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

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**ABSTRACT**

Hoverflies (Syrphidae) are essential pollinators, and their severe decline jeopardizes their enormous 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 investigate the spatial genetic structure and spatial variation in genetic diversity of two species of hoverflies in two urban areas. Using thousands of specimens collected by hand netting from two Western Europe urbanized study areas of 490 km2 and 460 km2 in 2021, we identified XX and 24 microsatellite SNP loci for *Syritta pipiens* and *Myathropa florea*, respectively. Using STRUCTURE, DAPC and IBD analyses, we found evidence for high genetic connectivity for both species, suggesting effective dispersal at scales larger than metropoles, despite urbanization. Although anthropogenic land cover changes generally have dramatic consequences on biodiversity, some 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 a key ecosystem service to agricultural crops and wild plants, but they are declining across the world. 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 of 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 clear-cut: 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, and the spread 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 many of pollinator habitats (Seibold et al., 2019). Because of demographic growth, anthropogenic development is expected to increase, which will lead to further loss and fragmentation of natural and semi-natural habitats (Jaeger et al., 2016). In order to counteract the negative effects of habitat fragmentation however, it is important to understand the functional connectivity of the landscape from the viewpoint of the pollinator (Dreier et al., 2014; Rands, 2014). However, there is still a considerable lack of knowledge on the mechanisms underlying the responses of invertebrate pollinators to fragmentation resulting from land-use change (Dicks et al., 2013; Gill et al., 2016; Simmons et al., 2019; Winfree et al., 2011).

Although dispersal is a key trait to deal with habitat fragmentation, we only have a limited understanding of dispersal for most insect pollinators.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 ability generally are better able to move efficiently between suitable habitat patches and may exploit fragmented resources more efficiently (Öckinger et al., 2010). Nevertheless, the results from work on Apiformes suggest that even good dispersers can be impacted by habitat fragmentation. Bumblebee (Bombus) species normally exhibit very little 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). Even at larger spatial scales, urban areas can be a substantial gene flow barrier for pollinators (Davis et al., 2010). A particular difficulty with evaluating the impact of land-use change relates to the fact that flying ability and response to habitat fragmentation differs significantly between pollinators, 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, but they are understudied relative to bees, and little is known about their dispersal and their response to landscape fragmentation. Hoverflies are a biologically very diverse family of flower-visiting flies (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.

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 a quantification of gene flow, 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.

In this study, we investigated the genetic diversity, structure, IBD, isolation-by-environment (IBE) and demographic history of two species of hoverflies, *S. pipiens* and *M. florea*, based onthousands of individuals in two urbanized landscapes in Western Europe.We expected some population-wide and fine-scale genetic structure due to the large extent and the anthropogenic nature of the study areas, notably the large 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. We chose an extent o of around 400km2 for each study area. This specific extent is a key parameter because it should allow 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), notably stimulated 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 (Fig. 1) 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 *Syritta pipiens* (Linnaeus, 1758) and *Myathropa florea* (Linnaeus, 1758), 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 massive 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 detect 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). We caught 831 and 1226 *S. pipiens* individuals, and 559 and 394 *M. florea* individuals in Cologne and the Luxembourg study area, respectively (Fig. 1).

## | Laboratory procedures

DNA was 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 [*Syritta 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 Myathropa 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 *Syritta pipiens* we retained 14 microsatellite loci that were amplified in two multiplex PCRs, while the 24 microsatellite loci for *Myathropa florea* were amplified in three multiplex recations (Table 1)

**Figure 1** [PLACEHOLDER]. Maps of Cologne and Luxembourgshowing the diversity and quantity of disturbed land covers. White circle represent *S. pipiens* samples and black squares represent *M. florea*.

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 Syritta pipiens and at least 18 loci for Myathropa florea. Extreme outliers based on a preliminary PCA analysis were sent to sequencing to verify their species identification (Sup. X). They all belonged to our target species and were kept in the dataset

## | Genetic diversity

We conducted all analyses in this manuscript in R (R Core Team, 2022) using RStudio (RStudio Team, 2022), except for the STRUCTURE and BAPS analyses. We conducted basic analyses of our genetic datasets using the *adegenet* v. 2.1.7 (Jombart, 2008; Jombart and Ahmed, 2011) ,the *hierfstat* v.0.5.11 (Goudet, 2005), the *pegas* v. 1.1 (Paradis, 2010), R packages. We evaluated allelic richness, heterozygote deficiency, overall fixation indices with bootstrap confidence interval, fixations indices per locus, and the pairwise genetic distance between our study areas.

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 (Kamvar et al., 2014) R package 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 (Adamack and Gruber, 2014) R package. We considered that observed estimates of null allele frequency higher than 0.1 were problematic. The percentage of missing data was 2.24% for *S. pipiens* and 2.93% for *M. florea*.

## | Isolation-by-distance

To explore whether isolation-by-distance (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 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 calculated Loiselle’s kinship using the *EcoGenetics 1.2.1-6* R 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, which is admittedly less conservative than other options, giving us more chance of detecting spatial structure.

## | Isolation-by-environment

## | 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 non-correlated allele frequencies. An important parameter to set it α, the Dirichlet prior parameter for the degree of admixture (Hubisz et al., 2009) which conceptually represents the number of ancestral populations from which each individual’s alleles originate. We set the inference of a different α for each study area and allowed unequal representation of source populations in the sample (alternative ancestry prior). We also set initial values of α it to 1/K because this parametrization led to lower average assignment errors in a simulation study (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 five clusters. To determine the most likely number of clusters, we directly compared log-likelihoods for all *K* values across the ten runs, and we used the Δ*K* statistic which is based on the rate of change in the log probability of data given *K* (Evanno et al., 2005). We also surveyed the variation in STRUCTURE outputs and matched clusters across runs to avoid issues with label change and multimodality, using STRUCTURE HARVESTER (Earl and vonHoldt, 2012) and CLUMPAK (Kopelman et al., 2015). Second, we used BAPS (Cheng et al., 2013; Corander et al., 2008) and used both the spatial and non-spatial algorithms.

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 (2 study areas = 2 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 want 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.

# RESULTS

## | Genetic diversity and isolation-by-distance

Prior to running genetic structure analyses, we decided to remove only loci that presented both linkage disequilibrium and extreme heterozygote deficiency, as well as a frequency of null alleles at a locus significantly different from zero. Therefore, we removed one locus from the *S. pipiens* dataset (Spp141). 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*).

Regarding *S. pipiens*, while there is significant IBD between study areas when using the whole dataset, it is very low and has 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-resistance and clustering

Bayesian ancestry inference using STRUCTURE support *K*=1 for *S. pipiens* and *K*=2 with systematic partial assignments to two non-geographic clusters for *M. florea* . Different approaches to choose *K* generally supported *K*=2 for *M. florea* (Table 1) whereas one to three clusters were selected for *S. pipiens*. Importantly, inferred clusters were not geographically meaningful at all, with extreme genetic mixing as almost all individuals assign to both clusters with the second one representing only a minor fraction of the genetic variation within all individuals (Table 1; Fig. 2). There was no substantial difference in cluster assignment between areas.

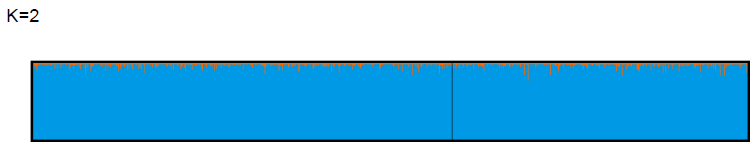
BAPS XXX

The single discrimination functions (responsible to distinguish clusters) for *a priori* DAPC showed a lot of overlap for both species (Fig. 3). 50 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).

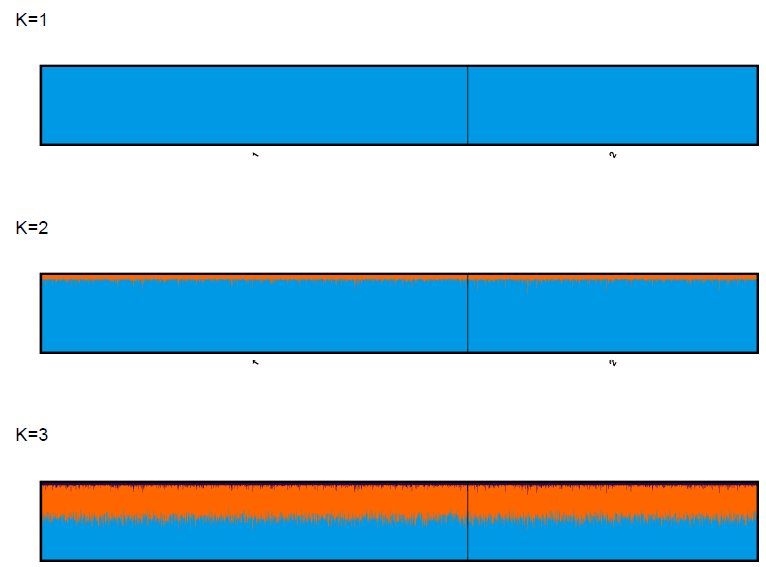
**Figure 2.** Log likelihood graphs

***Myathropa florea***

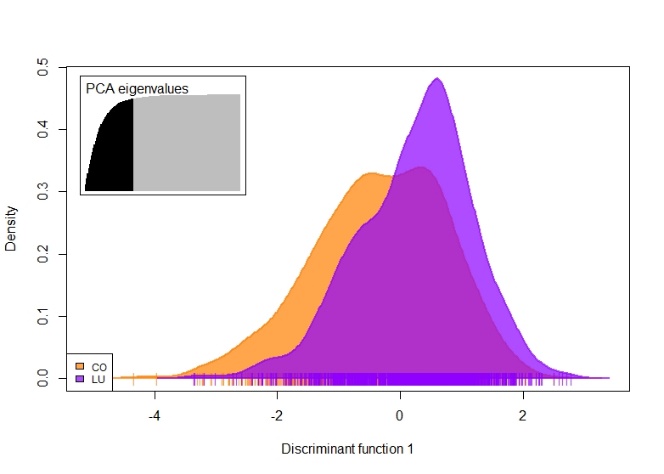
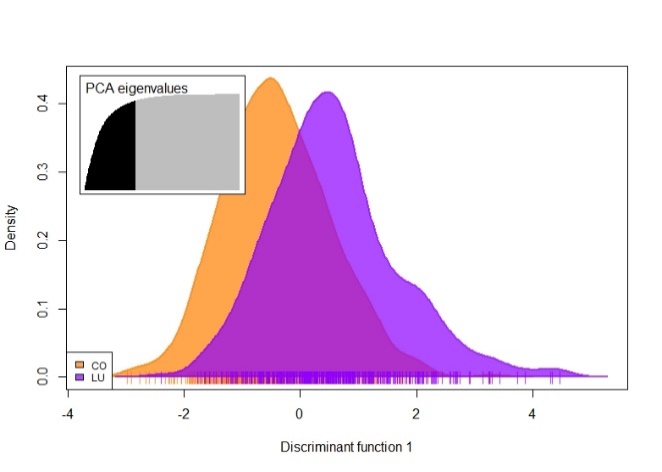
A)



Cologne Luxembourg

***Syritta pipiens***

Luxembourg Cologne



B)

Density

Discrimination function

**Figure 3.** A) Average cluster assignment the best solutions *K*=2 for *M. florea*; B) *K*=1, *K*=2, and *K*=3 for *S. pipiens*. B) 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% (*M.* florea) and 70% (*S.* pipiens) individuals from Luxembourg.

# 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 present 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.

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 suggest 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 did (wrongly) predict that 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 issue is that both the IBD and the structure detection results could be artefacts of high effective population size. Have a look at my 2009 paper on ibd and structure. The higher the effective density, the lower the IBD pattern. AAlthough simulations could help us better understand whether we could detect change rapidly, parameterization for non-model insect species with potentially huge population sizes.Similarly, there may be an effect of the landscape but the effective population size of these things will be too large for genetic drift to have an effect (relative to the time scale of the disturbance). You could mention that relatedness-based resistance modelling might detect an effect earlier (see Landguth et al. (2010) ). However, we cannot apply these methods here as not IBD to work with.

## | 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 that 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.

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**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 2**.

**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 | MF239 | MF25 | MF265 | MF270 | MF303 | MF59 | MF130 | MF197 |
| 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 | MF36 | MF419 | MF432 | MF486 | MF492 | MF103 | MF26 | MF261 |
| 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 | MF263 | MF269 | MF28 | MF323 | MF457 | MF491 | MF56 | MF70 |
| 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.

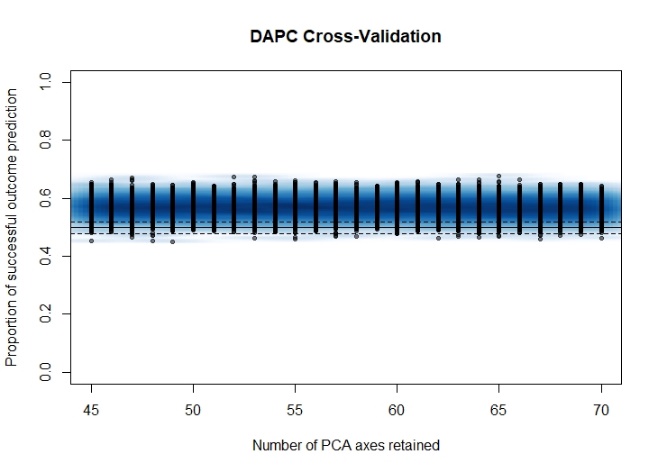
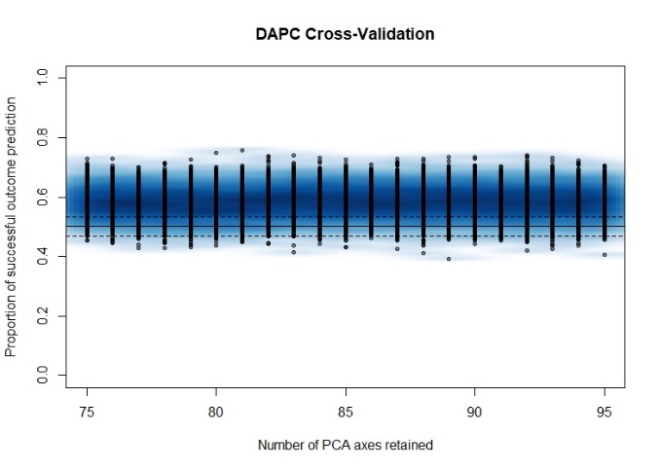
D) *M. florea* in Cologne

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| LOCUS | MF239 | MF25 | MF265 | MF270 | MF303 | MF59 | MF130 | MF197 |
| 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 | MF36 | MF419 | MF432 | MF486 | MF492 | MF103 | MF26 | MF261 |
| 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 | MF263 | MF269 | MF28 | MF323 | MF457 | MF491 | MF56 | MF70 |
| 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** |

**Supplementary table 4**. Clustering solutions and derivatives for values of *K* from 1 to 7, over ten runs for each *K* value. SD refers to standard deviation. Rates of changes are given as means. Δ*K* is calculated as the ratio of the absolute value of the second order rate of change over the standard deviation of the logarithm of the likelihood of K given the data.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Myathropa florea*** | |  |  |  |  |  |
| ***K*** | **Mean Log *Pr*(data|*K*)** | **SD Log *Pr*(data|*K*)** | **Median-based *Pr*(data|*K*)** | **Rate of change** | **Absolute 2nd order rate of change** | **Δ*K*** |
| 1 | -45682.48 | 0.114 | <0.0001 | *NA* | *NA* | *NA* |
| 2 | -45608.06 | 1.84 | 1 | 74.42 | 584.32 | 317.91 |
| 3 | -46117.96 | 397.91 | <0.0001 | -509.9 | 437.18 | 1.10 |
| 4 | -46190.68 | 616.80 | <0.0001 | -72.72 | 50.86 | 0.082 |
| 5 | -46212.54 | 293.07 | <0.0001 | -21.86 | 113.12 | 0.39 |
| 6 | -46347.52 | 153.69 | <0.0001 | -134.98 | 747.92 | 4.87 |
| 7 | -47230.42 | 680.77 | 0 | -882.9 | *NA* | *NA* |
| ***Syritta pipiens*** | |  |  |  |  |  |
| ***K*** | **Mean Log *Pr*(data|*K*)** | **SD Log *Pr*(data|*K*)** | **Median-based *Pr*(data|*K*)** | **Rate of change** | **Absolute 2nd order rate of change** | **Δ*K*** |
| 1 | -65213.43 | 0.0675 | 1 | *NA* | *NA* | *NA* |
| 2 | -66201.97 | 1607.07 | <0.0001 | -988.54 | 1584.29 | 0.99 |
| 3 | -65606.22 | 35.64 | <0.0001 | 595.75 | 1039.33 | 29.16 |
| 4 | -66049.8 | 193.78 | 0 | -443.58 | 702.34 | 3.62 |
| 5 | -67195.72 | 474.23 | 0 | -1145.92 | 332.66 | 0.70 |
| 6 | -68008.98 | 610.14 | 0 | -813.26 | 350.20 | 0.57 |
| 7 | -69172.44 | 1435.84 | 0 | -1163.46 | *NA* | *NA* |

*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.