Fonds National de la Recherche Luxembourg

CORE MULTI-ANNUAL THEMATIC RESEARCH PROGRAMME

PROJECT DESCRIPTION FORM

CORE 2020 CALL

Project Acronym	LandGen
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PROJECT DESCRIPTION

1. Description of the Proposed Research Project

1.1 Introduction

Pollinators provide a key ecosystem service to agricultural crops and wild plants and their importance for food security is widely acknowledged (Potts et al. 2015). It has been estimated that, globally, the economic value of pollination is worth a total of €153 billion (Gallai et al. 2009). The vast majority of European crops and of temperate wildflowers benefit from insect pollination by, in particular, bees (Apiformes) and hoverflies (Syrphidae) (Potts et al. 2015). Besides being essential to the maintenance of plant diversity, pollinators also provide enormous added-value by indirectly supporting an immense range of other organisms, from microbes and parasites, to specialist predators, herbivores, fruit- and seed-eating animals, among others (Ollerton 2017). The maintenance of pollinator abundance and diversity is therefore of critical importance for both ecosystems and agricultural services. However, evidence of the loss of pollinators is clear-cut: wild pollinators are declining at local, regional and global scales, in both diversity and abundance. Numerous species are threatened with extinction, primarily as consequence of human activities (Potts et al. 2010, Gill et al. 2016, Ollerton 2017). This is a serious cause for concern because pollinators are an integral part of ecosystems and their precipitous decline presents a crisis for food security and human wellbeing.

Pollinator declines are frequently attributed to multiple interacting causes, with the intensification of land-use, climate change, the spread of invasive species and parasites/pathogens usually considered as the main underlying drivers (Vanbergen et al. 2013, Gill et al. 2016). Especially land-use change and the resulting habitat loss and homogenisation are often seen as major individual factors causing the problem (Vanbergen et al. 2013). The spread of urban areas and the intensification of agriculture have resulted in the destruction and fragmentation of many of the natural habitats that pollinators depend on for feeding and nesting resources (Potts et al. 2010; Vanbergen et al. 2013). While it appears that the rapid man-made landscape alterations of recent decades were associated with declines in the species richness of insect pollinators (Senapathi et al. 2015), there is a considerable lack of knowledge on the mechanisms underlying the responses of invertebrate pollinators to land-use change (Winfree et al. 2011). This makes it difficult to reliable quantify the interactive effects with other drivers of decline and to develop effective approaches to conserve pollinator populations and the associated ecosystem services (Gill et al. 2016).

Dispersal capability is a key life-history trait affecting an organism's ability to deal with habitat fragmentation. It is required to maintain connectivity between distant habitat patches, to colonise new habitats and to allow re-colonization after local extinction. It thus impacts species distribution, community structure, (meta-)population dynamics, gene flow and extinction risk (Bowler & Benton 2005). Species with high dispersal ability generally are better able to move efficiently between suitable habitat patches and may exploit fragmented resources more efficiently (Öckinger et al. 2010). Beyond a species' intrinsic dispersal ability, however, dispersal between remnant habitat patches will be influenced by the functional connectivity of the landscape, which refers to how the behaviour of an organism is affected by the distance between patches and the composition of the intervening habitat matrix (Baguette & Van Dyck 2007, Jauker et al. 2009).

Until now, we only have a limited understanding of the dispersal ability of most insect pollinators and about the functional connectivity of fragmented habitats (Dicks et al. 2013, Vanbergen et al. 2013). Specifically, further insights are needed into distances moved in different landscapes as well as the features and environmental factors that hinder or facilitate

movement through the habitat matrix (Vanbergen et al. 2013, Dicks et al. 2013, Gill et al. 2016). A particular difficulty with evaluating the impact of land-use change relates to the fact that flying ability differs significantly between different insect pollinator species (Greenleaf et al. 2007). Even closely-related species can respond differently to habitat fragmentation (Steffan-Dewenter et al. 2002, Jauker et al. 2009). Further studies on the impact of fragmentation on different pollinator groups/species in contrasting landscapes are thus needed. These would be of key importance for answering questions relative to the landscape structure and composition necessary to support insect pollinators, as well as the geographic scale at which maintenance measures should be implemented.

One important group of plant pollinators are hoverflies (Syrphidae), a biologically diverse family of flower-visiting flies (Speight 2017). Adults feed on nectar to gain energy and adult females on pollen to gain nutrients for egg production. Species rarely display preferences for specific flower species (Branquart & Hemptinne 2000). Their dependence on floral resources makes hoverflies the most important pollinators besides bees, providing a major contribution to plant diversity and agricultural production (Ssymank et al. 2008). Hoverfly larvae utilise a diverse array of habitats and feeding modes, including fungal fruiting bodies, nests of social Hymenoptera, decaying wood, dung and different water bodies. Many larvae also feed on aphids and are very effective biocontrol agents, especially in agricultural landscapes (Speight 2017). Given the ecological and economic importance of hoverflies, it would thus be important to gain a more thorough understanding of their movement ecology in fragmented landscapes.

1.2 Relevant state-of-the art and your own contribution to it

Little is known about dispersal of hoverflies in general and the effects of landscape fragmentation on their dispersal in particular. In Europe, some 30 species migrate southwards in the autumn, covering long distances and crossing mountain ranges in the process (Gatter & Schmid 1990). The majority of hoverfly species, however, are non-migratory (Speight 2017) and not much is known about their intrinsic dispersal ability. Schweiger et al. (2007) assumed that hoverflies with large body sizes had high dispersal abilities. In a mark-recapture study on the aspen hoverfly (*Hammerschmidtia ferruginea*, Fallén 1817), Rotheray et al. (2014) found wing length to be positively correlated with dispersal, but only in males. There is also little knowledge about dispersal distances. While Rotheray et al. (2014) recovered some *H. ferruginea* individuals at up to 5 km from the release site, during their normal foraging activity, hoverflies move a few hundred meters at most and tall vegetation, bare soil (dirt tracks, asphalt roads or ploughed fields) can act as barriers (Lövei et al. 1998, Wratten et al. 2003). Studies investigating hoverfly richness in relation to habitat patch isolation suggest that hoverflies are significantly impacted by habitat fragmentation (Ouin et al. 2006, Moquet et al. 2018).

Molecular genetic methods are powerful tools to investigate the effect of fragmentation on target species where dispersal capability cannot be studied directly, or only with great difficulty. In particular, investigating the spatial organization of genetic variation can help to clarify the degree of isolation of different habitat patches and identify landscape elements that hinder or facilitate gene flow (Guillot et al. 2005; Peterman 2018). Frequently, abrupt genetic discontinuities are linked with elements in the landscape that may disrupt dispersal in a species of interest. For example, during my research I have shown that motorways and large water bodies can act as gene flow barriers for different vertebrate species (Frantz et al. 2010b, Frantz et al. 2012). However, methods that detect abrupt genetic discontinuities can provide only limited information on how animals move through a landscape. By statistically relating the distribution of genetic similarities among individuals to landscape characteristics, it is possible to relate gene-flow patterns to landscape structure and develop rigorous empirical models of the functional connectivity of a landscape (Peterman 2018). Recently, I have applied this genetics-based resistance modelling to show that urban foxes in Berlin used railways and motorways as main dispersal corridors within the cityscape, while avoiding

densely built-up areas. Based on these results, it was possible to model landscape resistance to fox dispersal (Kimmig et al. 2020). I have also applied resistance modelling to assess the effects of landscape features on large ungulates in Belgium and Germany (Renner et al. 2015, Dellicour et al. 2019). Finally, by comparing the characteristics of spatial genetic structure it is possible to infer difference in dispersal patterns of populations or sexes, as I have shown for badgers in the UK and Switzerland (Frantz et al. 2010a).

There are only very few studies that use genetic methods to investigate the effect of habitat fragmentation on dispersal of insect pollinators, particularly at the landscape scale. Studies on hoverflies either looked at large spatial scales and/or did not statistically evaluate the effect of environmental features on dispersal (Raymond et al. 2013, Schauer et al. 2018). Nevertheless, the results from work on Apiformes suggest that even good dispersers can be impacted by habitat fragmentation. Bumblebee (*Bombus*) species normally exhibit little genetic structure at smaller spatial scales (10s of km; Lozier et al. 2011, Dreier et al. 2014). Jha & Kremen (2013) nevertheless showed that impervious cover associated with built-up areas significantly limited gene flow in a North American *Bombus* species. Even though working at a larger spatial scale, Davis et al. (2010) similarly showed that urban areas were a substantial gene flow barrier for a rare solitary bee.

Luxembourg is the European country with the highest landscape fragmentation or, more specifically, where wildlife movement across the landscape is the most frequently interrupted by human infrastructure (Jaeger & Madrinan 2011). Moreover, the country is expected to almost double its number of inhabitants by 2060[1]. As a consequence of this demographic growth, land use change for new infrastructure and urban development is expected to be considerable. This, in turn, will lead to further loss and fragmentation of natural and semi-natural habitats (Jaeger & Madrinan 2011). Luxembourg has recognised that habitat loss and fragmentation are threatening its biodiversity in general and insect pollinators in particular. Key strategies to counteract the negative effects of habitat fragmentation include the design of a network of ecological corridors as well as land set-a-side to support pollinators within the agricultural landscape (Ministère du Développement durable et des Infrastructures 2017). In order for these mitigating measures to be successful, however, it is important to understand the functional connectivity of the landscape from the viewpoint of the pollinator (Dreier et al. 2014, Rands 2014).

1.3 Hypotheses, project objectives and contribution to knowledge development in the research field

The overall objective of the proposed study is to analyse the functional connectivity of typical Luxembourg and western European landscapes from the viewpoint of hoverflies.

Working in different habitat types and with four different hoverfly species, we aim to understand the influence of landscape structure and composition on hoverfly dispersal in order to understand the influence of habitat fragmentation and to design effective mitigation strategies. While our main focus will be on Luxembourg, we want to include study areas in a neighbouring region, to ensure that the observed patterns are not a specificity of the Luxembourg context. We will use four non-migrating species with different life histories and individual-specific genetic profiling techniques coupled with landscape resistance modelling to address the following objectives:

[1] Eurostat (2020) https://ec.europa.eu/eurostat/databrowser/view/tps00002/default/table?lang=en (accessed 11/03/2020)

Objective 1: Understanding the functional connectivity of urbanised landscapes from the viewpoint of hoverflies. We will select three urban areas (Luxembourg City, Southwest Luxembourg and the German city of Cologne) to test whether

Hypotheses:
a) Urbanised areas provide a high resistance for dispersal and limit gene flow in hoverflies.
b) Larger hoverflies have better flight ability and are therefore better able to cross urbanised areas.
c) The urbanised environment presents little resistance to species known to utilise urban and sub-urban habitats.

Expectations:
While built-up areas have been shown to limit gene flow even in highly mobile insects at the regional scale (e.g., Davis et al. 2010, Jha & Kremen 2013), no study, to the best of our knowledge, investigated functional connectivity of insects within an urbanised area and the impact of urban development on hoverfly dispersal is completely unknown. Similarly to other mobile insects, urban areas may limit hoverfly dispersal. They may not navigate well through urban areas and use more rural pathways. Conversely, the capability of hoverflies to disperse through urbanised areas may be strongly impacted by physiology. Small, potentially less mobile hoverflies may be more strongly impacted by urbanisation than larger species. While foraging distances are positively related to body size in Apiformes (Greenleaf et al. 2007), very little information is available for hoverflies, other than the mark-recapture study by Rotheray et al. (2014), which found wing length to be positively correlated with dispersal in males of *H. ferruginea*. Finally, the impact of urbanisation may depend on a species' behaviour. For example, looking at range-expanding butterflies, Gilchrist et al. (2016) found that among species with similar dispersal capability, only the most adaptable generalists managed to expand through an urbanised landscape. A number of hoverfly species are known to utilise the resources of urban and suburban gardens and parks. Urbanised landscapes may therefore offer little resistance to their movement. Here, we therefore analyse four species of differing sizes, two of which are commonly found in urban and suburban habitats.

Objective 2: Understanding whether hoverfly dispersal in agroecosystems is affected by landscape diversity. Landscape elements identified to be influencing gene flow patterns can differ between regions with differing landscape structure (Short Bull et al. 2011). We will select four rural study areas with opposing degrees of landscape structural diversity and flower resources to test whether

Hypotheses:
a) The functional connectivity of the landscape is higher in the structurally more diverse landscapes, as it provides a larger range of habitat elements and landscape features needed for dispersal.
b) The functional connectivity of the landscape depends on the habitat specialisation of the target species.
c) The flight ability of small hoverflies is limited. Small hoverflies are therefore more strongly impacted by the landscape with reduced structural diversity.

Expectations:
Very little is known about the dispersal ecology of hoverflies in agroecosystems. Studies on hoverfly richness have shown, however, that more hoverfly species are present in an agricultural landscape with larger amounts of flower-rich semi-natural habitats (Kleijn & van Langevelde 2006, Meyer et al. 2009). While not directly investigating dispersal, these studies thus suggested that structurally diverse landscapes facilitate dispersal between optimal habitats (Meyer et al. 2009). Alternatively, the degree of habitat specialisation may influence functional connectivity. Land use characteristics certainly affect the richness of different groups of hoverflies differently (Schweiger et al. 2007, Meyer et al. 2009). For instance, the

richness of forest specialists within open habitats is influenced by proximity to woodlands (Meyer et al. 2009, Moquet et al. 2017). Also, similarly as above, small, potentially less mobile hoverflies may be more strongly impacted by the lack of structural diversity than larger animals.

Objective 3: Gaining a better understanding of the dispersal behaviour of hoverflies.
We will analyse isolation-by-resistance (IBR) patterns (genetic relatedness as a function of geographic distance corrected for landscape resistance) of all four species in the three urban and four rural study areas to test:

Hypothesis:
Dispersal distances of hoverflies are lower in areas with reduced connectivity.

Expectations:
Dispersal rates of certain butterfly species have been shown to decline with increasing habitat fragmentation and reduced connectivity (e.g., Baguette et al. 2003, Mennechez et al. 2003). On the other hand, dispersal distances might also increase as a result of habitat fragmentation (e.g., Mennechez et al. 2003, Redhead et al. 2016).

Objective 4: Modelling the functional connectivity of Luxembourg for hoverflies.
Preserving, improving and restoring connectivity and movement corridors in fragmented landscapes are essential elements in any conservation strategy. However, empirical studies on how landscapes influence species' movement are, by necessity, limited in the extent of the study area and not available for locations where measures for improving connectivity are planned. Consequently, connectivity models designed locally have been extrapolated to larger regions or different regions altogether (e.g., Cushman et al. 2009). Working with four different hoverfly species in seven structurally different study regions, our project would provide an ideal study case to test:

Hypothesis:
The concept of extrapolation of resistance models between study areas with differing landscape diversity is not a valid approach.

Expectations:
Because landscape elements identified to be influencing gene flow patterns can differ between regions with differing landscape structure (Short Bull et al. 2011), it is likely that extrapolation between study areas with different degrees of landscape complexity is generally invalid or at least invalid for only those species whose dispersal ecology is impacted by differing landscape structure.

1.4 Methods and approach

Study species

We plan to analyse the functional connectivity of the seven different landscapes form the viewpoint of four non-migratory hoverfly species. It is therefore necessary to choose target species that are widespread and likely to occur in all study areas. The species should be sufficiently common and easily identifiable in the field, with flying periods covering multiple months. In order to test our different hypotheses, the species ought to differ in their wing lengths (as a proxy for dispersal ability, see Rotheray et al. 2014), ecological characteristics and association with humans. We chose species from the same subfamily (Eristalinae). We will focus on the following four species (species characteristics based on Ball & Morris 2015, Leopold et al. 1996, Schulten 2018, Speight 2017):

1) *Myathropa florea* (Linnaeus, 1758)
Occurrence: Widespread and abundant. Seventh most common hoverfly species in the observation database of National Natural History Museum Luxembourg (MNHNL); the species has been trapped in different suburban areas of Cologne; Flight time in the Netherlands/Belgium: April to October. Identification: distinctively patterned thorax and abdomen. Wing length: 7-12 mm; Preferred habitat: deciduous forest, humid pasturage and suburban gardens. Larvae: wet hollows with leaves and twigs, also wet cow dung and compost heaps.

2) *Rhingia campestris* (Meigen, 1822)
Occurrence: Widespread and common. MNHNL: 5th most common species; the species has been trapped in different suburban areas of Cologne; Flight time NL/B: from April to October, but with two generations flying end of April to June, the second August to September; Identification: Unmistakable hoverfly with long rostrum. *Rhingia rostrata* (Linnaeus, 1758) may also occur in Luxembourg (Speight 2017); see genetic methods below. Wing length: 6-9.5 mm; Preferred habitat: Common in woodland and field edges, but can be found in all (semi-)natural habitat. Larvae: live in fresh cattle dung and other enriched wet media.

3) *Syritta pipiens* (Linnaeus, 1758)
Occurrence: Widespread and abundant. MNHNL: 14th most common species; the species has been trapped in different suburban areas of Cologne; Flight time NL/B: April to October, with peaks in abundance in June to September. Identification: Unmistakable, greatly enlarged hind femora, thorax dusted ash-grey. Wing length: 4.25-7 mm; Preferred habitat: wetland, most farmland, suburban gardens and urban parks. Larvae: live in moist, decaying, vegetable matter, including cow dung and garden compost heaps.

4) *Volucella pellucens* (Linnaeus, 1758)
Occurrence: Widespread and abundant. MNHNL: 19th most common species; in Cologne, this species was only trapped in some suburban areas; Flight time NL/B: May to September. Identification: very obvious large hoverfly with large white markings on the abdomen. Wing length: 10-15.5 mm. Preferred habitat: Deciduous forests, scrub, tree-lined paths. Larvae: scavengers in nests of social wasps or bumblebees.

Study sites

We plan to analyse functional connectivity of three urbanised landscapes (Luxembourg City, Southwest Luxembourg and the city of Cologne) and of four rural landscapes (two in Luxembourg, two in the Cologne hinterland) with different configurations. Based our current knowledge on hoverflies, it is difficult to decide on the size of the study areas. Landscape genetic analysis should not be performed at spatial extents greatly exceeding common dispersal distances, as environmental factors influencing gene flow will mainly be analysed at the tail end of the dispersal curve, where effective movement only occurs rarely (Angelone et al. 2011). As explained above, there is, however, little knowledge on dispersal distances of non-migrating hoverflies. While one study recovered some *H. ferruginea* individuals at up to 5 km from the release site (Rotheray et al. 2014), Kleijn & van Langevelde (2006) found that hoverfly density optimally related to landscape context at spatial scales of 500-1000m within agricultural landscapes. In the case of the agricultural landscapes, we therefore selected study areas of 10 x 10 km as a compromise between sampling density (we aim to sample one specimen per species per km^2, see below) and likelihood of detecting landscape genetic structure. In the case of the two urban areas in Luxembourg, we extended this to a 200 km^2 study area to ensure that large parts of the urban sprawl as well as sufficient amount of adjoining countryside were included. For the same reasons, we extended the study area further still in the case of Cologne.

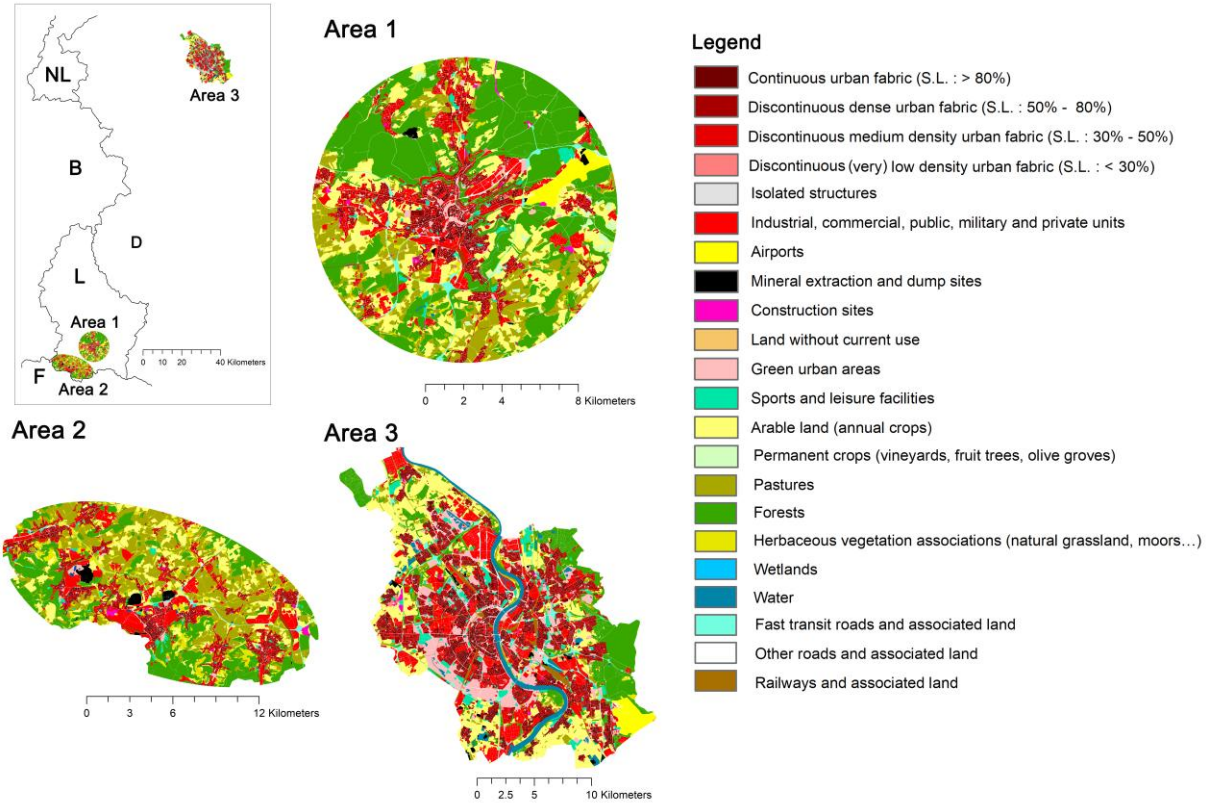

Fig. 1: Proposed urbanised study areas. Area 1: Luxembourg City; Area 2: Southwest Luxembourg; Area 3: Cologne. Top left inset: Geographic location of the study areas (B=Belgium, D=Germany, F=France, L=Luxembourg, NL=The Netherlands). The landscape structure of neighbouring regions (white surfaces in each study area) will be considered in the analyses, but sampling will be limited to the study area. Both study areas within Luxembourg have an area of 200 km^2 (minus the white surfaces within the ellipse in case of study area 2), while the Cologne has an area of 405 km^2. Land-cover data based on Copernicus Urban Atlas.

There are two major urbanised agglomerations in Luxembourg: Luxembourg City and its surroundings, as well as a cluster of different towns in the southwest of Luxembourg (Fig. 1). Both urban areas are relatively small, with the municipality of Luxembourg City, for example, having an area of 51 km^2 and the southwestern agglomeration having a similar amount of built-up areas (Fig. 1). We therefore also propose to gain insights into hoverfly dispersal in a large metropolitan area and aim to include the city of Cologne in the study. Based on its surface of 405 km^2, Cologne is the third largest city in Germany. It has a large number of green surfaces, protected areas, riparian forest fragments and wetland (Sabovljevic & Sabovljevic 2009). In the case of Luxembourg City and the southwest of Luxembourg, we will sample an area of 200 km^2, while in the case of Cologne, we will sample the whole of the city (see comments below regarding sampling regime).

We also aim to compare two rural landscapes that both have high habitat diversity with two more homogenous landscapes dominated by agriculture. One of each will be located in Luxembourg, while the other pair will be near the city of Cologne. We provisionally chose the two Luxembourg study areas based on the Luxembourg Biophysical Land Use database and

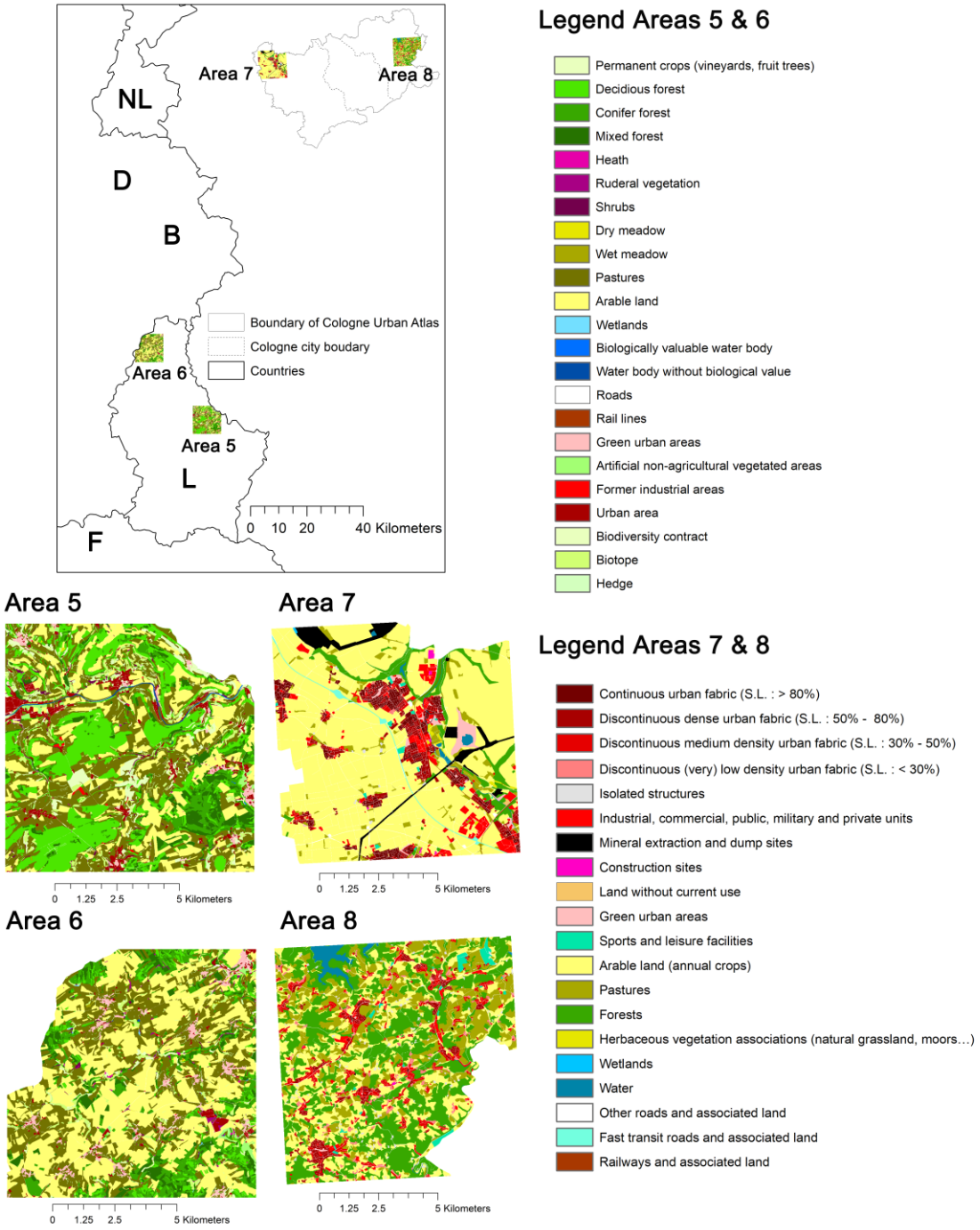

Fig. 2: Provisional choice of rural study areas. Areas 5 & 8: areas with diverse habitats in Luxembourg and near Cologne, respectively; Areas 6 & 7: agriculture-dominated landscapes in Luxembourg and near Cologne, respectively. The 10 x 10 km study areas were chosen to maximise specific landscape characteristics (see Methods section). In the case of areas 5 & 6, the choice of landscapes was based on the Luxembourg Biophysical Land Use database, while in the case of areas 7 & 8 it was based on the Copernicus Urban Atlas database of Cologne and its rural hinterland (whose outline can be found in the top inset). Areas 7 & 8 appear crooked because of the geographic projection. In the Luxembourg study areas, the landscape structure of neighbouring regions (white surfaces) will be considered in the analyses, but, because of trapping permits, sampling will be limited to the study area. Sampling will cover the whole of the German study areas, and information on the landscape structure of the white surfaces will be added. Top inset: Geographic location of the study areas (B=Belgium, D=Germany, F=France, L=Luxembourg, NL=The Netherlands).

the two rural areas near Cologne based on the Copernicus Urban Atlas database for Cologne and its hinterland (Fig. 2). We moved a 10 x 10 km window across Luxembourg and across Cologne and its hinterland (at 2 km intervals) and calculated landscape metrics for each subsampled landscape (Fig. 2) using the R package *SDMTOOLS*[2]. The more 'diverse' area was chosen in order to maximise each of the following criteria: large number of habitat patches, size of habitat patches small and equitable, small size of urban areas. Conversely, the agriculture-dominated landscape was chosen to maximise each of the following criteria: large agricultural surface, non-agricultural patches small with low equitability, small size of urban areas. At a later stage, we will repeat the selection including a single database that includes the same land cover information for all study areas (e.g. CORINE), allowing us to avoid gaps in the spatial coverage of the chosen study sites, and also include information on the location of biotopes in the selection of sites.

Sampling design and field work

The field sampling design can have an important impact on the accuracy of the inference in landscape genetics (Oyler-McCance et al. 2013). We therefore propose a systematic sampling design where we will collect samples at regular intervals throughout a study area. This design performed well in a simulation study, correctly identifying the environmental features driving gene flow in a continuously distributed species (Oyler-McCance et al. 2013). As a general guideline, we propose to sample one animal per species per km^2 of study area. Analogous to the choice of the sizes of the study areas, this choice is based on our limited knowledge of the dispersal distances of non-migratoy hoverflies (see above) and a compromise between a sampling density permitting the detection of the underlying landscape genetic processes and the feasibility of collecting the required number specimens in the field (3200 flies in the three urbanised study areas and 1600 flies in the four rural habitats).

Our initial plan is to collect species along a daily walk across multiple squares by a combination of pan trapping (i.e. using small coloured pans filled with soapy water that passively trap flying pollinators; O'Connor et al. 2018) and hand-netting. For example, one person will sample five adjoining squares by walking across the five squares every day for five days. On the morning of the first day, the field worker will set up four pan trap stations in different locations in the first square and then hand-net flies on a walk across all five squares. On the second day, the person will start by setting up four pan traps in the second square, then net flies in all five squares and remove the pan traps in the first square at the end of the day and so on. By changing the daily order in which the square will be sampled, we will ensure that each square is visited during different times of the day. For each study area grid cell, we will choose four pan trapping and netting sites based on land cover and biotope maps as well as satellite imagery. Depending on the success rate of both approaches, we might increase the number of pan traps or the netting effort. The latter is likely to be necessary in the urban areas due to pan traps probably being vandalised.

To sample the urbanised areas, we require at least six fieldworkers, with each person collecting samples from the four target species in ~130 square kilometres during a six months field season. To sample the rural areas, we need at least four fieldworkers (two in Luxembourg and two near Cologne), with each person sampling 100 square kilometres during the field season. Specimens collected in parallel in our study areas as part of an ongoing Luxembourg wild bee atlas project (2019-2022) will also be integrated in the present study.

[2] http://www.rforge.net/SDMTools/ (accessed 24/03/2020)

Genetic methods

Given the large number of hoverflies that we want to analyse (4800 in total), the only cost-efficient and feasible method is to use microsatellite-based genotyping. Microsatellite loci are still powerful tools when neutral markers are needed to investigate dispersal, gene flow and landscape genetic patterns (Kimmig et al. 2020). Because, to the best of our knowledge, no microsatellites have been developed for our four target species, this will be a necessary first step. We will use approximately 1 µg of genomic DNA of a single individual of each species to generate an unenriched Illumina® paired-end library that will be sheared to fragments between 300 and 500 base pairs (bp) in size. Fragments will be sequenced using a single lane with a read size of 250 bp on an Illumina® MiSeq benchtop sequencer (Illumina, San Diego, USA). This initial work will be outsourced to the University of Sheffield. We will then use the PRIMER3 v.0.4.0 software (Untergasser et al., 2012) to develop primer pairs for 75 regions flanking microsatellite repeats. The amplification success of all new primers will be tested using unlabelled primers and the forward primer of 45 markers that will gave rise to a clear polymerase chain reactions (PCR) product will be 5'-labelled with fluorescent dyes and amplified using eight good-quality DNA samples. Loci that will be polymorphic, will give rise to clear peaks and that can be combined into two multiplex PCRs will be retained for analysis. We have experience developing microsatellite panels using this method (Osten-Sacken et al. 2018) and aim for between 13 and 20 valid microsatellite markers per species.

Population and landscape genetics

We will first perform a factorial correspondence analysis (She et al.1987) to look for genetic outliers in all the datasets. The species identity of the outliers will be cross-checked using mitochondrial DNA COI barcodes (Ståhls et al. 2009). We will use the programs *STRUCTURE* (Pritchard et al. 2000), *GENELAND* (Guillot et al. 2005) and *EEMS* (Petkova et al. 2016) to identify the location of abrupt genetic discontinuities, i.e. gene flow barriers. The functional connectivity of the landscapes will be assessed using the R package *ResistanceGA* (Peterman 2018). Rather than letting the user subjectively assign a value representing the extent to which a feature impedes or facilitates connectivity, *ResistanceGA* computes pairwise resistance distances between individual animals and uses a linear mixed effects model relying on genetic algorithms to maximize the fit of resistance surfaces to the data. The process is based on stochastic search algorithms that solve optimisation problems by mimicking processes of natural selection. The optimisation process uses log-likelihood as the objective function. Mixed models are fitted using the maximum likelihood population effects (MPLE) parameterization. A simulation study by Shirk et al. (2018) has shown that this linear-mixed-effects-model-based method had a high accuracy in model selection. We have shown recently that, when using an eigenvector-based multivariate genetic distance measure, ResistanceGA has a high power to infer the functional connectivity of a landscape, even when the genetic signal is relatively weak (Kimmig et al. 2020).

Hypothesis testing

If gene flow is impacted by the landscape configuration, we 1) expect to identify gene flow barriers in a study area. In other words we expect to find abrupt genetic discontinuities that can be associated with landscape features. Alternatively, 2) if a specific environmental feature facilitates or hinders gene flow, we expect that the landscape resistance model testing the effect of that specific environmental feature will explain the distribution of pairwise genetic distances significantly better than Euclidean distance between samples alone.

Objective 1: For this analysis, we will use the latest available version of the Copernicus Urban Atlas (https://land.copernicus.eu/), which provides detailed land cover data of larger European urban areas and their hinterland. The urban atlas contains information on the degree of imperviousness of built-up areas, building height and even the location of street trees. If urbanised areas represented a significant impediment to gene flow to all species, we

would always expect distinct genetic populations separated by the urbanised area and/or high and significant resistance-to-movement associated with built-up areas. Alternatively, we may only see this pattern for species which are not usually associated with built-up areas (*R. campestris* and *V. pellucens*), with built-up areas offering little or no resistance to species common in sub-urban and urban areas (*M. florea*, *S. pipiens*). Finally, built-up areas may offer little or no resistance to larger species of hoverflies (*M. florea*, *V. pellucens*), while smaller species (*R. campestris*, *S. pipiens*) may be more impacted by urbanisation.

Objective 2: If structurally diverse landscapes facilitate dispersal of all species of hoverflies, we would expect very few individual environmental features to explain the distribution of pairwise genetic distance significantly better than Euclidean distance. In the more homogenous landscape, in contrast, we expect intensively used agricultural land to present significant resistance to gene flow, with biotopes channelling gene flow significantly. Alternatively, habitat generalists/open habitat specialists (*R. campestris*, *S. pipiens*) may exhibit limited landscape genetic structure in either type of agricultural landscape, while species mainly associated with forests or tall vegetation (*M. florea*, *V. pellucens*) will exhibit significant landscape genetic structure in the homogenous landscape. Finally, the larger species of hoverflies (*M. florea*, *V. pellucens*) may be good dispersers and not greatly impacted by the structure of agriculture-dominated habitats, while dispersal of the smaller species (*R. campestris*, *S. pipiens*) may be strongly impacted by the agricultural habitat, independently of its degree of structuring.

Objective 3: Within-population dispersal patterns can be analysed by performing a regression analysis between measures of genetic kinship and spatial distance (Frantz et al. 2010a). Lower levels of dispersal lead to more pronounced fine-scale genetic structure as individuals living in close proximity will be, on average, more related than individuals taken at random from the population. We will calculate species-specific isolation-by-resistance relationships for each study area. Following the methods outlined in Frantz et al. (2010a), we will test whether the increase in genetic differentiation among individuals with resistance-distance will be more pronounced (as indicated by a significantly steeper slope of the isolation-by-resistance regression) in the study area(s) where the landscape genetic structure is pronounced (and hence functional connectivity reduced). Circuit theory (McRae et al. 2013) will be applied to the optimised resistance surfaces to correct pairwise geographic distances for landscape resistance

Objective 4: We will take the resistance values of the features shown to be influencing gene flow in one study area and assign these values to the same features in another study area. We will use *gdistance* (van Etten 2017) to calculate the resistance distances for the extrapolated surfaces and the RESIST.BOOT() function of *ResistanceGA* to compare the model support of the extrapolated resistance surface with the composite resistance surface inferred for each study area. We will perform this for all pairs of study areas and species.

1.5 Ethical considerations (only if applicable)

It is extremely unlikely that sampling one individual of each hoverfly per km^2 of study area will have any impact on population persistence since all four target species are abundant and widespread. Moreover, a single female can lay eggs in the range of hundreds to thousands, depending of the species. Also, while being non-selective, pan trapping is unlikely to have a negative impact on the different populations of flying insects in the target square, even when using multiple traps or leaving the traps in the field for a few days (Gezon et al 2015). We will request a formal sampling permission from the relevant authorities in Luxembourg and Germany. Access to genetic resources in Germany is free[3].

[3]https://www.bfn.de/themen/nagoya-protokoll-nutzung-genetischer-ressourcen/abs-in-deutschland.html (accessed 29/04/2020)

2. Project plan

Work package (WP) 1: Landscape genetic analysis of hoverfly dispersal across three urban areas

WP leader: Postdoctoral Researcher; Start date: 01/02/2021, End date: 31/12/2022

Objective

We will test the hypotheses of Objective 1 by identifying the landscape features influencing hoverfly gene flow across two urbanised areas in Luxembourg as well as across the Cologne metropolitan area (Fig. 1). We will subdivide all three areas into 1 km^2 grid cells and attempt to collect and genotype one individual per species per grid cell. The modelling of the environmental variables potentially influencing gene flow will be performed using the Copernicus Urban Atlas, first testing and optimising the resistance of single environmental predictors and then combining the relevant variable into composite resistance surfaces. Model fit will be assessed using corrected Akaike information criterion (AICc) values (see Kimmig et al 2020 for detailed methodological details).

Dr Mengual will provide training in regards to species identification and optimal sampling strategies. He will also assist with his knowledge on hoverfly biology during analysis. The postdoctoral researcher will develop primers for microsatellite loci while a team of field workers led by two technicians will perform the field work. The laboratory work will be performed by the postdoctoral researcher and the technician. Dr Frantz will assist with administration and the statistical analyses.

Tasks

-Task 1.1: Preparation of field work

Before starting field work, it will be necessary to gather all the required permits, including official authorisations to catch the insects and permission by landowners to access their land. Based on land cover and biotope maps as well as satellite imagery we will chose suitable trapping sites in each grid cell of the study areas.

-Task 1.2: Development of laboratory methods

The postdoctoral researcher will develop the microsatellite multiplex PCRs. For each species an Illumina® library will be generated and sequenced before the start of the project so that sequences containing microsatellites will be available.

-Task 1.3: Field work

Because we will attempt to net one individual per species per grid cell, we aim to sample 200 individuals per species in the two urbanised study areas in Luxembourg and 405 individuals per species in Cologne. In order to sample this large number of insects, the equivalent of six persons working full-time will have to perform the sampling. The field work team in Luxembourg will consist of the main technician who will also coordinate the field work, technical staff of the National Natural History Museum Luxembourg (NNHML) as well as free-lancers and student research associates working for the NNHML. The field work in Cologne will be coordinated by a field technician, with the remaining man power provided by student research assistants.

-Tasks 1.4 & 1.5: Laboratory work

After the methods are established, the postdoctoral researcher will start with DNA extraction and genotyping. Both the postdoctoral researcher and the technician (after the end of field work) will genotype the individuals, generating microsatellite genotyping for all 3200 specimens.

-Task 1.6: Archiving of specimens and DNA samples

The technician will make sure that the all the specimens and DNA samples are adequately labelled and archived.

-Task 1.7: Landscape genetic analyses

The postdoctoral researcher will start with the landscape genetic modelling of one species before the data of all species are generated. Because of the relatively large size of the metropolitan area and the relatively fine grain required for the analysis, the landscape genetic analysis of the city of Cologne is expected to last for several months (during which the field work for WP2 will be performed).

-Task 1.8: Preparation of manuscript

Will be performed after the field work for WP2

Interdependence with other word packages

The field, laboratory and analysis methods developed and applied in WP1 will also be used in WP2, making the work on WP2 faster and more efficient. The data generated here will be used in WP3 for further analysis.

Deliverables and milestones

Deliverables:

Task 1.6: Specimens and samples labelled and archived (Project Month PM: 13)

Task 1.7: Resistance map of the study areas for all four species (PM: 20)

Task 1.8.: Submission of manuscript (PM: 23)

Milestones:

Task 1.1: Relevant permits and authorisations (PM: 2)

Task 1.2: Laboratory methods developed (PM: 6)

Task 1.3: 3200 animals collected (PM: 8)

Task 1.4: DNA extracted and quantified (PM: 9)

Task 1.5: All microsatellite genotypes generated (PM: 11)

Task 1.7: List of factors influencing gene flow (PM: 20)

Human resources:

1. Unnamed postdoctoral researchers (Qualification level: PhD), Person*months: 23 (overlap with WP2)
2. Unnamed technician (Qualification level: MSc), Person*months: 12
3. Unnamed field technician (Qualification level: MSc), Person*months 6
4. Four field workers, finances requested (Subcontracting) for two: Person*months: 24
5. Alain C Frantz (Qualification level: PhD), Person*months: 2

Work Package 2: Landscape genetic analysis of hoverfly dispersal across agricultural landscapes

WP leader: Postdoctoral Researcher; Start date: 01/01/2022, End date: 30/06/2023

Objective

We will test the hypotheses of Objective 2 by identifying the landscape features influencing hoverfly gene flow across four agricultural landscapes with differing landscape structure (Fig. 2). We will use the same methods as in WP1, netting one specimen per species per 1 km^2 grid cell, genotyping all 1600 individuals using microsatellite loci and performing the same landscape genetic methods. We will use land cover maps that contain detailed information on different biotopes present in the study areas.

Both the field and laboratory work will be performed by the postdoctoral researcher and the technician. One technician and student research assistant will help with the fieldwork. Dr Mengual will assist with his knowledge on hoverfly biology during analysis. Dr Frantz will assist with the statistical analyses.

Tasks

-Task 2.1: Preparation of field work

The technician will obtain the permits and authorisations for the filed work under WP2 and chose suitable trapping sites in each grid cell of the study areas to allow the postdoctoral researcher to start with the data analysis for WP1.

-Task 2.2: Field work

Because we will attempt to net one individual per species per grid cell, we aim to sample 400 individuals per species per study area. To sample 1600 animals, the equivalent of four persons working full-time (the postdoctoral researcher, two technicians, and student helpers) will have to perform the sampling during the flight periods of the species.

-Tasks 2.3 & 2.4: Laboratory work

During the summer months (June, July, August, September) student research assistants working at the NNHML will additionally help with the field work in Luxembourg, allowing the technician to start with the laboratory work. After all specimens have been collected both the technician and the postdoctoral researcher need to work in the laboratory to finish the work in the allotted time.

-Task 2.5: Archiving of specimens and DNA samples

All the samples will be archived, including leftover samples from WP1.

-Task 2.6: Landscape genetic analyses

After the field season, the postdoctoral researcher will focus on writing a manuscript based on the results of WP1. He/she will also launch the landscape genetic modelling of the agricultural study areas, starting with one species before the genotypes of the other three species are generated.

-Task 2.7: Preparation of manuscript

Interdependence with other word packages

The field, laboratory and analysis methods were developed in WP1. The data generated here will be used in WP3 for further analysis.

Deliverables and milestones

Deliverables:

Task 2.5: Specimens and samples labelled and archived (PM: 24)

Task 2.6: Resistance maps of study areas for all four species (PM: 27)

Task 2.7: Submission of manuscript (PM: 29).

Milestones:

Task 2.1: Relevant permits and authorisations (PM: 14)

Task 2.2: 1600 animals collected (PM: 20)

Task 2.3: All DNA samples extracted and quantified (PM: 21)

Task 2.4: Microsatellite genotypes for all 1600 specimens (PM: 23)

Task 2.6: List of factors influencing gene flow (PM: 27)

Human resources:

1. Unnamed postdoctoral researchers (Qualification level: PhD), Person*months: 17 (overlap with WP1)
2. Unnamed technician (Qualification level: MSc), Person*months: 12
3. Field technician (Qualification level: MSc), Person*months 6
4. Three field workers, finances requested (Subcontracting) for one: Person*months: 15
5. Alain C Frantz (Qualification level: PhD), Person*months: 1

Work Package 3: Data analysis: Hoverfly dispersal & validity of extrapolation

WP leader: Postdoctoral Researcher; Start date: 01/07/2023, End date: 31/01/2024

Objective

In order to gain a better understanding of the dispersal behaviour of hoverflies in different landscapes, we will calculate species-specific isolation-by-resistance relationships for each study area. We will compare the model support of extrapolated resistance surfaces with the composite resistance surface inferred for each study area. We plan to use different land cover maps for WP1 and WP2 because they differ in the detail of information provided for certain land class types (e.g. built-up areas). We will therefore perform all the extrapolation tests using the two different land cover maps.

The postdoctoral researcher will perform the statistical analyses and write the manuscripts. Dr Mengual will assist with his knowledge on hoverfly biology during analysis. Dr Frantz will assist with the statistical analyses.

Tasks

-Task 3.1: Analysis of within-population dispersal patterns

-Task 3.2: Preparation of manuscript

-Task 3.3: Analysis of validity of extrapolating resistance surfaces

-Task 3.4: Preparation of manuscript

-Task 3.5: Report for conservation managers presenting the result of the study

Interdependence with other word packages

The postdoctoral researcher will take the genetic data and the resistance maps generated during WP1 & WP2 to perform the analyses

Deliverables and milestones

Deliverables:

Task 3.2 & 3.4: Scientific manuscripts (PM: 33 & PM: 36)

Task 3.5.: Report for conservation managers (PM: 36)

Milestones:

Task 3.1: Fine-scale genetic structure of each species in each study analysed and compared (PM: 30)

Task 3.3: The validity of extrapolating resistance surface between study areas will be tested (PM: 34)

Human resources:

1. Unnamed postdoctoral researchers (Qualification level: PhD), Person*months: 7
2. Alain C Frantz (Qualification level: PhD), Person*months: 1

3. Risk management and quality assurance

The global coronavirus COVID-19 pandemic, if not subsided by 2021, will not impact the field work, as it is to be performed individually. Extended periods of bad weather with few insect flight days would have an impact on the project, as there is a risk of netting too few individuals. At the start of the field work season, we will train the technical staff of the Museum on how to hand-net and identify the study species, allowing an increase in the number of field workers on days with good weather and translocation of the main technician to help in the Cologne area. In an initial phase, we plan to set up four pan traps for a ~36 hour period in each grid cell. In case of sustained periods of unfavourable weather, we can either use a larger number of pan traps and/or leave the pan traps for multiple days when conditions are more favourable. If pan traps are to be employed over several days, they can have overflow openings and be filled with propylene glycol, a chemical with a very restricted evaporation rate that also preserves DNA over an extended period of time and is non-toxic to mammals (Brown & Matthews 2016).

It is likely that we will have to deviate from the principle of sampling one sample per species in each km^2 of study area on a number of occasions. Firstly, even presuming a continuous distribution, it is very likely that we will sample multiple individuals from one species in some squares, while failing to sample the species altogether in other squares. Given the large number of samples overall, we will genotype the number of specimens corresponding to the number of km^2 of study area, while ensuring an optimal geographic spread of the samples. In their simulation study, Oyler-McCance et al. (2013) showed that a random sampling design, where the authors subsampled a specified number of individuals at random from all the simulated continously-distributed animals, performed well in landscape genetic inference when using similar sample sizes and number of loci as proposed here. In other words, the occasionally lack of specimens from some squares should still allow a robust analysis of the underlying drivers of landscape genetic structure.

Secondly, it is likely that all four hoverflies, but especially the two species not usually associated with built-up areas (*R. campestris* and *V. pellucens*), will be absent from a number of squares of the core of the urban areas because of lack of suitable (larval) habitat. Given the relatively small size of the dense urban fabric and the high proportion of rural area included in the two urban study sites in Luxembourg (Fig. 1) we do not believe this to be a problem in those areas (see paragraph above). In Cologne, we will start the field work in the densely built-up areas, where the paucity of suitable habitat will allow the sampling of an increased number of squares during a daily walk. The time thus gained can be invested in more thoroughly investigating the green urban and suburban areas (where all four species have been shown to occur, Leopold et al. 1996) by increasing the number of pan traps employed and/or be increasing the frequency with which each square is visited. During the course of the field season, we will additionally expand the size of the study area, should we trap some species in a small number of sites within Cologne only. Similarly, if too small a number of hoverflies will be netted in the core of the intensively used agricultural areas (e.g. Fig 2, study area 7), the extent of the study area will be increased (while maintaining sampling density) to achieve the desired number of samples.

Based our current knowledge on hoverflies, it is really difficult to suggest an optimal field sampling design. We currently believe that the design we propose (sampling one individual per species per 1x1 km^2 of study area) is the best compromise to ensure feasibility of the project and a correct match between the scale of the study and the underlying processes impacting the relationship between gene flow and landscape structure. If spatial autocorrelation patterns of the genetic data generated during the first year suggest that dispersal distances are substantialler larger (or smaller) than anticipated, we will of course adjust sampling scheme in the second year accordingly, by decreasing the sampling density but sampling over larger areas (or sampling more animals in smaller study area).

The genotyping of a maximum 3200 animals in one year is relatively time-consuming. We presume that will need to develop at least 13 microsatellite loci per species and ideally more (Landguth et al. 2012). While the maximum product size will be limited to 250 bp (due to the size of the fragments sequenced using the Illumina® technology), it is possible to define fragment size during primer development in program PRIMER3. Thus, by developing a range of microsatellites with different product sizes, similar annealing temperatures and by labelling the primers using a system based in five fluorescent dyes, we believe that all the markers can be PCR-amplified in two multiplex reactions. While the laboratory already has a PCR machine allowing 384-well-based genotyping, we budget for another 384-well block for an available PCR machine, thus allowing the genotyping of a large number of samples simultaneously. Furthermore, the laboratory is set up to allow the extraction of up to 200 samples in parallel and the ABI 3730XL (Applied Biosystems) allows processing of 96 samples during a single run. In summary, we believe that the genotyping of a larger number of samples with ≥13 microsatellite loci will be achieved within the available time frame. Based on previous experience (Kimmig et al. 2020), analysing the functional connectivity of a study area using ResistanceGA is a very time-consuming process. We therefore included a high-end desktop computer in the budget.

In addition to Dr Ximo Mengual, we will set up scientific committee consisting of a further expert in landscape genetics (Prof. Niko Balkenhol, University of Göttingen). Regular internal meetings will allow the PI to check on the progress of the project.

4. Project Outputs

Similar to other countries, Luxembourg has agricultural management schemes aiming at the creation of semi- or near-natural habitat within the agricultural landscape (Anonymous 2017). These areas are believed to support pollinator persistence, either by creating refuge areas or by creating stepping stone patches that facilitate dispersal. While set-aside land has been shown to enhance biodiversity, their value as corridors is debatable, however (Rands 2014). One problem is that the precise impact of certain habitat features on dispersal is often not clear (Rands 2014) and that the landscape context also has to be considered (Gill et al. 2016). The proposed project has the potential to make an important contribution in the design of effective habitat management practices for insect pollinators, since we propose to empirically test dispersal capabilities in different landscapes, taking different biological and ecological characteristics into account. Furthermore, the resistance surfaces that we will generate and our conclusions about the utility of extrapolation should provide the basis for the design of effective movement corridors. Throughout the research project, an annual meeting will be scheduled with staff from the Ministry of the Environment, Climate and Sustainable Development, national agencies and bodies of nature conservation to facilitate knowledge exchange. By the end of the project in early 2024, we will produce a report aimed at all the relevant stake holders which contains a detailed summary of our results, as well as management recommendations derived from the results.

Our scientific results will be published Open Access in international peer-reviewed journals focussing on molecular, animal and landscape ecology as well as on insect biology. We aim to publish four papers that will focus on a) the impact of urbanised areas on hover fly dispersal and gene flow (targeted journal: Molecular Ecology), b) the impact of structural diversity of an agricultural landscape and hoverfly dispersal (targeted journal: Journal of Animal Ecology) c) dispersal distances of hoverflies in different habitats (targeted journal: Ecological Entomology), d) validity of extrapolation of resistance surfaces in the context of the proposed study (targeted journal: Landscape Ecology). Furthermore, our results will be disseminated through international conferences such as the European Congress of Entomology, the International Congress of Dipterology, the International Congress for Conservation Biology, the Conservation Genetics meeting and the BENELUX Zoology meeting, as well as in the framework of local meetings of naturalists.

The scientists involved in the project will take part in the Luxembourg Science Festivals 2021 and 2023, the leading event in promoting science to the general public in Luxembourg. The team will present a simple hands-on activity that will illustrate a more generally aspect about the ecology of insect pollinators. The scientists will also take part in the Luxembourg Researchers Days 2022, a two-day event where researchers can present their scientific projects to a lay audience with simple hands-on workshops. We will publish regular updates on the progress of the project on the social media channels of the Natural History Museum. Towards the end of the project a press conference and public lectures will be held to publicise the specific results of the project. The research project will also be popularised in the children's magazine of the Luxembourg Natural History Museum.

The Zoology Section of the Luxembourg Natural History Museum has taken the strategic decision to focus on the diversity and distribution of Luxembourg's insect pollinators in the next few years. The first major project, set up during the course of 2019 and started in 2020, will be the generation of an atlas of wild bees (Apiformes), with future atlas projects also focussing on Syrphidae. This project would therefore fit well within our general strategy and allow a fruitful exchange between all the members of the group. The project would also help to further establish the Zoology group at the Natural History Museum, as well as the postdoctoral researcher, as experts in genetics-based resistance modelling.

5. Project Participants and Management

5.1 Description of the consortium, communication and decision-making

Dr Alain Frantz, head of the Zoology department of the National Natural History Museum Luxembourg (MNHNL), is coordinating the project as principle investigator (PI). The main areas of interest of his research group include population, conservation and landscape genetics, phylogeography and parasitology. Dr Frantz is very experienced in the development and genotyping of microsatellite loci and the analysis of landscape genetic data. The Museum has a well-equipped molecular biology laboratory.

The Fondation Faune-Flore will be hosting the postdoctoral researcher scheduled to work on the project, the main technician and the field technician. The as yet unnamed postdoctoral researcher will need to have experience in laboratory work, the genotyping of microsatellite loci and their statistical analysis, as well as a proven interest in entomology. The main technician needs to have laboratory experience and be willing to also perform field work, while the field technician needs to be experience in entomology field work. We will advertise all positions on relevant internet forums (e.g. evoldir) – and, in the case of the technicians – internally in order to ensure that they are filled swiftly.

Dr Ximo Mengual is a world expert in phylogenetic systematics of flies (Diptera) with a special focus on the family Syrphidae. He is interested in the ecology and the larval feeding modes of hoverflies. He uses morphology and genetic techniques to answer phylogenetic questions.

By establishing a consortium of the postdoctoral researcher of the Fondation Faune Flore, Alain Frantz (microsatellite genotyping and landscape genetics) and Ximo Mengual (hoverfly biology and ecology), experts will be availble for the three major technical aspects of the project. Dr Alain Frantz will be responsible for the general execution of the project, coordinate the development of microsatellite loci and help with the analysis of the landscape genetic data, while the postdoctoral researcher will coordinate the project on daily basis and perform the majority of the work. Dr Ximo Mengual will provide training and support for the field work (netting and identification of species) as well as provide help with the interpretation of the data.

5.2 Summaries (term sheets) of the Consortium agreement and/or the Intellectual Property Rights (IPR) agreement

It was agreed between all the members of the consortium that:

1. Ownership and protection of results

All results shall be the exclusive property of the Contractor generating it. For instance:

1.1) Intellectual property rights on the DNA samples collected as part of this project shall remain the property of the MNHNL until publication of the first scientific paper making use of the samples. After the first publication, samples can be shared with other members of the consortium, as well as to third parties, based on a sample sharing agreement.

1.2) Intellectual property rights on the raw genetic data collected as part of this project shall remain the property of the MNHNL until publication of the first scientific publication making use of the genetic data. Afterwards, the data shall be in the public domain.

1.3) The faunistic data obtained via this project (e.g. occurrence of all animals recorded during the course of the project) shall be entered into the MNHNL's database.

2. Publication of Results

2.1. A partner may publish results mainly produced by himself and partly by another partner (referred to as "Joint Results") with the other partner's prior written approval. However, partners will strive to obtain as many joint papers (in which at least two (2) Contractors are involved) as possible.

2.2. Prior to submission for publication or presentation of Joint Results the issuing partner will provide the other co-owner partner(s) with at least thirty (30) days for review of a manuscript. The co-owner partner(s) will arrange expedited reviews for abstracts, poster presentations or other materials. Notwithstanding the foregoing, no paper or manuscript that incorporates another partners confidential information will be submitted for publication without prior written consent of this partner. If requested in writing, the issuing partner will withhold such publication for up to an additional forty-five (45) days to allow for filing of a patent application. The issuing partner shall disclose in the publication the co-owner partner in a manner consistent with prevailing editorial standards. Objection from a partner who considers reasonably that the proposed abstract or publication could affect his results shall be made both to the issuing partner, with a copy to the coordinator.

2.3. Authorship for publications should be based on having made a substantial, direct, intellectual contribution to the work, including conception, design, data collection, analysis and/or interpretation of data.

5.3 Track record of the PI and applicant team (competence in the domain, publications, past fundings as PI)

Alain Frantz has published numerous scientific papers analysing the population and landscape genetic structure of various organisms.

Five most relevant publications:
a) Kimmig SE, Beninde J, Brandt M, Schleimer A, Kramer-Schadt S, Hofer H, Börner K, Schulze C, Wittstatt U, Heddergott M, Halczok T, Staubach C, Frantz AC (2020) Beyond the

landscape: Resistance modelling infers physical and behavioural gene flow barriers to a mobile carnivore across a metropolitan area. Mol Ecol 29:466-84
b) Dellicour S, Prunier JG, Piry S, Eloy MC, Bertouille S, Licoppe A, Frantz AC, Flamand MC (2019) Landscape genetic analyses of *Cervus elaphus* and *Sus scrofa*: comparative study and analytical developments. Heredity, 123:228-41
c) Stillfried M, Fickel J, Boerner K, Wittstatt U, Heddergott M, Ortmann S, Kramer-Schadt S, Frantz AC (2017) Urban wild boar (*Sus scrofa*) population structure: do cities represent sources, sinks or isolated islands? J Appl Ecol 54:272-81
d) Renner SC, Suarez-Rubio M, Wiesner KR, Drögemüller C, Gockel S, Kalko EKV, Ayasse M, Frantz AC (2016) Using multiple landscape genetic approaches to test the validity of genetic clusters in a species characterized by an isolation-by-distance pattern. Biol J Linn Soc 118:292-303
e) Frantz AC, Bertouille S, Eloy MC, Licoppe A,Chaumont F, Flamand MC (2012) Comparative landscape genetic analyses show a Belgian motorway to be a gene flow barrier for red deer (*Cervus elaphus*), but not wild boars (*Sus scrofa*). Mol Ecol 21:3445-57

Relevant funding:

Microsatellite marker development:

Project: Population genetic structure of Geoffroy‘s bat in Luxembourg and neighbouring countries
Year: 2016/2017
Funding Source: Luxembourg Ministry of the Environment, Climate and Sustainable Development.
Amount: 50000 €

Project: Using population genetics to understand the evolutionary potential of an introduced parasitic nematode
Year: 2015/2016
Funding Source: Fonds National de la Recherche, Luxembourg
Amount : 111172 €

Landscape genetics

Project: Using genetic methods to understand the movement ecology of the crested newt (*Triturus cristatus*) in Luxembourg.
Year: 2019/2021
Funding Source: Luxembourg Ministry of the Environment, Climate and Sustainable Development.
Amount : 119600 €

Project: Using comparative landscape genetics to assess connectivity in fragmented landscapes and identify functional ecological networks
Year: 2012/2013
Funding Source: Fonds National de la Recherche, Luxembourg
Amount : 58750 €

Ximo Mengual has published several scientific papers studying hoverfly larval biology and phylogenetics and phylogenomics of Syrphidae.

Five most relevant publications:
a) Aracil A, Pérez-Bañón C, Mengual X, Radenković S, Ståhls G, Vujić A, Rojo S (2019) New information about the pre-imaginal morphology of genus Graptomyza (Diptera, Syrphidae, Volucellini): description of third-instar larva and re-description of puparium of G. signata (Walker, 1860). Afr Invertebr 60:15-30

b) Pauli T, Burt T, Meusemann K, Bayless K, Donath A, Podsiadlowski L, Mayer C, Kozlov A, Vasilikopoulos A, Liu S, Zhou X, Yeates D, Misof B, Peters RS, Mengual X (2018) New data, same story: phylogenomics does not support Syrphoidea (Diptera: Syrphidae, Pipunculidae). Syst Entomol, 43, 447-59
c) Mengual X, Ståhls G, Láska P, Mazánek L, Rojo S (2018) Molecular phylogenetics of the predatory lineage of flower flies Eupeodes-Scaeva (Diptera: Syrphidae), with the description of the Neotropical genus Austroscaeva gen. nov. J Zool Syst Evol Res 56:148-69
d) Arcaya E, Pérez-Bañón C, Mengual X, Zucoff-Vallejo JJ, Rojo S (2017) Life table and predation rates of the syrphid fly Allograpta exotica, a control agent of the cowpea aphid Aphis craccivora. Biol Control 115:74-84
e) Young AD, Lemmon AR, Skevington JH, Mengual X, Ståhls G, Reemer M, Jordaens K, Kelso S, Lemmon EM, Hauser M, De Meyer M, Misof B, Wiegmann BM (2016) Anchored enrichment dataset for true flies (order Diptera) reveals insights into the phylogeny of flower flies (family Syrphidae). BMC Evol Biol 16:143

Relevant funding:

<u>New-Generation sequencing and systematics</u>:

Project: BIG4 - Biosystematics, Informatics and Genetics of the big four insect groups: training tomorrow's researchers and entrepreneurs
Year: 2015/2018
Funding Source: European Comission, H2020-MSCA-ITN-2014.
Amount: 234816 €

<u>DNA barcoding</u>:

Project: German Barcode of Life, phase II
Year: 2015/2017
Funding Source: Bundesministerium fuer Bildung und Forschung
Amount: 271044 €

Project: German Barcode of Life, phase III
Year: 2020/2022
Funding Source: Bundesministerium fuer Bildung und Forschung
Amount: 1354406 €

6. Comments on Resubmission (only if applicable)

All the reviewers agreed that the lack of replication at the landscape level was a major drawback previously. Furthermore, the three reviewers took different views as to what might constitute the best genetic marker system for the proposed project. The two questions are of course related. Previously, we suggested to use a reduced representation method to analyse hundreds to thousands of single nucleotide polymorphisms (SNPs) per species. However, this method is still expensive and time-consuming when used to genotype a large number of specimens. The proposal was therefore limited to three study areas.

Microsatellite loci are relatively cost-effective and they are still valid neutral markers for the analysis of gene flow and dispersal. Using microsatellite markers, we expect it to be possible to genotype 4800 animals, permitting replication at the landscape level. As a compromise between the different sampling strategies suggested by the reviewers and to keep the study feasible, we propose to compare three urbanised areas – including a metropolitan area outside of Luxembourg – and two pairs of rural areas with different degrees of habitat diversity.

Based on the literature, our main hypothesis regarding rural areas is that structurally diverse landscapes facilitate dispersal between optimal habitats (Meyer et al. 2009). We therefore

did not put the focus on habitat fragmentation by roads. Because our main hypothesis is related to the diversity of the landscape we stuck to the method of choosing our rural study areas.

Choosing hoverfly species that represent “the key pollinator species and important habitats” would be impossible given the lack of information on flower visitation rates, behaviour, pollenload and delivery of pollen to stigmas in hoverflies in general and relative to other flower visitors (Orford et al. 2015, Carvell et al. 2016). Moreover, given the large number of essential criteria that our target species need to fulfil (non-migratory; common and widespread; easily identifiable in the field; flying periods covering multiple months; different wing lengths, ecological characteristics and association with humans) the choice becomes rather restricted.

The different habitat requirements of the larvae, or even those of the adults, do not represent a problem as we are not modelling habitat suitability, but the resistance of the landscape to movement.