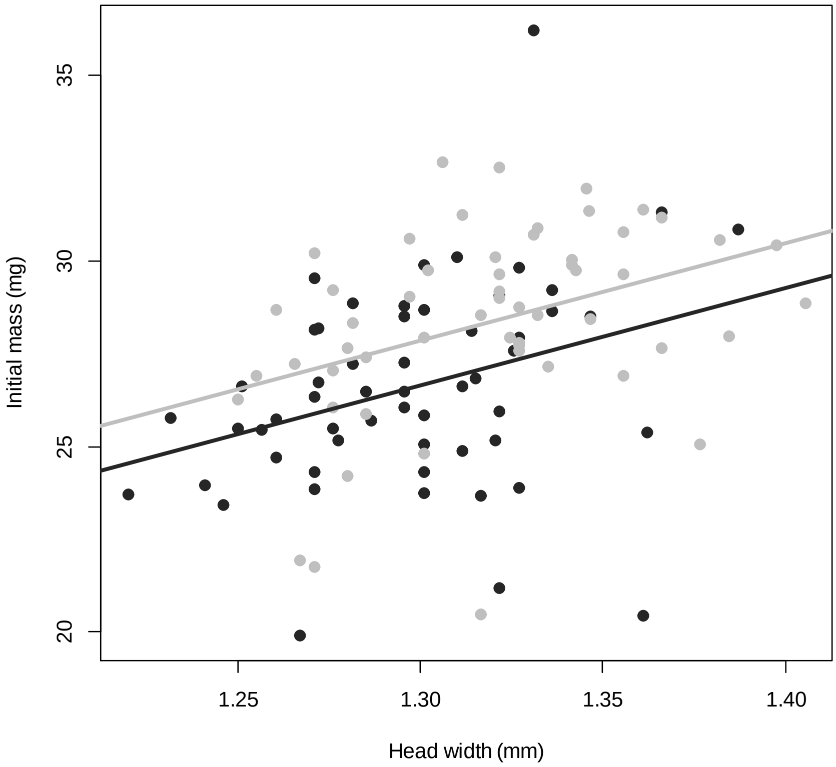

Figure 1: Linear regression describing the relationship between initial mass, head width and origin of *L. niger* queens. Grey and black dots are rural and urban queens respectively. Grey and black lines represent the regression between initial mass and head width for rural (grey) and urban (black) queens.

Heat stress – Hsp60 and Hsp90 were up-regulated in 45°C treatments and only Hsp60 expression was related to queen origins, as urban queens expressed lower levels of Hsp60 than rural queens for both control and treatment. The expression of Hsp75 was not related to any factor for queens (Fig. 2).The additive model (temperature + origin) was the best to explain Hsp60 expression levels for queens. Temperature and origin had a significant effect on Hsp60 expression ($n_{urban} = 9$, $n_{rural} = 9$, $P < 0.001$ and $P = 0.003$ respectively, $R^2 = 0.94$). The model with only temperature was the best to explain Hsp90 expression levels in queens. ($n_{urban} = 10$, $n_{rural} = 10$, $P < 0.001 R^2 = 0.83$).

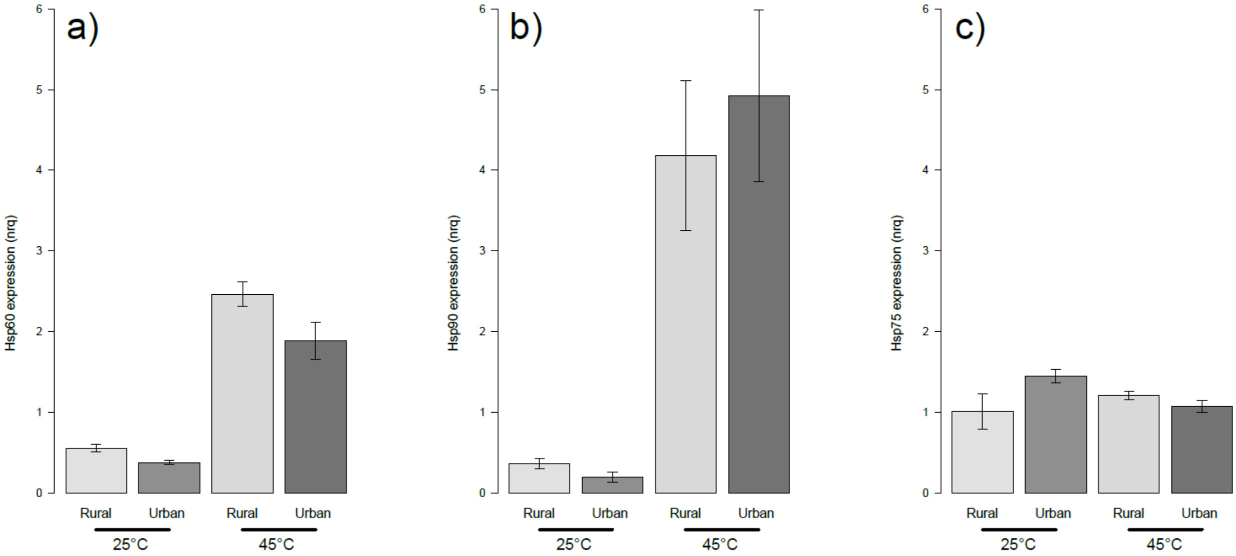

Figure 2: Expression of a) Hsp60, b) Hsp90 and c) Hsp75 in rural and urban queens for 25°C, 1h and 45°C, 1h treatment. The expression levels are normalized relative quantities (NRQs). Bars are standard errors of the mean.

Mating behavior – 75.6% of urban queens (31 out of 41 queens) and 61.5% of rural queens (24 out of 39 queens) had mated twice. This difference (14%) was significant according to the permutation test (10,000 permutations, $P = 0.054$).

Colony founding success

Queen mortality – 12.4% of queens died during the first 44 days of colony founding, 11.8% for rural queens (17 out of 144) and 14.3% for urban queens (7 out of 49). The probability of death during colony founding was negatively related to initial mass but not related to origin nor to the interaction between initial mass and origin (best model: logistic regression with initial mass alone, explained deviance = 8.2%, $P = 0.0007$).

Mass loss – After 44 days of colony founding, urban and rural queen average weights had reached 15.01 mg and 15.33 mg respectively, corresponding to 12.35 mg and 13.34 mg of average mass loss respectively. Mass loss in urban and rural queens were not significantly different (10,000 permutation, $W_{obs} = 822.5$, $P = 0.21$), neither was the proportion of initial mass loss (respectively 44.9% and 46.1%, 10,000 permutation, $W_{obs} = 698$, $P = 0.59$).

Brood development – Brood development in urban and rural incipient colonies were similar for all stages but some differences emerged. The number of eggs was significantly higher in urban colonies during the first 15 days. The number of larvae was never different between urban and rural colonies. The number of pupae was always significantly higher in urban colonies (i.e. the number of pupae in urban colonies was in average 75% of the number of pupae in rural colonies, Fig. 3). The first worker emerged 40 ± 2 days after incipient colonies started, with no difference between urban and rural incipient colonies and no association with the initial weight of queens.

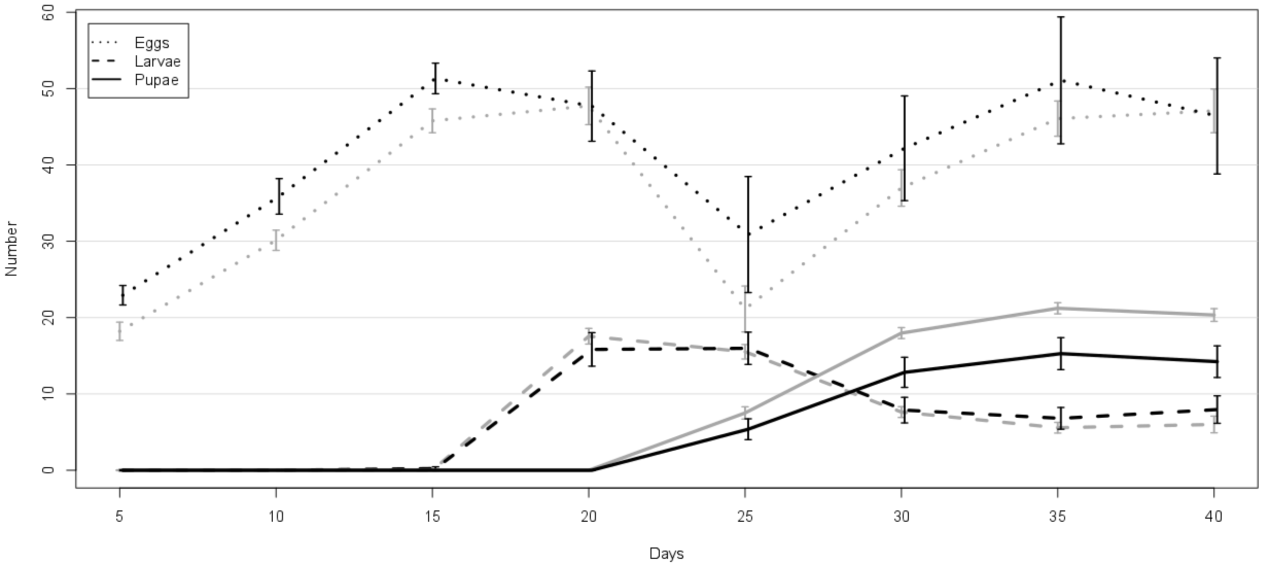

Figure 3: Comparison of the evolution of the number of eggs, larvae and pupae in *L. niger* incipient colonies of urban (black lines) and rural (grey lines) queens ($n_{urban} = 38$ and $n_{rural} = 127$). Vertical bars are 95% confidence intervals on the mean.

Workers phenotype

Size – We found no difference in average worker head width between urban and rural colonies (linear mixed model, $n_{urban} = 70$, $n_{rural} = 91$, P = n.s.). The variance in colony average head width was significantly higher in urban compared to rural colonies (F = 0.287, P = 0.015). The intra-colony coefficient of variation (cv) was not significantly different between urban and rural colonies ($cv_{urban} = 2.23 \pm 0.99$, $cv_{rural} = 1.83 \pm 0.5$, P = 0.17).

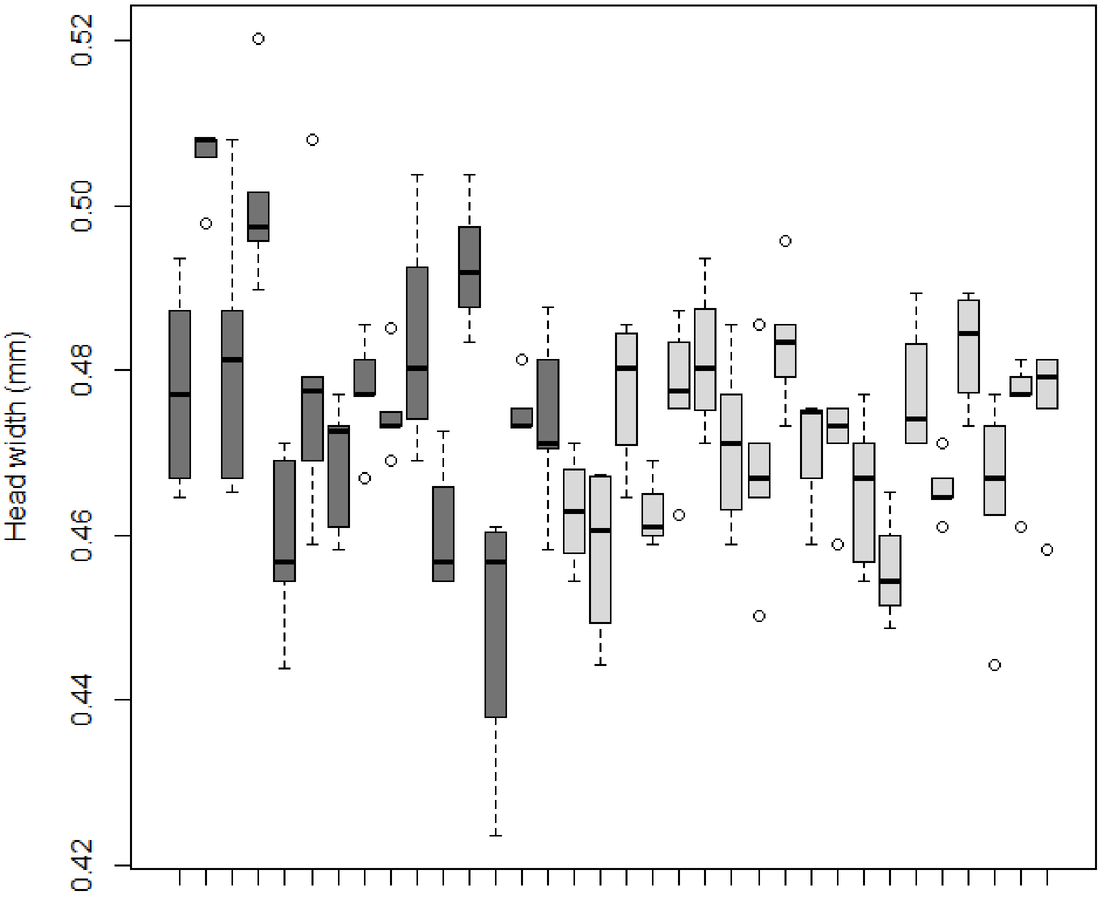

Figure 4: Intra- and inter-colony workers head size variation. Each box represents a colony. Four to five workers were measured for each colony. Dark grey and light grey boxes correspond to urban and rural colonies respectively.

Heat stress – All three HSP genes were affected by temperature, but none were affected by origin. While expression levels were higher after heat exposure for Hsp60 and Hsp90, they were slightly lower for Hsp75. The model with only temperature was the best explaining expression levels for workers for all three HSPs (Hsp60: $n_{urban} = 22$, $n_{rural} = 27$, $P < 0.001$, $R^2 = 0.87$, Hsp90: $n_{urban} = 24$, $n_{rural} = 28$, $P < 0.001$, $R^2 = 0.7$; Hsp75: $n_{urban} = 24$, $n_{rural} = 27$, $P < 0.02$, $R^2 = 0.71$) (Fig. 5).

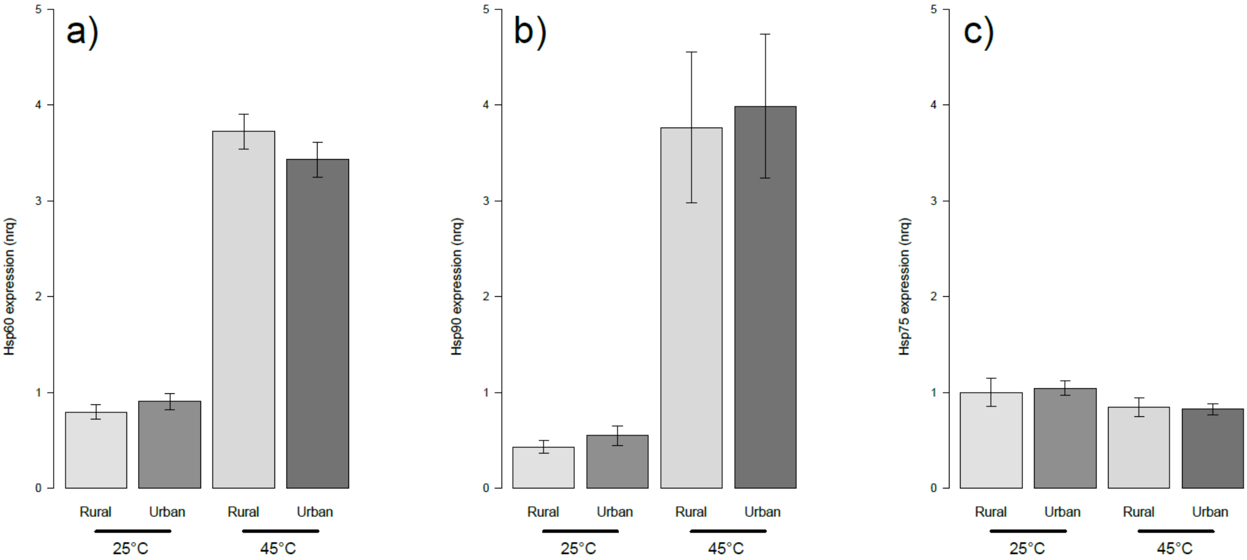

Figure 5: Expression of a) Hsp60, b) Hsp90 and c) Hsp75 in rural and urban workers for 25°C, 1h and 45°C, 1h treatment. The expression levels are normalized relative quantities (NRQs). Bars are standard errors of the mean.

Genetic differentiation

We found no genetic differentiation between urban and rural queens (GENEPOP: Weir and Cockerham (1984) Fst = -0.0007, G-test for genic differentiation: Chi-square: 41.24295, P=0.416059), nor any indication of sub-structuring between or within rural and urban queens (STRUCTURE results indicate that K= 1 is the best clustering option, as other cluster numbers offer equal Q values for all individuals at all clusters (Porras et al. 2013), Supp. Mat.).

Discussion

Urban tolerant ants, and more broadly urban tolerant insects, are still poorly studied despite the opportunities they represent for investigating phenotypic shifts, contemporary adaptation and biological diversification in urban environments. In this study, we investigated phenotypic changes and possible contemporary evolution in a widespread urban tolerant species: the black garden ant *Lasius niger*. We found differences in morphology, physiology and behavior between urban and rural queens, as well as contrasted patterns in colony founding and offspring morphology. Our results showed that urbanization can induce phenotypic changes in all traits categories simultaneously (morphology, physiology and behavior) and suggested that these changes could depend on each other (e.g., queen mating strategy and worker size variation). Our findings confirm the potential of urbanization to cause rapid ecological and evolutionary changes, and raised questions about their consequences on urban biodiversity dynamics and conservation (Shochat et al. 2006, Kotze et al. 2011, Paris 2016).

Queens phenotype

Size and weight – In *L. niger*, body weight and size have been related to queen fitness (Aron et al. 2009, Fjerdingstad and Keller 2004). We found that urban queens were lighter than rural ones (mean ± SD: respectively 26.4 ± 2.8 mg and 28.29 ± 2.6 mg) and also smaller (mean ± SD: respectively 1.32 ± 0.04 mm and 1.34 ± 0.04 mm) In addition, we found a positive relationship between head width and weight, significantly depending on queens' origins, indicating that, for a given size, rural queens were heavier than urban ones. These results can have multiple non-exclusive causes. First, environmental constraints in urban environments, especially temperature and alimentary resources (quality and quantity) could affect both the developmental conditions of queens during their larval stage and the accumulation of metabolic reserves before nuptial flight, respectively limiting body size and the amount of stored fat (Chown and Gaston. 2010, Haatanen and Sorvari 2013). Second, the observed patterns can also be the result of changes in reproductive strategies induced by the same environmental constraints. Ants are known to make quality-quantity tradeoffs in gyne or male production depending on environmental conditions (Molet et al. 2008), which could explain the production of smaller and lighter queens in urban environments. A recent theoretical model based on the ability of ant colonies to make this tradeoff (Cronin et al. 2016) showed that, in temporally and spatially heterogeneous environments, colonizers are selected over competitors, which means that producing many low quality queens in heterogeneous urban environments should be favored over producing fewer high quality queens.

As body size and weight should also be impact dispersal capabilities, investigating wing size and shape differences between urban and rural *L. niger* queens should be the next step into investigating the effect of urbanization on ant traits. If lighter, smaller queens were colonizers favored in urban environments (Cronin et al. 2016), selective pressures on dispersal abilities should induce evolutionary change in dispersal related traits in urban populations. Comparing wing morphology in young queens sampled from inside colonies of origin before nuptial flights would test this hypothesis.

The existence of smaller and lighter queens in urban populations could also have consequences on behavioral changes during colony founding. The propensity to undergo pleometrosis, i.e. colony founding by multiple queens, has been related to the production of smaller and lighter young queens in *Messor pergandei* (Helms Cahan and Rissing 2005). This behavioral and morphological shift occurred over an 8.5 km-wide transition zone and was related to environmental change. Pleometrosis was positively associated with reduced precipitation, lower vegetation biomass and lower adult colony density (Helms Cahan 2001). As urban areas are also characterized by increased dryness and reduced vegetation biomass compared to adjacent rural areas (Forman 2014), the same behavioral and morphological changes might have been favored in urban vs rural queens. As pleometrosis is known to occur in *L. niger*, experiments testing the propensity of queens to associate should be conducted. In L. ngier, pleometrosis is a temporal polygyny where after the first workers emerge, associated queens fight until only one survives. While Aron et al. (2009) showed that in *L. niger*, heavier queens have a higher probability to survive fights, smaller queens may still find an advantage to pleometrosis because they have an even higher probability to die during haplometrosis (single queen colony founding) than larger queens (Aron et al. 2009, Fjerdingstad and Keller 2004). Further investigation will therefore be necessary to disentangle the causes and consequences of urban conditions on interacting biological traits such as founding strategy and body size.

Heat stress – Recent studies demonstrated higher heat tolerance in urban ants (Angilletta et al. 2007, Foucaud et al. 2013). Using HSPs expression levels as indicators of heat acclimation or adaptation, we showed that urban queens expressed lower Hsp60 levels than rural queens when thermally stressed (45°C, 1h), indicating that urban queens were less stressed by heat than rural ones, as expected. The same patterns of HSPs expression levels were observed in other ectotherm species along natural temperature gradients (desert versus montane population in redband trout, Narum et al. 2013; land snail populations along a climatic gradient, Mizrahi et al. 2015). Interestingly, constitutive expression of Hsp60 was also significantly lower in urban queens. This is the third evidence of changes in heat tolerance related trait between urban and rural conspecific ants (Angilletta et al. 2007, Foucaud et al. 2013) and the first time these changes are demonstrated in the reproductive caste. Queens in our study were sampled after nuptial flight, which means that they experienced field temperature during their larval development as well as during the nuptial flight. As constitutive and inducible expression levels of Hsp60 were measured 40 days after nuptial flight, our results suggested either long term acclimation or adaptation to warm environments in urban queens. To our knowledge, this is the first time HSP expression under thermal stress is related to urban life.

This pattern was only visible for Hsp60, as Hsp90 expression was only affected by temperature and Hsp75 expression was affected neither by temperature nor by origin.

Hsp60, Hsp75 and Hsp90 expression were assessed in the ant *Formica cinerea* foraging on cold and hot surfaces (Ślipiński et al. 2015). While the authors found no effects of the foraging surface temperature on HSPs expression in workers, they showed that all three HSPs expression levels were positively related to temperature in workers experimentally exposed to 23°C (control), 40°C and 45°C. They also found that Hsp75 reached its maximum expression levels at 40°C and was thus more sensitive to heat stress than the two other HSPs. Nguyen et al. (2016) investigated expression levels of several HSPs in two ant species under temperatures ranging from 25°C to 46°C. They showed that, above a critical temperature, HSPs expression was similar to basal expression. This phenomenon could explain our patterns of expression of Hsp75 and indicate that 45°C is either below or far above the critical limit of expression for Hsp60 and Hsp90. Further studies investigating urban-rural differences in HSPs expression levels under heat stress should measure HSPs expression across a range of temperatures in order to assess finer responses and better understand urban-rural differences in heat tolerance. In addition, HSP are also involved in responses to other stresses, including low temperature, heavy metals, pesticides, salinity, desiccation and oxidative stress (Sorensen et al. 2003). As urban areas are multi-stressor environments, HSP coding genes might undergo complex interactive selective pressures which should be taken into account when investigating HSPs expression levels in relation to urbanization.

Mating behavior – Among 80 queens for which sperm was genotyped, we found only single and twice mated queens. Corley and Fjerdingstad (2011) found that *L. niger* queens mated in average with 1.11 males in Sweden, 1.18 males in Ireland, 1.73 males in Switzerland and to 2 males in southern France. In our study, the average number of mates per queens was of 1.62 (61.5% of double mated queens) in rural areas and 1.77 (75.6% of double mated queens) in urban areas. Our results are consequently consistent with what would be expected at the latitude of Lyon (150 km distant from Lausanne, Switzerland) given the latitudinal patterns in number of mating described by Corley and Fjerdingstad (2011). We showed that multiple mating was more frequent in urban queens than in rural queens. These findings corroborated theoretical models (Rüppell et al. 2008, Gove et al. 2009) predicting that multiple mating should be favored in milder and more complex environments because the characteristics of these habitats should increase the fitness of colonies with more genetically (and thus phenotypically) variable workers. To our knowledge, this is the first time urbanization is related to changes in mating strategies in insects and espcially in ants for which it could have significant consequences on social structure and whole colonies ecology (Hölldobler and Wilson 1990, Strassmann 2000).

Success in colony founding

Survival during colony founding was positively related to initial weight, accordingly to previous findings by Fjerdingstad and Keller (2004) in *L. niger*, but did not differ between urban and rural incipient colonies. However, as urban queens were lighter than rural queens, it should be expected that they would fail colony founding more often. After 44 ± 2 days of colony founding, urban and rural queens did not differ in mass (mean ± SD: 15.01 ± 3.68 mg and 15.33 ± 2.6 mg respectively), in absolute mass loss (12.35 ± 4.13 mg and 13.34 ± 2.7 mg respectively), nor in percentage of initial mass (44.9% and 46.1% respectively). As urban queens demonstrated smaller initial weight, it could have been expected that they could not invest as much internal reserves as rural queens. However, as lighter queens died more often during colony founding, the differences in initial weight between surviving urban and rural queens decreased (27.36 mg and 28.46 mg respectively for surviving queens against 26.4 mg and 28.29 mg respectively initially), which is likely to explain our findings.

The first worker emerged 40 ± 2 days after colony founding started, with no difference between urban and rural incipient colonies and no association with the initial weight of queens. Brood development of urban and rural queens was very similar for eggs and larvae, but contrasted for pupae. Urban incipient colonies had fewer pupae (25% less) than rural incipient colonies, which logically lead to fewer workers in urban than rural incipient colonies. High initial worker production is associated with early colony fitness because producing more worker (i) decreases the probability of being predated by adult ant colonies (Jerome et al. 1998) or suffering brood raids from other incipient colonies (Bartz and Hölldobler 1982), (ii) increases the probability of raiding success (Adams and Tschinkel 1995) and (iii) increases foraging success (Helms Cahan 2001).

Success in colony founding of urban and rural queens was quite similar and only worker production was affected by the origin of queens, with fewer workers for urban colonies. Because urban queens likely experienced more chemical pollution than rural queens during their larval development and nuptial flight, we could have expected deleterious effect on the probability of survival of young queens (De La Riva and Trumble 2016). *L. niger* was found to rapidly accumulate phthalates (Lenoir et al. 2014), which in chronic exposure decreased the laying rate of *L. niger* young queens while short term exposure activated their immune system (Cuvillier-Hot et al. 2014). Additionally, *L. niger* workers have been shown to accumulate and tolerate high amounts of heavy metals (Grzes and Okrutniak 2016b), however, to our knowledge, no study has investigated their impacts on founding queens. Pollution associated with urbanization has the potential to affect brood development of *L. niger* colonies either directly by disrupting brood larval development (Abidi et al. 2016) or indirectly by imposing cost on urban life. Such costs may lead to tradeoffs between brood production and, for example, immune defense (Sorvari et al. 2006, Borowska and Pyza 2011) or oxidative stress (Kafel et al. 2012, Wilczek et al. 2013, Isaksson 2015).

Kipyatkov et al. (2004) found differences in the duration of brood development between northern and southern Russian populations of young queens of *L. niger*. The authors showed that northern and southern queens differed in their norm of response to temperature, linking physiological thermal adaptation to brood development. Rearing urban and rural queens under different temperatures regimes would be useful to determine if the differences we found in brood production could be the result of distinctive thermal optima between urban and rural populations. In addition, interactive effects of metal pollution and temperature on metabolism have been documented in aquatic ectotherms (Sokolova and Lannig 2008, Kimberly and Salice 2014, Haap et al. 2016). Urban areas, as they combine both warmer temperature and higher pollution levels, represent great opportunities to test such interactive effects and investigate their ecological and evolutionary consequences.

Worker phenotypes

Size – L. niger is a species with monomorphic workers, i.e. workers have similar size and shape within the same colony (Hölldobler and Wilson 1990). In some monomorphic ant species, the head width of workers was found to differ between colonies (e.g., Laskis and Tchinkel 2009), sometimes in association with environmental differences (e.g., between colonies from edges and interior of forest, Sorvari and Hakkarainen 2009; between colonies from seaside and vineyard, Clemencet and Doums 2007). Our results showed that urban and rural incipient colonies did not differ in average head size of workers but that urban incipient colonies demonstrated higher inter-colonies variance in head size. Such difference in variability between urban and rural incipient colonies is likely to spring from more complex differences between urban and rural queens than previously thought. Differences in workers average size among urban colonies might arise from physiological differences in fat stored or behavioral differences in founding strategies of urban queens. Whatever the cause, such increase in inter-colony phenotypic variation should result in broader phenotypic range in urban populations compared to rural populations and could thus have consequences on urban biotic interactions and populations demographic parameters (for example) (Bolnick et al. 2011). We also found that the coefficient of variation of workers head width (intra-colony variation) was higher in urban incipient colonies than in rural ones (although not significantly), in accordance with our initial hypothesis. Increased within-colony variation in worker size is often associated with colony performance (Porter and Tschinkel 1984, but see Jandt and Dornhaus 2014) and might be selected in complex environments (Rueppell et al. 2008) but the mechanistic causes of this variation are still to be determined. Our previous results on number of mating of queens constitute an interesting clue. Indeed, we predicted and verified that queens originating from more complex and milder environments (urban) mated with two males more often than queens originating from simpler and harsher environments (rural). This prediction was based on the fact that multiple mating increases offspring genetic and sometimes phenotypic variance (Bargum et al. 2004), and thus should be selected in complex and milder environments (Corley and Fjerdingstad 2011, Rueppell et al. 2008 and Gove et al. 2009). Our findings corroborated this hypothesis, but would need confirmation by more closely investigating the relationship between multiple mating and offspring variability in size.

Variations in ant worker size have already been associated with environmental gradients (Sorvari and Hakkarainen 2009, Clemencet and Doums 2007), but to date, most studies concluded that the observed variations were mostly due to environmental factors and little to genetic factors (Bargum et al. 2004, Purcell et al. 2016). In addition, because size of offspring is predicted to be affected by the investment strategy of parents (Gilboa and Nonacs 2006), the initial condition of founding queens might be related to the phenotypic variation observed in their workers.

Heat stress – Because queens and workers perform different functions in the colony, they experience different environmental conditions and are thus likely to suffer different selective pressures (Kovacs et al. 2010). Workers perform brood care and nest excavation, but also nest cleaning, foraging and fighting (Hölldobler and Wilson 1990). Consequently, workers experience all the environmental conditions found outside the buffered environment of the nest. Among such conditions, air and ground temperature were shown to greatly affect ant foraging activity (circadian rhythm and competitive success: Cerda et al. 1997; pheromones trails and foraging strategy: van Oudenhove et al. 2012, Ruano et al. 2000). Consequently, heat tolerance is predicted to play a crucial role in colonies fitness in warm environments (Andrew et al. 2013, Nguyen et al. 2016). This hypothesis has been investigated for two tropical ant species (*Atta sexdens,* Angilletta et al. 2007; *Wasmannia auropunctata,* Foucaud et al. 2013) and results showed that urban ants tolerated higher temperatures than rural ones. While our results demonstrated similar trends for queens, no differences in heat stress response was found in urban vs rural workers. Modifications of the circadian rhythm or annual phenological changes (Andrew et al. 2013) in colony activity could enable workers to avoid heat stress by foraging at milder air and ground temperature, which is likely to release selective pressures on physiological adaptation to heat stress. Investigating foraging behavior of urban and rural *L. niger* colonies *in natura* and in laboratory should answer this question. However, before directly excluding physiological adaptation in urban workers of *L. niger,* more energy should be invested in the investigation of HSP expression levels between urban and rural colonies. As for queens, further studies should investigate constitutive and inducible expression of HSPs coding genes across different temperature treatments (Nguyen et al. 2016, Sorensen 2010). Diamond et al. 2013 found that the range of thermal tolerance for colony growth was narrower than for workers survival. If urban warmer temperatures impose selective pressures on *L. niger* colonies even when workers stay inside the nest, they might have stronger impacts on colony growth than on worker survival.

Genetic differentiation

As could be expected from Boomsma and Van der Have (1998) (no genetic differentiation between *L. niger* populations distant from hundreds of kilometers) we found no genetic differentiation between our urban and rural populations (see Supplementary Material II, Figure S1). These findings suggest either that gene flow is not disrupted between urban and rural populations, or that it has been disrupted too recently to be observable. As genetic diversity was high in *L. niger* (see allelic richness in Supplementary Material II, Table S3) and its population sizes are very large (Gippet et al. *unpublished*), a recent disruption of gene flow might have prevented urban and rural populations to genetically drift from each other. If gene flow is effectively not disrupted, our situation might constitute an opportunity to study the establishment of local adaptation with gene flow (Tigano et al. 2016). Intermediate levels of gene flow were shown to maximize local adaptation in temporally fluctuating environments (compared to temporally stable environments) based on evolutionary simulations (Blanquart et al. 2013). Based on these findings, urban populations should benefit from intermediate levels of gene flow as urban environments tend to be more spatio-temporally heterogeneous than rural or natural environments (Shutters et al. 2006, Cheng and Chan 2014, Earl et al. 2016).

Further investigation using landscape genomics (Lowry et al. 2010, Jones et al. 2013) along the urban to rural gradient (Harris et al. 2016) should allow the identification of patterns of isolation by distance, by resistance (e.g., Munshi-South et al. 2016), by adaptation (Nosil et al. 2008) or by drift (Prunier et al. 2015 *BioRxiv*).

Conclusion

Our study demonstrated clear phenotypic shifts between urban and rural conspecifics. These differences were morphological, physiological and behavioral and occurred in both queens and workers of the black garden ant *Lasius niger*. No evidence of evolutionary changes was detected in workers, but the change in mating behavior observed in queens suggested behavioral adaptation in urban environments. Multiple mating results in increased genetic and thus phenotypic variability in offspring (workers but also gynes and males). This suggests that urban populations should tend to demonstrate higher phenotypic variability at both within colony and within population levels, compared to rural populations. This hypothesis was corroborated by our results on urban worker morphology. Such changes are likely to affect biotic interactions, community structure and even ecosystem functioning as *L. niger* is an abundant species interacting with many taxa and able to modify local conditions (e.g., soil properties, Holec and Frouz 2006). Additionally, physiological differences in heat stress response (HSPs expression) between urban and rural queens suggested that warmer temperature in urban environments impacted the physiological state of ants, but, as no differences were observed in workers reared in common garden, the causes (acclimation or adaptation) are still unclear. Local adaptation to urban environments and its possible consequences on population genetic divergence and biological diversification associated with urbanization need further investigation and *Lasius niger* seems to constitute a promising biological model to do so. Recent sequencing of the complete genome of *L. niger* should make possible a deeper understanding of how selection, gene flow and drift shape the genetic evolution of urban populations (Harris and Munshi-South 2016 *BioRxiv*) and are affected by eco-evolutionary dynamics (Alberti 2015).

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SUPPLEMENTARY MATERIAL

I- HSPs expression: material and methods

RNA extraction and cDNA Synthesis

Each sample was crushed (2min at 30 Hz in Fisher Bioblock Scientific) into a tube containing Tri-reagent (Euromedex). The lysate was transferred to a phase lock gel heavy (DUTSCHER). Total RNA was extracted with following manufacturer's instructions. After the RNA samples were treated with Turbo DNAse (Fischer Scientific Life Technologies) in order to remove any traces of DNA. The RNA integrity was verified using a BioAnalyzer 2100 (Agilent Technologies). RNA concentrations were measured in duplicate by fluorometry using Quant-iT^{TM} RNA assay kit (Fischer Scientific Life Technologies).

First-strand synthesis was performed using an SuperScript III First-Strand Synthesis System (Fischer Scientific Life Technologies) and an oligo(dt)$_{20}$. 0,4 µl of RNAse H (Invitrogen) was added into each cDNA. After 30 min at 37°c, samples were stored at -20°c while waiting qPCR assay.

Primers design

For target genes (Hsp60, Hsp75) and EF1 reference gene, first amplification was realized with primers from genomic DNA of *Formica cinerea* (Ślipiński et al. 2015). For the second reference gene, α-tubulin, the first primers were designed based on *Camponotus floridanus* genome (GENBANK: GCF_000147175.1). PCR products were sequenced and new specific primers to *L.niger* were designed. For Hsp90, the primers were designed based from alignment of different Hymenoptera, with similar sequences.

qPCR

In order to calculate PCR efficiencies, standard curves were plotted using eight dilutions (10–10^6 copies) of a previously amplified PCR product purified using QIAquick gel extraction kit (QIAGEN). The qPCR conditions were improved until all genes had an amplification efficiency between 90% and 110%. Each sample was measured in three replicates with a total reaction volume of 10 µl containing 250nM of each primer, 1X SsoADV Univer SYBR Green Supermix (BIORAD) and 20 ng of cDNA. qPCR was performed using CFX96 (BIORAD) with conditions as follows : 3 min at 95°c and 40 cycles of 10 sec at 95°c, 30 sec at varying annealing temperature (Table S1), followed by a final melting curve analysis. The absence of DNA was validated with no-reverse transcription assays. For each run, no template controls (NTC) were included to detect possible contamination. The specificity of the PCR products was confirmed by melting curve analysis for all samples.

Table S1: HSPs coding genes and reference genes used in heat stress experiments. Initial and designed primers are presented.

Gene	**Amplicon size (bp)**	**Annealing Temperature**	**Primer sequence (5’→ 3’)**
EF1	402	52°C	F : ATCATCGACGCTCCTGGACAC R : TTTGCCTTCCTTGCGCTCCAC
HSP60	1013	52°C	F : GTTCGTGCCTTGATGTTGCAA R : TGATCGGCTCTYCTATCGATATC
HSP75	503	52°C	F : AAAGAGGGCATTGTCACBAGTA R : ATCATCTCGCATTTCTTTCTC
α-tubulin	213	52°C	F : AAGACGAGCCCAAGCTTCCGCAAT R : CGGTGACGTCGTGCCCAAAGATGTC
EF1 *L.niger*	256	64°c	F : GTACTGGCGAATTCGAGGCTGGTATC R : GGCTCCAGCATGTTGTCTCCATGCC
HSP60 *L.niger*	251	64°c	F : GAGCAAACCAGTCACGACACCTGAG R : CGTCTTGAAACTCGACTTTGGCTCC
HSP75 *L.niger*	256	64°c	F : GCATTGTCACCAGTACTGATCAATC R : CAATACTAATTCATCGTACGGTTCG
HSP90 *L.niger*	278	60°c	F : GAGTATGGTGAATTCTACAAGAGC R : CGAGARATATTCAAAGGMAGATCTTC
α-tubulin *L.niger*	158	62°c	F : CGGTGGTATTTGACAGCATGCAAAC R : AGCGATTGCCACTATCAAGACCAAG

II- Population Genetics: material and methods

Morphological and molecular identification

DNA extraction

DNA was extracted from queen head and thorax only (to remove formic acid). Ants were crushed and then mixed with 150 µl of hot (90°C) Chelex® 100 then cooled;10 µL of proteinase K (15mg/mL) were added and the solution was incubated at 55°C overnight (Casquet et al. 2012). Spermathecal contents were mixed with 100 µl of hot (90°C) Chelex® 100 then cooled;10 µL of proteinase K (15mg/mL) were added and the solution was incubated at 55°C at 2 hours (Casquet et al. 2012). Extraction of spermathecal contents followed the same procedures with the same amount of reagents.

Microsatellite markers development

We developed new microsatellites markers for *Lasius niger*. Total genomic DNA was isolated from the heads of 6 queens collected from nuptial flights in Lyon in 2014. DNA was extracted following crushing (3 min at 30Hz in a Retsch MM200 ball mill), standard digestion (proteinase K [75 µg] + 200 µl of TNES buffer [0.05 M Tris, 0.1 M NaCl, 0.01 M EDTA, 0.5% SDS]) and salt-chloroform purification (adapted from Nürnberger et al. 2003). Total genomic DNA was sent to GenoScreen, Lille, France (www.genoscreen.fr). 1µg was used for the development of microsatellites libraries through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries as described in Malausa et al. (2011). Briefly, total DNA was mechanically fragmented and enriched for AG, AC, AAC, AAG, AGG, ACG, ACAT and ATCT repeated motifs. Enriched fragments were subsequently amplified. PCR products were purified, quantified and GsFLX libraries were then carried out following manufacturer's protocols and sequenced on a GsFLX PTP.

Genotyping

Twenty microsatellites markers from (Table S2) were organized into 3 genotyping mixes ran on a 3730xl DNA Analyzer (Applied Biosystems) by a service provider (GENTYANE, Clermont-Ferrand). Mix3 comprised primers pairs from the literature with end-labeled forward primers: Lng-1, Lng-3 (Fjerdingstad et al. 2003), L10-174 (Ugelvig et al., 2008), La33b, La35h, La36d (Steiner et al, 2007). Forward primers in mix1 and mix2 were end-labelled with universal tails following Blacket & al. (2012); 4 different tails were used, with following fluorescent dyes (Tail_A_6FAM [6FAM]GCCTCCCTCGCGCCA, Tail_B_HEX [HEX]GCCTTGCCAGCCCGC, Tail_C_ATTO550 [ATTO550]CAGGACCAGGCTACCGTG, Tail_D_ATTO565 [ATTO565]CGGAGAGCCGAGAGGTG. Which primer uses which tail is indicated in Table

S1. PCR mix composition were as follows : 1) mix 1, total volume of 10 µl, 0.1 µM of each primer Forward and tails, plus 0.2 µM of primer reverse, 1X Master Mix Type-it microsatellite PCR kit (QIAGEN, 206246), and 2µl extracted DNA; 2) mix 2, total volume of 10µl, 0.1-0.2 µM of each primer Forward and tails, plus 0.2-0.4 µM of primer reverse, 1X Master Mix Type-it microsatellite PCR kit (QIAGEN, 206246), and 2µl extracted DNA; 3) mix 3 total volume of 10µl, 0.2 µM of each primer Forward, plus 0.2 µM of primer reverse, 1X Master Mix Type-it microsatellite PCR kit (QIAGEN, 206246), and 2µl extracted All PCRs were run with the same cycling program : 5 min. annealing at 95°C, 36 cycles with denaturation (30 s) at 95°C, annealing (3 min) at 60°C, extension (30 s) at 72°C, and a final 30 min extension at 60°C. Electrophoregrams were read and interpreted with Genemarker 1.95 (Softgenetics).

Genetic analyses

Methods

Basic population genetics statistics were calculated using Genalex 6.5 (Peakall & Smouse, 2012), F_{is} values and exact tests for deviations from Hardy-Weinberg equilibrium were computed using Genepop 4.0 (Rousset, 2008); estimation of null alleles frequencies were obtained using FreeNA (Chapuis & Estoup, 2007), as were F_{st} estimations with or without null alleles. To check for hidden structuring between or within populations, we used STRUCTURE 2.3.4, (burnin length of 100000 steps for a total 1000000 steps, number of clusters from K=1 to 5, 10 runs per K value). STRUCTURE outputs were then analyzed with Structure Harvester (Earl and vonHoldt, 2012) to estimate Delta K following Evanno's method (Evanno et al. (2005). CLUMPP 1.1 was used to summarize STRUCTURE output, averaging Q-values over 10 structure runs of the best K value.

Results: Basic population genetics

Over 95 primer pairs tested following library development, 14 new microsatellite markers were selected for genotyping. 40 individuals from rural populations and 42 from urban populations were genotyped at 20 microsatellite markers. Allele numbers ranged from 2-27, with means over all loci of 9,85 (Rural) and 9,55 (Urban); expected heterozygosity ranged from 0.13 to 0.95. Significant deviations from Hardy-Weinberg equilibrium were detected in 2 markers for rural ants, and in three different markers in urban ants, and were probably caused by null alleles at low frequencies (around 5%). Multi-locus deviations from Hardy-Weinberg were small (F_{is} rural = 0.0113, F_{is} urban = 0.0465) but significant for both populations, and mostly due to loci with probable null alleles. F_{st} was close to 0 using and without using the ENA correction described in Chapuis and Estoup (2007) (respectively F_{st} = -0.0003, 95% interval [-0.0029, 0.0027] and F_{st} = -0.0007, 95% interval [-0.0033, 0.0024]) (Table S3).

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Table S2. Characteristics of the microsatellite loci developed and used for the study. Markers with * are from the literature.

Locus name	Accession number	Primer sequence 5'_3' F	Primer sequence 5'_3' R	Repeat motif	Allele size range (bp)	PCR Mix	Fluorochrome
Lni_C01		GCCTCCCTCGCGCCACGTCGTATGCGATATTTATGCT	ATATACGATCGTTATTAAGACG	$(AG)_{12}$	130-142	1	Tail_A_6FAM
Lni_F10		GCCTCCCTCGCGCCACGCTACGTTATTCTGCGACATTTCG	GGTTCGATATCAGCATCGGCAGGAT	$(CA)_{18}$	166-182	1	Tail_A_6FAM
Lni_G07		GCCTTGCCAGCCCGCATCATAATCCGATCTAGCTGCCAAA	TATCGCAATCATATGTCTTCGCTCC	$(AC)_{9}$	155-219	1	Tail_B_HEX
Lni_F09		GCCTTGCCAGCCCGCACGTTTCCGCATATTCGTTCGAGTC	CAAATACACATCCCTCTTTCTATCT	$(CA)_{9}$	324-338	1	Tail_B_HEX
Lni_A03		CAGGACCAGGCTACCGTGCCGTAAAATTGTCACTTGTTCATTC	CACGATATACTTTGTCGTCGACTT	$(TG)_{10}$	141-153	1	Tail_C_ATTO550
Lni_C08		CAGGACCAGGCTACCGTGAGATCGAACCATCAGGCATCGTTGC	AGCGAGCTTTTCGTTACAGACTCGC	$(GA)_{10}$	190-240	1	Tail_C_ATTO550
Lni_B10		CGGAGAGCCGAGAGGTGTTATCACGACGTAGAAGACCAAGGA	AGATTGGGAGTCTCTGCGAATCCGC	$(AG)_{12}$	176-194	1	Tail_D_ATTO565
Lni_F02		CGGAGAGCCGAGAGGTGGCACCGTACTTGCACTCAGACATAC	GCATGTGGGTGGCTATTCTTATG	$(CA)_{11}$	120-132	1	Tail_D_ATTO565
Lni_F04		GCCTCCCTCGCGCCATTATCTACATACACAACGAGCCAGC	TATTTCGTCTCCCATTCGTCATCG	$(CT)_{10}$	179-191	2	Tail_A_6FAM
Lni_C11		GCCTTGCCAGCCCGCATCATCGTCCCAACATCGTCTTCC	TATTGATTGGAAATGTAAGCCGGAG	$(CT)_{11}$	116-156	2	Tail_B_HEX
Lni_H05		GCCTTGCCAGCCCGCGGCTTGCGTTCGAAAATCTACTCG	GCATGCGCTTAAAGGGCAGATGTC	$(GA)_{10}$	195-199	2	Tail_B_HEX
Lni_D03		CAGGACCAGGCTACCGTGACGAAAGATATGGAAGTACGTAC	TAACACCCGTTGTACGCTCAATTG	$(TG)_{9}$	131-145	2	Tail_C_ATTO550
Lni_G05		CAGGACCAGGCTACCGTGCTATAATACATTATCGGAGCCAGC	GCTCACTGGAGCGTCCTTACGTAC	$(AC)_{12}$	248-252	2	Tail_C_ATTO550
Lni_E03		CGGAGAGCCGAGAGGTGTTCGCGAGAATGAATCGAAAGAA	TCTCCATCATGCTGGAGTCAGGAA	$(GA)_{11}$	163-177	2	Tail_D_ATTO565
Lng-1*		TCTCGCTCCAACTACTTAAA	TTGTCTTCAAATTGTCCAAC		210-258	3	HEX
Lng-3*		GATGCCAAGTTTACATGG	CACAATACTCACATATTCACAA		99-115	3	PET
L10-174*		CACGCTCGCGTTTACATAAC	GAAAATCTTTGCCAATTCACG		202-262	3	PET
La33b*		CATTCCTGCCTTCGCATTATC	AGCGGAGGTGTGAAACAGTC		228-292	3	NED
La35h*		AACGGTAGCCGGGACATC	CTGGTGGAAGGTAAGTCTCG		178-206	3	6FAM
La36d*		CGATCTCGTGTCGTTCAAG	CAACGGATGTGTGATTTTGC		134-152	3	NED

Table S3. Basic population genetics for the microsatellite loci used in the study. N = Number of diploid individuals genotyped, Na = number of alleles, Ne = number of efficient alleles, Ho = observed heterozygosity, uHe = unbiased expected heterozygosity, Fis W& C (1984) = Fis as calculated using the method by Weir & Cockerham (1984) with * indicating significant deviation from HW, NA = frequency of null alleles (program FreeNA, Chapuis & Estoup, 2007), ** indicates null allele frequencies above 0.05.

	N		**Na**		**Ne**		**Ho**		**uHe**		**Fis W&C (1984)**		**NA (FreeNA)**	
	R	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*
Lni_C01	39	41	5	7	2,5780	3,2672	0,4872	0,6341	0,6200	0,7025	0.2165	0.0984	0.0767**	0.0409
Lni_F10	40	41	6	7	3,1746	2,6431	0,8000	0,5854	0,6937	0,6293	-0.1556	0.0707	0.0000	0.0237
Lni_G07	40	40	27	22	14,5455	15,5340	0,9500	0,9500	0,9430	0,9475	-0.0075	-0.0027	0.0000	0.0000
Lni_F09	40	42	6	4	1,9048	2,0405	0,5750	0,5000	0,4810	0,5161	-0.1984	0.0315	0.0000	0.0000
Lni_A03	40	42	6	6	3,3264	3,5174	0,6250	0,6667	0,7082	0,7243	0.1188	0.0805	0.0330	0.0399
Lni_C08	40	42	11	10	6,1303	4,3990	0,8500	0,7143	0,8475	0,7820	-0.0030	0.0875	0.0146	0.0429
Lni_B10	40	42	9	9	6,2622	5,6812	0,8750	0,7619	0,8509	0,8339	-0.0286	0.0873	0.0000	0.0252
Lni_F02	40	42	6	7	3,6158	4,1851	0,6750	0,7143	0,7326	0,7702	0.0795	0.0734	0.0313	0.0147
Lni_F04	39	37	5	6	2,7806	2,7967	0,7179	0,5676	0,6487	0,6512	-0.1083	0.1300*	0.0000	0.0548**
Lni_C11	40	41	9	12	4,7619	5,9190	0,8750	0,7073	0,8000	0,8413	-0.0951	0.1609*	0.0000	0.0535**
Lni_H05	40	42	3	3	1,7988	1,8776	0,3750	0,4762	0,4497	0,4730	0.1679	-0.0068	0.0500	0.0000
Lni_D03	40	42	6	4	1,4447	1,6618	0,3250	0,4524	0,3117	0,4030	-0.0432	-0.1241	0.0000	0.0000
Lni_G05	40	42	5	4	2,8369	2,6172	0,4750	0,6190	0,6557	0,6254	0.2781*	0.0102	0.0929**	0.0000
Lni_E03	40	42	6	5	2,5039	2,5110	0,6000	0,5714	0,6082	0,6090	0.0137	0.0624	0.0000	0.0000
Lng-1	40	42	21	21	8,9385	10,5629	0,8000	0,8810	0,8994	0,9162	0.1117*	0.0390	0.0542**	0.0000
Lng-3	40	41	2	3	1,4060	1,1589	0,3500	0,1463	0,2924	0,1388	-0.2000	-0.0549	0.0000	0.0000
L10-174	40	42	23	22	11,9403	9,8000	0,9250	0,8571	0,9278	0,9088	0.0031	0.0575	0.0006	0.0000
La33b	39	42	24	22	11,4361	14,3415	0,9231	0,9286	0,9244	0,9415	0.0015	0.0139	0.0053	0.0000
La35h	40	42	10	8	4,6852	4,9274	0,8250	0,7619	0,7965	0,8067	-0.0362	0.0561	0.0000	0.0083
La36d	40	42	7	9	4,6310	4,9620	0,8000	0,8810	0,7940	0,8081	-0.0077	-0.0914	0.0000	0.0000
multi-locus mean	39,85	41,45	9,85	9,55	5,0351	5,2202	0,6914	0,6688	0,6993	0,6930	0.0113*	0.0465*		

Figure S1. STRUCTURE results with K=2 and K=3 , after averaging Q-values over 10 structure runs (burnin length of 100000 steps for 1000000 steps total) using CLUMPP 1. and drawn using Distruct 1.1 (1 (Jakobsson & Rosenberg, 2007).

CONCLUSION AND PERSPECTIVES

Summary of the results

Urbanization is a process which modifies environmental conditions by affecting simultaneously several factors. Chapter One of my thesis assessed the consequences of such multiple changes by demonstrating that ant responses to urbanization were species-specific (Gippet et al. 2016). Because each species is characterized by a unique set of environmental optima (associated with their unique set of biological traits), the consequences of urbanization on their spatial distribution are unique as well. In addition, we have identified three response syndromes to urbanization that are well-described in the literature: urban exploiter, urban avoider and urban tolerant species. Four out of the seven ant species we studied were, to different degrees, urban avoider species. One species was an urban exploiter (*Tetramorium* spE) and two were urban tolerant species, including the native black garden ant *Lasius niger* and the invasive garden ant *Lasius neglectus*. We additionally showed that the spatial distribution of *L. neglectus* was related to human activities involved in soil transportation.

As *L. neglectus* has no natural long-distance dispersal abilities, this result raised questions about the process of human-mediated dispersal, and more particularly its role in secondary spread of exotic species. Chapter Two of this manuscript therefore focused on human-mediated dispersal. We first developed a new conceptual framework investigating human-mediated dispersal by focusing on human activities rather than directly on alien species. This conceptual approach should bring new insights to understanding and modelling human-mediated dispersal at different spatial scales. It also points out that human-mediated dispersal implicated in landscape-scale secondary spread of invasive species is the most neglected topic in theoretical as well as in modeling approaches. To implement the conclusions of this conceptual framework, we constructed a spatially explicit stochastic model, MoRIS, which simulates human-mediated dispersal by materials transportation events and by taking into account the topology of the network involved in transportation events. MoRIS was used to estimate the dispersal parameters of *L. neglectus* in our study area: for instance, we found mean dispersal distances of approximately 10km. Our results therefore demonstrated that human-mediated dispersal could explain invasive species spread even at small spatial scale (short distance dispersal), contrarily to common assumptions (i.e. that human-mediated dispersal is mostly implicated in long distance dispersal) (Chapter 2, article 3: submission in *Methods in Ecology and Evolution* for December 2016).

Because urbanization increases alien species introduction events and accelerates secondary spread dynamics, the probabilities of encounter between independently introduced species should be higher in urban areas. In Chapter Three, we investigated the interactions between *L. neglectus* and another alien species, the ectoparasitic fungus *Laboulbenia formicarum*. As is the case for the study of many ecological mechanisms, urban areas represent opportunities to investigate new biotic interactions between species which have not co-evolved but also to investigate potential changes in species interactions between urban and rural areas. We found that *L. neglectus* was heavily infected by *L. formicarum* in our study area, as 58% of the invasive ant colonies were infected by the fungus. In urban areas, *L. neglectus* colonies were smaller and *L. formicarum* prevalence higher in infected colonies than in rural areas. These findings suggest that urbanization might modify biotic interactions, and consequently inflict costs to urban life, even in an apparently urban tolerant species. We additionally found that in our study area, among six other *Lasius* ant species, only *L. niger* was also infected by the fungus, with 5% of colonies found infected. (Chapter 3, article 4, in revision for *Biological Invasions*).

The environmental conditions of adjacent urban and rural (or semi-natural) areas differ in many abiotic as well as biotic factors. Species occurring in both kinds of areas, i.e. urban tolerant species, are therefore excellent biological models to understand the consequences of contrasting environmental conditions on phenotypic and evolutionary divergence of populations. In the fourth and ultimate chapter of this manuscript we investigated phenotypic and evolutionary changes associated with urbanization. The ant *Lasius niger*, being an urban tolerant species occupying 75% of the sampling locations used in Chapter One, is an ideal biological model for studying phenotypic and evolutionary shifts between urban and rural populations. We investigated morphological, physiological and behavioral differences between urban and rural newly mated *L. niger* queens. We found that urban queens were lighter and smaller, had lower expressed levels of heat-shock proteins (Hsp60) when subjected to heat stress and mated multiply more often than rural queens. Colony founding parameters (queen mortality and mass loss, brood development) were not very different between urban and rural queens, except for urban queens producing fewer pupae than rural queens. Average size of workers and intra-colony worker size were more variable in urban compared to rural incipient colonies.

Ecological dynamics in an urbanizing world

Establishing general patterns of the consequences of urbanization on biological communities (Brown et al. 2009) remains a challenge. From Chapter One of this manuscript, two necessary steps toward elucidating these patterns can be identified.

(i) Species-specific responses to urbanization showed it is necessary to adopt a more mechanistic approach of species-environment relationship in urban ecology (Shochat et al. 2006, Verberk et al. 2013), because species responses to urbanization are likely to be determined by species morphological, physiological and behavioral traits which control the species' environmental preferences (Verberk et al. 2013). Identifying how traits, or combinations of traits, drive species responses to environmental changes associated with urbanization (e.g., light pollution, habitat fragmentation, urban heat island effect) should make patterns clearer. They should also provide realistic predictions of changes in biological communities associated with urbanization (Brown et al. 2009, Verberk et al. 2013).

(ii) The environmental context is likely to influence the impact of urbanization on species' spatial distributions and community composition patterns. Recent studies demonstrated and discussed complex interactive effects between climate and urbanization on biological communities (Nelson et al. 2009, Radinger et al. 2015, Gibb et al. 2015). Such interactions should be further explored in the coming decades and the integration of biological traits (Diamond et al. 2015) should allow the construction of efficient predictive models of species responses to urbanization worldwide. Other environmental factors, such as human social and economic activities (which are likely to vary among political territories), should also be considered as they are likely to generate contrasting effects of urbanization on biodiversity patterns (Grimm et al. 2008b).

Urban areas often feature higher invasive species richness than adjacent rural areas (Gaston 2010). However, while native species distributions tend to approach equilibrium at landscape scale, invasive species are generally still in a spread process and have yet to fill in their potential distributions (Bradley et al. 2015). This suggests that in urbanized landscapes, relevant models of biodiversity distributions and thus biological community dynamics should obligatorily integrate invasive species spread dynamics to produce relevant scenarios and predictions. As human-mediated dispersal of invasive species at landscape scale is often neglected, Chapter Two brings insights and methodologies to fill this gap. Indeed, more than solely simulating and predicting single species secondary spread, MoRIS could be used to provide predictive distributions of several invasive species in any single landscape. Coupled with existing species distribution models and natural dispersal models, this may greatly improve our ability to predict the dynamics of biological communities in urbanized landscapes. In addition, as invasive species spread, the number co-occurrences between species (invasive-invasive and invasive-native) and the number of potential biotic interactions (e.g., predation, competition, mutualism) should increase, which is likely to affect biological community composition patterns over time and space (Guisan and Thuiller 2005). In Chapter Three, I documented such a novel interaction and showed that, more than adding complexity by creating novel biotic interactions (*Lasius neglectus – Laboulbenia formicarum, Lasius niger – Laboulbenia formicarum*), urbanization can also affect the strength of these interactions (Irwin et al. 2014).

Finally, intraspecific trait variations can affect intra- and interspecific interactions as well as demographic parameters in complex ways (see Bolnick et al. 2011). We showed that phenotypic shifts occurred between urban and rural populations in both mean (for queen size, heat stress and mating behavior) and variance (for worker size). These changes may affect biotic interactions by, for example, modifying prey-predator relationships or food preferences as colonies with more variable ant workers adopt a broader foraging range. Our results on intra-colony variation in size were not supported statistically, but, even if we consider that only inter-colony variation in worker size differed between urban and rural populations, higher between-colony variation in urban populations might broaden the foraging regime of urban ants, making them more generalist even if individuals remain specialized (Bolnick et al. 2011). How urbanization, by inducing intraspecific variations in biological traits, can impact demographic parameters of populations and biotic interactions remains an open question in urban ecology which will require further theoretical as well as empirical studies.

Our ability to understand the present and predict future patterns of biodiversity in urban ecosystems also depends on the development of theoretical approaches and modeling methodologies aiming to combine species-environments relationships, natural and human-mediated dispersal of invasive species, ecological outcomes of species interactions (Guisan and Thuiller 2005) and the effects of intraspecific variations (Bolnick et al. 2011).

This broad and diverse combination of research avenues offers several perspectives to this thesis. First, at the biological community level, ant abundances and densities should be investigated in order to access more precise species-environment relationships and detect potential positive or negative species associations (Cushman et al. 1998, Kanizsai et al. 2013). Keeping our focus on open vegetated habitats, such sampling should be performed on patches all along the urban to rural gradient. Ideally, sites where *L. neglectus* occurred should represent a significant proportion of all sampling sites in order to properly assess the impacts of this invasive species on native species densities (in particular *L. niger*, see chapter one). Another stimulating research direction would be to measure species biological traits in order to identify how biological characteristics are related to environmental conditions associated to urbanization. Most studies investigating environment-traits relationships along environmental gradients used trait values found in the scientific literature (e.g., Vallet et al. 2010). However, as phenotypes often vary along species range, using traits values measured in another part of the species range (or mean values over the species range) may not be relevant. A solution would be to measure traits directly on sampled specimens along the urbanization gradient. Chosen traits should comprise traits most likely to be affected by urbanization including mean size and intra-colonial variance in size in all castes (queens, males and workers), thermal niche for foraging and for brood development (Diamond et al. 2013), food preferences, circadian rhythm, social structure, resistance to chemical pollution (e.g., heavy metals: Grześ 2010a) and oxidative defenses. Ideally, these traits should be estimated for all species occurring along the urbanization gradient and between populations of urban tolerant species.

In 2014 and 2015, I sampled ant nest densities in urban, peri-urban, agricultural and rural open vegetated habitat. This work is not included in this thesis as taxonomical identifications and data analyses have yet to be completed. However, in a near future, I will have precise, reproducible measures of nests densities and spatial organization of ant nests in the major environmental contexts along urbanization gradients, as well as morphological measurement of workers for all species and populations along the urbanization gradient.

Heavy metal pollution is common in urban areas (Imperato et al. 2003) and have already been related to increases in oxidative stress caused by enhanced generation of Reactive Oxygen Species (ROS Cells under oxidative stress display various dysfunctions due to lesions caused by ROS to lipids, proteins and DNA. We recently initiated the investigation of the relationship between heavy metal pollution, bioaccumulation and oxidative stress defenses and damages in *L. niger* workers originating from urban and rural populations in Lyon and Saint-Etienne. This should tell us how *L. niger* responds to chemical pollution generated by urban living, which is surprisingly novel, as urbanization, heavy metals and oxidative stress have never been investigated simultaneously in ants.

Evolutionary changes induced by urbanization

As more evidence of phenotypic shifts between urban and rural populations is documented in many taxa (e.g., insects: San Martin y Gomez and Van Dyck 2012; lizards: Winshell et al. 2016; mammals: Snell-Rood and Wick 2013; plants: Ushimaru et al. 2014), intraspecific variations induced by urbanization appear to be a common and widespread phenomenon. It is however difficult to evaluate the implication of genetic evolution in these phenotypic shifts. Even if they are primarily plastic response to urban conditions, phenotypic shifts in urban populations could drastically impact the nature and strength of selective pressures imposed on urban individuals, by affecting survival and reproductive rates (Alberti 2015) or intra and interspecific interactions (e.g., parasite loads, Giraudeau et al. 2014). Such new selective pressures may act on morphology, physiology and behavior, and provoke evolutionary changes in urban populations (Fig. 12, Palkovacs and Hendry 2010, Alberti 2015). Understanding such eco-evolutionary feedbacks is an obligatory step in our ability to model and predict evolutionary changes induced by urbanization and more broadly biodiversity dynamics in urban ecosystems (Alberti 2015).

This thesis provides novel insights on the ecological impacts of urbanization at different levels of biological organization in ants. Urbanization is here shown to shape biological communities in complex ways as they affect native and invasive species distributions idiosyncratically, to promote the emergence of new biotic interactions and to provoke phenotypic shifts. These ecological changes are predicted to cascade between all levels of biological organization and to lead to genetic evolution and evolutionary changes (Fig. 12, Alberti 2015). Our results suggest that eco-evolutionary dynamics might be at play in our study landscape as urban ant populations undergo strong ecological changes at organization levels from community structure to individual phenotype (Fig. 12). We have however no clear evidence of genetic evolution in urban populations.

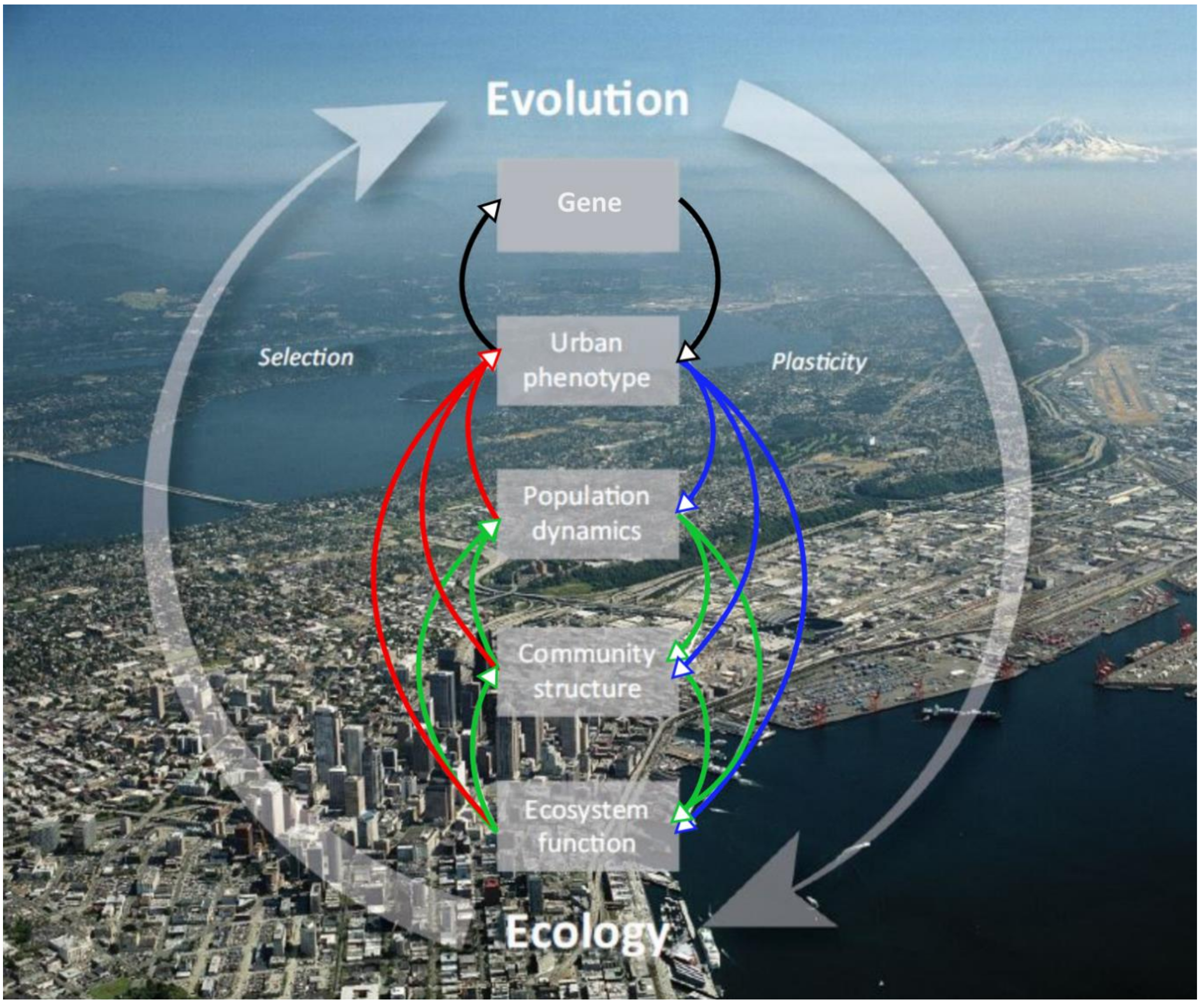

Figure 12: Eco-evolutionnary feedbacks in urban ecosystems. Boxes represent biological organization levels. Phenotypic shifts (genetically induced or not) in urban populations (in term of morphology, physiology, behavior) can affect demographic rates (e.g., reproduction, survival), community structure (e.g., species richness, biological interactions) and ecosystems functionning (e.g., primary productivity, nutrient cycling) (blue lines). Changes in one of these organization levels can have consequences on the other organization levels (green lines), including on urban phenotypes (red lines) and finally influence genetic evolution (loops represented by black lines) (Modified from Palkovacs and Hendry 2010 and Alberti 2015).

Genomics in the Anthropocene

A promising way forward to investigate genetic evolution in urban areas might lie in genomic approaches. While classic neutral genetic markers such as microsatellites can highlight genetic differentiation between urban and rural populations (Munshi-South and Kharchenko 2010), except if deployed in massive numbers, they cannot be used to identify selective pressures on coding parts of the genome. Alternatives could be transplant and common garden experiments, as well as crossbreeding. However, these methods are not applicable to most of taxa, except some particular species (e.g., *Turdus merula*, Partecke et al. 2006).

Genomics have already been used to identify loci under selection along environmental gradients (Stinchcombe and Hoekstra 2008, Prunier et al. 2011, Nunes et al. 2011, Tsumura et al. 2012, Sork et al. 2013, Feng et al. 2015) and could thus be applied to urbanization gradients in most species. Munshi-South et al. (2016) and Harris et al. (2016) were first to investigate genome-wide single nucleotide polymorphism variation along an urbanization gradient (New York, USA). These two studies focused on white-footed mice (*Peromyscus leucopus*) populations and investigated landscape genetic changes linked to dispersal limitations and colonization history in urban areas. The same research group recently detected signatures of positive selection and local adaptation to urbanization in the same species using transcriptome data (Harris and Munshi-South, *pre-published in bioRxiv*). Among the outlier loci they found, many were implicated in metabolic functions, including diet specialization.

Their work demonstrated that important genetic differentiation resulted from reduced gene flow, drift and selection. The interplay between these three forces were discussed in the introduction of this manuscript and genome datasets constitute a most promising and exciting window into how gene flow, drift and selection interact with each other (Tigano and Friesen 2016) leading to genetic divergence and potentially to speciation. *Lasius niger*, as an urban tolerant species occurring in thousands of cities all over the Holarctic, is a singularly relevant biological model for genomic investigations. Comparing evolutionary changes in *L. niger* urban and rural populations in several distant urbanized landscape would shed precious light on the impact of urbanization on genetic evolution. Indeed, even under similar selective pressures, independent urban populations should exhibit independent genetic evolution because of the multitude of possible evolutionary trajectories, either exploring divergent adaptive landscapes or converging towards similar adaptations. However, as described in Chapter Two, even spatially distant cities are now connected via human-mediated dispersal. Therefore, evolutionary changes occurring in one city could rapidly be transferred between cities. Under a scenario of strong positive selection (Harris and Munshi-South, *pre-published in bioRxiv*), transfer of adaptations between cities would greatly accelerate the establishment of local adaptations. However, it could also lead to the genetic homogenization of all urban populations connected by human activities.

Landscape genomics can be used to identify adaptive variations along urbanization gradients by investigating genotype-environment correlation (Sork et al. 2013). Common methods would consist in (i) identifying outlier loci between urban and rural populations (loci for wich genetic differenciation (Fst) between populations is maximized), (ii) assessing which of these outlier loci allelic frequencies are significantly associated with factors related to urbanization (e.g., Harris and Munshi-South, *pre-published in bioRxiv*) and (iii) finding the gene family of these candidate loci and their putative biological function (Prunier et al. 2011). After genomic analysis, experimentally testing the relative importance and relevance of predicted functions should conclusively reveal the mechanisms by which urbanization acts on evolution.

An annotated genome for *Lasius niger* has been published in 2016 by Konorov and colleagues and made available in Genbank (Konorov et al. 2015). It joins there other ant genomes (to date, more than ten annotated ant species genomes are available), which should greatly facilitate genome-wide investigation of genetic evolution in this species and others for most laboratories in the coming years (Morandin et al. 2016).

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