**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 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).

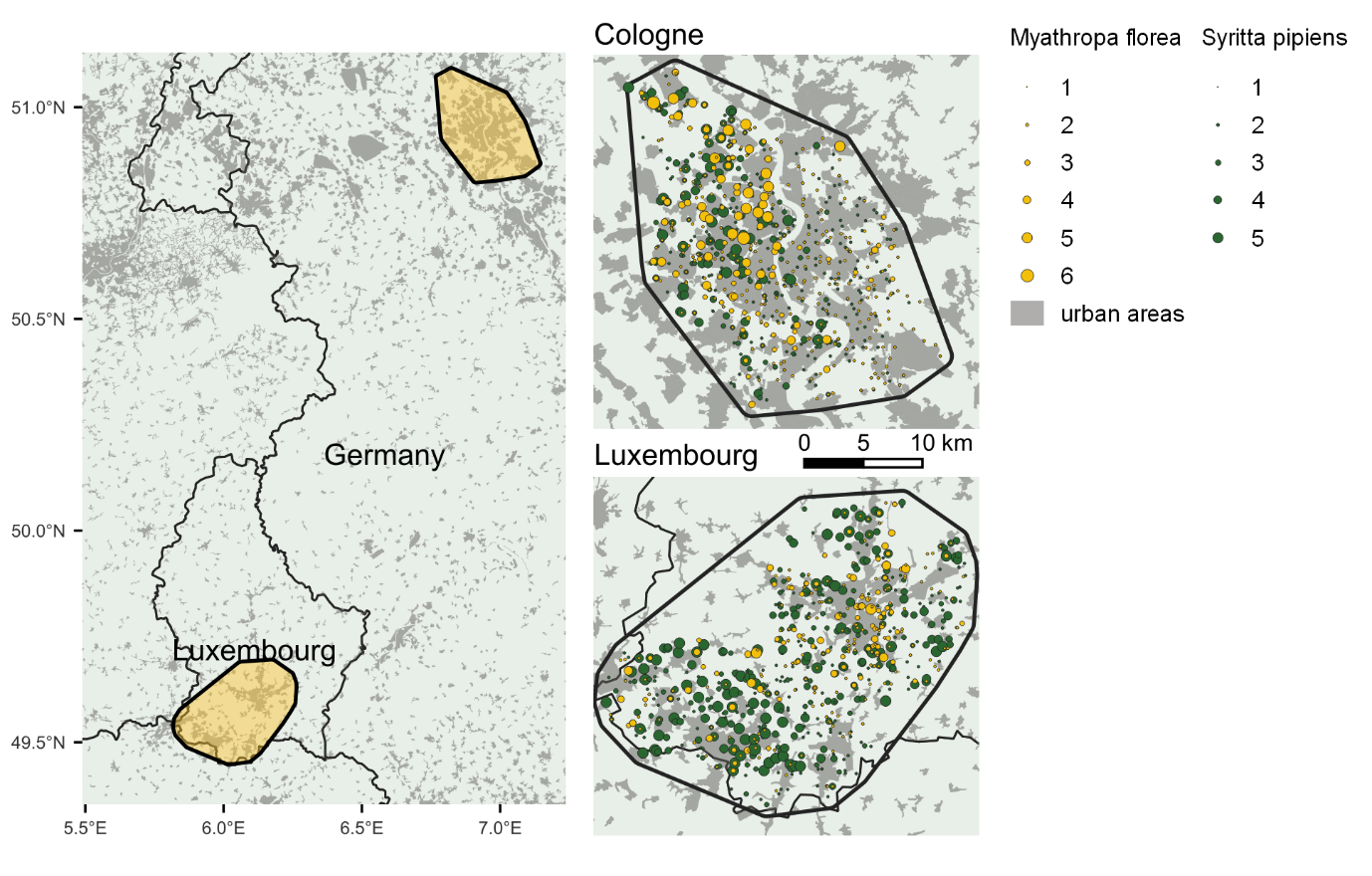
In this study, we investigated the genetic diversity, structure, IBD, isolation-by-environment (IBE) of two species of hoverflies, *Syritta pipiens* (Linnaeus, 1758; henceforth abbreviated to SP) and *Myathropa florea* (Linnaeus, 1758; MF), based onthousands of individuals in two urbanized landscapes 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 v. 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 (HWE), we conducted 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. We used this liberal threshold because we also tested HWE in an aspatial test (no pooling per commune/districts) considering the scenario where there is no population structure and no loci was considered in disequilibrium.

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 *FST* 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., 2008a, 2008b). 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 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 to avoid overfitting while preserving discrimination power given that the first few components represent most of the genetic variation. 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

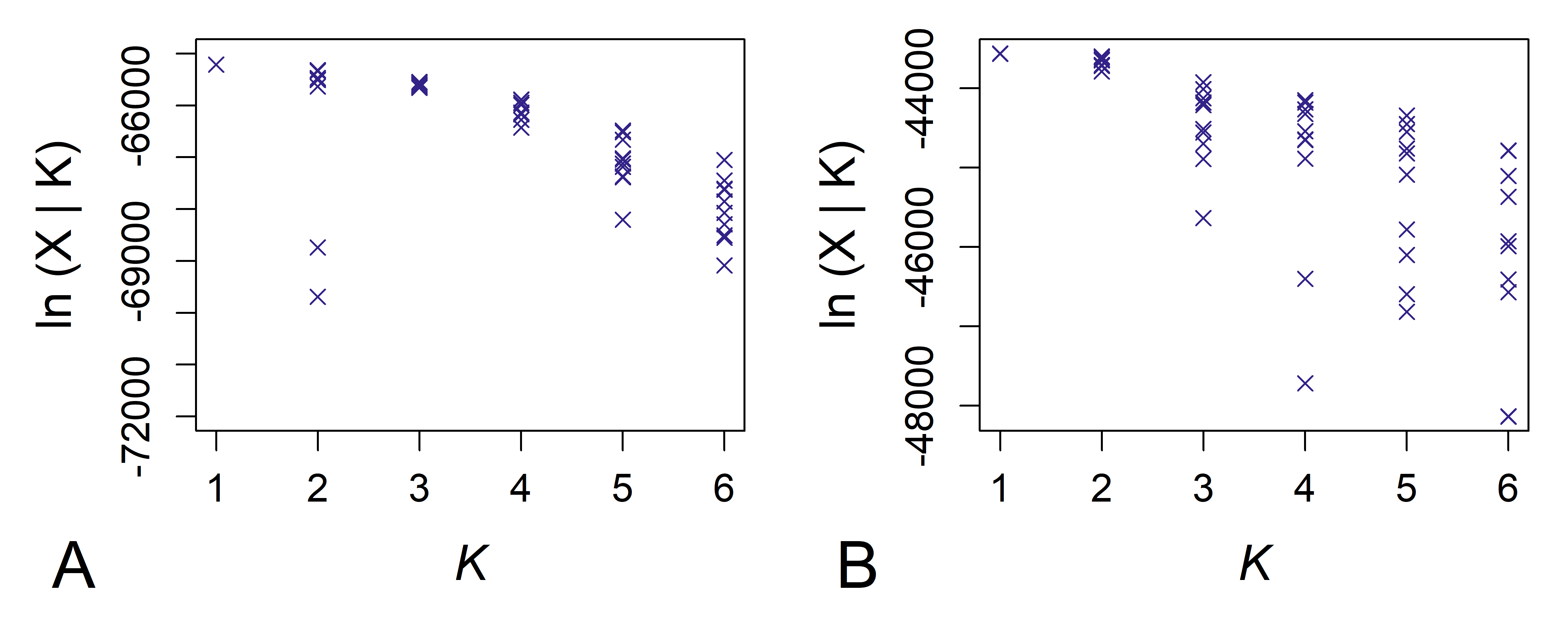
Generalised Dissimilarity Modelling (GDM) was employed to simultaneously test for isolation by geographic and environmental distance with the *gdm* R package v. 1.5.0-9.1 (Fitzpatrick, M et al., 2022). By performing matrix regression, the approach fits non-linear relationships between the response variable, 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 (QGIS Development Team, 2022) 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

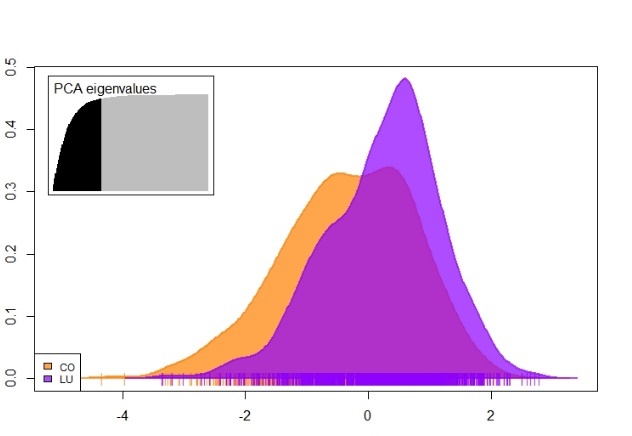
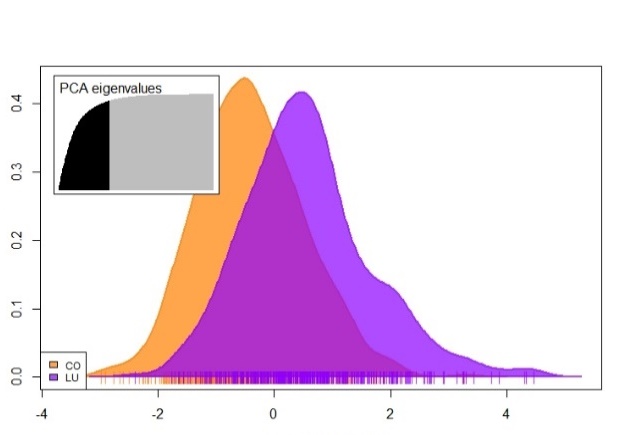
A total of 831 and 1226 *S. pipiens*, and 559 and 394 *M. florea* individuals were caught in Cologne and the Luxembourg study area, respectively (Fig. 1). The locus Spp141 was removed from the *S. pipiens* dataset due to heterozygote deficiency (possible null alleles) and linkage disequilibrium. The 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).

STRUCTURE analyses support *K* = 1 in both 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., 2022). 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. A 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.

The high effective dispersal ability of *M. florea* suggested in our study suggests could spread promptly in new suitable landscapes. species could become established quickly once introduced. *M. florea* has 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) which supports our conclusion that dispersal ability may be high in this species. Non-native hoverflies can potentially outcompete native species due to their high polyphagy, territoriality, and dispersal abilities (Doyle et al., 2020; Hong et al., 2012; Thompson, 2008; Wellington and Fitzpatrick, 1981). Understanding connectivity is key to understand, prevent, and mitigate the negative impacts of those introductions.

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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 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

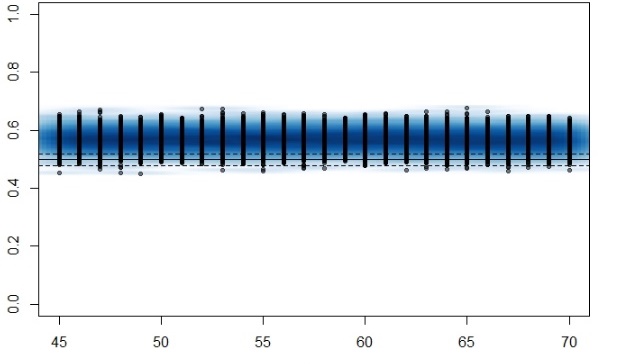
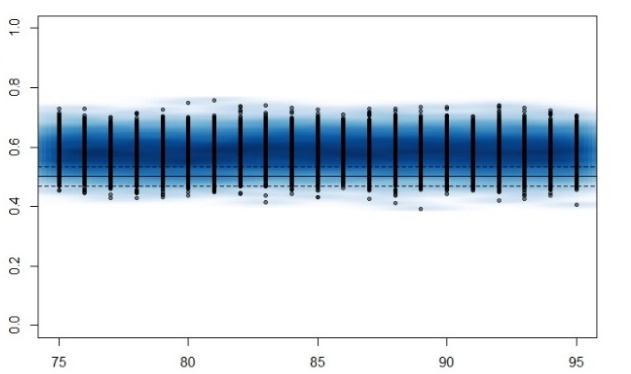
|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| LOCUS | Mfl239 | Mfl25 | Mfl265 | Mfl270 | Mfl303 | Mfl59 | Mfl130 | Mfl197 |
| COMMUNE |  |  |  |  |  |  |  |  |
| Bettembourg | 0.07558 | 0.03681 | 0.55508 | 1 | **0.002023** | 0.0759 | 0.10143 | 1 |
| Hesperange | 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.