Strong gene flow across an urbanized coastal landscape in a dune specialist digger wasp

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Abstract

Genetic connectivity is often disrupted by anthropogenic habitat fragmentation, and therefore often a focus in landscape-scale conservation. Landscape genetics methods allow for studying functional connectivity in heterogenous landscapes in detail to inform conservation measures for a species' regional persistence. Yet, for insects, functional connectivity through landscape genetics remains largely unexplored. We studied the functional connectivity in the dune-specialist digger wasp *Bembix rostrata* in a human-altered coastal region in Belgium based on landscape genetics methods. We used an optimization approach to correlate individual genetic distances with landscape resistance distances to deduce the conductance of natural and anthropogenic landscape categories to gene flow. Overall, the populations of this dune-specialist insect are genetically well-connected. Through multi-model inference we could detect—on top of the prominent background process of isolation-by-distance—a weak but consistent signal of urban features facilitating gene flow. However, because urbanisation leads to larger scale fragmentation, its impact on the distribution of populations in the landscape and related effective regional gene flow remains substantial. We discuss the results in the context of movement behaviour and conservation. As this species depends on early-succession dune vegetations, restoring and increasing sand dynamics at the local and landscape scale should be the focus of conservation aimed at the regional species' persistence. This would be more effective for *B. rostrata* than trying to increase habitat connectivity at the landscape scale in the focal human-altered dune ecosystem.

Keywords: landscape genetics, landscape resistance modelling, resistanceGA, resistance optimization, dispersal, Hymenoptera, Crabronidae

Introduction

Movement of individuals through landscapes is an inherent component of dispersal and can lead to exchange between populations, resulting in gene flow and connectivity. Connectivity can ultimately support a species' regional persistence, as it maintains or supports a wide range of natural processes, such as resource use, demographic rescue, genetic diversity, range expansion, or metapopulation dynamics (Hanski 1998; Crooks and Sanjayan 2006; McRae et al. 2008; Hodgson et al. 2022). The environment in between habitat patches (i.e. the matrix) can impede or facilitate the movement or dispersal of an organism and is in reality more heterogenous than a practical binary simplification of habitat versus homogeneous matrix, especially in human-altered landscapes (Hein et al. 2003; Manel and Holderegger 2013). How organisms react to unfamiliar or inhospitable habitat in fragmented landscapes can be manifold and depends on the specific landscape context and varies between species and individuals (Baguette and Van Dyck 2007; Knowlton and Graham 2010). More than the structural connectivity—the landscape structure itself, independent from any organism—or distances between populations, such behavioural responses will determine the realized connectivity between populations. The functional connectivity—the connectivity from the species' perspective (Tischendorf & Fahrig, 2003)—is therefore crucial to understand the dynamics of spatially structured populations (or metapopulations) and to inform effective conservation measures (McRae 2006).

A general view on functional connectivity is that many organisms will avoid unfamiliar habitat altogether—they exhibit high reluctance to cross gaps and favour instead considerable detours—making unfamiliar habitat a barrier to movement (Develey and Stouffer 2008; Knowlton and Graham 2010; Driscoll et al. 2013). This is the general assumption in for instance least cost mapping (Adriaensen et al. 2003). Unfamiliar habitat can, however, also act as a facilitator to movements when search behaviour of individuals in unfamiliar habitat is more directional—and thus faster—than in favoured habitat (Van Dyck and Baguette 2005; Schtickzelle et al. 2006; Kuefler et al. 2010; Knowlton and Graham 2010). Or conversely, movements in more suitable habitats can be impeded

because of the availability of resources, leading to less directed—and thus slower—exploratory movements than in unfamiliar habitat (Van Dyck and Baguette 2005; McRae et al. 2008; Keller et al. 2012). Independent of the exact mechanism behind this potential (relative) increase in movement through the matrix, the eventual functional connectivity will remain dependent on the distance between habitats and thus the scale of the fragmentation. The same matrix typology or landscape category can therefore potentially facilitate or constrain connectivity in different landscapes, depending on the eventual distance-related costs in terms of survival and energy-use (Segelbacher et al. 2010; Spear et al. 2010; Bonte et al. 2012; Richardson et al. 2016; Haran et al. 2017).

How a landscape influences the mobility of an organism has implications for considering regional conservation measures in human-altered landscapes. If animals show high reluctance towards crossing habitat boundaries, creating corridors might be an effective structural connectivity measure to increase functional connectivity between subpopulations. Such regional landscape-scale conservation measures, including corridors and stepping-stones, which are based on logical and sound scientific principles, have been widely embraced by conservation practitioners and policy-makers (Crooks and Sanjayan 2006; Watts et al. 2016). However, there is much debate about the effectiveness of landscape-scale conservation and the emphasis on increasing connectivity for species' regional persistence in conservation decisions (Hodgson et al. 2009; Humphrey et al. 2015; Watts et al. 2016). The emphasis of practitioners and policy-makers on landscape-scale conservation is probably biased by a focus towards larger terrestrial vertebrates, for which structural connectivity—such as corridors—seems effective (Humphrey et al. 2015). Empirical evidence for many other groups is more limited and equivocal (Ovaskainen et al. 2008; Watts et al. 2016). For instance, it is expected that for many insect species other processes might dominate the regional persistence of spatially structured populations, such as demographic processes or effective population sizes (Richardson et al. 2016; Drake et al. 2022).

In such cases, site-based conservation focusing on improving habitat area and quality could be more effective than increasing structural connectivity on the landscape-scale. To effectively direct the limited resources available for conservation measures towards implementations with the highest success rate or biodiversity gain, implementations should be founded in empirical evidence (Sutherland et al. 2004; Ferraro and Pattanayak 2006; Samways et al. 2020). Especially for insects—whose decline and biological value get widespread attention (Didham et al. 2020; Wagner et al. 2021)—scientific evidence on the effects of landscapes on functional connectivity and gene flow is still limited (Keyghobadi et al. 1999; Wilcock et al. 2007; Keller et al. 2012; Pérez-Espona et al. 2012; Watts et al. 2016; Haran et al. 2017; Trense et al. 2021).

An indirect way of measuring functional connectivity is through molecular genetic data. With landscape genetics methods, one can quantify the effects of landscape composition between habitat patches on gene flow between populations (Manel et al. 2003; Manel and Holderegger 2013; Balkenhol et al. 2015). Patterns of gene flow reflect realized or successful dispersal and depend on post-dispersal survival and reproduction (Spear et al. 2010). Landscape genetics methods allow for inferences regarding connectivity without the need to collect individual movement data. Collecting such field data—for example from telemetry or mark-recapture methods—requires intensive effort or is even unfeasible for many organisms, such as the majority of insects (Spear et al. 2010; Batsleer et al. 2020). Only some larger insect species have been employed with tracking devices, and even for these, the biases on the resulting measurements remain insufficiently considered (Batsleer et al. 2020). Both mark-recapture and telemetry rarely detect, and thus underestimate, long-distance dispersal (Ugelvig et al. 2012; Trense et al. 2021; De Ro et al. 2021). The advancing tools in molecular and landscape genetics can help us understand connectivity and landscape use in species for which direct estimates of habitat use and movement are difficult to obtain with more classical field methods (Spear et al. 2010).

An interesting ecosystem to thoroughly study functional connectivity and its consequences for nature management is coastal dunes. These dune habitats harbour a specific insect biodiversity typical for early-succession and pioneer vegetations subject to very dry and hot conditions, nourished by the natural processes of wind and sand dynamics (Maes and Bonte 2006). Coastal habitats in northwest Europe, and more specifically Flanders (Belgium), have gone through extensive landscape changes and fragmentation during the past century (Provoost et al. 2011). Coastal dunes in Flanders are geological young formations with calcareous sandy soils forming a narrow, linear ecosystem along the coast (Provoost et al. 2004; Decleer 2007). The fragmentation of coastal sandy habitats had two main impacts: loss of total area and decrease in habitat quality of the remaining patches. Urbanization from the interbellum period onwards decreased the total dune area by half during the previous century and separated the major dune entities physically (Provoost and Bonte 2004). The resulting obstruction of wind and sand dynamics, combined with the loss of agricultural practices (such as grazing of livestock), stimulated the succession and scrub development, decreasing the amount of open dune habitats (Provoost et al. 2011). Large herbivores have been introduced in many coastal dune reserves to revitalize dune dynamics as a substitute for natural wind dynamics, but have—due to trampling—a variable effect on local arthropod species (Bonte and Maes 2008; van Klink et al. 2015; Batsleer et al. 2022c). Altogether, coastal dunes in Flanders are a human-altered landscape with complex nature management considerations to take into account in both landscape scale and site-based nature management. In such a landscape, understanding functional connectivity—with its many specialist dune species—is vital to make informed conservation decisions.

Here, we study the genetic (functional) connectivity of a dune-habitat specialist digger wasp *Bembix rostrata* in a human-altered coastal landscape in Belgium using landscape genetics methods. Although a population genetics study shows that the subregion is genetically well connected (Batsleer et al. 2022a), the influence of the surrounding landscape on genetic relatedness between individuals remains unexplored. We use a resistance raster approach to quantify resistance values of

the distinctive landscape types in our study area. The resistance values of each landscape type are established through an optimization algorithm, which maximizes correlation between the landscape resistance distances and observed genetic distances between all pairwise individuals. All possible combinations of landscape categories were optimized to be able to perform multi-model inference. This allows us to deduce possible barriers or facilitators in the landscape for this dune-specialist digger wasp. Based on this estimate of functional connectivity, we can make recommendations on the balance between landscape- and site-based conservation.

Material & Methods

Study species

Bembix rostrata (Linnaeus, 1785; Hymenoptera, Crabronidae, Bembicinae) is a univoltine dune-specialist, gregariously nesting digger wasp from sandy habitats with sparse vegetation, sensitive to grazing management (Larsson 1986; Klein and Lefeber 2004; Bonte 2005; Batsleer et al. 2022c). Adults are active in summer, showing protandry: females are immediately mated upon emergence by the guarding males, who emerge one to five days earlier (Wiklund and Fagerström 1977; Schöne and Tengö 1981; Evans and O'Neill 2007). Females show brood care: one individual constructs one nest burrow at a time in which it progressively provisions a single larva with flies (Nielsen 1945; Field et al. 2020). An estimated of up to 5 nests (in northern regions) are produced, each with one offspring (Larsson and Tengö 1989). There are no overlapping generations, as the species overwinters as prepupa and there is only one generation per year. *Bembix rostrata* is often considered to be a philopatric species, as they do not easily colonize vacant habitat and exhibit site fidelity throughout the nesting season (Nielsen 1945; Larsson 1986; Bogusch et al. 2021; Batsleer et al. 2022b). However, a population genetics study across Belgium shows that this does not preclude gene flow between existing populations, including dispersal to distant, more isolated populations (Batsleer et al. 2022a).

Genetic sampling

Sampling took place in the summer of 2018 in the dunes along the Belgian west coast (Fig. 1), from the French-Belgian border (De Panne) to the Yser estuary (Nieuwpoort), stretching 14 km along the coast and reaching maximum 4 km inland. As nests are clustered together at nesting aggregates in grey dunes (EU Habitats Directive habitat 2130), we tried to spatially cover all known nesting locations in the landscape and locally took samples spread within and across nesting areas. The coordinates of each individual sample were saved. Only females were sampled, to solely use diploid individuals in the genetic analyses for this haplodiploid mating system. To minimize the impact of sampling, we used a non-lethal sampling procedure with wing clips, a method shown to produce

good-quality DNA for microsatellite PCR amplification (Châline et al. 2004). Forewing tips from live digger wasps were cut on both sides and stored in absolute ethanol, kept at 4°C after each day of sampling and transferred to a freezer (-18°C) for longer term storage.

DNA extraction and PCR amplification was performed as described in (Batsleer et al. 2022a) and summarized hereafter. DNA is extracted from the wing tips with a protocol based on Chelex (Biorad). 33 polymorphic microsatellites are PCR-amplified for each sample and electropherograms are scored using Geneious Prime (Biomatters). Microsatellites that were excluded after assumption testing (Hardy-Weinberg, null alleles and linkage disequilibrium) in the population genetics study in (Batsleer et al. 2022a) were also excluded here, resulting in 21 microsatellites for the subsequent genetic analysis. A total of 539 samples were successfully amplified.

Land cover categories

We combined two types of land cover datasets available for the study area. For landscape categories within dune areas, we used detailed vegetation maps (resolution 0.4m) available for the region derived from remote sensing data with a classification machine learning technique (Bonte et al. 2021). For landscape categories outside dune areas, we used the ground cover map 2018 provided by the Flemish government ('Bodembedekkingskaart' BBK 1m resolution, source: www.geopunt.be). We aligned, reclassified and combined the raster datasets (see online code; supplementary S1 for detailed information on reclassificaitons) to have one raster file with resolution of 1m of the study area with 7 simplified landscape categories: urbanized (*urban*), *water*, agricultural (*agric*), *beach*, *scrub*, *trees* and open dune (*opend*) vegetation (Fig. 1). Category *beach* was added by rasterizing a manually drawn shapefile based on aerial maps and the high water line. For further analyses, we resampled the rasters to work with resolutions of 20m and 50m. We deem these resolutions (spatial grains) adequate to capture the turnover in heterogeneity of the focal landscape and to be linked with the ecological processes investigated (McRae et al. 2008; Cushman and Landguth 2010).

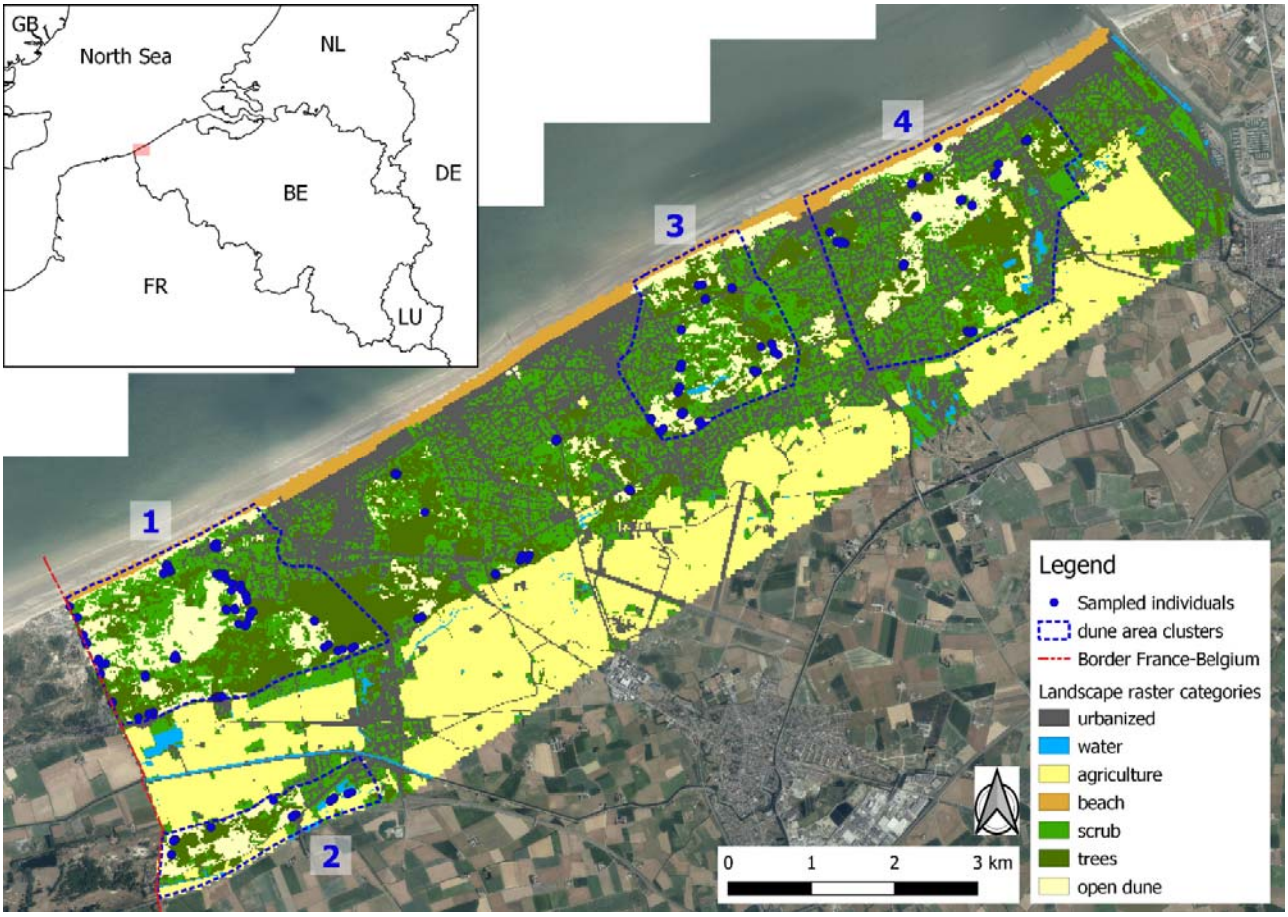

Figure 1: overview map of study area: west coast in Belgium from the French-Belgian border (west; dashed red line) to the Yser estuary (east). Sample locations of each individual (blue dots) are shown. and four dune area clusters (blue dashed lines) are shown. Four dune area clusters (blue dashed lines) are ecological clusters deemed relevant to nature management; 1) Westhoek 2) Cabour 3) Doornpanne 4) Ter Yde. The landscape raster categories are shown with a 20m × 20m resolution (cell size). Neighboring countries of Belgium (BE): France (FR), Luxembourg (LU), Germany (DE), The Netherlands (NL), United Kingdom (GB). Map made with QGIS v3.22 (QGIS Development Team 2020). Background aerial photographs (summer 2018) from Agency for Information Flanders (source: geopunt.be).

Genetic distance measure

To quantify the genetic distance between pairwise individuals, we used a metric based on multiple axes of principle components analysis (PCA). Such PCA-based individual genetic distances perform better in landscape genetics analyses, especially when sample size and genetic structure are low (Shirk et al. 2017; Kimmig et al. 2020). Principal components (PC) were calculated from a multiple contingency table (0, 1, 2 values for each allele for each individual), for which the Euclidean distance among the first 16 principle components was calculate to create a distance matrix (Shirk et al. 2017).

Resistance distance measure

To quantify the resistance distance between pairwise individuals (i.e. distance based on the landscape), we used the commute resistance distance for individuals in the RESISTANCEGA R package (van Etten 2017; Peterman 2018). The commute distance is the expected time of a random walker between a pair of points (sampled individuals) along a circuit-based resistance surface. It is a measure directly related to circuit-theory-based resistance distances (McRae et al. 2008; Peterman 2018).

RESISTANCEGA uses a linear mixed effects model with maximum likelihood population effects (MLPE) parameterization to fit pairwise resistance distances (predictor) to pairwise genetic distances (response). MLPE parameterization accounts for the non-independence among pairwise data (Clarke et al. 2002; Bates et al. 2015).

Landscape resistance optimization

To determine the resistance values of each landscape type with RESISTANCEGA (Peterman 2018), we ran an optimization procedure. The pairwise resistance distances are recalculated at each iteration based on the resistance values of the landscape types during the previous iteration. The optimization procedure iteratively searches many possible resistance value combinations to maximize the MLPE model fit (based on log-likelihood), resulting in the best fit between resistance distances and genetic distances (Peterman 2018). This optimization procedure is based on a genetic algorithm (Scrucca 2013). For each raster, we used the SS_OPTIM function in RESISTANCEGA to optimize the resistance

values (see online code). Parameters were set to default, only the maximum number of iterations (*max.iter*) was set to 300. There are no a priori expectations for the resistance values of the landscape categories, as no information is present regarding habitat selection and movement behaviour at the landscape scale for this species (Knowlton and Graham 2010).

Multicategorical rasters. We optimized multi-categorical rasters, which take into account several landscape categories at once. Although more complex to parameterize, multi-categorical raster optimization adds more biological realism to the analysis than considering only one single category at a time, which assumes an organism's perception of a landscape to be binary (Spear et al. 2010). We ran optimizations for each possible combination of all 7 categories. A raster then consists of one to seven focal landscape categories and all else or 'the rest' (for example when categories *urban* and *scrub* are considered, all five other categories are lumped into a third category). The total number of all possible combinations of 7 categories (*urban*, *water*, *agric*, *beach*, *scrub*, *trees* and *opend* vegetation) is 127: $\sum_{k=0}^{7} \binom{7}{k}$. Ascii-files needed as input for RESISTANCEGA were created for each possible combination. Thus, optimizations are run for each possible combination for the 7 categories.

Bootstrap analysis. To compare the performance of each of the 127 optimized rasters—inlcuding Isolation-By-Distance (IBD) as null model—we performed a bootstrap analysis. With the RESIST.BOOT function, 75% of the individuals are subsampled and the MPLE model refitted for each of the 127 optimized rasters and IBD, this for 1000 iterations. Relative support for each model is then compared using the difference in corrected Akaike information criterion (AICc, correction for small sample sizes), number of times the model was the top model across all bootstrap samples, and the average Akaike weight across bootstrap samples. Average marginal R^2 and log-likelihood across bootstrap samples are also given.

Independent optimizations runs. As the optimization with RESISTANCEGA is a stochastic process, optimized values can differ from run to run, and it is advised to run all optimizations at least twice to confirm convergence and relative relationships among resistance values (Peterman 2018). To check

this convergence and account for a possible bias due to the starting cell values of a category in multi-categorical rasters (i.e. the initial order: urban, water, agricultural, beach, scrub, trees, open dune) (Peterman 2018; Kimmig et al. 2020), we ran the multi-categorical raster optimizations and bootstrap analysis a second time with the order of categories inverted (starting with *opend*, ending with *urban*). To confirm convergence more conclusively, we ran optimizations and bootstrap analyses of the seven single category rasters (that consider only one category) four times each (supplementary S2). This is computationally less intensive then running all possible combinations, but gives anyway a broad idea of the robustness of the optimization.

Multi-model inference. As all possible combinations of categories were optimized to obtain resistance values, we were able to use a multi-model inference approach to obtain robust estimates of average weight for each category and average relative resistance value. Multi-model inference is a procedure to account for the uncertainty of parameter estimates related to model selection and is especially useful when no single model is overwhelmingly supported by the data (Johnson and Omland 2004). We used Akaike weights from the bootstrap analysis considering all the models (i.e. optimized rasters) for each area type. From these, we calculated the summed Akaike weight per category by taking into account a model if the focal category was included. These summed Akaike weights give an indication for the relative importance of each category to explain the pairwise genetic distances. We also calculated relative resistance values of each category. As optimized resistance values depend on the other categories included in a multi-categorical model, interpretation for a focal category should be done relatively to these other categories. To be able to do this for each landscape category, resistance values were rescaled per optimized raster by subtracting the resistance value of the focal category from the other resistance values that were simultaneously optimized. As such, the value of the focal category becomes zero and is considered facilitating to gene flow compared to categories with values larger than zero and a barrier to gene flow compared to categories with values smaller than zero. All models and the relative resistance values per focal category were then summarized by plotting boxplots for each focal category.

To get an idea of the possible small scale variation in landscape effects on gene flow we repeated the analyses on four dune area clusters within our study area. These dune areas that are seen as ecological clusters relevant for nature management (Fig. 1). These optimizations for the dune area clusters are run with a resolution of 20m (in contrast to 50m for the complete study area). Details and results can be found in supplementary S4.

The total computational time (runs for all possible combinations of categories) was very high and could not be run on a single desktop. Therefore, we made use of the high performance computing infrastructure of Ghent University (HPC-UGent) to run the optimizations and bootstrapping analyses. By doing this, we could run all possible combinations in the multi-categorical raster optimizations, which is the most ideal for robust statistical inference and allows for model averaging to assess the importance of the different landscape categories. Previous studies reported computational constraints for optimizations with RESISTANCEGA considering all combinations and used alternative approaches for model comparison based on model selection (e.g. Lourenço et al. 2019; Kimmig et al. 2020).

The best-supported models (i.e. optimized rasters) for the complete study area were used to visualize the putative gene flow currents using Circuitscape (McRae et al. 2013; Kimmig et al. 2020). Currents were inferred between all pairs of sample locations and the resulting conductance (reverse of resistance) of each grid cell are summed for the final map.

The main analyses were performed in RSTUDIO V1.4 (RStudio Team 2022) using R v4.1.3 (R Core Team 2020), see online code. Except for calculations for the current map with the GUI of Circuitscape v.4.0.5 (McRae et al. 2013) and cartographic visualizations in QGIS v3.22 (QGIS Development Team 2020).

Results

Results of the top five best supported models with optimizations of multi-categorical models (all possible combinations of landscape categories) after bootstrapping can be found in table 1 (full table in supplementary S3). The summed Akaike weights per landscape category are in table 2 and the relative resistance values are shown in figure 2.

In general, there is no overwhelming support for one or a few models, as isolation-by-distance (null model) is one of the best supported models (Distance; ΔAICc=1.20 run 1 and 1.42 run 2; table 1) and is a competing model considering ΔAICc < 2 with *urban* (the best supported model). In general, R^2m's are low (table 1; overall <1%), Akaike weights are small (avg.weight in table 1) and all ΔAICc's are relatively small (table 1; maximum ΔAICc was 14.58; supplementary S3); and models with ΔAICc ranging between 2-7 have some support and should not be dismissed (Burnham et al. 2011). When no single model is overwhelmingly supported by the data, multi-model inference is ideal to deduce average weights and relative resistance values of categories (Johnson and Omland 2004). From such a multi-model inference based on the optimization of all possible combinations, we can deduce more general patterns from the data (table 1, Fig. 2). *Urban* is an important explanatory category to discriminate pairwise genetic distances (table 2), which acts mainly as facilitator to gene flow compared to other landscape categories (table 1, Fig. 2). Natural landscape categories (*beach*, *scrub*, *trees*, *open dune*; bottom row in Fig. 2) are in general more resistant to gene flow than anthropogenic categories (*urban*, *agriculture, water*; top row in Fig. 2) in the focal landscape. As most categories, apart from urban, are of little (and inconclusive) importance to discriminate pairwise genetic distances (table 2), the uncertainty of relative resistance values between these categories (especially natural categories, Fig. 2 second row) should be considered high. The second independent optimization run (included in table 1 and 2; Fig. 2) confirms these patterns and shows that category *urban* is consistently present in the top best supported models (table 1) and the most important (and only conclusive) category to discriminate pairwise genetic distances (table 2). This is also confirmed

by the four independent runs of the single categories, which have consistently urban as the first supported model and IBD as the second best competing model (supplementary S2).

Circuitscape current maps (Fig. 3) were made for the three best supported models (table 1). As all ΔAICc's are relatively small (supplementary material S3), there are many more models worth considering (as done in the multi-model inference, Fig. 2 and table 2). However, these Circuitscape current maps give good visualisations of how the landscape likely influences genetic connectivity within the study area.

The analysis repeated on the dune area clusters (Fig. 1) show Isolation-By-Distance (IBD) is the overall best supported model on this smaller scale (supplementary material S4 and S5). Some specific patterns arise depending on the focal dune area cluster, based on the best supported models and the multi-model inference (supplementary material S4). These in general confirm the overall pattern from the complete study area: anthropogenic categories (urban, agric, water) are more facilitating to gene flow than natural landscape categories. There are two exceptions: for the dune area Doornpanne (dune cluster 3 in Fig. 1), *trees* is a facilitating landscape type to gene flow, and for Cabour (dune cluster 2 in Fig. 1), *agriculture* is a barrier to gene flow. This is probably related to the specific habitat composition and landscape context for these dune areas: Doornpanne has relatively much encroached area (trees and shrubs), while Cabour is the dune area most embedded in agricultural land (Fig. 1).

Table 1: Top 5 bootstrap results for the multi-categorical model optimizations for the complete study area for the two independent optimization runs.

Independent run	Model	k	avg.AICc	ΔAICc	avg.weight	avg.rank	avg.R^2m	avg.LL	n.top	Res.value 1st	Res.value 2nd	Res.value other
	urban	3	239704.83	0.00	0.10	4.50	0.00010	-119849.38	326	1	-	2500
	urban.water	4	239705.86	1.03	0.06	7.47	0.00015	-119848.88	94	8	1	2500
Run 1	Distance	2	239706.03	1.20	0.05	6.75	0.00001	-119851.00	180	-	-	-
	beach.scrub	4	239706.67	1.84	0.05	11.92	0.00005	-119849.29	136	1	2500	776
	urban.beach	4	239706.87	2.04	0.04	12.29	0.00010	-119849.38	0	1	2500	2438
	urban	3	239650.74	0	0.10	4.83	0.00011	-119822.34	343	1	-	2500
	water.urban	4	239651.83	1.09	0.06	8.52	0.00016	-119821.87	94	1	2.5	2500
Run 2	Distance	2	239652.16	1.42	0.05	8.43	0.00001	-119824.07	180	-	-	-
	beach.urban	4	239652.78	2.04	0.04	13.16	0.00011	-119822.34	0	2500	1	2488.5
	opend.urban	4	239652.89	2.14	0.03	13.79	0.00011	-119822.39	0	1884	1	2500

Notes: combinations of 7 landscape categories compared in bootstrap analysis after optimization of multi-categorical models. The two independent optimization runs (Independent run) have an inverted order of categories, to check convergence and account for a possible bias due to the starting cell values. Urbanized (*urban*), *beach, water, trees,* agriculture (*agric*), *scrub,* open dune (*opend*). Distance is the isolation-by-distance null model: increasing genetic distance with increasing Euclidean geographic distance. Predictor, landscape category or univariate model; k, number of parameters; avg.AICc, average AICc across all bootstrap iterations; ΔAICc, difference in avg.AICc compared to the lowest avg.AICc (the best supported model); avg.weight, average Akaike weight across iterations; avg.rank, average rank across iterations; avg.R^2m,

355 average marginal R^2 across iterations; avg.LL, average log-likelihood across iterations; n.top, number of times the model was the top model across iterations (does not sum op to 1000, as many more models were considered, see supplementary 3); Res.value 1st, the optimized resistance value for the first mentioned landscape category (e.g. *urban* in the second row); Res.value 2nd, the optimized resistance value for the second mentioned landscape category (e.g. water in the second row); notice the inverted order of categories in run 2 compared to run 1; Res.value other, optimized resistance value for all else (combined into one landscape variable).

Table 2: Summed Akaike weights per category for multi-categorical model optimizations, indicating the importance of a category in all possible multi-categorical optimized rasters to explain the pairwise genetic distances.

Category	**Sum weights run 1**	**Sum weights run 2**
urban	0.496	0.501
agric	0.357	0.335
beach	0.356	0.376
trees	0.338	0.307
water	0.297	0.315
scrub	0.286	0.259
opend	0.246	0.258

Notes: the Akaike weight for an optimized model was added to the summation (Sum weights) if the focal category (Category) was in it.

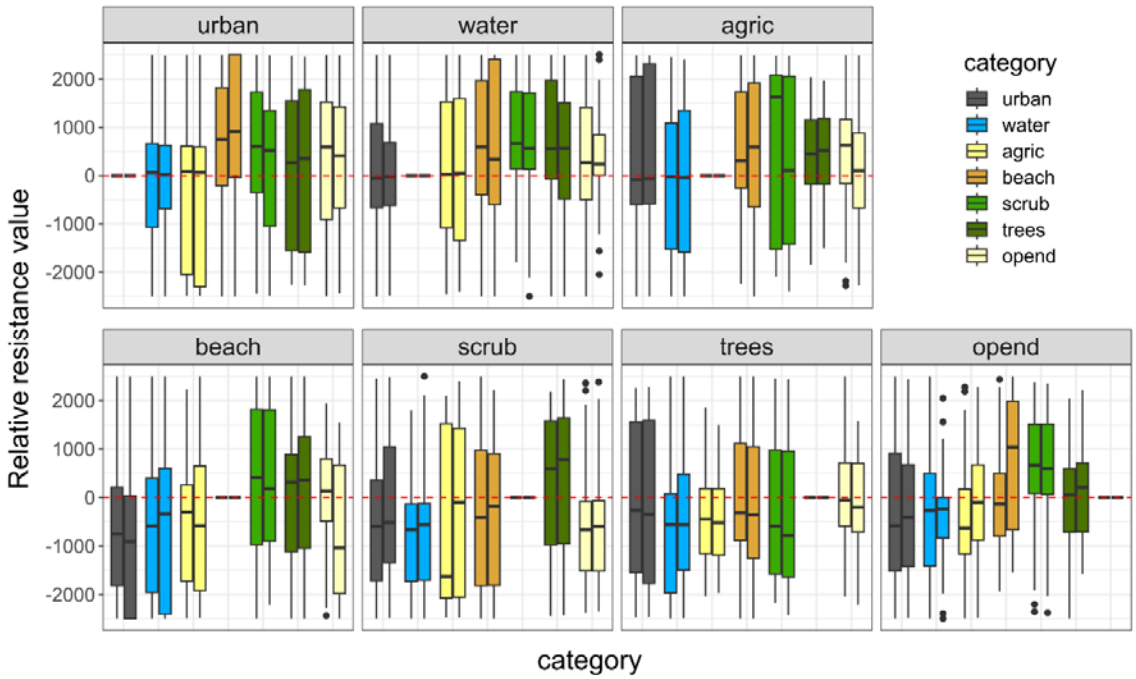

Figure 2: Relative resistance values from the optimization of multi-categorical models for the complete study area. Anthropogenic landscape categories (*urban*, *water*, *agric*=agriculture) are in the top row, natural landscape categories (*beach*, *scrub*, *trees*, *opend*=open dune) in the bottom row. The two independent runs are combined as paired boxplots for each category in each panel (left run 1, right run 2). As optimized resistance values depend on the other categories included in a model, interpretation for a focal category (panel) should be done while holding it constant and rescale the resistance values for all other categories in each optimized raster (resistance values – resistance value of focal category). Each panel should then be interpreted by checking what lies above zero (focal category more facilitating) and below zero (focal category more resisting). The red dashed line is zero, the boundary of equal resistance values between the focal category (panel) and other categories. For example, in the urban panel (left upper corner), the medians of beach, scrub, trees and open dune are situated above the red line, consequently, urban is in general more facilitating than those four categories. As most categories, apart from urban, prove to be of little importance to discriminate pairwise genetic distances (table 2), the uncertainty on the relative resistance values of these categories should be considered high.

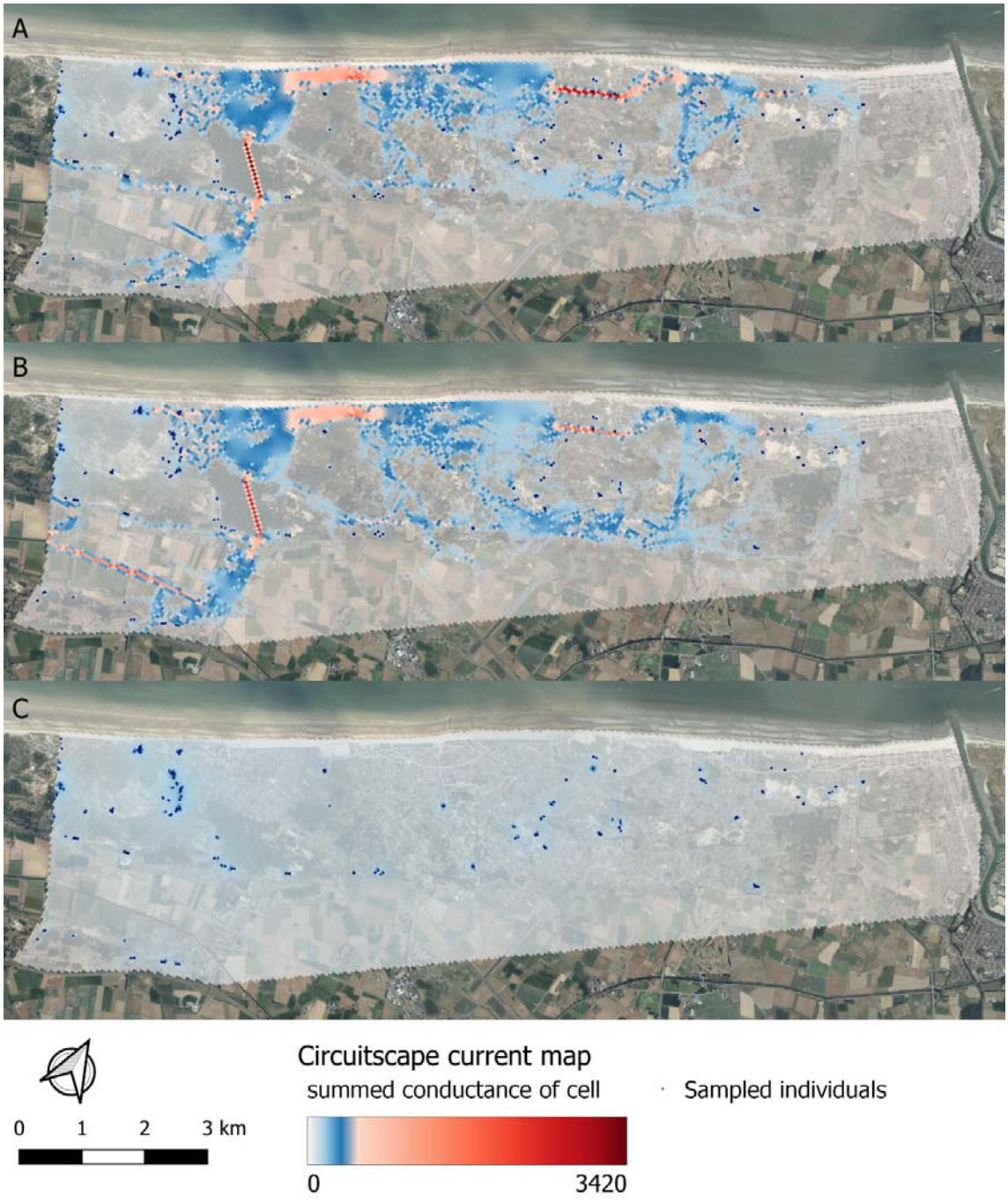

Figure 3: CIRCUITSCAPE current maps for the complete study area based on the three best supported models. Current maps of A) best supported model with *urban* as facilitator B) 2nd best supported model *urban* and *water* combined, both facilitators C) 3rd best supported model Isolation-by-distance (resistance values for all cells was set to 1). The map visualizes conductance (high conductance—or low resistance—dark red, average conductance or resistance blue, low conductance—or high resistance—white-transparent) to gene flow based on the summed currents between pairwise sampled individuals (small black dots). Map made with QGIS v3.22

(QGIS Development Team 2020). Background aerial photographs (summer 2018) from Agency for Information Flanders (source: geopunt.be).

Discussion

We studied functional connectivity based on landscape genetics methods of the digger wasp *Bembix rostrata* in a human-altered coastal region. Overall, the populations of this dune-specialist insect are genetically well-connected in this region (Batsleer et al. 2022a). Despite this overall genetic admixture, we could detect a consistent signal of urban features facilitating gene flow, in addition to the prominent isolation-by-distance (IBD). In general, anthropogenic landscape categories (urban, agriculture, water) seem more facilitating than natural habitat types in this human-altered dune landscape context.

Isolation-by-distance (IBD) was a prominent pattern in our results: genetic distances increase with geographic distance between individuals or populations (Batsleer et al. 2022a). An IBD pattern emerges naturally when dispersal is limited at a certain spatial scale, i.e. dispersal is more likely to nearby populations than distant ones, independent of the landscape (Kimura and Weiss 1964). Consequently, IBD is seen as a biological realistic null model (opposed to panmictic gene flow) in landscape genetics when considering the influence of the landscape on gene flow, i.e. isolation-by-resistance (IBR) (Wagner and Fortin 2015). Our results showed that IBD was consistently a competing model and one of the best supported models (table 1, considering ΔAICc < 2 with *urban*). Because overall support for models was low, as models with ΔAICc ranging between 2-7 have some support and should not be dismissed (Burnham et al. 2011), we applied multi-model inference to deduce average weights and relative resistance values of categories (Johnson and Omland 2004). This confirmed the importance of *urban* for discriminating pairwise genetic distances and deduce that generally anthropogenic landscapes seem more facilitating than natural habitat types. Therefore, considering all possible combinations for landscape resistance optimization can be powerful when landscape resistance signals are weak and hard to detect.

The current maps in figure 3 visualize the potential realized conductance of all sample locations according to three best supported and competing models (*urban*, *urban-water*, IBD; table 1). We can

413 see that these models should be considered as complementary rather than opposing. First,
414 waterways often run parallel to roads and waterbodies are embedded along or within urban areas
415 (Fig. 1). Such water—which is also a scarce landscape type—adds only a small extra effect on top of
416 the urban areas for the resulting current map (Fig. 3B). Consequently, we cannot easily disentangle
417 the effect of water from that of urban within the given landscape (Angelone et al. 2011; Keller et al.
418 2012). The Akaike weights from multi-model inference (table 2) indicate that the urban category is
419 overall the most important to discriminate pairwise genetic distances. Second, the IBD pattern (Fig.
420 3C) is also present within the dune areas in the current map for the *urban* and *urban-water* models
421 (Fig. 3A, 3B). In the latter models, the natural habitat categories (*open dune*, *scrub*, *trees*, *beach*) all
422 have the same resistant values (relatively higher than urban and/or water; table 1), resulting in a
423 homogenous resistance landscape within dunes, resulting in an average IBD pattern. The importance
424 of IBD on this scale is also confirmed by the analyses repeated on the dune area clusters within our
425 study area (supplementary material S4). This interpretation is also in agreement with the multi-
426 model inference, as natural habitat categories proved to be of little importance to discriminate
427 pairwise genetic distances (table 2) and the uncertainty on the relative resistance values of these
428 categories was high (Fig. 2). Consequently, IBD can be considered as the main background process,
429 wherein gene flow only depends on the pairwise distances. The extra signal from anthropogenic
430 features is then at play between dune areas where especially urban areas give a weak but consistent
431 extra signal of conductance.

432 It is counterintuitive that gene flow of a specialized digger wasp nesting in grey dune habitat can be
433 facilitated by urban features in a dune landscape. Importantly, patterns of gene flow only reflect a
434 certain component of movement in a species and interpretation of results in a larger ecological
435 framework should consider other crucial components of a species survival and resource
436 requirements (Spear et al. 2010; Cushman and Lewis 2010). As *B. rostrata* is a dune-specialist insect,
437 urban areas should be considered as inhospitable environments. As the deduced resistance values
438 per category are relative values (Fig. 2), the pattern can be explained by both slower, confined

439 movement in dune habitat and/or faster, compensatory movement in inhospitable areas. Indeed,
440 search behaviour in unfamiliar environments can be more directional and faster than in natural,
441 familiar habitats, where movements are more exploratory (Van Dyck and Baguette 2005; Schtickzelle
442 et al. 2006; Knowlton and Graham 2010). Suitable habitat intervening the landscape might hinder
443 gene flow between more distant populations, as individuals are likely to settle at encountered
444 habitat during dispersal (Adriaensen et al. 2003; McRae et al. 2008; Keller et al. 2012). On the other
445 hand, compensatory movement through inhospitable habitats can result from a lack of resources or
446 can be a strategy to reduce mortality risk (Schtickzelle et al. 2006; Peterman et al. 2014). Both can be
447 counterbalanced by moving faster in inhospitable environments, resulting in increased genetic
448 connectivity between fragmented habitats (Schtickzelle et al. 2006). As isolation-by-distance is a
449 persistent underlying pattern in our results, the behavioral responses to anthropogenic or natural
450 landscape types are only relevant on top of the fundamental isolation-by-distance process.

451 The deduced resistance values are values per grid cell and relative compared to the other considered
452 landscape types. Consequently, the deduced resistance values of a category will depend on the
453 habitat composition and the spatial configuration of how populations are embedded within the
454 landscape (Richardson et al. 2016; Haran et al. 2017). In our study, urbanisation induces large-scale
455 fragmentation compared to more natural processes like shrub and woodland encroachment. Hence,
456 a low resistance does not conflict with the retrieved low gene flow caused by an increased isolation
457 by distance from this urban infrastructure. Differences in a species' ecology (and evolutionary
458 history) across regions could further complicate and diversify possible responses to a certain
459 landscape type in different landscapes, especially at larger scales and across regions (Segelbacher et
460 al. 2010; Spear et al. 2010). Therefore, results regarding the influence of anthropogenic landscape
461 types on the gene flow of *B. rostrata* cannot easily be extrapolated or generalized. Nevertheless, our
462 results for this species will reasonably remain valid for similar landscapes at comparable scales and
463 with similar compositions, such as several human-altered coastal landscapes of northwest Europe.
464 But extrapolation to inland sandy regions, where the species occurs as well, would be invalid.

465 We find that the connectivity of *B. rostrata* does not seem limited by the matrix composition. Even
466 on the contrary, on top of the general process of IBD, inhospitable urban features seem to facilitate
467 gene flow in this landscape for a given distance between different habitats. This indicates that for *B.*
468 *rostrata*'s persistence in this study area, landscape-based conservation measures aimed at increasing
469 connectivity between dune areas (e.g. with habitat corridors) would not be an effective management
470 measure unless they are able to give rise to the establishment of new populations. It is probably
471 more critical to increase or maintain large population sizes, by increasing habitat area and habitat
472 quality (Richardson et al. 2016; Watts et al. 2016). The effectiveness of increasing connectivity in this
473 landscape might be worth more consideration for cursorial species experiencing high mortality costs
474 when crossing urban infrastructure (e.g., natterjack toad, Cox et al. 2017). However, benefits gained
475 from landscape-scale and site-based conservation should always be balanced to maximize
476 biodiversity benefits with the limited resources at hand (Watts et al. 2016). For the conservation of *B.*
477 *rostrata*, other processes linked with dune habitat fragmentation are anyway more fundamental to
478 consider than landscape connectivity, such as the decrease in habitat area itself and shifts in
479 ecological processes (e.g. edge effects and 'ecosystem decay' such as inhibition of sand dynamics in
480 dunes; Pfeifer et al. 2017; Chase et al. 2020). This fragmentation due to urbanization decreases the
481 overall potentially suitable locations, increases isolation and affects regional levels of exchange
482 among populations in a spatially altered population network (Cheptou et al. 2017). As urban areas
483 are not forming a barrier to direct gene flow, these more indirect effects of fragmentation should be
484 the focus to secure persistence of *B. rostrata* in the human-altered dune landscape. In the
485 contemporary context of dune nature management, a site-based conservation approach to maintain
486 and improve open dune habitat is recommended, for which grazing with large herbivores (cattle,
487 horses) is a crucial management tool. However, a reconciliation is needed between short-term
488 negative effects of trampling by grazers on nest densities of *B. rostrata* and long-term positive effects
489 of maintaining open dune vegetations within dune areas. Therefore, a local adaptive management
490 approach with sheep grazing is recommended (Bonte 2005; Batsleer et al. 2022c). Additionally,

491 improving habitat quality of open dune vegetations through landscape-scale conservation aimed at
492 restoring and increasing sand dynamics—rather than connectivity—would be a larger scale,
493 sustainable measure for the persistence of *B. rostrata* in the coastal dunes in Belgium.

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Author contributions

All authors contributed to the study conception and design. Data collection and laboratory work were performed by FB. Data analyses were done by FB and FD. The first draft of the manuscript was written by FB and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

Scripts and data are made available on github (https://github.com/FemkeBatsleer/LandGenBembix) for review and will be published on zenodo upon acceptance.

Conflict of Interest

The authors have no conflict of interests.

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738