**High pollinator population connectivity in heavily disturbed landscapes: substantial gene flow despite large urbanized areas in two hoverflies**

Julian Wittische 1,2,\*, Stéphanie Lippert 1,2, Amanda Luttringer 1,2, Hinatea Ariey 1,2, Anna Schleimer 1,2, Frank Drygala 3, Joerg Mehnert 3, Ximo Mengual 4, Alain Frantz 1,2

1: Musée National d’Histoire Naturelle, 25, rue Muenster, L-2160, Luxembourg, Luxembourg

2: Fondation Faune-Flore, 24, rue Muenster, L-2160, Luxembourg, Luxembourg

3: Association for Nature and Biodiversity, 16 Birsteiner Strasse 60386 Frankfurt am Main, Germany

4: Zoologisches Forschungsmuseum Alexander Koenig, Leibniz‐Institut zur Analyse des Biodiversitätswandels, Adenauerallee127, D‐53113 Bonn, Germany

\*: Corresponding author (julian.wittische@mnhn.lu)

Potential destination:

BMC Ecology and Evolution

**ABSTRACT**

Hoverflies (Syrphidae) are essential pollinators, and their severe decline jeopardizes their invaluable contribution to plant diversity and agricultural production. However, we know little about the dispersal abilities of hoverflies in urbanized landscapes, limiting our understanding of the spatiotemporal dynamics of plant–pollinator systems, and reducing our ability to preserve biodiversity in the context of global changes. Previous work has not addressed how urbanization affects the functional connectivity of hoverflies, and whether dispersal is a limiting factor in their population dynamics. In this study, we investigated the spatial genetic structure of two species of hoverflies in two urban areas. More than a thousand specimens of *Syritta pipiens* and *Myathropa florea* were collected by hand-netting from two western European urbanized study areas of 490 km2 and 460 km2 in 2021 and genotyped at 14 and 24 microsatellite loci, respectively. Based on spatial and non-spatial Bayesian clustering methods, we failed to reject the null hypothesis of panmixia, suggesting that both species exhibited high genetic connectivity despite urbanization. The distribution of allele frequencies was not correlated to geographic distance, implying that isolation-by-distance was negligible at the investigated spatial scale in both species. Although anthropogenic land cover changes generally have dramatic consequences on biodiversity, these hoverfly species retain high connectivity, which suggests that dispersal is not a strong limiting factor in their metapopulational dynamics. Provided we maintain or restore habitat, recolonization should therefore be prompt even in urban areas.

**KEYWORDS**

Landscape genetics; Spatial ecology; Diptera; Urbanization; Machine learning

# INTRODUCTION

Pollinators provide key ecosystem services to agricultural crops and wild plants. It has been estimated that, globally, the economic value of pollination is worth hundreds of billions of US dollars (Doyle et al., 2020; Gallai et al., 2009). The vast majority of crops (Klein et al., 2007; Reilly et al., 2020) and wildflowers (Ollerton et al., 2011) benefit from insect pollination by, in particular, bees and hoverflies (Potts et al., 2015). Pollinators also support an immense range of other organisms (Ollerton, 2017). However, evidence of the loss of pollinators is mounting: wild pollinators are declining at local, regional and global scales, in both diversity and abundance (Biesmeijer et al., 2006; Hallmann et al., 2017; Sánchez-Bayo and Wyckhuys, 2021, 2019; Senapathi et al., 2015). The main underlying drivers behind declines are the intensification of land-use, climate change, pesticides, and the introduction of invasive species and parasites/pathogens (Dicks et al., 2021; Ollerton, 2017; Potts et al., 2010; Vanbergen et al., 2013). The spread of urban areas and the intensification of agriculture have resulted in the destruction and fragmentation of vast expanses of natural pollinator habitat (Seibold et al., 2019); a trend which is stood to endure with continued human population growth and development (Jaeger et al., 2016). To counteract the negative effects of habitat fragmentation, it is therefore important to understand the functional connectivity of pollinators across altered landscapes and notably the extent to which urban areas form barriers to pollinator dispersal (Dreier et al., 2014; Rands, 2014). (Dicks et al., 2013; Simmons et al., 2019). (Gill et al., 2016; Winfree et al., 2011)

Dispersal is required to maintain connectivity in the face of landscape fragmentation, to colonize new habitats and to allow re-colonization after local extinction. Dispersal therefore impacts species distribution, community structure, (meta-)population dynamics, gene flow and extinction risk (Bowler and Benton, 2005). Species with high dispersal capacity generally have a greater ability to move efficiently between suitable habitat patches and may exploit fragmented resources more efficiently (Öckinger et al., 2010). For example, bumblebee species (*Bombus* spp.) normally exhibit very little spatial genetic structure (Dreier et al., 2014; Lozier et al., 2011). However, impervious cover associated with built-up areas significantly limited gene flow in a North American bumblebee (Jha and Kremen, 2013) which suggest that even good fliers may be impacted by urbanization. Urban areas can be a substantial barrier to gene flow in pollinators at even larger spatial scales (Davis et al., 2010). However, given the large range of flying abilities and species-specific responses to habitat fragmentation, it is difficult to generalize the impact of land-use changes on pollinator dispersal, even between closely related species (Greenleaf et al., 2007; Jauker et al., 2009; Steffan-Dewenter et al., 2002). We thus need to better understand the effect of landscape disturbance on the connectivity of pollinators (Taylor et al., 1993), the geographic scale at which mitigation measures should be implemented, and which element of the population dynamics of pollinators is the most sensitive to anthropogenic disturbance.

Hoverflies (Syrphidae) are an important group of pollinators. Hoverflies are a biologically very diverse family of flower-visiting flies with more than 6000 recorded species (Bickel et al., 2009; Speight, 2017; Wardhaugh, 2015). Their dependence on floral resources makes hoverflies the most important pollinators besides bees, providing a major contribution to plant diversity and agricultural production (Hodgkiss et al., 2018; Jauker et al., 2009; Pekas et al., 2020; Rader et al., 2016; Ssymank et al., 2008). Species do not display strict selectivity for specific flower species (Branquart and Hemptinne, 2000; Lucas et al., 2018) which make them especially important in disturbed landscapes (Jauker et al., 2009). Many hoverfly larvae feed on aphids and are effective biocontrol agents, especially in agricultural landscapes (Pekas et al., 2020; Speight, 2017), which adds to their large contribution to human food security. Although, some studies have been conducted about the population dynamics of hoverflies, often focusing their migrations, hoverflies are understudied relative to bees. In particular, little is known about the dispersal of non-migratory hoverflies and their response to landscape fragmentation.

Molecular genetic methods are powerful tools to investigate the effect of fragmentation on target species where dispersal capability is hard to evaluate directly, but such methods have seldom been used on hoverflies. Capture-mark-recapture (CMR) methods have been used to study hoverfly dispersal in the past (Aubert et al., 1969; Aubert and Goeldlin de Tiefenau, 1981; Rotheray et al., 2014). However, given the limitations of conducting CMR across a large area for abundant small insects, landscape connectivity is easier to investigate using molecular genetic methods. Genetic connectivity is evaluated through genetic similarity between individuals, which is directly related to dispersal as genes are propagated by individuals or propagules which disperse before reproduction (Broquet and Petit, 2009; Cayuela et al., 2018). Therefore, the greater the genetic connectivity is, the easier it is to disperse through the landscape. One population genetics study of hoverflies described continental-scale patterns for a migratory species (Raymond et al., 2013). As expected due to the extreme genetic mixing associated with mass migration, they found no substantial isolation-by-distance (IBD). Another more local study found no substantial barriers to gene flow, though they used a small number of hoverfly individuals, from a fraction of a low disturbance forest landscape (Schauer et al., 2018). However, the effect of urbanization on hoverfly functional connectivity has never been studied to our knowledge.

Germany and Luxembourg both present very high landscape fragmentation as measured by the number of meshes per km2 and the proportion of the country’s total surface experiencing high to very high fragmentation (European Environment Agency, 2016). Because of demographic growth, land use change for new infrastructure and urban development is expected to be considerable even in already heavily urbanized. This, in turn, will lead to further loss and fragmentation of natural and semi-natural habitats (Jaeger et al., 2016). Cologne is the fourth-most populous city in Germany and recently commissioned a major inventory of pollinators (Stadt Köln, 2022), notably stimulated by recent findings about country-wide insect declines (Hallmann et al., 2017; Seibold et al., 2019). Luxembourg has recognized that habitat loss and fragmentation are threatening its biodiversity in general and insect pollinators in particular (Ministère de l’Environnement, du Climat et du Développement durable, 2022). 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. 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).

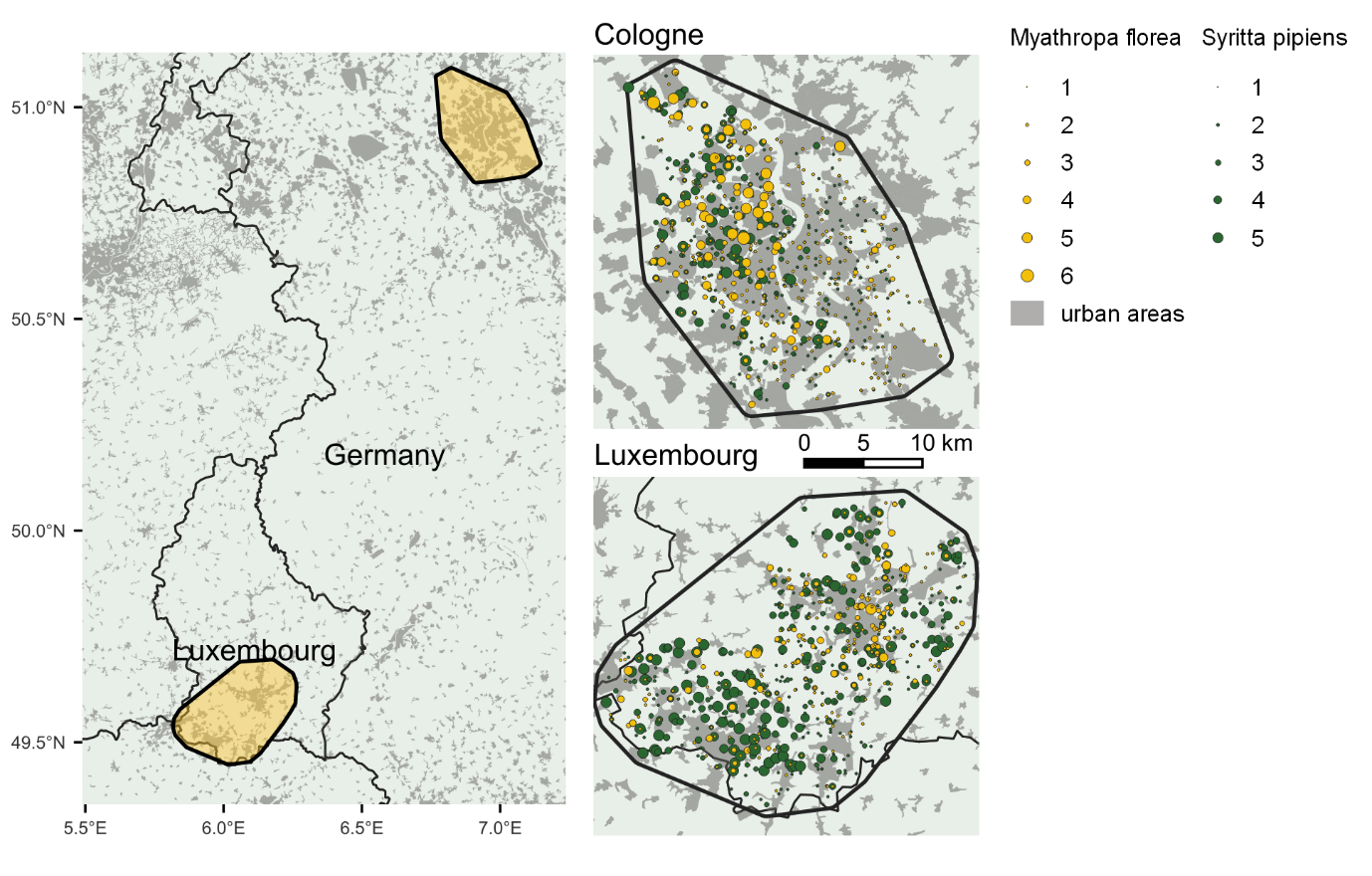
In this study, we investigated the genetic diversity, structure, IBD, isolation-by-environment (IBE) of two species of hoverflies, *Syritta pipiens* (Linnaeus, 1758; henceforth abbreviated to SP) and *Myathropa florea* (Linnaeus, 1758; MF), based onthousands of individuals in two urbanized landscapes in Germany and Luxembourg. We hypothesized that 1) given the non-migratory nature of our two species and the large distance between study areas (ca. 150 km), substantial genetic differentiation and IBD exist between study areas and the large extent of unvegetated impervious areas present in and around cities.

# METHODS

## | Study areas, study organisms, and sampling

To evaluate the genetic connectivity of hoverflies in the face of disturbance, we chose two urbanized study areas (Fig. 1). We chose an extent of around 400km2 for each study area. This specific extent is a key parameter because it allowed us to sample the whole landscape to improve the accuracy of our inferences, while being large enough to detect potential effects of large-scale anthropogenic disturbance on genetic variation. Cologne recently commissioned a major inventory of pollinators (Stadt Köln, 2022), stimulating recent findings about country-wide insect declines (Hallmann et al., 2017; Seibold et al., 2019). Luxembourg has recognized that habitat loss and fragmentation are threatening its biodiversity in general and insect pollinators in particular (Ministère de l’Environnement, du Climat et du Développement durable, 2022). The shape of the Luxembourg study area was chosen to include most parts of the urban sprawl between the two largest urban agglomerations in the country (Luxembourg and Esch-sur-Alzette), as well as sufficient amount of adjoining countryside. The Cologne study area focused on administrative city limits as it fit our requirements. Indeed, although Cologne is the fourth most populous and the third largest city in Germany, it has a large number of green surfaces, protected areas, riparian forest fragments and wetlands (Braun and Herold, 2004; Curdes, 1998; Mitter and Weber, 2011).

As study organisms, we chose two hoverfly species with long flight seasons and likely to occur across the whole study areas based on known preferred habitats, preliminary field experience, and previous inventories (Leopold et al., 1996). We avoided migratory species because their genetic variation is less likely to bear signal of isolation-by-distance and structure (Raymond et al., 2013) given their sometimes extensive ability to spread (Jia et al., 2022). Our sampling design was to catch at least one individual per squared kilometer in order to have as few gaps in geographical coverage as possible, following a uniform grid. The analytical purpose of this sampling design was to decrease bias and improve our accuracy in detecting influential landscape features, if there were any (Oyler-McCance et al., 2013; Schwartz and McKelvey, 2009). The size of the sampling unit (1km2) reflects the spatial scale at which hoverfly density optimally relates to landscape context (Kleijn and van Langevelde, 2006). Samples were caught using hand-netting and stored in 96% ethanol in the field and kept in a freezer until further processing. Species identity was confirmed visually as both species bear obvious and unique characters, which made misidentification unlikely. Namely, those were a thick overdeveloped hind femur with a row of spines and a pair of small pale marks on the dorsal side of the thorax just behind the head for SP, and bright yellow color and a black “Bat-Signal” shape on the dorsal posterior part of the thorax for MF.



**Figure 1**. Study areas in Luxembourg and Germany with the location of *Myathropa florea* (yellow) and *Syritta pipiens* (green) sample locations. Point size reflects sample size from one to six. Shaded areas represent urban areas with impervious soils.

## | Laboratory procedures

One leg of each sample was placed in an Eppendorf tube and grinded by vortexing with two ceramic beads. Genomic DNA was subsequently extracted using an ammonium acetate-based salting-out procedure (Miller et al., 1988). DNA extracts were quantified using a Drop-Sense 16 spectrophotometer (Trinean, Gentbrugge, Belgium). We used blast-2.11.0+ to perform a stand-alone blast of each of the 500 microsatellite sequences against the genome of [*S. pipiens* (assembly idSyrPipi1.1)](https://www.ncbi.nlm.nih.gov/genome/98123?genome_assembly_id=1557693) and against an ‘in-progress assembly’ of the genome of *M. florea* (20200119.hicanu.purge) obtained from Darwin Tree of Life Project (<https://github.com/darwintreeoflife/darwintreeoflife.data>, accessed 08/11/2022), respectively. For each species, we tested fifty microsatellite loci that only matched one site in the respective reference genome and that differed in their number of microsatellite repeats relative to the reference genome. We tested the amplification success of all 50 primers using a universal tail approach for fluorescent labelling of Polymerase Chain Reaction (PCR) products (Culley et al., 2013), and eight good-quality DNA samples originating from individuals sampled across both study areas. Each PCR contained 1x GoTaq Master Mix (Promega, Walldorf, Germany), 0.2 μM of each primer and 10 ng of DNA. After a 3-min denaturation at 95 °C, the PCR consisted of 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 45 s and an extension at 72 °C for 30 s. The PCR was ended with a final extension for 10 min at 72 °C. The PCRs were performed in a Mastercycler nexus (Eppendorf, Hamburg, Germany). Loci that were polymorphic and that gave rise to clear peaks were retained for further analysis. We then used the PRIMER3 software to develop new primer pairs that gave rise to products of differing length to allow multiplexing. The primers were specified to have a melting temperature of 59-61°C (optimum 60°C), a length of 18 to 26 base pairs (20 bp optimum), the presence of a G/C clamp, a maximum poly-X of three tandemly repeating nucleotides (e.g. TTT), with all other parameters set to default. For *S. pipiens* we retained 14 microsatellite loci that were amplified in two multiplex PCRs, while the 24 microsatellite loci for *M. florea* were amplified in three multiplex reactions (Sup. Table 1). Each PCR contained 1x GoTaq Master Mix (Promega, Walldorf, Germany), and between 0.1-0.4 μM of each primer (Table X). PCRs started with 3 min denaturation at 95 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60°C for 45 s and extension at 72 °C for 30 s. The final incubation was at 72 °C for 10 min. Allele sizes were determined using GENEMAPPER version 4.0 (Applied Biosystems). The genetic profiles of all samples consisted of at least 11 loci for *S. pipiens* and at least 18 loci for *M. florea*.

## | Genetic diversity

To evaluate deviations from the Hardy-Weinberg equilibrium, we first split the dataset into two geographic populations corresponding to the study areas, and further divided genetic samples by communes (Luxembourg) and districts (Cologne). We only kept spatial units with more than 20 individuals for *S. pipiens* and more than 11 individuals for *M. florea*. Within those spatial units, we used an exact test based on 10000 Monte Carlo permutations (Guo and Thompson, 1992) implemented in the *pegas* v. 1.1 R package (Paradis, 2010). We considered that loci presenting a disequilibrium (p-value < 0.05 after false discovery rate correction) in more than a third of spatial units were problematic. To explore linkage disequilibrium in our dataset, we also calculated standardized indices of association over all loci with a one-sided permutation test, as well as pairwise indices among all loci (Agapow and Burt, 2001; Kamvar et al., 2014). We used the *poppr* v. 2.9.3 R package (Kamvar et al., 2014) to calculate and associated p-values. We considered that there was significant linkage disequilibrium when the permutation-derived p-value was below 0.05, and when that was the case, searched for high pairwise association index values. We also evaluated whether null alleles were likely using a resampling-based test (Brookfield, 1996) implemented in the *PopGenReport* v. 3.0.7 R package (Adamack and Gruber, 2014). We considered that loci with observed estimates of null allele frequency higher than 0.1 were problematic. The retained microsatellite loci were used to estimate allelic richness, heterozygote deficiency, overall fixation indices with bootstrap confidence interval, fixations indices per locus, and the pairwise genetic distance between our study areas as implemented in the *adegenet* v. 2.1.7 R package (Jombart, 2008; Jombart and Ahmed, 2011).

## | Clustering

We used two different Bayesian model-based approaches to estimate the most likely number of distinct genetic clusters (*K*). First, we used STRUCTURE v. 2.3.4 (Pritchard et al., 2000), and chose the admixture model and correlated allele frequencies. The population-specific ancestry prior and α = 1/*K* were applied following (Wang, 2017). We conducted ten independent runs with 200 000 Markov Chain Monte Carlo burn-in iterations followed by 1 000 000 iterations for one to six clusters. The estimated posterior probability for the data for each K was assessed to determine the most likely number of plausible clusters. In addition to the Bayesian clustering method in STRUCTURE, we also employed the spatially-explicit genetic clustering method implemented in BAPS (v.6.0; Corander et al., 2008). The algorithm considers both the genetic data and the specific geographic coordinates and modally assigns each individual to its putative cluster of origin. The most likely number of clusters , in terms of highest log marginal likelihood, was inferred from 100 replicate runs at = 20.

As a complement to the Bayesian approaches, we considered a model-free approach which is less reliant on assumptions and used discriminant analysis of principal components (DAPC; Jombart et al., 2010, 2009). To evaluate whether there was spatial genetic structure, we considered a grouping prior based on study areas (two study areas = two potential clusters). We followed the up-to-date recommendations from the development team regarding the appropriate steps to conduct DAPC (Jombart and Collins, 2022). We chose the best number of components to retain for the DAPC based on both cross-validation (1000 iterations) and *a*-score optimization. This is a necessary step because the first few components represent most of the genetic variation, we wanted to find a balance to preserve discrimination power while avoiding overfitting. We systematically used all discriminant functions for the assignment of individuals into clusters, and used cross-validation to evaluate the general performance of the DAPC and compared it with a random classifier.

## | Isolation-by-distance

To explore whether IBD is responsible for genetic differentiation in our study landscapes, we first evaluated the linear relationship between the natural logarithms of geographic distance and Loiselle’s kinship values (Loiselle et al., 1995) which measure the genetic relatedness between pairs of individuals. We created linear models to detect the overall trend for IBD, as well as within study areas. We chose Loiselle’s kinship because this genetic similarity metric is considered a less biased estimator with low sampling variance (Vekemans and Hardy, 2004). We estimated Loiselle’s kinship using the *EcoGenetics* v. 1.2.1-6R package (Roser et al., 2017). Finally, to understand the scale at which genetic structure is shaped by dispersal we created a Mantel correlogram using Sturge’s rule to define distance classes and used a Monte Carlo procedure to test whether Mantel correlation (Mantel, 1967) values are significant. We used a progressive (Legendre and Legendre, 2012) Holm correction for multiple testing for the Mantel correlograms.

## | Isolation-by-environment

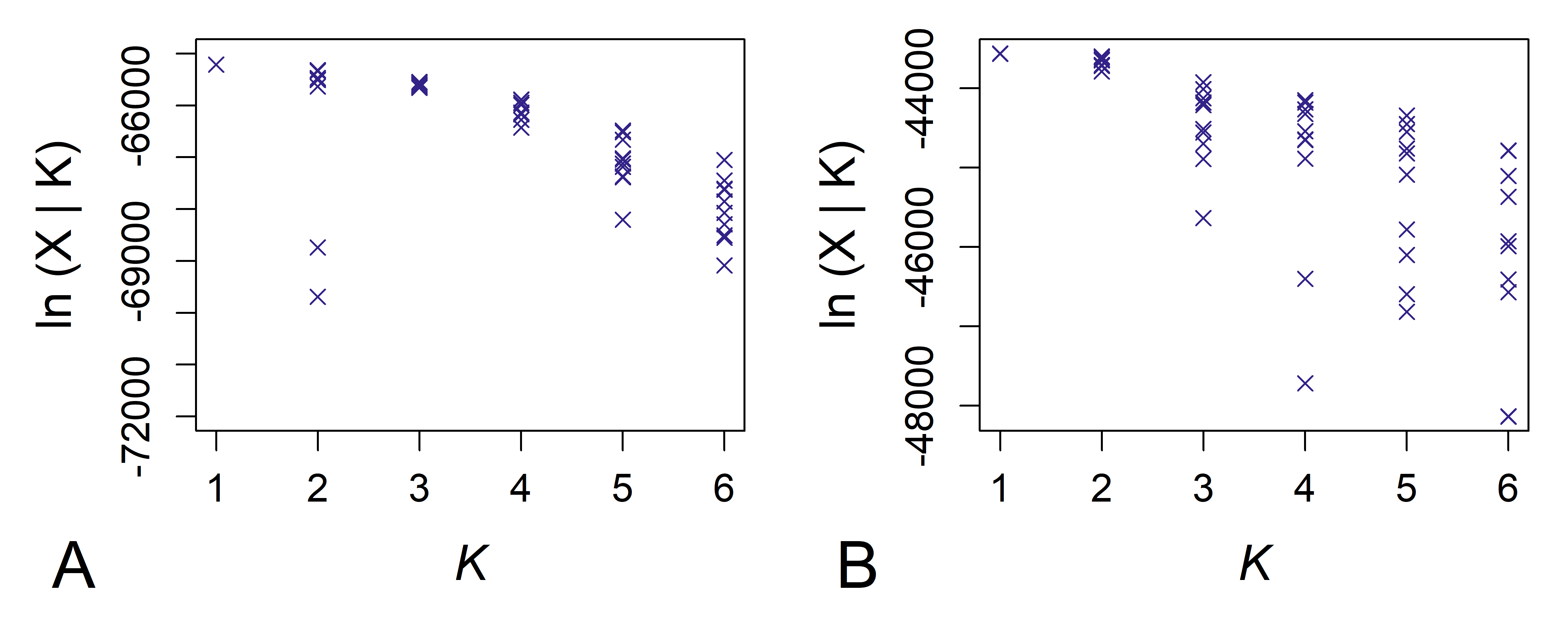
Generalised Dissimilarity Modelling (GDM) was employed to simultaneously test for isolation by geographic and environmental distance with the *gdm* R package (v. 1.5.0-9.1; REF). By performing matrix regression, the approach fits non-linear relationships between the response variable, i.e. pairwise genetic distance, and predictor variables, i.e. pairwise geographic and environmental distances. The analysis was limited to individuals sampled in Luxembourg. Given the lack of clear genetic clusters (see below), pairwise genetic distances were estimated between individuals. Three different genetic measures were tested, namely the proportion of shared alleles, (Bowcock 1994), pairwise distance on the first two principal component axes, and Loiselle’s kinship coefficient. Potential environmental covariates were derived from topographic data (EU Digital Elevation Model, Copernicus, 25m resolution), i.e. elevation, slope, roughness, and terrain ruggedness index, from climatic data (WorldClim, 30 second resolution), i.e. average spring temperature, spring precipitation, and summer precipitation, and land coverage data (CORINE), i.e. percentage agricultural and urban coverage, distance to urban areas, and forest height (GEDI). We tested for correlation among environmental raster surfaces and excluded surfaces with a correlation coefficient greater than 0.6. Sample locations with identical field coordinates were randomly displaced by 10m in QGIS (v. 3.28.1, REF) to generate unique locations. Percentage agricultural and urban coverages were estimated as percentage coverage within a 125 by 125 m grid cell. Three different models were computed for each species and measure of genetic distance: (1) straight-line geographic distance only, (2) environmental distance only, (3) combined geographic and environmental model. The percentage deviance explained was employed to assess the explanatory power of the model.

# RESULTS

## | Genetic diversity and population genetic structure

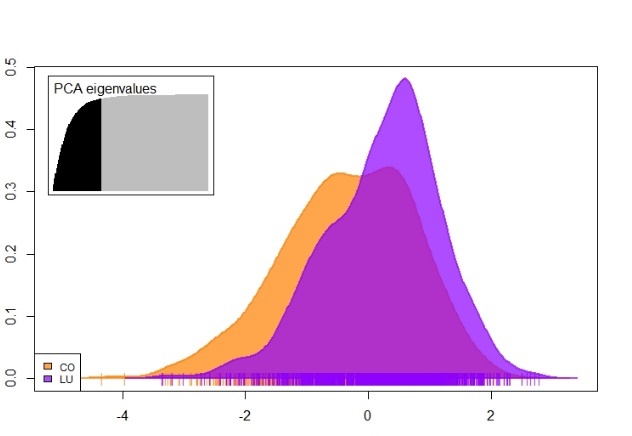
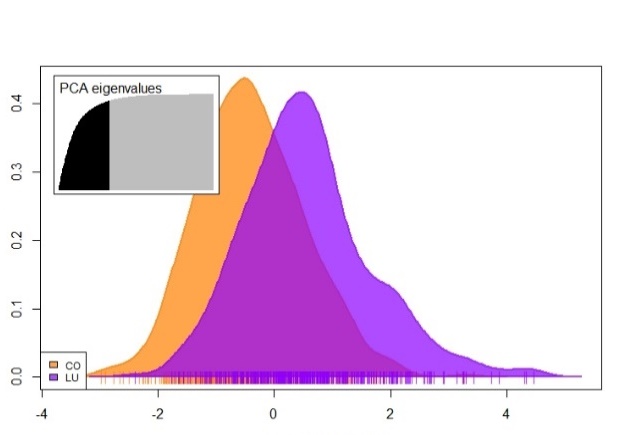
A total of 831 and 1226 *S. pipiens*, and 559 and 394 *M. florea* individuals were caught in Cologne and the Luxembourg study area, respectively (Fig. 1). The locus Spp141 was removed from the *S. pipiens* dataset due to heterozygote deficiency (possible null alleles) and linkage disequilibrium. The locus Mfl303 was excluded from the *M. florea* dataset, because of significant deviations from Hardy-Weinberg proportions in numerous tested sample partitions (Suppl. Table 3CD). The remaining multilocus genotypes were characterized by 2.24% and 2.93% missing data in *S. pipiens* and *M. florea*, respectively. Average expected (He ± sd) and observed heterozygosity (Ho ± sd) were comparable in both species 0.62 ± 0.13 and 0.57 ± 0.13 for *S. pipiens*; 0.49 ± 0.24 and 0.46 ± 0.23 for *M. florea*.

Bayesian ancestry inference using STRUCTURE inferred a single partition as most likely clustering solution for both *S. pipiens* and *M. florea* (Fig. 2). Similarly, when performing spatial clustering of individuals, BAPS inferred a probability of p(S) = 1 for the presence of one genetic population in both study species.



**Figure 2.** The estimated posterior probability for the data for each K across ten independent runs for *Syritta pipiens* (A) and *Myathropa florea* (B).

The single discrimination functions (responsible to distinguish clusters) for *a priori* DAPC showed a lot of overlap for both species (Fig. 3). Fifty and 83 PCA axes were found to be the number of axes achieving both the highest success and the lowest mean squared error for *S. pipiens* and *M. florea*, respectively. *A priori* grouping individuals by their geographic origin (i.e., Cologne and Luxembourg) performed very poorly across species (Fig. 3). Indeed, cross-validation results showed that a classifier based on DAPC, even after a-score optimization, did not reach a high precision (56.97% for *S. pipiens* and 55.91% for *M. florea*), partially overlapping with the success of a random chance classifier (Sup. Fig. 2).



Density

Discrimination function

**Figure 3.** Discrimination functions for DAPC with *a priori* geographic population groups; high overlap demonstrates poor distinction between geographic populations. Sample sizes ratios are divided among pop as follows: 32% (*Myathropa florea*) and 70% (*Syritta pipiens*) individuals from Luxembourg.

## | Isolation-by-distance

Regarding *S. pipiens*, while there was significant IBD between study areas when using the whole dataset, it was very low and had negligible explanatory power (estimate = -0.0005; p-value < 2e-16; adjusted R2 = 7e-05). There was no IBD within study areas (Cologne: estimate = -0.00004; p-value = 0.87; adjusted R2 = -3e-06; Luxembourg: estimate = 0.0001; p-value = 0.53; adjusted R2 = -8e-07). Similarly, very low IBD existed between study areas for *M. florea* (estimate = -0.0002; p-value < 2e-16; adjusted R2 = 2e-05). For this species there was also no IBD within Cologne (estimate = -0.0001; p-value = 0.68; adjusted R2 = -5e-06) or Luxembourg (estimate = 0.0001; p-value = 0.70; adjusted R2 = -1e-05). Mantel correlograms did not show a significant correlation in any distance classes within study areas (all p-values > 0.09).

## | Isolation-by-environment

For both *M. florea* and *S. pipiens*, GDM explained less than 1% of deviance across all tested models and genetic distance measures, providing no support for isolation by geographic or environmental distance among Luxembourg-sampled individuals.

# DISCUSSION

This study aimed to increase our knowledge about hoverfly connectivity in heavily disturbed ecological contexts. Briefly, our study showed that two species of hoverflies presented remarkably high genetic connectivity across tens of kilometers of urbanized landscapes bearing potential natural and artificial barriers. This putatively high ability to disperse in urbanized landscapes has implications for hoverfly conservation and maintaining pollination as an ecosystem service.

## | High large-scale population connectivity

The characteristics of genetic structure measured in this study indicates no strong recent effect of the landscape on gene flow *S. pipiens* and *M.* *florea* (Fig.2-3).

Superficially, one might be tempted to conclude towards the higher number of genetic clusters. However, several elements belie this simple conclusion. First, the structure did not map at all on geographic origins, within or even between study areas (Fig. 2-3). This can be seen in the spatially random and mixed assignments in the STRUCTURE analyses (Fig. 2). Not a single cluster is restricted to a specific study area. Similarly, DAPC outcomes show very poor performance for *a priori* DAPC (Fig. 3A) where the grouping corresponds to study areas, and again assignments seem to be randomly distributed across study area (Fig. 3C). Second, although both approaches partially supported the same number of clusters, the inferred clusters are very different with no apparent concordance between approaches. *De novo* runs selected a wide range of *K* values, especially for *M. florea*. Finally, some performance metrics for the Bayesian analysis did select lower number of clusters (*K*=1 for *S.* pipiens and *K*=2 for *M. florea*) which further highlights the likely spatial structure of those two species. IBD analyses support the conclusions drawn from structure analyses. An extremely low IBD is detected for both species when using both study areas, which denotes that local individuals (e.g., Cologne) are slightly more similar to each other. However, the significance of this relationship between genetic similarity and geographic distance is likely driven by the large number of individuals, and therefore, of pairwise measures, and high significance values should be contrasted with the low goodness-of-fit. No such relationship exists within study areas, even when splitting pairwise indices of similarity and distance into geographic distance classes. Taken altogether, one could conclude based on our results that there is no strong structure and that hoverflies sampled in those two study areas distant by 160km, currently belong to the same genetic population.

Previous studies highlighted that isolation by environmental distance can occur in the absence of isolation by geographical distance or by resistance (Glück et al. 2005). Glück et al. 2022 found that environmental differences accounted for over 30% of the genetic divergence observed among buff-tailed bumblebee (*Bombus terrestris*) populations across Romania and Bulgaria, although population structure was subtle (*FST* < 0.07) and not detected by Bayesian clustering. Environmental heterogeneity was suggested to act as a selective pressure against dispersers, which would result in a disruption in genetic connectivity whereby divergence in neutral markers can arise through genetic drift. Here, we found no evidence for isolation by geographic or environmental distance, suggesting that environmental heterogeneity, e.g. urban versus rural habitat, is not exerting strong selective pressures on the investigated spatial and temporal scale in these two hoverfly species in Luxembourg.

While unexpected given the large amount of disturbance and apparent costs of dispersal in urban contexts, high genetic connectivity is not unheard of in hoverflies. Hoverflies usually move a few hundred meters and tall vegetation and bare soil including ploughed fields and roads can act as barriers (Lövei et al., 1998; Wratten et al., 2003). Similarly, studies investigating hoverfly richness in relation to habitat patch isolation suggested that hoverflies are significantly impacted by habitat fragmentation (Jauker et al., 2019; Moquet et al., 2018; Ouin et al., 2006). This had led us to expect an effect of fragmentation on genetic variation. However, other studies have highlighted the high dispersal ability of hoverfly species. Some individuals are able to cover more than 100 km in less than 3 days during migration (Aubert et al., 1969; Aubert and Goeldlin de Tiefenau, 1981), and potentially more than a thousand kilometer over the whole migration season (Jia et al., 2022; Ouin et al., 2011), especially when aided by wind (Gao et al., 2020; Wotton et al., 2019). Those last seven studies focused on migratory species, which have very different life history traits. Hence, we (wrongly) predicted some level of IBD within study areas because our study species are not migratory. Given the high prevalence of hoverfly species presenting a partial migration syndrome (Doyle et al., 2022; Menz et al., 2019; Speight, 2017), the genetic and structural pathways to efficient dispersal might also be present in non-migratory hoverflies such as *S. pipiens* and *M. florea*. Indeed, even rare non-migratory species may fly several kilometers away from their emergence sites (Rotheray et al., 2014).

## | Methodological limits and future directions

Detecting genetic structure is rarely a straightforward endeavor and there a known limits to certain approaches. Although STRUCTURE may perform better than DAPC in some scenarios because DAPC may be sensitive to IBD, DAPC performs well for scenarios with low IBD (Blair et al., 2012) which was the case in our study. There are known biases towards selecting *K*=2 when using STRUCTURE (Janes et al., 2017), but we are confident that we lowered this bias by using more flexible parameters in our runs and by comparing outcomes with DAPC. The somewhat intriguing pattern displayed in the DAPC scatterplot for *S. pipiens* disappeared when dropping other alleles. Because the structure is so low, only a few alleles may be driving the visual grouping of some observations on the first two axes. The general conclusions about clustering were maintained after removing several loci to disrupt this pattern. Therefore, we kept all loci except the one mentioned in the methods. Given the large number of *de novo* DAPC runs we conducted, we had to choose the best number of genetic clusters programmatically, based on a fixed criterion rather than using the visual “elbow in the curve” or the minimum methods. However, it is important to note that in most runs, using the visual heuristic (or minimum approach) led to much higher numbers of clusters, notably for *S. pipiens* (Sup. Fig. 1). Such a situation where STRUCTURE selects fewer clusters than DAPC has been described for other pollinators (Frantine-Silva et al., 2021; Glück et al., 2022).

Another potential methodological limit is that both structure and IBD results (i.e., no IBD or structure detected) could be associated with high effective population size. Indeed, very high effective population size may hide the signal of clustering, IBD, or landscape effects on dispersal (Frantz et al., 2009; Gauffre et al., 2008). Although simulations could help us better understand whether we could detect the actual signals of structure or isolation (Frantz et al., 2009; Gauffre et al., 2008; Landguth et al., 2010), information about population dynamics may not be easily extracted from microsatellite data for non-model insect species with potentially very large population sizes such as hoverflies. In other words, while there may be an effect of the landscape on the stratification and connectivity of hoverfly populations, their high effective population size could be too large for genetic drift to have a detectable effect. More genetic information (e.g., using thousands of variable markers), could help to detect effects of the landscape at the spatial and temporal scales relevant for the disturbance (Landguth et al., 2012).

## | Implications for hoverfly biodiversity and pollination services

We could not use sophisticated landscape genetics models, however, there might still be effects of the landscape on movement and on population health. Indeed, while we could not identify features associated with a hindrance on gene flow, there may be costs to dispersal (Bonte et al., 2012). For example, there could be high mortality rates in some urban or peri-urban agricultural habitats, which would likely lower population density and genetic diversity although a large number of local dispersers could offset their genetic signal. Finally, although we did not find constraints on gene flow within urbanized landscapes for those two species, they are likely to exist in other systems, including for hoverflies.

Based on our results, given proper habitats, hoverfly with similar life history traits as the ones we studied could quickly colonize the landscape. Habitat quality and quantity are likely more limiting than isolation between habitat patches. Wildflower strips distributed homogenously in agricultural or urban landscapes could support some hoverfly species and would foster their pollinator services and their large contribution to aphid control. For some species, urban centers could act as a refuge (Hall et al., 2017; Theodorou et al., 2020) when the surrounding landscape is unfavorable due to heavy pesticide use or lack of floral resources. However, urban areas may not often support species-rich hoverfly communities (Svenningsen et al., 2020, 2021). Gene flow associated with high connectivity might not be sufficient to compensate for low urban genetic diversity and, low genetic diversity could limit resilience when facing catastrophic events or gradual environmental changes. Indeed, genetic diversity is the raw material for evolutionary adaptation necessary to overcome environmental constraints on survival and growth. The hoverfly populations we studied could be at risk such as a new disease, or the ongoing threat of climate change, which may affect the sequential use of flower by the hoverfly community throughout the season by affecting plant phenology.

Some introduced hoverflies can potentially outcompete native species due to their high polyphagy and dispersal abilities and understanding connectivity is key to understand, prevent, and mitigate their negative impacts. The high effective dispersal ability of *M. florea* suggested in our study suggests that this species could become established quickly once introduced. *M. florea* has already been introduced on the west coast of North America pre-2005 (BugGuide, 2022), likely through the timber trade because their larvae often develop among decaying roots or in rot-holes of trees, or with associated decaying matter (Rotheray, 1993). Unfortunately, but unsurpringsly given our conclusios, *M. florea* has quickly spread towards the east in its introduced range (GBIF.org, 2022; Miranda et al., 2013). *M. florea* were seen feeding on more than 10 species of flowers during the fieldwork for this study (Wittische, unpublished); many hoverflies are known to be highly polyphagous (Branquart and Hemptinne, 2000). Furthermore, given a similar climatic niche, widespread larval habitat, high dispersal ability and its tolerance for disturbance and urbanization suggested by our study, we expect *M. florea* to spread further East in North America. *Merodon equestris* is a European species now present in East Asia, North America, and Oceania (Hong et al., 2012; Thompson, 2008) and is a major pest of daffodils. *Eristalis tenax*, another European species, is a strong competitor due to its polyphagy, strong dispersal and aggressive territorial behavior towards other pollinators (Wellington and Fitzpatrick, 1981). *E. tenax* has spread through North America and New Zealand where they reach high abundances. Finally, *Simosyrphus grandicornis* is an Australasian species which has been introduced to Hawaii and French Polynesia where no previous hoverfly species occurred (Doyle et al., 2020), which may have affected the floral and pollinator communities. The lack of knowledge about hoverfly introductions and their potential consequences on biodiversity is dire and further highlight why it is crucial to understand hoverfly dispersal and their population dynamics.

**ACKNOWELDGEMENTS**

We thank Balint Andrasi, Dylan Thissen, António Cruz, Caroline Grounds, Fernanda Andrea Herrera Mesías, Monique Kirsch, Jérôme Herr, and the other people who helped on the field or in the lab. This study was funded by a CORE grant (C20/SR/14748041) from the Luxembourg National Research Fund (FNR). We thank local authorities for granting us netting permits.

# REFERENCES

Adamack, A.T., Gruber, B., 2014. PopGenReport: simplifying basic population genetic analyses in R. Methods in Ecology and Evolution 4.

Agapow, P.M., Burt, A., 2001. Indices of multilocus linkage disequilibrium. Molecular Ecology Notes 1, 101–102. https://doi.org/10.1046/j.1471-8278.2000.00014.x

Aubert, J., Goeldlin de Tiefenau, P., 1981. Observations sur les migrations de Syrphides (Dipt.) dans les Alpes de Suisse occidentale. Journal of the Swiss Entomological Society 54. https://doi.org/10.5169/SEALS-402013

Aubert, J., Goeldlin, P., Lyon, J.-P., 1969. Essais de marquage et de reprise d’insectes migrateurs en automne 1968. Journal of the Swiss Entomological Society 42. https://doi.org/10.5169/SEALS-401588

Bickel, D., Pape, T., Meier, R. (Eds.), 2009. Diptera Diversity: Status, Challenges and Tools. Brill.

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J., Kunin, W.E., 2006. Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. Science 313, 351–354. https://doi.org/10.1126/science.1127863

Blair, C., Weigel, D.E., Balazik, M., Keeley, A.T.H., Walker, F.M., Landguth, E., Cushman, S., Murphy, M., Waits, L., Balkenhol, N., 2012. A simulation-based evaluation of methods for inferring linear barriers to gene flow. Molecular Ecology Resources 12, 822–833. https://doi.org/10.1111/j.1755-0998.2012.03151.x

Bonte, D., Van Dyck, H., Bullock, J.M., Coulon, A., Delgado, M., Gibbs, M., Lehouck, V., Matthysen, E., Mustin, K., Saastamoinen, M., Schtickzelle, N., Stevens, V.M., Vandewoestijne, S., Baguette, M., Barton, K., Benton, T.G., Chaput-Bardy, A., Clobert, J., Dytham, C., Hovestadt, T., Meier, C.M., Palmer, S.C.F., Turlure, C., Travis, J.M.J., 2012. Costs of dispersal. Biological Reviews 87, 290–312. https://doi.org/10.1111/j.1469-185X.2011.00201.x

Bowler, D.E., Benton, T.G., 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biological Reviews 80, 205–225. https://doi.org/10.1017/S1464793104006645

Branquart, E., Hemptinne, J.-L., 2000. Selectivity in the exploitation of floral resources by hoverflies (Diptera: Syrphinae). Ecography 23, 732–742. https://doi.org/10.1111/j.1600-0587.2000.tb00316.x

Braun, M., Herold, M., 2004. Mapping imperviousness using NDVI and linear spectral unmixing of ASTER data in the Cologne-Bonn region (Germany), in: Remote Sensing for Environmental Monitoring, GIS Applications, and Geology III. Presented at the Remote Sensing for Environmental Monitoring, GIS Applications, and Geology III, SPIE, pp. 274–284. https://doi.org/10.1117/12.510978

Brookfield, J.F.Y., 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. Molecular Ecology 5, 453–455.

Broquet, T., Petit, E.J., 2009. Molecular Estimation of Dispersal for Ecology and Population Genetics. Annual Review of Ecology, Evolution, and Systematics 40, 193–216. https://doi.org/10.1146/annurev.ecolsys.110308.120324

BugGuide, 2022. Species account - Myathropa florea.

Cayuela, H., Rougemont, Q., Prunier, J.G., Moore, J.S., Clobert, J., Besnard, A., Bernatchez, L., 2018. Demographic and genetic approaches to study dispersal in wild animal populations: A methodological review. Molecular Ecology 27, 3976–4010. https://doi.org/10.1111/mec.14848

Culley, T.M., Stamper, T.I., Stokes, R.L., Brzyski, J.R., Hardiman, N.A., Klooster, M.R., Merritt, B.J., 2013. An efficient technique for primer development and application that integrates fluorescent labeling and multiplex PCR. Applications in Plant Sciences 1, 1300027. https://doi.org/10.3732/apps.1300027

Curdes, G., 1998. Urban form and innovation: The case of Cologne. Urban Morphology 2, 11–18.

Davis, E.S., Murray, T.E., Fitzpatrick, Ú., Brown, M.J.F., Paxton, R.J., 2010. Landscape effects on extremely fragmented populations of a rare solitary bee, Colletes floralis. Molecular Ecology 19, 4922–4935. https://doi.org/10.1111/j.1365-294X.2010.04868.x

Dicks, L.V., Abrahams, A., Atkinson, J., Biesmeijer, J., Bourn, N., Brown, C., Brown, M.J.F., Carvell, C., Connolly, C., Cresswell, J.E., Croft, P., Darvill, B., De Zylva, P., Effingham, P., Fountain, M., Goggin, A., Harding, D., Harding, T., Hartfield, C., Heard, M.S., Heathcote, R., Heaver, D., Holland, J., Howe, M., Hughes, B., Huxley, T., Kunin, W.E., Little, J., Mason, C., Memmott, J., Osborne, J., Pankhurst, T., Paxton, R.J., Pocock, M.J.O., Potts, S.G., Power, E.F., Raine, N.E., Ranelagh, E., Roberts, S., Saunders, R., Smith, K., Smith, R.M., Sutton, P., Tilley, L.A.N., Tinsley, A., Tonhasca, A., Vanbergen, A.J., Webster, S., Wilson, A., Sutherland, W.J., 2013. Identifying key knowledge needs for evidence-based conservation of wild insect pollinators: a collaborative cross-sectoral exercise. Insect Conservation and Diversity 6, 435–446. https://doi.org/10.1111/j.1752-4598.2012.00221.x

Dicks, L.V., Breeze, T.D., Ngo, H.T., Senapathi, D., An, J., Aizen, M.A., Basu, P., Buchori, D., Galetto, L., Garibaldi, L.A., Gemmill-Herren, B., Howlett, B.G., Imperatriz-Fonseca, V.L., Johnson, S.D., Kovács-Hostyánszki, A., Kwon, Y.J., Lattorff, H.M.G., Lungharwo, T., Seymour, C.L., Vanbergen, A.J., Potts, S.G., 2021. A global-scale expert assessment of drivers and risks associated with pollinator decline. Nat Ecol Evol 5, 1453–1461. https://doi.org/10.1038/s41559-021-01534-9

Doyle, T., Hawkes, W.L.S., Massy, R., Powney, G.D., Menz, M.H.M., Wotton, K.R., 2020. Pollination by hoverflies in the Anthropocene. Proceedings of the Royal Society B: Biological Sciences 287, 20200508. https://doi.org/10.1098/rspb.2020.0508

Doyle, T., Jimenez-Guri, E., Hawkes, W.L.S., Massy, R., Mantica, F., Permanyer, J., Cozzuto, L., Hermoso Pulido, T., Baril, T., Hayward, A., Irimia, M., Chapman, J.W., Bass, C., Wotton, K.R., 2022. Genome-wide transcriptomic changes reveal the genetic pathways involved in insect migration. Molecular Ecology 31, 4332–4350. https://doi.org/10.1111/mec.16588

Dreier, S., Redhead, J.W., Warren, I.A., Bourke, A.F.G., Heard, M.S., Jordan, W.C., Sumner, S., Wang, J., Carvell, C., 2014. Fine-scale spatial genetic structure of common and declining bumble bees across an agricultural landscape. Molecular Ecology 23, 3384–3395. https://doi.org/10.1111/mec.12823

European Environment Agency, 2016. Landscape fragmentation Effective Mesh Density: major and medium anthropogenic fragmenting elements (FGA2-S) - version 2.0, Nov. 2016.

Frantine-Silva, W., Augusto, S.C., Tosta, T.H.A., Pacheco, A.S., Kotelok-Diniz, T., Apolinário da Silva, C., Sofia, S.H., 2021. Genetic diversity and population structure of orchid bees from the Brazilian savanna. Journal of Apicultural Research 60, 385–395. https://doi.org/10.1080/00218839.2021.1898788

Frantz, A.C., Cellina, S., Krier, A., Schley, L., Burke, T., 2009. Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: Clusters or isolation by distance? Journal of Applied Ecology 46, 493–505. https://doi.org/10.1111/j.1365-2664.2008.01606.x

Gallai, N., Salles, J.-M., Settele, J., Vaissière, B.E., 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecological Economics 68, 810–821. https://doi.org/10.1016/j.ecolecon.2008.06.014

Gao, B., Wotton, K.R., Hawkes, W.L.S., Menz, M.H.M., Reynolds, D.R., Zhai, B.-P., Hu, G., Chapman, J.W., 2020. Adaptive strategies of high-flying migratory hoverflies in response to wind currents. Proceedings of the Royal Society B: Biological Sciences 287, 20200406. https://doi.org/10.1098/rspb.2020.0406

Gauffre, B., Estoup, A., Bretagnolle, V., Cosson, J.F., 2008. Spatial genetic structure of a small rodent in a heterogeneous landscape. Molecular Ecology 17, 4619–4629. https://doi.org/10.1111/j.1365-294X.2008.03950.x

GBIF.org, 2022. Occurrence Download - Myathropa florea - North America (05 September 2022) https://doi.org/10.15468/dl.ctqqr2.

Gill, R.J., Baldock, K.C.R., Brown, M.J.F., Cresswell, J.E., Dicks, L.V., Fountain, M.T., Garratt, M.P.D., Gough, L.A., Heard, M.S., Holland, J.M., Ollerton, J., Stone, G.N., Tang, C.Q., Vanbergen, A.J., Vogler, A.P., Woodward, G., Arce, A.N., Boatman, N.D., Brand-Hardy, R., Breeze, T.D., Green, M., Hartfield, C.M., O’Connor, R.S., Osborne, J.L., Phillips, J., Sutton, P.B., Potts, S.G., 2016. Chapter Four - Protecting an Ecosystem Service: Approaches to Understanding and Mitigating Threats to Wild Insect Pollinators, in: Woodward, G., Bohan, D.A. (Eds.), Advances in Ecological Research, Ecosystem Services: From Biodiversity to Society, Part 2. Academic Press, pp. 135–206. https://doi.org/10.1016/bs.aecr.2015.10.007

Glück, M., Geue, J.C., Thomassen, H.A., 2022. Environmental differences explain subtle yet detectable genetic structure in a widespread pollinator. BMC Ecol Evo 22, 8. https://doi.org/10.1186/s12862-022-01963-5

Greenleaf, S.S., Williams, N.M., Winfree, R., Kremen, C., 2007. Bee foraging ranges and their relationship to body size. Oecologia 153, 589–596. https://doi.org/10.1007/s00442-007-0752-9

Guo, S.W., Thompson, E.A., 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48, 361–372.

Hall, D.M., Camilo, G.R., Tonietto, R.K., Ollerton, J., Ahrné, K., Arduser, M., Ascher, J.S., Baldock, K.C.R., Fowler, R., Frankie, G., Goulson, D., Gunnarsson, B., Hanley, M.E., Jackson, J.I., Langellotto, G., Lowenstein, D., Minor, E.S., Philpott, S.M., Potts, S.G., Sirohi, M.H., Spevak, E.M., Stone, G.N., Threlfall, C.G., 2017. The city as a refuge for insect pollinators. Conservation Biology 31, 24–29. https://doi.org/10.1111/cobi.12840

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., Kroon, H. de, 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLOS ONE 12, e0185809. https://doi.org/10.1371/journal.pone.0185809

Hodgkiss, D., Brown, M.J.F., Fountain, M.T., 2018. Syrphine hoverflies are effective pollinators of commercial strawberry. Journal of Pollination Ecology 22, 55–66. https://doi.org/10.26786/1920-7603(2018)five

Hong, K.-J., Lee, J.-H., Lee, G.-S., Lee, S., 2012. The status quo of invasive alien insect species and plant quarantine in Korea. Journal of Asia-Pacific Entomology 15, 521–532. https://doi.org/10.1016/j.aspen.2012.06.003

Jaeger, J.A.G., Soukup, T., Schwick, C., Madriñán, L.F., Kienast, F., 2016. Landscape Fragmentation in Europe, in: European Landscape Dynamics. CRC press, Boca Raton, Florida, USA, p. 42.

Janes, J.K., Miller, J.M., Dupuis, J.R., Malenfant, R.M., Gorrell, J.C., Cullingham, C.I., Andrew, R.L., 2017. The K = 2 conundrum. Molecular Ecology 26, 3594–3602. https://doi.org/10.1111/mec.14187

Jauker, F., Diekötter, T., Schwarzbach, F., Wolters, V., 2009. Pollinator dispersal in an agricultural matrix: opposing responses of wild bees and hoverflies to landscape structure and distance from main habitat. Landscape Ecol 24, 547–555. https://doi.org/10.1007/s10980-009-9331-2

Jauker, F., Jauker, B., Grass, I., Steffan-Dewenter, I., Wolters, V., 2019. Partitioning wild bee and hoverfly contributions to plant–pollinator network structure in fragmented habitats. Ecology 100, e02569. https://doi.org/10.1002/ecy.2569

Jha, S., Kremen, C., 2013. Urban land use limits regional bumble bee gene flow. Molecular Ecology 22, 2483–2495. https://doi.org/10.1111/mec.12275

Jia, H., Liu, Y., Li, X., Li, H., Pan, Y., Hu, C., Zhou, X., Wyckhuys, K.A., Wu, K., 2022. Windborne migration amplifies insect-mediated pollination services. eLife 11, e76230. https://doi.org/10.7554/eLife.76230

Jombart, T., 2008. Adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., Ahmed, I., 2011. adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. Bioinformatics 27, 3070–3071. https://doi.org/10.1093/bioinformatics/btr521

Jombart, T., Collins, C., 2022. A tutorial for Discriminant Analysis of Principal Components (DAPC) using adegenet 2.1.6.

Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC genetics 11, 94. https://doi.org/10.1186/1471-2156-11-94

Jombart, T., Pontier, D., Dufour, a-B., 2009. Genetic markers in the playground of multivariate analysis. Heredity 102, 330–341. https://doi.org/10.1038/hdy.2008.130

Kamvar, Z.N., Tabima, J.F., Gr̈unwald, N.J., 2014. Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2014, 1–14. https://doi.org/10.7717/peerj.281

Kleijn, D., van Langevelde, F., 2006. Interacting effects of landscape context and habitat quality on flower visiting insects in agricultural landscapes. Basic and Applied Ecology 7, 201–214. https://doi.org/10.1016/j.baae.2005.07.011

Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences 274, 303–313. https://doi.org/10.1098/rspb.2006.3721

Landguth, E.L., Cushman, S.A., Schwartz, M.K., McKELVEY, K.S., Murphy, M., Luikart, G., 2010. Quantifying the lag time to detect barriers in landscape genetics: QUANTIFYING THE LAG TIME TO DETECT BARRIERS IN LANDSCAPE GENETICS. Molecular Ecology 19, 4179–4191. https://doi.org/10.1111/j.1365-294X.2010.04808.x

Landguth, E.L., Fedy, B.C., OYLER-McCANCE, S.J., Garey, A.L., Emel, S.L., Mumma, M., Wagner, H.H., Fortin, M.-J., Cushman, S.A., 2012. Effects of sample size, number of markers, and allelic richness on the detection of spatial genetic pattern. Molecular Ecology Resources 12, 276–284. https://doi.org/10.1111/j.1755-0998.2011.03077.x

Legendre, P., Legendre, L., 2012. Numerical Ecology. Elsevier, Amsterdam, Netherlands.

Leopold, J., Schöne, M., Cölln, K., 1996. Zur Kenntis der Schwebfliegen (Diptera, Syrphidae) der Stadt Köln und ihrer Randgebiete. Decheniana - Beihefte (Bonn) 35, 433–458.

Loiselle, B. a, Sork, V.L., Nason, J., Graham, C., 1995. Spatial Genetic Structure of a Tropical Understory Shrub. American Journal of Botany 82, 1420–1425.

Lövei, G.L., Macleod, A., Hickman, J.M., 1998. Dispersal and effects of barriers on the movement of the New Zealand hover fly Melanostoma fasciatum (Dipt., Syrphidae) on cultivated land. Journal of Applied Entomology 122, 115–120. https://doi.org/10.1111/j.1439-0418.1998.tb01471.x

Lozier, J.D., Strange, J.P., Stewart, I.J., Cameron, S.A., 2011. Patterns of range-wide genetic variation in six North American bumble bee (Apidae: Bombus) species. Molecular Ecology 20, 4870–4888. https://doi.org/10.1111/j.1365-294X.2011.05314.x

Lucas, A., Bodger, O., Brosi, B.J., Ford, C.R., Forman, D.W., Greig, C., Hegarty, M., Jones, L., Neyland, P.J., de Vere, N., 2018. Floral resource partitioning by individuals within generalised hoverfly pollination networks revealed by DNA metabarcoding. Sci Rep 8, 5133. https://doi.org/10.1038/s41598-018-23103-0

Mantel, N., 1967. Cancer research. Mantel 27, 34.

Menz, M.H.M., Reynolds, D.R., Gao, B., Hu, G., Chapman, J.W., Wotton, K.R., 2019. Mechanisms and Consequences of Partial Migration in Insects. Frontiers in Ecology and Evolution 7.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16, 1215.

Ministère de l’Environnement, du Climat et du Développement durable, 2022. Plan Pollinisateurs Luxembourg [WWW Document]. Plan Pollinisateurs. URL https://www.planpollinisateurs.lu (accessed 9.16.22).

Miranda, G.F.G., Young, A.D., Locke, M.M., Marshall, S.A., Skevington, J.H., Thompson, F.C., 2013. Key to the Genera of Nearctic Syrphidae. CJAI 23.

Mitter, H., Weber, G., 2011. Green Belt(s)—a Challenge for Urban Policy of Expanding Cities. Regions Magazine 282, 18–20. https://doi.org/10.1080/13673882.2011.9697692

Moquet, L., Laurent, E., Bacchetta, R., Jacquemart, A.-L., 2018. Conservation of hoverflies (Diptera, Syrphidae) requires complementary resources at the landscape and local scales. Insect Conservation and Diversity 11, 72–87. https://doi.org/10.1111/icad.12245

Öckinger, E., Schweiger, O., Crist, T.O., Debinski, D.M., Krauss, J., Kuussaari, M., Petersen, J.D., Pöyry, J., Settele, J., Summerville, K.S., Bommarco, R., 2010. Life-history traits predict species responses to habitat area and isolation: a cross-continental synthesis. Ecology Letters 13, 969–979. https://doi.org/10.1111/j.1461-0248.2010.01487.x

Ollerton, J., 2017. Pollinator Diversity: Distribution, Ecological Function, and Conservation. Annual Review of Ecology, Evolution, and Systematics 48, 353–376. https://doi.org/10.1146/annurev-ecolsys-110316-022919

Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by animals? Oikos 120, 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Ouin, A., Menozzi, P., Coulon, M., Hamilton, A.J., Sarthou, J.P., Tsafack, N., Vialatte, A., Ponsard, S., 2011. Can deuterium stable isotope values be used to assign the geographic origin of an auxiliary hoverfly in south-western France? Rapid Communications in Mass Spectrometry 25, 2793–2798. https://doi.org/10.1002/rcm.5127

Ouin, A., Sarthou, J.-P., Bouyjou, B., Deconchat, M., Lacombe, J.-P., Monteil, C., 2006. The species-area relationship in the hoverfly (Diptera, Syrphidae) communities of forest fragments in southern France. Ecography 29, 183–190. https://doi.org/10.1111/j.2006.0906-7590.04135.x

Oyler-McCance, S.J., Fedy, B.C., Landguth, E.L., 2013. Sample design effects in landscape genetics. Conserv Genet 14, 275–285. https://doi.org/10.1007/s10592-012-0415-1

Paradis, E., 2010. pegas : an R package for population genetics with an integrated – modular approach 26, 419–420. https://doi.org/10.1093/bioinformatics/btp696

Pekas, A., De Craecker, I., Boonen, S., Wäckers, F.L., Moerkens, R., 2020. One stone; two birds: concurrent pest control and pollination services provided by aphidophagous hoverflies. Biological Control 149, 104328. https://doi.org/10.1016/j.biocontrol.2020.104328

Potts, S., K., B., Bommarco, R., Breeze, T., Carvalheiro, L., Franzén, M., González-Varo, J.P., A., H., Kleijn, D., Klein, A., Kunin, Lecocq, T., Lundin, O., Michez, D., Neumann, P., A., N., Penev, L., Rasmont, P., Ratamäki, O., Schweiger, O., 2015. Status and trends of European pollinators. Key findings of the STEP project. Pensoft Publishers, Sofia, Bulgaria.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution 25, 345–353. https://doi.org/10.1016/j.tree.2010.01.007

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of Population Structure Using Multilocus Genotype Data.

Rader, R., Bartomeus, I., Garibaldi, L.A., Garratt, M.P.D., Howlett, B.G., Winfree, R., Cunningham, S.A., Mayfield, M.M., Arthur, A.D., Andersson, G.K.S., Bommarco, R., Brittain, C., Carvalheiro, L.G., Chacoff, N.P., Entling, M.H., Foully, B., Freitas, B.M., Gemmill-Herren, B., Ghazoul, J., Griffin, S.R., Gross, C.L., Herbertsson, L., Herzog, F., Hipólito, J., Jaggar, S., Jauker, F., Klein, A.-M., Kleijn, D., Krishnan, S., Lemos, C.Q., Lindström, S.A.M., Mandelik, Y., Monteiro, V.M., Nelson, W., Nilsson, L., Pattemore, D.E., de O. Pereira, N., Pisanty, G., Potts, S.G., Reemer, M., Rundlöf, M., Sheffield, C.S., Scheper, J., Schüepp, C., Smith, H.G., Stanley, D.A., Stout, J.C., Szentgyörgyi, H., Taki, H., Vergara, C.H., Viana, B.F., Woyciechowski, M., 2016. Non-bee insects are important contributors to global crop pollination. Proceedings of the National Academy of Sciences 113, 146–151. https://doi.org/10.1073/pnas.1517092112

Rands, S.A., 2014. Landscape fragmentation and pollinator movement within agricultural environments: a modelling framework for exploring foraging and movement ecology. PeerJ 2, e269. https://doi.org/10.7717/peerj.269

Raymond, L., Plantegenest, M., Vialatte, A., 2013. Migration and dispersal may drive to high genetic variation and significant genetic mixing: the case of two agriculturally important, continental hoverflies (Episyrphus balteatus and Sphaerophoria scripta). Molecular Ecology 22, 5329–5339. https://doi.org/10.1111/mec.12483

Reilly, J.R., Artz, D.R., Biddinger, D., Bobiwash, K., Boyle, N.K., Brittain, C., Brokaw, J., Campbell, J.W., Daniels, J., Elle, E., Ellis, J.D., Fleischer, S.J., Gibbs, J., Gillespie, R.L., Gundersen, K.B., Gut, L., Hoffman, G., Joshi, N., Lundin, O., Mason, K., McGrady, C.M., Peterson, S.S., Pitts-Singer, T.L., Rao, S., Rothwell, N., Rowe, L., Ward, K.L., Williams, N.M., Wilson, J.K., Isaacs, R., Winfree, R., 2020. Crop production in the USA is frequently limited by a lack of pollinators. Proceedings of the Royal Society B: Biological Sciences 287, 20200922. https://doi.org/10.1098/rspb.2020.0922

Roser, L.G., Ferreyra, L.I., Saidman, B.O., Vilardi, J.C., 2017. EcoGenetics: An R package for the management and exploratory analysis of spatial data in landscape genetics. Molecular Ecology Resources 17, e241–e250. https://doi.org/10.1111/1755-0998.12697

Rotheray, E.L., Bussière, L.F., Moore, P., Bergstrom, L., Goulson, D., 2014. Mark recapture estimates of dispersal ability and observations on the territorial behaviour of the rare hoverfly, Hammerschmidtia ferruginea (Diptera, Syrphidae). J Insect Conserv 18, 179–188. https://doi.org/10.1007/s10841-014-9627-7

Rotheray, G.E., 1993. Colour Guide to Hoverfly Larvae (Diptera: Syrphidae). Dipterists Digest 9.

Sánchez-Bayo, F., Wyckhuys, K.A.G., 2021. Further evidence for a global decline of the entomofauna. Austral Entomology 60, 9–26. https://doi.org/10.1111/aen.12509

Sánchez-Bayo, F., Wyckhuys, K.A.G., 2019. Worldwide decline of the entomofauna: A review of its drivers. Biological Conservation 232, 8–27. https://doi.org/10.1016/j.biocon.2019.01.020

Schauer, B., Bong, J., Popp, C., Obermaier, E., Feldhaar, H., 2018. Dispersal limitation of saproxylic insects in a managed forest? A population genetics approach. Basic and Applied Ecology 32, 26–38. https://doi.org/10.1016/j.baae.2018.01.005

Schwartz, M.K., McKelvey, K.S., 2009. Why sampling scheme matters: the effect of sampling scheme on landscape genetic results. Conserv Genet 10, 441–452. https://doi.org/10.1007/s10592-008-9622-1

Seibold, S., Gossner, M.M., Simons, N.K., Blüthgen, N., Müller, J., Ambarlı, D., Ammer, C., Bauhus, J., Fischer, M., Habel, J.C., Linsenmair, K.E., Nauss, T., Penone, C., Prati, D., Schall, P., Schulze, E.D., Vogt, J., Wöllauer, S., Weisser, W.W., 2019. Arthropod decline in grasslands and forests is associated with landscape-level drivers. Nature 574, 671–674. https://doi.org/10.1038/s41586-019-1684-3

Senapathi, D., Carvalheiro, L.G., Biesmeijer, J.C., Dodson, C.-A., Evans, R.L., McKerchar, M., Morton, R.D., Moss, E.D., Roberts, S.P.M., Kunin, W.E., Potts, S.G., 2015. The impact of over 80 years of land cover changes on bee and wasp pollinator communities in England. Proceedings of the Royal Society B: Biological Sciences 282, 20150294. https://doi.org/10.1098/rspb.2015.0294

Simmons, B.I., Balmford, A., Bladon, A.J., Christie, A.P., De Palma, A., Dicks, L.V., Gallego-Zamorano, J., Johnston, A., Martin, P.A., Purvis, A., Rocha, R., Wauchope, H.S., Wordley, C.F.R., Worthington, T.A., Finch, T., 2019. Worldwide insect declines: An important message, but interpret with caution. Ecology and Evolution 9, 3678–3680. https://doi.org/10.1002/ece3.5153

Speight, M.C.D., 2017. Species account of European Syrphidae, Syrph the Net, the database of European Syrphidae (Diptera). Syrph the Net publications, Dublin, Ireland.

Ssymank, A., Kearns, C.A., Pape, T., Thompson, F.C., 2008. Pollinating Flies (Diptera): A major contribution to plant diversity and agricultural production. Biodiversity 9, 86–89. https://doi.org/10.1080/14888386.2008.9712892

Stadt Köln, 2022. Insektenschutz [WWW Document]. URL https://www.stadt-koeln.de/leben-in-koeln/klima-umwelt-tiere/insektenschutz (accessed 9.16.22).

Steffan-Dewenter, I., Münzenberg, U., Bürger, C., Thies, C., Tscharntke, T., 2002. Scale-Dependent Effects of Landscape Context on Three Pollinator Guilds. Ecology 83, 1421–1432. https://doi.org/10.1890/0012-9658(2002)083[1421:SDEOLC]2.0.CO;2

Svenningsen, C., Bowler, D.E., Hecker, S., Bladt, J., Grescho, V., van Dam, N.M., Dauber, J., Eichenberg, D., Ejrnæs, R., Fløjgaard, C., Frenzel, M., Guldberg Frøslev, T., Hansen, A.J., Heilmann-Clausen, J., Huang, Y., Colling Larsen, J., Menger, J., Liyana Binti Mat Nayan, N., Pedersen, L.B., Richter, A., Dunn, R.R., Tøttrup, A.P., Bonn, A., 2020. Contrasting impacts of urban and farmland cover on flying insect biomass.

Svenningsen, C.S., Frøslev, T.G., Bladt, J., Pedersen, L.B., Larsen, J.C., Ejrnæs, R., Fløjgaard, C., Hansen, A.J., Heilmann-Clausen, J., Dunn, R.R., Tøttrup, A.P., 2021. Detecting flying insects using car nets and DNA metabarcoding. Biology Letters 17, 20200833. https://doi.org/10.1098/rsbl.2020.0833

Taylor, P.D., Fahrig, L., Henein, K., Merriam, G., 1993. Connectivity Is a Vital Element of Landscape Structure. Oikos 68, 571. https://doi.org/10.2307/3544927

Theodorou, P., Radzevičiūtė, R., Lentendu, G., Kahnt, B., Husemann, M., Bleidorn, C., Settele, J., Schweiger, O., Grosse, I., Wubet, T., Murray, T.E., Paxton, R.J., 2020. Urban areas as hotspots for bees and pollination but not a panacea for all insects. Nat Commun 11, 576. https://doi.org/10.1038/s41467-020-14496-6

Thompson, F.C., 2008. A conspectus of New Zealand flower flies (Diptera: Syrphidae) with the description of a new genus and species. Zootaxa 1716, 1. https://doi.org/10.11646/zootaxa.1716.1.1

Vanbergen, A.J., 1, 2, 3, 4, 2013. Threats to an ecosystem service: pressures on pollinators. Frontiers in Ecology and the Environment 11, 251–259. https://doi.org/10.1890/120126

Vekemans, X., Hardy, O.J., 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. Molecular Ecology 13, 921–935. https://doi.org/10.1046/j.1365-294X.2004.02076.x

Wang, J., 2017. The computer program structure for assigning individuals to populations: easy to use but easier to misuse. Molecular Ecology Resources 17, 981–990.

Wardhaugh, C.W., 2015. How many species of arthropods visit flowers? Arthropod-Plant Interactions 9, 547–565. https://doi.org/10.1007/s11829-015-9398-4

Wellington, W.G., Fitzpatrick, S.M., 1981. Territoriality in the drone fly, Eristalis tenax (Diptera: Syrphidae). The Canadian Entomologist 113, 695–704. https://doi.org/10.4039/Ent113695-8

Winfree, R., Bartomeus, I., Cariveau, D.P., 2011. Native Pollinators in Anthropogenic Habitats. Annual Review of Ecology, Evolution, and Systematics 42, 1–22. https://doi.org/10.1146/annurev-ecolsys-102710-145042

Wotton, K.R., Gao, B., Menz, M.H.M., Morris, R.K.A., Ball, S.G., Lim, K.S., Reynolds, D.R., Hu, G., Chapman, J.W., 2019. Mass Seasonal Migrations of Hoverflies Provide Extensive Pollination and Crop Protection Services. Current Biology 29, 2167-2173.e5. https://doi.org/10.1016/j.cub.2019.05.036

Wratten, S.D., Bowie, M.H., Hickman, J.M., Evans, A.M., Sedcole, J.R., Tylianakis, J.M., 2003. Field boundaries as barriers to movement of hover flies (Diptera: Syrphidae) in cultivated land. Oecologia 134, 605–611. https://doi.org/10.1007/s00442-002-1128-9

**SUPPLEMENTARY MATERIAL**

**Supplementary table 1.** Primer information. Dilutions for PCR products of *Myathropa florea* were 1/75 for Multiplex 1, 4/50 for Multiplex 2 and 1/120 for Multiplex 3. Pcr Products of *Syritta pipiens* were diluted 1/20. PCR products were genotyped using a capillary sequencer (ABI 3730XL, Applied Biosystems). Allele sizes were determined using GENEMAPPER version 4.0 (Applied Biosystems).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| M.plex | Lous | Forward Primer | Reverse Primer | Pigtail | Dye | Primer conc. | Size range |
| 1 | Spp\_193 | CATGAACCGACTCCAGAATG | CGGGAGACGAGACCTGAG |  | FAM | 0,2 | 80-130 |
| 1 | Spp\_010 | CACATCTCCTCAGCTTCCATC | GTCCACTAATGGGCCAAATG |  | FAM | 0,2 | 140-180 |
| 1 | Spp\_146 | TTACATCGGCAATCCACTTG | ACGAGAACGAGAACGAGGAC |  | FAM | 0,2 | 230-300 |
| 1 | Spp\_476 | TTATGGTCTGGCTCGAATGC | CGTCTCTTCGTGAGGTCGTC |  | HEX | 0,1 | 90-130 |
| 1 | Spp\_053 | TGATTAGCGAAGAGACCGAATC | CAACCAGCCAGCCATCTC | Pigtail | HEX | 0,15 | 135-175 |
| 1 | Spp\_273 | GCTCCCTCCTTGAATGCTC | CCTGCCTCTTAATGGTCCTG |  | HEX | 0,2 | 225-330 |
| 1 | Spp\_142 | TCACTGCCCGTTTCTTTCTC | TGGGTGAAGGCAAATTAAGG |  | TAMRA | 0,2 | 70-110 |
| 1 | Spp\_231 | GATGGTGTGCTCTCGATGTC | GGTTGGGTACCTTCAGGTTG |  | TAMRA | 0,2 | 120-144 |
| 1 | Spp\_080 | CGTTTCGTCATTCATTGCTG | AAGGCCAACAGGTCCTCTG |  | TAMRA | 0,2 | 145-180 |
| 2 | Spp\_033 | GGACAATTGTTCACTTGACAGG | CTGTTGGTCCTTTGTCTGTGTC | Pigtail | FAM | 0,15 | 65-100 |
| 2 | Spp\_141 | TCTCCACCCACTTCCCTTATC | CAAATTGACTTTCGGCCAAG | Pigtail | FAM | 0,2 | 103-120 |
| 2 | Spp\_416 | ATCTTGGAGTGCCCAGTTTG | CCACTCAACCCAGCCTTG | Pigtail | FAM | 0,1 | 130-160 |
| 2 | Spp\_108 | TCATCGACTTCCTGATGCTG | TTAAACGTCCACGGTGTGAG | Pigtail | FAM | 0,2 | 160-200 |
| 2 | Spp\_313 | CAGGTCAAACCTCCATCACC | AGGAGCTCCAAGGAAGAAGG |  | FAM | 0,2 | 215-250 |
| 2 | Spp\_410 | GGCTCATTTCACGCTTGTTG | GATCATTTGCACGCGTCTG |  | HEX | 0,075 | 70-100 |
| 2 | Spp\_360 | ACAATGTGTCCCAATGTCG | TCGGGAGTCTCTTGCCTAC | Pigtail | HEX | 0,2 | 115-150 |
| 2 | Spp\_391 | CGTGCGATAGATGTCTGGTG | CTCGCCTCTGAAATCATTGAC |  | HEX | 0,2 | 150-185 |
| 2 | Spp\_048 | CTCGCTGAAATGGTTGCTC | AAACCTGGAAGCCCTATTCC | Pigtail | TAMRA | 0,2 | 65-105 |
| 2 | Spp\_051 | TCGCACATTTACGACTTCTCC | CAAATTGACTTTCGGCCAAG | Pigtail | TAMRA | 0,2 | 110-145 |
| 2 | Spp\_387 | TCGAATGTGCATGGCTAATC | CGAGATCCGAGGTAGACAGG | Pigtail | TAMRA | 0,2 | 155-200 |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| M.plex | Lous | Forward Primer | Reverse Primer | Pigtail | Dye | Primer conc. | Size range |
| 1 | Mfl\_341 | CAATGACAACACAAAGTCATTCC | GAACTGAAGGCGAGTCGTG | Pigtail | TAMRA | 0,2 | 80-140 |
| 1 | Mfl\_059 | CAAACGACCCACATTTGATG | GGCACTAGGTCTCGTCGTTC | Pigtail | TAMRA | 0,2 | 150-190 |
| 1 | Mfl\_025 | ATGTTGGCACGGACATGG | CCATCTCGGACTTCAGTTTGTC | Pigtail | TAMRA | 0,2 | 210-280 |
| 1 | Mfl\_303 | TGGAATGTGGCTTTCATCTC | CCAATTGATTGTTGCTCCAC | Pigtail | HEX | 0,2 | 70-130 |
| 1 | Mfl\_301 | CCAATTGTCTGCTCAGCATC | GAAATATTGGGTGCGCTTG | Pigtail | HEX | 0,2 | 150-170 |
| 1 | Mfl\_270 | TGTCAGGAAATCCGTTCATTC | TCACTCCCGAAACAATCCTC | Pigtail | HEX | 0,3 | 190-230 |
| 1 | Mfl\_322 | AACTTGGGAACGAACGTCTG | CTCAGCAATCCTTCAATCTCG | Pigtail | HEX | 0,3 | 235-300 |
| 1 | Mfl\_337 | TTTCTATGGTCATACGCAAACG | CATACGCACGCTAACAGCAC | Pigtail | FAM | 0,2 | 70-90 |
| 1 | Mfl\_253 | TTCCGATTCATTCACTTGACC | CGACAGTTCGGAAGGTTAGC | Pigtail | FAM | 0,2 | 105-130 |
| 1 | Mfl\_239 | CTCTCGCATTCCCTGTCTTC | GACGCGTCCAACTAATAGGC | Pigtail | FAM | 0,2 | 150-190 |
| 1 | Mfl\_265 | ATTGGCTACACTTCGGTTGG | TGCATCAGTTCCCGAAATC | Pigtail | FAM | 0,2 | 210-275 |
| 2 | Mfl\_036 | CAGCACTGGAGACGTTCG | GGGTCATCTTGGAATGGTG | Pigtail | FAM | 0,3 | 80-115 |
| 2 | Mfl\_130 | ACATTTCACACCGCAAACG | AACCTTCCGTTTCCAGTTCC | Pigtail | FAM | 0,3 | 150-225 |
| 2 | Mfl\_419 | TGGTCCAAAGTTCCGTTCTC | AACAGCGTGAGCTTGATGG | Pigtail | FAM | 0,4 | 228-275 |
| 2 | Mfl\_358 | TATGTTGCTGTTCCCTGCTG | GGAATACATCACCGCGTTTC | Pigtail | HEX | 0,2 | 70-120 |
| 2 | Mfl\_197 | CTTATCGCGCTAATCCAAGC | CAACTCGCTCCACTCAAGC | Pigtail | HEX | 0,15 | 130-160 |
| 2 | Mfl\_486 | GGTGCATCACTTGATGTTGG | AACCGAACACATTCCGTCTC | Pigtail | HEX | 0,3 | 188-235 |
| 2 | Mfl\_432 | ATCAGCAACAGCAACATTCG | AGGTTCCCACCAATGCAG | Pigtail | HEX | 0,2 | 245-280 |
| 2 | Mfl\_159 | CGCGCTACTTACCGATGAC | GTTCATTAGGCTGCGAACG | Pigtail | TAMRA | 0,3 | 83-110 |
| 2 | Mfl\_492 | GGGCTGTTAACAAGATGTAAAGG | ACGACTCGCTAAGGTCACG | Pigtail | TAMRA | 0,4 | 130-160 |
| 3 | Mfl\_028 | GAACAAGGCTCTTCGCAAAC | CGAGATGGTGGCTATAAAGGAC | Pigtail | FAM | 0,2 | 70-115 |
| 3 | Mfl\_103 | ACTCGGTTATGGCTCCACTG | GGTTGCATGCGATTAGTGTG | Pigtail | FAM | 0,2 | 130-155 |
| 3 | Mfl\_323 | CCGCACAGTTTGTGAGTGTC | CAGCCTATATTTGGGTGTTTGC | Pigtail | FAM | 0,2 | 165-190 |
| 3 | Mfl\_261 | GGTCAAGGGTGTCATCCATC | CATGAGAACCCGCTGGAG | Pigtail | FAM | 0,2 | 205-270 |
| 3 | Mfl\_026 | AATGGAAACGAGGTGGGATAC | GCTTGCAGAATGGAAACTACG | Pigtail | HEX | 0,2 | 120-153 |
| 3 | Mfl\_457 | TCAACGTGCAGCAACTATCTG | GAGGGCAAAGGACAAACTCTC | Pigtail | HEX | 0,2 | 160-195 |
| 3 | Mfl\_269 | TTCTCTTCACATCTGCGATCC | AATGGATGTCCGCAATGG | Pigtail | HEX | 0,3 | 205-280 |
| 3 | Mfl\_263 | AAATGCGCTGAAATTGTGG | AACCCAAGCAACAGTCAACC | Pigtail | TAMRA | 0,3 | 70-110 |
| 3 | Mfl\_056 | TTGCCACCAAAGGTTAGTCC | AGTCATCCTTCGGTTGTTGC | Pigtail | TAMRA | 0,3 | 115-150 |
| 3 | Mfl\_070 | CGACCGCATAGATTCCATAG | AATTTCGTTGCGCATTTG | Pigtail | TAMRA | 0,4 | 160-190 |
| 3 | Mfl\_491 | CTGTCGATGGACTCCGATG | GCTTACCCGTTGGTTGAGAG | Pigtail | TAMRA | 0,2 | 195-240 |

**Supplementary table 3.** Hardy-Weinberg results for within commune/district tests. Underlined values describes significance of the raw exact Monte Carlo p-values and values in bold describes significance after Benjamini-Hochberg correction.

A) *S. pipiens* in Luxembourg

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| LOCUS | Spp010 | Spp053 | Spp080 | Spp142 | Spp231 | Spp273 | Spp476 |
| COMMUNE |  |  |  |  |  |  |  |
| Bertrange | 0.46865 | 0.95024 | 0.69754 | 0.0163 | 0.19582 | 0.59045 | 0.62023 |
| Bettembourg | 1 | 0.10449 | 0.11397 | 0.2949 | 0.84115 | 0.05685 | 0.67288 |
| Differdange | 0.55521 | 0.44525 | 1 | 0.62001 | 0.23886 | 0.0113 | 0.15871 |
| Hesperange | 0.30481 | 0.91293 | 0.50448 | 0.84056 | 0.36222 | 0.23736 | 0.04438 |
| Leudelange | 1 | 0.94832 | 1 | 0.23009 | 0.04667 | 0.42926 | 0.57406 |
| Luxembourg | 0.4571 | 0.9754 | **0.00397** | 0.02425 | 0.21308 | 0.02378 | 0.06994 |
| Mondercange | 0.27194 | **0.00285** | 0.39906 | 0.36459 | 0.38697 | 0.33725 | 0.59137 |
| Reckange.sur.Mess | 0.20473 | 0.39483 | 0.79744 | 0.15975 | 0.0772 | 0.48224 | 0.89006 |
| Roeser | 0.03363 | 0.54848 | 0.19854 | 0.85538 | 0.8941 | 0.20986 | 0.84413 |
| Sanem | 0.37714 | 0.76817 | 0.66863 | 0.08061 | 0.90576 | 0.08795 | 0.81115 |
|  |  |  |  |  |  |  |  |
| LOCUS | Spp051 | Spp108 | Spp141 | Spp313 | Spp360 | Spp391 | Spp416 |
| COMMUNE |  |  |  |  |  |  |  |
| Bertrange | 0.13729 | 0.43302 | 0.04397 | 0.04236 | 0.02565 | 0.14458 | 0.45367 |
| Bettembourg | 0.82098 | 0.02719 | 0.06297 | 1 | 0.36233 | 0.21826 | 0.46175 |
| Differdange | 0.25831 | 0.01227 | 0.52862 | 0.0498 | 0.84132 | 0.77332 | 1 |
| Hesperange | 0.17594 | 0.0289 | **0.00755** | 0.09473 | 0.09085 | 0.75882 | 0.00783 |
| Leudelange | 0.51488 | 0.74713 | 0.4415 | 0.75031 | 0.81947 | 0.52532 | 1 |
| Luxembourg | **0.0034** | 0.11569 | **2.50E-05** | 0.78179 | 0.08737 | 0.94801 | 0.17845 |
| Mondercange | 0.38665 | 0.73393 | 0.06728 | 0.70222 | **0.00067** | 0.66159 | 0.60818 |
| Reckange.sur.Mess | 0.0188 | 0.28713 | **0.00128** | 1 | 0.22058 | 0.02668 | 0.13222 |
| Roeser | 0.60075 | 0.44421 | 0.71558 | 1 | 0.20036 | 0.98877 | 0.28223 |
| Sanem | 0.40735 | 0.1923 | 0.4325 | 0.94902 | **0.0098** | 0.61927 | 0.34174 |

**Supplementary table 3 (continued).** Hardy-Weinberg results for within commune/district tests. Underlined values describes significance of the raw exact Monte Carlo p-values and values in bold describes significance after Benjamini-Hochberg correction.

B) *S. pipiens* in Cologne

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| LOCUS | Spp010 | Spp053 | Spp080 | Spp142 | Spp231 | Spp273 | Spp476 |
| DISTRICT |  |  |  |  |  |  |  |
| Chorweiler | 0.12752 | 0.60563 | 0.150394 | 0.10686 | 0.53282 | 0.88323 | 0.99145 |
| Ehrenfeld | 1 | 0.02225 | 0.020745 | 0.91336 | 0.34489 | 0.51535 | 0.82652 |
| Innenstadt | 0.28081 | 0.04494 | 0.093206 | 0.7953 | 0.83661 | 0.30467 | 0.34926 |
| Kalk | 0.02072 | 0.85614 | 0.011822 | 0.9951 | 0.72311 | 0.52521 | 0.72626 |
| Lindenthal | 1 | 0.5187 | 0.301973 | 0.31506 | 0.24409 | 0.08357 | 0.32403 |
| Mülheim | 0.50703 | 0.25692 | 0.099152 | 0.0474 | 0.97048 | 0.09012 | 0.31427 |
| Nippes | 0.79712 | 0.13555 | 0.753436 | 0.04397 | 0.1436 | 0.3169 | 0.49341 |
| Porz | 0.80316 | 0.08885 | 1 | 0.32349 | 0.80507 | 0.16589 | 0.95115 |
| Rodenkirchen | 0.73581 | 0.61336 | 0.371361 | 0.16444 | 0.0455 | 0.60921 | 0.13224 |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| LOCUS | Spp051 | Spp108 | Spp141 | Spp313 | Spp360 | Spp391 | Spp416 |
| DISTRICT |  |  |  |  |  |  |  |
| Chorweiler | 0.52708 | 0.11054 | 0.130841 | 0.12865 | **0.00732** | 0.21973 | 0.97576 |
| Ehrenfeld | 0.24925 | 0.43465 | **0.006584** | 0.60381 | 0.26081 | 0.67129 | 0.13751 |
| Innenstadt | 0.80358 | 0.26216 | 0.803424 | 0.62112 | 0.87794 | 0.21896 | 0.79259 |
| Kalk | 0.37096 | 0.50108 | 0.147073 | 0.13964 | 1 | 0.77844 | 0.11707 |
| Lindenthal | 0.09665 | 0.52755 | **0.000811** | 0.05372 | **0.0009** | 0.75324 | 0.09903 |
| Mülheim | 0.12872 | 0.04677 | **0.000504** | 0.20815 | 0.03027 | 0.27453 | 0.34293 |
| Nippes | 0.03816 | 0.24386 | 0.062558 | 0.86321 | 0.14086 | 0.60568 | 0.37727 |
| Porz | 0.29718 | 0.92317 | **4.80E-05** | 0.59336 | **0.00266** | 0.72204 | 0.21004 |
| Rodenkirchen | 0.25726 | 0.02631 | 0.099492 | 0.51345 | 0.00092 | 0.01676 | 0.34083 |

**Supplementary table 3 (continued).** Hardy-Weinberg results for within commune/district tests. Underlined values describes significance of the raw exact Monte Carlo p-values and values in bold describes significance after Benjamini-Hochberg correction.

C) *M. florea* in Luxembourg

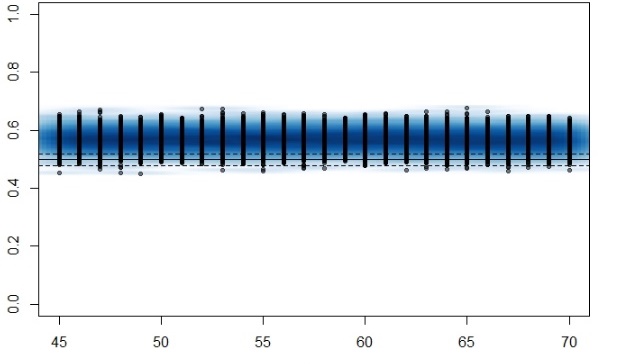
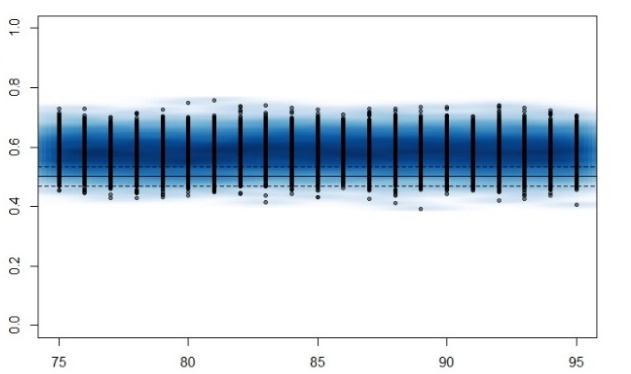
|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| LOCUS | Mfl239 | Mfl25 | Mfl265 | Mfl270 | Mfl303 | Mfl59 | Mfl130 | Mfl197 |
| COMMUNE |  |  |  |  |  |  |  |  |
| Bettembourg | 0.07558 | 0.03681 | 0.55508 | 1 | **0.002023** | 0.0759 | 0.10143 | 1 |
| Hesperange | 0.02763 | 1 | 0.67279 | 1 | 0.106715 | 0.11435 | 0.01009 | 1 |
| Leudelange | 1 | 1 | 1 | 1 | **0.00111** | 1 | 0.71799 | 1 |
| Luxembourg | 0.11521 | 1 | 1 | 1 | 0.198534 | 0.51079 | 0.23935 | 0.0303 |
| Reckange.sur.Mess | 1 | 1 | 1 | 1 | 0.053632 | 0.72936 | 0.36682 | 0.1308 |
| Roeser | 0.25473 | 1 | 1 | 1 | 0.326839 | 0.34339 | 0.32761 | 1 |
| Strassen | **0.00619** | 1 | 0.55451 | 1 | 1 | 0.71896 | 0.31547 | 1 |
|  |  |  |  |  |  |  |  |  |
| LOCUS | Mfl36 | Mfl419 | Mfl432 | Mfl486 | Mfl492 | Mfl103 | Mfl26 | Mfl261 |
| COMMUNE |  |  |  |  |  |  |  |  |
| Bettembourg | 0.44377 | 0.79134 | 0.59938 | 0.321578 | 1 | 0.62913 | 1 | 0.04357 |
| Hesperange | 0.55053 | 0.38686 | 0.96488 | 0.482147 | 0.027192 | **0.0019** | 0.77069 | 0.16877 |
| Leudelange | 0.25204 | 0.14541 | 0.53412 | 0.206174 | 1 | 1 | 0.73694 | 0.13348 |
| Luxembourg | 0.01672 | 0.00923 | 0.19389 | 0.683537 | 1 | 0.0425 | 0.0407 | 0.02688 |
| Reckange.sur.Mess | 0.19539 | 0.1333 | 0.41685 | 0.676678 | 1 | 1 | 0.16447 | 0.11173 |
| Roeser | 0.6249 | 0.48451 | 0.09023 | 0.059087 | 1 | **0.00208** | 0.37182 | 0.29657 |
| Strassen | 1 | 0.30896 | 0.57766 | 0.505063 | 1 | 1 | 0.14976 | 0.32637 |
|  |  |  |  |  |  |  |  |  |
| LOCUS | Mfl263 | Mfl269 | Mfl28 | Mfl323 | Mfl457 | Mfl491 | Mfl56 | Mfl70 |
| COMMUNE |  |  |  |  |  |  |  |  |
| Bettembourg | 0.78548 | 1 | 0.11327 | 1 | 0.827774 | 0.50377 | 0.69959 | 0.04357 |
| Hesperange | 0.94047 | 1 | 0.18588 | 0.057074 | 0.052615 | 0.10988 | 0.5176 | 0.0431 |
| Leudelange | 0.18433 | 1 | 0.28086 | 0.779567 | 0.12357 | 0.03761 | 0.60136 | 1 |
| Luxembourg | 0.68909 | 0.69099 | **0.00044** | 0.046591 | 0.899094 | 0.11115 | 0.00895 | 0.06129 |
| Reckange.sur.Mess | 0.60471 | 1 | 0.38699 | 0.741548 | 0.390443 | 1 | 0.08808 | 1 |
| Roeser | 0.97177 | 1 | 0.03594 | 0.101381 | 0.309156 | 1 | 0.67393 | 1 |
| Strassen | 1 | 0.40337 | 0.56581 | 0.575691 | 0.811412 | **0.00518** | 0.87737 | 1 |

**Supplementary table 3 (continued).** Hardy-Weinberg results for within commune/district tests. Underlined values describes significance of the raw exact Monte Carlo p-values and values in bold describes significance after Benjamini-Hochberg correction.

D) *M. florea* in Cologne

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| LOCUS | Mfl239 | Mfl25 | Mfl265 | Mfl270 | Mfl303 | Mfl59 | Mfl130 | Mfl197 |
| DISTRICT |  |  |  |  |  |  |  |  |
| Chorweiler | 0.187385 | 1 | 0.463712 | 1 | **0.000664** | 0.697397 | 0.008297 | 0.617675 |
| Ehrenfeld | **0.000223** | 0.137541 | 1 | 1 | **0.000574** | 0.448749 | 0.874605 | 1 |
| Innenstadt | 1 | 1 | 0.23504 | 1 | **0.04258** | 0.578779 | 0.145654 | 1 |
| Kalk | 1 | 1 | 1 | 1 | **0.003848** | 0.134567 | 0.916517 | 1 |
| Lindenthal | 0.117569 | 0.110426 | 0.255473 | 1 | **3.90E-05** | 0.521362 | 0.062563 | 1 |
| Mülheim | 0.53351 | 1 | 0.259954 | 1 | **0.022502** | 0.430113 | 0.364526 | 0.034712 |
| Nippes | 0.467935 | 0.024438 | 1 | 1 | **0.022504** | 0.673133 | 0.185257 | 0.098858 |
| Porz | 1 | 1 | 0.779743 | 1 | **0.000197** | 0.899219 | 0.20642 | 0.097777 |
| Rodenkirchen | **0.00062** | 1 | 0.76402 | 1 | **0.002095** | 0.683952 | 0.952999 | 1 |
|  |  |  |  |  |  |  |  |  |
| LOCUS | Mfl36 | Mfl419 | Mfl432 | Mfl486 | Mfl492 | Mfl103 | Mfl26 | Mfl261 |
| DISTRICT |  |  |  |  |  |  |  |  |
| Chorweiler | 0.360955 | 0.259445 | 0.340875 | 0.528724 | 0.896736 | 0.561282 | 0.271476 | 0.014024 |
| Ehrenfeld | 0.266969 | 0.423088 | 0.103394 | 0.188563 | 0.010739 | 1 | 1 | 0.522125 |
| Innenstadt | 0.173158 | 0.995328 | 0.212558 | 0.559298 | 0.097795 | 0.025502 | 1 | 0.284256 |
| Kalk | 0.702266 | 0.65358 | 0.746243 | 0.423449 | 0.440705 | 0.630899 | 0.853903 | 0.076917 |
| Lindenthal | 0.039085 | 0.321135 | 0.612372 | 0.831892 | 0.820113 | 0.088288 | 0.343321 | 0.260863 |
| Mülheim | 0.496119 | 0.048248 | 0.761891 | 0.938751 | 0.485052 | 0.7515 | 0.111797 | 0.08677 |
| Nippes | 0.465714 | 0.309903 | 0.091547 | 0.882453 | 0.429053 | 0.631676 | 1 | **0.000719** |
| Porz | 0.687181 | 0.548659 | 0.738018 | 0.190963 | 0.156992 | 0.148395 | 0.159586 | 0.531328 |
| Rodenkirchen | 0.078088 | 0.468644 | 0.498658 | 0.539762 | 0.575624 | 1 | 0.440321 | 0.242125 |
|  |  |  |  |  |  |  |  |  |
| LOCUS | Mfl263 | Mfl269 | Mfl28 | Mfl323 | Mfl457 | Mfl491 | Mfl56 | Mfl70 |
| DISTRICT |  |  |  |  |  |  |  |  |
| Chorweiler | 0.115321 | 0.262396 | 0.035804 | 0.349732 | 0.816721 | 0.066249 | 0.199926 | **0.004995** |
| Ehrenfeld | 0.153483 | 0.022494 | 0.080211 | 0.061459 | 0.291601 | 1 | 0.175751 | **0.027115** |
| Innenstadt | 1 | 1 | 0.099554 | 1 | 0.932374 | 0.343226 | **0.02294** | 0.168998 |
| Kalk | 0.689417 | 0.26974 | 0.189664 | 0.197719 | 0.54294 | 0.636117 | **0.005139** | 1 |
| Lindenthal | 0.477847 | 1 | 0.013073 | 0.415752 | 0.79858 | 0.938806 | **0.010607** | **0.014454** |
| Mülheim | 0.495912 | 0.345558 | 0.80957 | 0.912547 | 0.261778 | 0.073996 | 0.371235 | **0.001256** |
| Nippes | 0.550962 | 0.073153 | 0.411703 | 0.655542 | 0.852669 | 0.432001 | **0.004846** | 0.080643 |
| Porz | 0.860106 | 0.384907 | 0.045418 | 0.015527 | 0.807261 | 0.889576 | **0.007018** | 0.060243 |
| Rodenkirchen | 0.580439 | 1 | 0.161837 | 0.33633 | 0.534054 | 0.006784 | 0.624522 | **0.005602** |

*M. florea S. pipiens*

Number of PCA axes retained

Proportion of successful outcome prediction

**Supplementary figure 1.** DAPC cross-validation results. The solid and dashed lines represent the median and confidence interval for a random chance classifier; high overlap between this interval and the estimated values highlights poor performance.