Asymmetrical gene flow between coastal and inland dunes in a threatened digger wasp

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Abstract

Connectivity is a species- and landscape-specific measure that is key to species conservation in fragmented landscapes. However, information on connectivity is often lacking, especially for insects that are known to be severely declining. Patterns of gene flow constitute an indirect measure of functional landscape connectivity. We studied the population genetic structure of the rare digger wasp *Bembix rostrata* in coastal and inland regions in and near Belgium. The species is restricted to sandy pioneer vegetations for nesting and is well known for its philopatry as it does not easily colonize vacant habitat. It has markedly declined in the last century, especially in the inland region where open sand habitat has decreased in area and became highly fragmented. To assess within and between region connectivity, we used mating system independent population genetic methods suitable for haplodiploid species. Despite *B. rostrata* being a poor colonizer, gene flow between existing populations appeared not highly restricted. We found more pronounced genetic structure in the small and isolated inland populations as compared to the well-connected coastal region, including a pattern of asymmetrical gene flow from coast to inland. Most likely, demography within different landscape contexts is the main factor explaining the discrepancy in connectivity and asymmetrical gene flow between the different regions. This illustrates the importance of large populations for the species' metapopulation persistence. Therefore, to improve the conservation status *B. rostrata* through increased connectivity, large sites with sand dunes need to be preserved or created, especially in landscapes with small and isolated populations.

Keywords: haplodiploid; microsatellites; insect conservation; dunes; coastal; sandy habitats; Hymenoptera; Crabronidae; *Bembix rostrata*

Introduction

The decline in abundance and distribution of many insects has raised a widespread public and political awareness on their biological value (Harvey et al. 2020; Didham et al. 2020; Wagner et al. 2021; Welti et al. 2021). Habitat loss and fragmentation have been identified as major drivers of this decline (Cardoso et al. 2020), rendering the focus on connectivity conservation for (meta-)population persistence essential (Hanski et al. 1996; Hanski and Ovaskainen 2002; Haddad et al. 2015). Connectivity is a biological concept, in which fluxes of individuals between patches in heterogenous landscapes are determined by both landscape structure and the species' dispersal capacity. Such exchange processes are difficult to quantify in a direct way in invertebrates. Mark-recapture or tracking approaches typically fail when populations are large and widely spread (Chadœuf et al. 2018). Patterns of gene flow do, however, reflect realized dispersal across multiple generations and are informative on the functional landscape connectivity especially at large spatial scales (Kim and Sappington 2013; Hodgson et al. 2022; Maes and Van Dyck 2022). Because drivers of fragmentation can be diverse in identity, scale and intensity, responses to habitat loss and fragmentation are species- and context-dependent (Cheptou et al. 2017). Consequently, every landscape and its regional context is unique for each species. Therefore, habitat connectivity—as perceived by different species—remains difficult to generalize, even across different regions for the same species.

Levels of general natural habitat fragmentation in Flanders (Belgium) are among the highest in Europe (European Environment Agency et al. 2011). Dune habitats harbor a specific insect biodiversity with typical species of conservation interest (Maes and Bonte 2006; Provoost et al. 2011; De Ro et al. 2021). These sandy habitats, both at the coast and inland, have gone through extensive—but different—landscape changes and fragmentation during the past decades or centuries. They differ regarding their size, extent, history and nature of fragmentation. Firstly, coastal dunes in Flanders are geological young formations with calcareous sandy soils (Provoost et al. 2004; Decleer 2007) that form a narrow, linear system along the coast. Coastal sandy habitats became

fragmented at two scales. Primarily, urbanization from the interbellum period onwards decreased the total area of dunes significantly and resulted in a physical separation of the larger dune entities. In parallel, loss of agricultural practices (including grazing of livestock) and obstruction of sand dynamics due to urbanization stimulated the succession and shrub development, at the cost of the open, early-succession or pioneer dune habitats (Provoost et al. 2011). Large herbivores have been introduced in many coastal dune reserves during the last three decades to revitalize dune dynamics (Provoost et al. 2004), but might have mixed effects on local arthropod species due to intense trampling (Bonte and Maes 2008; van Klink et al. 2015; Batsleer et al. 2022b). Second and contrastingly, inland sandy soils in Flanders are large postglacial relicts. In this region, large open heathland and land dune systems were present, mainly on acidic sandy soils (Decleer 2007), which were heavily afforested since the 19th century (De Keersmaeker et al. 2015). Later, in the second half of the 20th century—parallel to, but more severe than at the coast–the remaining open habitat patches became further build-up and hence, smaller and more fragmented. Secondary loss and fragmentation of the remaining sandy habitat patches took place, here due to acidification and eutrophication leading to a continuous grass encroachment of sparsely vegetated sand areas on lime-poor soils (Schneiders et al. 2020).

The digger wasp *B. rostrata* is a univoltine habitat-specialist associated with dynamic sandy habitats with early-successional vegetation: grey dunes in coastal areas (EU Habitats Directive habitat 2130) and dry sand heaths and inland dunes with open grasslands in inland areas (habitats 2310 and 2330 respectively). *Bembix rostrata* has strongly declined during the 20th century in Belgium, resulting in a distribution with local strongholds at the coast and more fragmented or isolated populations present inland. The same decline was seen in the Netherlands, whereby mostly inland populations were lost (Klein and Lefeber 2004). *Bembix rostrata* is labeled as a philopatric species that does not easily colonize vacant habitat and aggregates stay present at the same location for many consecutive years (Nielsen 1945; Larsson 1986; Bogusch et al. 2021). This presumed philopatry is likely deduced from the species gregarious life-style where females base their nest choice on the presence of conspecifics

96 and build consecutive nests—each with one provisioned larva—close to each other (Batsleer et al.
97 2022a). Given the typical dynamic character of the species' habitat (pioneer dune vegetations),
98 philopatry should be highly disadvantageous and eventually put the species' at risk if new early-
99 successional sites cannot be colonized (Bogusch et al. 2021). Hence, as for other species from early-
100 successional habitat, good dispersal capacities would be expected despite the overall sedentary life
101 style during breeding (e.g. *Andrena vaga*: Černá et al. 2013; Exeler et al. 2008). Correct information
102 on the species' dispersal capacity is therefore essential to guide future conservation strategies.

103 Given the species' conservation flagship status in Belgium as emblematic ground-nesting
104 hymenopteran, we studied connectivity through gene flow between *B. rostrata* populations within
105 and between coastal and inland fragmented sandy habitats in Belgium and bordering areas. We also
106 included a few recently discovered populations to deduce potential origin populations. We amplified
107 21 microsatellite markers and used a non-lethal sampling method with wing clips (Châline et al.
108 2004) to minimize the impact of sampling on the often small and/or geographically isolated
109 populations. As the species has a haplodiploid sex-determination system, we used population genetic
110 analyses that are assumption-free and mating system independent.

111 # Material and methods

112 ## 1. Study species

113 *Bembix rostrata* is a univoltine specialized, gregariously nesting digger wasp from sandy habitats with
114 sparse vegetation (Larsson 1986; Klein and Lefeber 2004). Adults are active in summer, showing
115 protandry: females are directly mated when emerging by the guarding males, who emerge one to
116 five days earlier (Wiklund and Fagerström 1977; Schöne and Tengö 1981; Evans and O'Neill 2007).
117 Females show brood care: one individual constructs one nest burrow at a time in which it
118 progressively provisions a single larva with flies (Nielsen 1945; Field et al. 2020). An estimated of up
119 to 5 nests are produced, each with one offspring (Larsson and Tengö 1989). There are no overlapping
120 generations, as the species overwinters as prepupa and there is only one generation per year.

121 ## 2. Study sites and sampling

122 Sampling took place during the summers of 2018, 2020 and 2021. Samples were taken across 49
123 Belgian populations, three French populations and one Dutch population (Figure 1). Detailed
124 information about each sampling site—region, name, coordinates, sample year(s), number of
125 samples—can be found in supplementary S1. A large part of the Belgian coastal populations and two
126 inland populations were sampled both in 2018 and 2020 (table S1) and are used to check if samples
127 from different years can be pooled in one analysis (see below, hierarchical Analysis of Molecular
128 Variance; AMOVA). Hence, all locations that were sampled in only one year could be compared in
129 one analysis across years. Only females were sampled, to solely use diploid individuals in the
130 population genetic analyses for this haplodiploid mating system.

131 As it is estimated that *B. rostrata* has 5 nests (or equivalent number of offspring) per female (Larsson
132 and Tengö 1989), population sizes grow slowly, especially in isolated fragments. Therefore, to
133 minimize the impact of sampling on the populations, we used a non-lethal sampling method with
134 wing clips, a method shown to give good-quality DNA for microsatellite PCR amplification (Châline et
135 al. 2004). Tips of both forewings from live digger wasp individuals were cut. Both wingtips were

stored in absolute ethanol, stored in a refrigerator at 4°C after sampling and transferred to a freezer (-18°C) for long-term storage. For each sample, individual coordinates of the capture position (most often a nest) were noted.

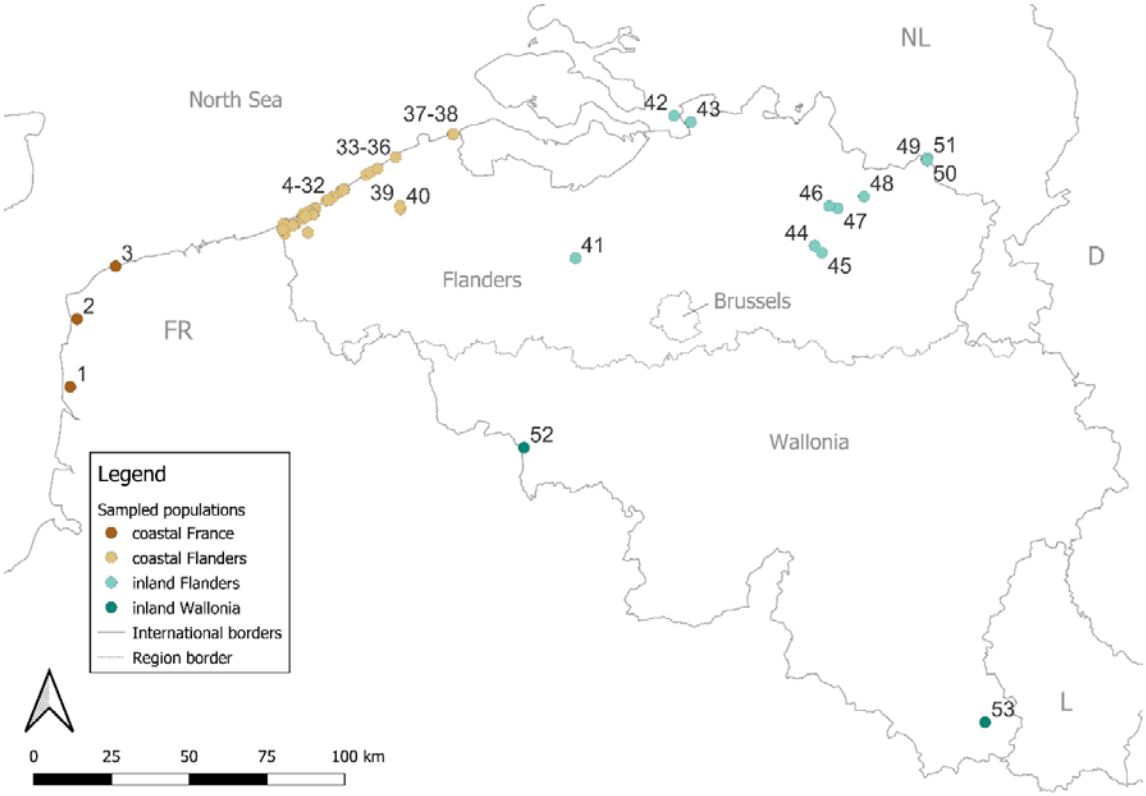

Figure 1: Overview map with locations of sampling. Populations 1-3 in coastal France (dark brown dots), 4-40 in coastal Flanders (light brown dots), 41-51 in inland Flanders (light green dots), 52-53 in inland Wallonia (dark green dots). Neighboring countries and the three administrative regions of Belgium are indicated: FR (France), NL (Netherlands), D (Germany), L (Luxembourg); Flanders (Flemish region), Wallonia (Walloon region) and Brussels (Brussels-capital region).

145 ## 3. DNA extraction and PCR amplification

146 Genomic DNA was extracted from the wing tips with a protocol based on Chelex (Biorad), detailed
147 information and steps can be found in supplementary S2. The development of species-specific
148 microsatellites was outsourced to AllGenetics® (A Coruña, Spain), who provided 500 non-tested
149 microsatellite primers and tested 72 of those biologically with 11 individuals. Of those, we selected
150 33 polymorphic microsatellites and rearranged them in 3 pairs of primer-multiplexes for PCR and
151 amplified these for each sample. Details on the PCR-conditions and multiplexes can be found in
152 supplementary S2; characteristics of the primers can be found in supplementary S3. PCR products
153 were run on an ABI 3500 analyzer with the GeneScan-600 LIZ size standard and the
154 electropherograms were scored using Geneious Prime (Biomatters). Samples from three recaptured
155 individuals were blindly and randomly added to the workflow. They popped up as duplicate
156 genotypes in the first part of the data analysis, so we were confident the scoring error was minimal.

157 ## 4. Genetic data analysis

158 ### 4.1 Hardy-Weinberg, linkage disequilibrium and null alleles

159 To exclude misbehaving microsatellites with artefacts, the assumptions of Hardy-Weinberg (HW)
160 equilibrium and null alleles at individual loci, and of no linkage disequilibrium (LD) across pairs of loci
161 were examined before subsequent genetic analysis (Waples 2015). HW deviations, null alleles and LD
162 deviations were calculated and examined with the R-packages *pegas* (Paradis 2010), *PopGenReport*
163 (Adamack and Gruber 2014) and *poppr* (Kamvar et al. 2014) respectively, for populations that had at
164 least 10 samples (see online code).

165 ### 4.2 Hierarchical AMOVA to check variation between years

166 To test if samples from populations of different years (2018, 2020 and 2021) could be pooled, a
167 hierarchical Analysis of Molecular Variance (AMOVA) was carried out using the package *poppr*
168 (Kamvar et al. 2014). To test the null hypothesis of no population structure between years, 23

169 populations that were sampled twice (2018 and 2020; table S1) were used in this test and year was
170 hierarchically nested within population (see online code).

171 **4.3 Population-level statistics**

172 For each population, we calculated the number of private alleles (NP) with the R-package *poppr*
173 (Kamvar et al. 2014). Rarefied allelic richness (AR), expected heterozygosity (H_e), observed
174 heterozygosity (H_o) and inbreeding coefficient (F_{IS}) were calculated with the r-package *hierfstat*
175 (Goudet and Jombart 2020). These measures should only be interpreted relatively within the studied
176 haplodiploid system.

177 **4.4 Genetic distance**

178 Genetic distance between populations was quantified using Nei's standard genetic distance D_S (Nei
179 1972) calculated with the package *hierfstat* (Takezaki and Nei 1996; Goudet and Jombart 2020). To
180 check which populations have on average the highest genetic distance to other populations, the
181 mean D_S per population was calculated. Similar calculations for genetic differentiation indices F_{ST} and
182 Jost's D (Weir and Cockerham 1984; Jost 2008; Keenan et al. 2013) can be found in supplementary
183 S4, but are considered less suitable for haplodiploids as they are by definition based on Hardy-
184 Weinberg assumption.

185 **4.5 Isolation-by-distance (IBD)**

186 We compared patterns of IBD between coastal and inland populations. To avoid bias due to
187 incomplete coverage (as is the case for France and Wallonia), only samples from Flanders were used,
188 where sampling was spatially covering all populations known to exist at that time. IBD was examined
189 with the multivariate approach distance-based redundancy analysis (dbRDA) (Diniz-Filho et al. 2013)
190 because the suitability of Mantel tests to examine IBD is highly debated (Meirmans 2015). First, a
191 Principal Coordinate Analysis (PCoA) to the Nei's genetic distance D_S matrix was applied. The
192 resulting PCoA-axes were then used as a response matrix in a Redundancy Analysis (RDA) to correlate
193 them with the geographic coordinates. The adjusted coefficient of determination R^2 of the RDA's (the

194 proportion explained by the constrained axes) was used to compare the strength of IBD for coastal
195 and inland populations. Permutations tests (n = 999) were used to check if the R^2 significantly
196 differed from zero. Nei's genetic distance D_S between populations was plotted against the pairwise
197 geographical distance for the two regions. To check if the intercept and/or slope differ between the
198 two regions, a permutation test was applied with the R package *lmPerm* with a maximum of 10,000
199 iterations (Wheeler and Torchiano 2016) with the formula D_S ~ distance + region + distance:region. In
200 general, realized gene flow is dependent on population sizes and dispersal capacity (~$N{\cdot}m$), with
201 spatial configuration of populations a confounding factor. A different intercept in IBD (for the same
202 species) for different regions would be mainly related to differences in population sizes (as *N*
203 decreases, all else being equal, differentiation will increase due to total number of migrants) and a
204 differing slope to different dispersal capacities.

205 **4.6 DAPC**

206 A Discriminant Analysis of Principal Components (DAPC) was performed with the R-package *adegenet*
207 to explore between-population structure and differentiation (Jombart 2008; Jombart et al. 2010).
208 DAPC is a multivariate statistical approach wherein data is first transformed using a principal
209 component analysis (PCA) and subsequently a discriminant analysis (DA) is performed. Genetic
210 variation is partitioned into a between-group and a within-group component, maximizing
211 discrimination between groups. DAPC can be seen as an alternative to Bayesian clustering algorithms
212 that does not assume a population genetic model, which is more suitable for a haplodiploid mating
213 system (Jombart et al. 2010; Grünwald and Goss 2011). We performed a DAPC for all populations
214 together and the coastal and inland populations separately. A cross-validation with 1,000 replicates
215 was performed for the three subanalyses to retain an optimal number of PC-axes with the function
216 *xvalDapc* from the R-package *adegenet* (Jombart 2008; Kamvar et al. 2015). We use scatterplots of
217 the first four principal components of the DAPC analyses to visualize within and between population
218 variation in the study area.

219 **4.7 Assignment tests**

220 To identify the most likely population of origin for all individuals based on the genetic profiles of
221 these individuals and the populations, we performed individual assignment with GENECLASS2 tests
222 to identify immigrant individuals or individuals that have recent immigrant ancestry (Rannala and
223 Mountain 1997; Piry et al. 2004). The most standard method currently is to perform first-generation
224 migrant detection (Paetkau et al. 2004). However, as this model is explicitly based on the sampling of
225 gametes from haploid or diploid populations, we considered this method inappropriate for a
226 haplodiploid mating system. Therefore, we used a Bayesian criterium to estimate likelihoods for each
227 individual to originate from any of the given populations based on allele frequencies, combined with
228 the probability computation from the same method using 10,000 Monte Carlo simulations and the
229 expected type I error rate (alpha) set to 0.01 (Rannala and Mountain 1997). These probability
230 computations are based on random drawing of alleles using allele frequencies directly estimated
231 from the reference population samples and are thus mating system independent. With this method,
232 we can identify individuals that are immigrant or have recent immigrant ancestry. However,
233 interpretation should be done with care, as these assignment or exclusion methods are (compared to
234 first-generation migrant detection) known to be prone to over-rejection of resident individuals and
235 thus might overestimate gene flow (Paetkau et al. 2004; Piry et al. 2004).

236 Recently discovered populations (and thus probably only recently established) were not included as
237 possible source populations for individuals, as they could bias results if they are from mixed origin.
238 We used the following criterion to specify newly established populations: Flemish populations were
239 indicated as new populations if no observations were known in a radius of 2.5km from before 16-10-
240 2017 (reported before the summer of first sampling on the citizen-science species observation
241 platform www.observations.be). French and Walloon populations were known to be historically
242 present from literature (Barbier 2007). The individuals from the new populations were added to the
243 assignments test, to deduce the possible source of these putative new populations. Results from
244 GENECLASS2 consisted of probabilities to belong to each of the possible source populations for each

245 individual. To compare possible within and between region genetic connectivity according to these
246 assignment tests, we summarized the results (excluding new populations) by calculating per region
247 (coast France, coast Flanders, inland Flanders and inland Wallonia) how many individuals were
248 assigned to their sampled population, sampled region or populations from other regions. We made a
249 flow chart (spatial directed network graph) in QGIS (QGIS Development Team 2009) representing the
250 links between sampled populations and putative origin population according to the assignment tests.
251 Arrows in such a graph start at the putative source population according to the assignment tests and
252 end in the sampled population.

253 # Results

254 In total, wing tips of 867 individuals from 53 populations were genotyped. Five microsatellite loci
255 showed a lot of stutter in the amplification profiles which were hard to score and were therefore
256 discarded from further analysis (AGBro486, -329, -196, -437, -298). Hardy-Weinberg (HW), linkage
257 disequilibrium (LD) and null alleles assumption testing identified a further 7 microsatellites that were
258 left out of the analysis (see online code): AGBro35, -57, -419 (HW and null alleles), AGBro111 (HW
259 and LD), AGBro20, -16 (null alleles) and AGBro138 (HW, LD, null alleles). This resulted in a total of 21
260 microsatellite loci for further population genetics analyses. If an individual had more than 8 loci with
261 missing data, it was discarded from the analysis beforehand (10.5%; 102 out of 969). A total of 133
262 alleles with an average of 6.3 alleles per locus (ranging from 3 to 14) were observed across the 21
263 microsatellite loci. The resulting dataset had an overall 3.48% of missing data for the 867 individuals.

264 522 samples (of 867) came from the 23 populations sampled both in 2018 and 2020. Hierarchical
265 AMOVA comparing genetic variation between populations and between years for this dataset,
266 showed that sampling year explained 0.19% of the variation. Thus, it was decided to pool the
267 different years for the subsequent population genetics analyses.

268 ## Population-level statistics

269 The complete table with number of private alleles (NP), allelic richness (AR), expected (H_e) and
270 observed (H_o) heterozygosity and inbreeding (F_{IS}) can be found in supplementary S5, table S6. Allelic
271 richness was in general lower in the inland populations (mean(AR inland)=2.33, range=[2.11; 2.61];
272 mean(AR coast)=2.61, range=[2.41;2.84]; two-sided t(14.84)=0.621, p<0.001). A similar pattern
273 occured for expected heterozygosity (mean(H_e inland)=0.51, range=[0.43;0.58]; mean(H_e coast)=0.59,
274 range=[0.53;0.64]; two-sided t(14.41)=5.820, p<0.001) and less strong for observed heterozygosity
275 (mean(H_o inland)=0.45, range=[0.33;0.54]; mean(H_o coast)=0.52, range=[0.36;0.62]; two-sided
276 t(19.38)=3.503, p=0.002). Inbreeding was in general high (maximum F_{IS}=0.36) and very variable
277 across all populations (range F_{IS}=[-0.025;0.36]; mean ± SD F_{IS}=0.11 ± 0.09), which is expected for a

haplodiploid system. Inbreeding was relatively high in some populations at the mid- and eastern part of the coast (populations 26-38, figure 1): from the 10 populations with the highest F_{IS}, six were from this eastern part of the coast and four from inland populations, including the two Walloon populations (populations 52-53).

Genetic distance

Figure 2 shows the pairwise D_S (Nei's standardized genetic distance) between all populations. The 10 populations with the highest mean D_S, which have the highest average differentiation from all other populations, were 8 inland populations and the two Walloon populations (Table S4). Similar figures for pairwise differentiation measures F_{ST} and Jost's D can be found in supplementary S4, giving similar results. Genetic distances were overall large among inland sampling sites (right upper corners Fig. 2) and small among coastal sites (left lower corner Fig. 2). The genetic distances between coastal and inland regions are medium to large.

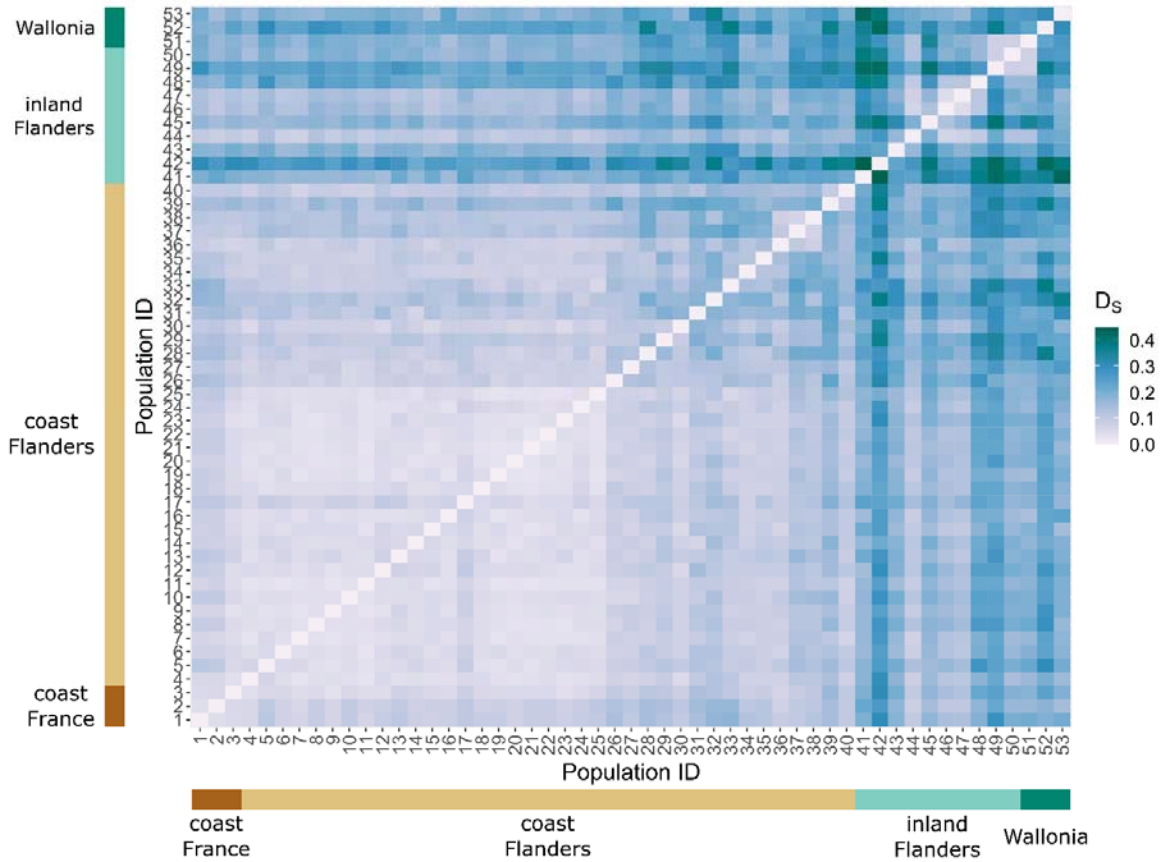

Figure 2: graphical matrix representation of Nei's standardized genetic distance (D_S): light blue are low, dark blue are mid, and dark green are high genetic distance values between pairwise populations. The x- and y-axes represent the population ID, subdivided in the four different regions. Genetic distances are symmetrical and consequently the matrix is mirrored along the diagonal. There is overall large genetic distances within the inland regions (right upper corner, populations 41-53) and small genetic distances within the coastal regions (left lower corner, populations 1-40). The genetic distances between coastal and inland regions (y-values 41-53 with x-values 1-40, or vice versa) are medium to large.

298 **Isolation-by-distance**

299 Isolation-by-distance (IBD) results are only given for coastal and inland Flanders, as the sampling
300 there was spatially covering all known populations and incomplete coverage (for France and
301 Wallonia) might bias results. A RDA performed with Nei's genetic distance D_S and the pairwise
302 geographical Euclidian distances indicate there is spatial genetic structure, and most strongly for the
303 Flanders inland region: proportion explained by the constrained axes (or R^2) is 22% for all populations
304 together (>0: p=0.001; Df=2), 23% for the coastal region (>0: p=0.001; Df=2) and 61% for the inland
305 region (>0: p=0.001; Df=2). Adjusted R^2 is 18%, 18% and 51% respectively. The relationship between
306 D_S and geographical distance is shown in Figure 3. Both the intercept (region: p<0.001; SS =0.342
307 (Type III); Df=1) and slope (distance-region interaction: p<0.001; SS =0.088 (Type III); Df=1) differed
308 significantly between regions according to the permutation test (adjusted R^2 of complete model was
309 58%). Some coastal datapoints (Fig. 3, in brown) are situated on the inland trendline. These are
310 populations from the eastern- and mid- part of the coast which have smaller population sizes
311 (populations 26-38, Fig. 1).

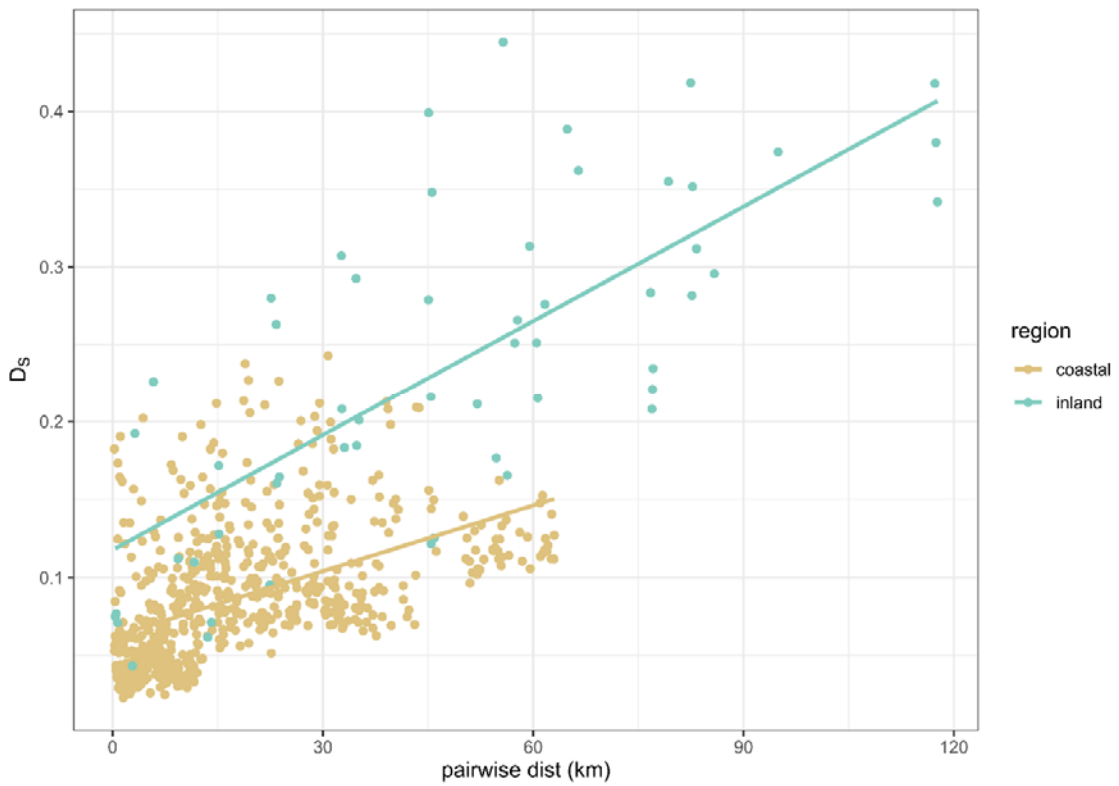

Figure 3: Isolation-by-distance (IBD) graph with Nei's genetic distance (D_S) plotted against Euclidian geographical distance (in km), separately for coastal and inland Flanders. Lines are smoothers plotted for indicating the trend, not regressions: statistical tests are done through RDA and a permutation test (see main text). These showed that the spatial genetic structure is higher for the inland regions and that both intercept and slope differ between the regions.

318 **DAPC**

319 Figure 4 gives scatterplots for the DAPC analysis for the complete dataset. Scatterplots for the coastal
320 and inland regions separately are given in supplementary S6. The coastal Flanders populations (light
321 brown in Fig. 4) clearly clump together, with coastal France (1-3, dark brown in Fig. 4) partially
322 overlapping. A similar pattern can be seen in the DAPC for the coastal regions separately (Fig. S3.A)
323 and are in line with a pattern of isolation-by-distance. Inland populations show more between-
324 populations structure (greens in Fig. 4; Fig. S3.B). This is also confirmed by the separate analyses for
325 both regions: for the coast, the first two principal components together explain 27.2% of the
326 variation, while for inland this is 54.6%.

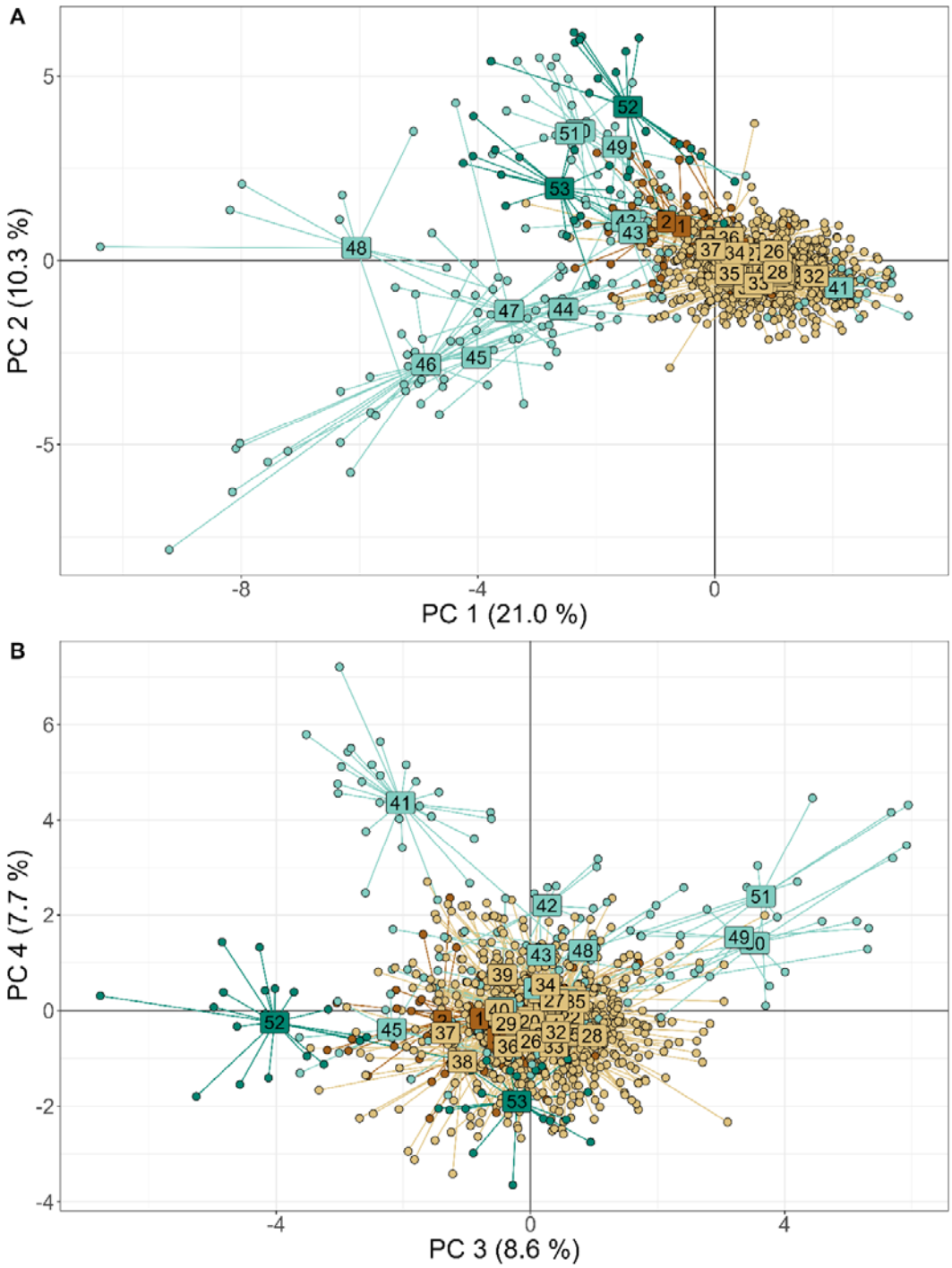

Figure 4: Scatterplot for DAPC results on complete dataset for A) the first two and B) the third and fourth principle components. Labels indicate population ID and point and label colors refer to the regions from Fig. 1: coastal France (dark brown), coastal Flanders (light brown), inland Flanders (light green), inland Wallonia (dark green). Coastal populations cluster together genetically in a large point cloud while inland regions show more genetic differentiation.

Assignment tests

Table 1 summarizes results of the assignment tests per region and figure 5 depicts the derived flow chart. These assignments depict immigrants or individuals with recent immigration ancestry, probably up to two generations (Rannala and Mountain 1997). As such, these results depicts accumulated gene flow from the last few generations and should not be interpreted as direct gene flow. Focusing on the original populations, inland regions have relatively higher numbers of individuals assigned to the original sampled populations (78% and 74%, table 1) compared to coastal Flanders (42%, table 1). However, they also have a higher number of individuals assigned to another region (16% and 26% compared to 4% in coastal Flanders), mainly coming from the western part of coastal Flanders and France (Figure 5). Thus, inland populations are genetically more isolated from each other, but there is a genetic influx from the coast. Within coastal Flanders, there is a lot of genetic connectivity between populations (Fig. 5), as a relative low number of individuals are assigned to their original sampled population (42%) but almost all are assigned within the region (96%). Individuals from coastal France are mainly assigned to the region itself, but there is connectivity with coastal Flanders in both directions (Fig. 5) and these two regions probably form one big region genetically. Isolation-by-distance, the skewed sample sizes (59 for coastal France and 540 for coastal Flanders) and incomplete sampling coverage, probably gave rise to less clear results for coastal France (table 1). New populations (Fig. 5, table 1) inland seem to be a balanced coastal-inland mixture, mainly from a nearby inland population (Geel-Bel, population 46) and western coastal Flanders and French populations. New population at the coast follow a similar ratio as the original coastal populations and are a mixture of populations from mainly the western part of coastal Flanders (Fig. 5, table 1).

The pairwise geographical distances between sampled and assigned populations show that 75% of the distances between sampled and putative source populations lie below 28 km, with the largest distance being 320 km from coastal France to the south of Belgium (supplementary S7, Fig. S5).

Table 1: summarized results of the assignment tests according to (Rannala and Mountain 1997) per sampled region, for coastal and inland Flanders, separately given for original (source) and new populations (Type pop). Variables given are total number of samples (N samples), number and ratio (%) of individuals assigned to the original sampled population within a region (Assigned to original population), number and ratio (%) of individuals assigned to original sampled population (Assigned to original population), to the original sampled region (Assigned within original region), or to another region (Assigned to other region; which is N samples – N assigned within original region). Not applicable = n.a.

Sampled region	**Type pop**	**N samples**	**Assigned to original population**	**Assigned within original region**	**Assigned to other region**
Coastal France	original	59	47 (80%)	50 (85%)	9 (15%)
Coastal Flanders	original	540	228 (42%)	518 (96%)	22 (4 %)
Inland Flanders	original	129	100 (78%)	108 (84%)	21 (16%)
Inland Wallonia	original	39	29 (74%)	29 (74%)	10 (26%)
Coastal Flanders	new	72	n.a.	70 (97%)	2 (3%)
Inland Flanders	new	28	n.a.	15 (54%)	13 (46%)

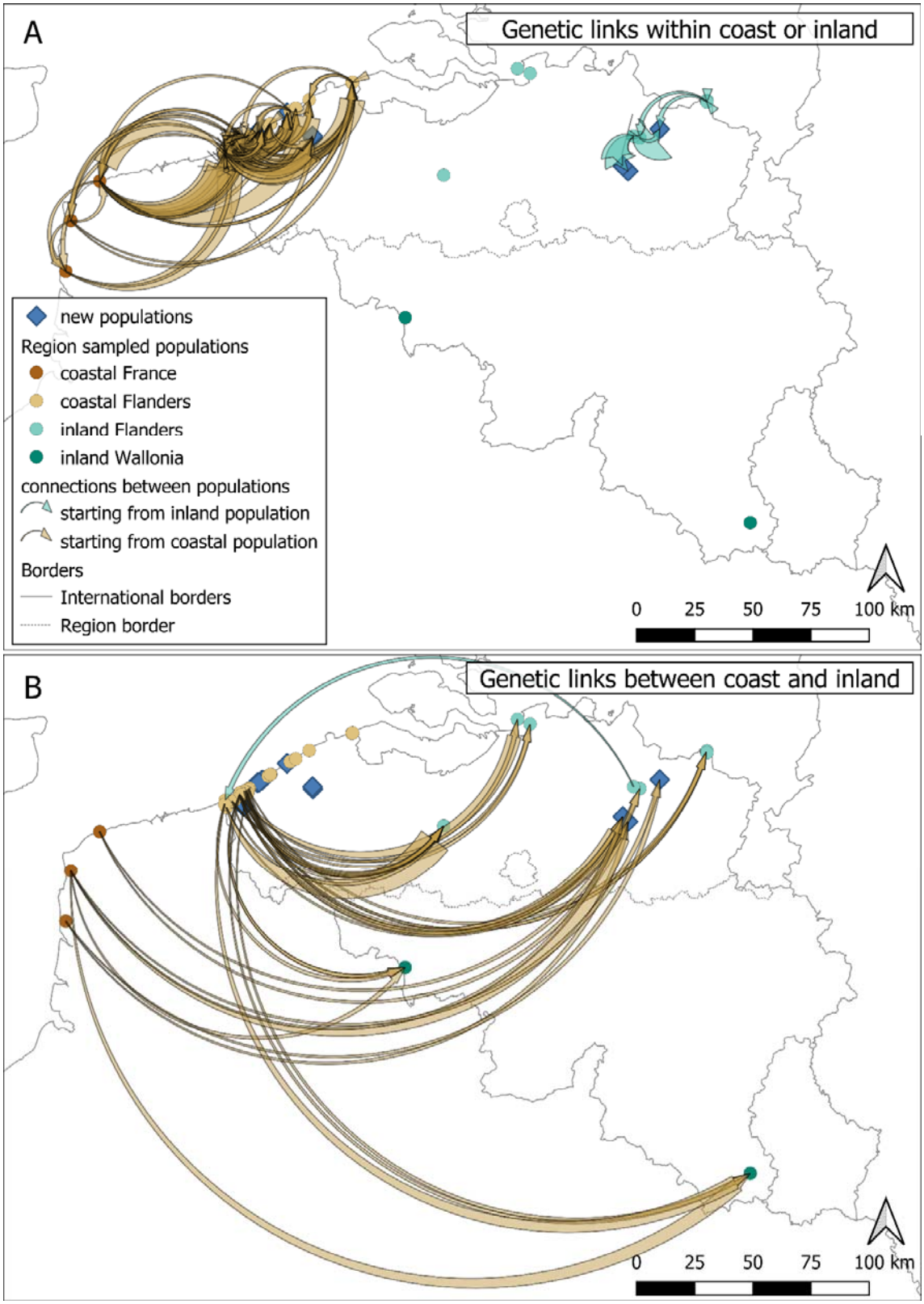

Figure 5: flow chart of genetic links (A) within the inland and coastal regions and (B) between the two regions for *B. rostrata* according to assignment to putative source (original) populations. These results depicts

accumulated gene flow from the last few generations and should not be interpreted as direct gene flow. New populations (blue squares) are populations discovered after summer 2017 further than 2.5km away from any earlier observation (observations.be), they are not used as possible source populations. The links represent the number of individuals assigned to a putative origin population (start of the arrow) that were caught in the sampled population (end of the arrow). Brown arrows are links starting from the coast, green arrows start from inland populations. The thicker the end of the arrow, the higher the number of individuals assigned to the putative source.

377 # Discussion

378 *Bembix rostrata* populations from inland sandy regions exhibited lower levels of gene flow and
379 genetic diversity and high genetic differentiation compared to those from the coastal region.
380 Asymmetrical gene flow from the coast to inland demonstrate that the species is—contrary to
381 expectations based on its behavior and poor colonization capacity—capable of dispersing to distant
382 existing populations.

383 *Bembix rostrata* has always been considered to be a philopatric species, not able to easily colonize
384 vacant habitat (Nielsen 1945; Larsson 1986). The retrieved pattern of genetic structure and gene flow
385 within and across sandy regions of Belgium clearly demonstrates this does not prevent gene flow
386 between already existing populations. The species is known to be gregarious, with on the one hand
387 local nest choice behavior showing positive density dependence because of conspecific attraction,
388 and on the other hand individuals making consecutive nests close to each other (Larsson 1986;
389 Batsleer et al. 2022a). This intragenerational individual site fidelity combined with the reported low
390 colonization capacity (Nielsen 1945; Bogusch et al. 2021), made this species a presumed poor
391 disperser. Our results show that dispersal between existing populations is not highly restricted,
392 especially in a well-connected, stepping stone landscape, such as in the Belgian coastal dunes. Likely,
393 female colonization capacities are not restricted by the species' movement capacity, but mainly by
394 the settlement phase of dispersal—with conspecific attraction of crucial importance in *B. rostrata*
395 (Batsleer et al. 2022a). When conspecifics are already present in existing populations, the settlement
396 phase is less restricted, which can explain the pattern of gene flow between existing populations.

397 Alternatively, as colonization capacity in *B. rostrata* is clearly disconnected from gene flow, the latter
398 may be equally or largely driven by male dispersal. Male-biased dispersal has indeed been found to
399 be most common in other bees and wasps (Johnstone et al. 2012). Given the protandry of the
400 species, such dispersal may be common in the period prior to female emergence, as a strategy to
401 avoid strong (kin) competition (Bonte et al. 2012; Baguette et al. 2013). As we only sampled females,

402 we cannot quantify a biased dispersal strategy, with for instance genetic spatial autocorrelation
403 analyses (Banks and Peakall 2012). Because of the haplodiploid mating system, indirect analyses by
404 the comparison of nuclear and mitochondrial markers are neither suitable because nuclear
405 introgression is reduced relative to mitochondrial introgression (Patten et al. 2015).

406 Since effective dispersal rates, resulting in establishment, depend on the species' (i.e. female)
407 capacity to move and settle, but also on the size of the source population (larger populations send
408 out more emigrants even under the same individual dispersal rate and densities), colonization at
409 short distance of vacant, new emerging pioneer habitats remains overall much more likely than
410 distant colonization. In addition, during periods of exceptional suitable environmental conditions,
411 e.g. warm summers or years with high resource abundance (nectar, prey), any local overshooting of
412 carrying capacities may further increase the magnitude of gene flow, both in terms of extent (the
413 threshold in density dependent dispersal; Kun and Scheuring 2006; Best et al. 2007) and spatial scale
414 (the fatness of the dispersal kernel; Bitume et al. 2013). We hence hypothesize that the
415 establishment of new populations may only succeed when Allee-effects are overcome by the
416 simultaneous settlement of multiple females into a single cluster. Such demographic contributions
417 are often overlooked in the dynamics of spatially structured populations in both population genetics
418 and connectivity studies (Lowe et al. 2017; Drake et al. 2022). We indeed found that the few new
419 populations included in our study originate from other large (meta)populations: at the coast from the
420 western coastal area and inland from a mixture of coastal populations and the one nearby old, large
421 inland population (Geel-Bel, 46). This confirms the very often neglected role of source population size
422 compared to absolute dispersal potential (the dispersal kernel) for gene flow in metapopulations and
423 their dynamics. The important role of demographics could also explain the observations of Bogusch
424 et al. (2021), who observed highly restricted colonization abilities of two small and isolated
425 populations in the Czech Republic.

426 The strong impact of the size of source populations on gene flow likely underlies the asymmetrical
427 gene flow from coastal to inland populations, with the latter overall smaller. Dunes from the west
428 coast (populations 1-25) are known to hold the oldest and largest populations of *B. rostrata* in
429 Belgium. In such a well-connected, stepping stone landscape, short-distance dispersal—dispersal
430 related to routine movements of resource exploitation (Van Dyck and Baguette 2005)—results in a
431 pattern of weak isolation-by-distance. As the genetic population structure is dominated by such
432 short-distance dispersers, the proportionally low numbers of long-distance dispersers do not leave a
433 detectable genetic signal within the coastal network. However, some long-distance dispersers –males
434 or females– from the coast appear to reach inland populations and leave a proportionally larger
435 signal of gene flow in these smaller populations. As mentioned higher, also within regions, such
436 demographic signals are picked-up with (1) populations from the west coast being the largest source
437 of gene flow to other populations along the coast and (2) largest and oldest known inland population
438 (Geel-Bel, population 46) leaving the strongest genetic signal in the surrounding inland populations.

439 In addition to the demographic causes, other mutually non-exclusive mechanisms may underlie the
440 retrieved pattern of asymmetrical gene flow. Although less likely in our case, wind has been put
441 forward as a dominant factor for long-distance flight behavior and migration in insects (Alerstam et
442 al. 2011; Knight et al. 2019; Leitch et al. 2021). In the focal study area, the predominant wind-
443 direction is from coast to inland (SW and WSW), which could reinforce the demographic process at
444 the coast. Alternatively, dispersal capacity itself could me more restricted in inland regions as well.
445 Indeed, our isolation-by-distance results suggest that apart from the intercept, he slope differs
446 between the regions as well. The intercept is related to population sizes: if N decreases, all else being
447 equal, differentiation will increase due to a lower total number of migrants and drift. The steeper
448 slope could be—apart from the influence of spatial habitat configuration (van Strien et al. 2015)—
449 due to dispersal capacity being more restricted in the inland region. A difference in dispersal capacity
450 within a species can result from evolved dispersal reductions in the most fragmented landscapes
451 (Cheptou et al. 2017) where costs of dispersal are highest or where spatiotemporal patch turnover is

452 lowest (Bowler and Benton 2005; Bonte et al. 2012; Duputié and Massol 2013). Such conditions may
453 indeed be more prominent for the more fragmented, and locally stable populations from the inland
454 sandy regions. Only a combination of behavioral experiments and/or quantifying physiological
455 differences in flight metabolic performance may shed light on the likelihood of such processes
456 (Hanski et al. 2004).

457 Pollinator conservation, and more specifically that of wild bees, is currently a major topic of interest
458 to policy and science. A major inherent factor complicating the interpretation of population genetics
459 results of hymenopteran species in a classical conservation genetics framework is the haplodiploid
460 mating system. Males are haploid (unfertilized) and females are diploid, which results in non-
461 symmetrical inheritance of genes across generations. In such a mating system, inbreeding
462 coefficients will be inherently high and effective population sizes low due to, for instance, purging
463 effects on deleterious alleles in haploid males (Zayed 2004, 2009). These specific attributes render
464 metrics based on assumptions of HW-equilibrium and population genetics models difficult to apply
465 and interpret, as different genetic processes will predominate in a haplodiploid conservation genetics
466 framework (Zayed 2009). While classical population genetics analyses may be used if not
467 overinterpreted (Černá et al. 2013; Sanllorente et al. 2015), we decided to report mating system
468 independent analyses, such as a multivariate approach DAPC and classical assignment tests. The
469 descriptive statistics provided should be interpreted with care and only be considered relatively
470 within the study system. In our opinion, future (modelling) studies should further elucidate the
471 potential biases for haplodiploid systems when using classical population genetics studies that are
472 based on diploid mating systems and related assumptions. In general, integration of haplodiploids in
473 the conservation genetics framework is lacking, although about 15% of all animal species are
474 haplodiploid (Evans et al. 2004; Lohse and Ross 2015).

475 Connectivity remains difficult to generalize among species and even for different landscapes within a
476 single species. In Flanders, the genetic connectivity of the grayling butterfly (*Hipparchia semele*)—

477 also occurring in sandy habitats—was in general much more restricted and gene flow slightly higher
478 in the inland region compared to the coast (De Ro et al. 2021). Differences in life history traits and
479 niche are the most likely reason for the contrasting results with *B. rostrata*. These diverging findings
480 for the focal region between two species from sandy habitat stress that connectivity is a trait of both
481 species and landscape combined—and should as such be considered in conservation policies.
482 Additionally, for *B. rostrata* itself, the asymmetrical gene flow and discrepancy in connectivity
483 between different regions were important to deduce the important role of demographics and discuss
484 the disconnection of gene flow from colonization capacity. As such, it is crucial to combine and
485 compare results from different regions for a single species to fully understand possible mechanisms
486 of gene flow.

487 Conservation implications

488 Our findings have direct implications for nature management and conservation of the flagship insect
489 species *B. rostrata* at both local and landscape scale. At the coast, a well-connected metapopulation
490 occurs, while inland populations show restricted gene flow in a fragmented sandy habitat landscape.
491 Moreover, there is an asymmetrical genetic influx from coast to inland. Our results stress the
492 importance of demographics (i.e. population sizes) for the genetic connectivity of this species. The
493 species' poor colonization capacity, resulting in a low establishment probability, should be
494 considered disconnected from gene flow between existing populations, as the latter seems much less
495 restricted.

496 To maintain the well-connected, large coastal populations, conservation should focus on local
497 management and internal processes to ensure a constant amount of suitable habitat through time.
498 Ideally, dunes are revitalized with aeolian (wind) dynamics at the landscape level (Provoost et al.
499 2011). However, in a fragmented and urbanized landscape, the current management framework
500 focuses on grazing used as a tool to locally revitalize sand dynamics (Provoost et al. 2004). It is
501 recommended to use a heterogenous approach for grazing and grazer type in space and time to

502 reconcile short-term negative (trampling) and long-term positive (open dune landscape) effects of
503 grazing on *B. rostrata* (Bonte 2005; Batsleer et al. 2022b). The genetically well-connected landscape
504 and large metapopulation context ensures population recovery and persisting connectivity when
505 implementing such a dynamic management approach.

506 Contrastingly, more isolated, small populations such as in the inland region, need a more cautious
507 approach and management should consequently focus on the protection of individual populations or
508 clusters of nearby populations. We suggest that the primary focus should be to enlarge population
509 sizes, as demographics seem highly important for this species' connectivity. Local and directly
510 surrounding habitat should be improved in quality, by creating pioneer open, sand dune habitat,
511 preferably manually as grazers should be used with caution in small, isolated populations (Batsleer et
512 al. 2022b). Creating extra stepping stones to increase landscape connectivity, which may already be
513 partially present but vacant, is only effective when combined with increasing population sizes to have
514 sufficient occasional short and long-distance dispersers. Apart from nesting resources, sufficient
515 neighboring floral resources for both nectar and prey hunting may also be important to sustain large
516 population sizes of *B. rostrata* (Kimoto et al. 2012; Buckles and Harmon-Threatt 2019).

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Author contributions

FB, DD, JM, AVB, DM, DB contributed to the study conception and design. Data collection was performed by FB, MG, MD, PV, FT; laboratory work and data analyses were performed by FB, MG, MD. 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/PopGenBembix.git) for review and will be published on zenodo upon acceptance.

Conflict of Interest

The authors have no conflict of interests.

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