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Retention of gene diversity during the spread of a non-native plant species

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Abstract

Spatial expansion, which is a crucial stage in the process to successful biological invasion, is anticipated to profoundly affect the magnitude and spatial distribution of genetic diversity in novel colonized areas. Here we show that, contrasting common expectations, Pyrenean rocket (*Sisymbrium austriacum*), retained SNP diversity as this introduced plant species descended in the Meuse river basin. Allele frequencies did not mirror between-population distances along the predominant expansion axis. Reconstruction of invasion history based on the genotypes of historical herbarium specimens indicated no influence of additional introductions or multiple points of entry on this non-gradual pattern. Assignment analysis suggested the admixture of distant upstream sources in recently founded downstream populations. River dynamics seem to have facilitated occasional long distance dispersal which brought diversity to the expansion front and, so maintained evolutionary potential. Our findings highlight the merit of a historical framework in interpreting extant patterns of genetic diversity in introduced species and underscore the need to integrate long distance dispersal events in theoretical work on the genetic consequences of range expansion.

Keywords: SNP, non-native plant, spatial expansion, genic diversity, population genetic, reconstruction, introduction history, herbarium specimen

Introduction

Spatial expansion, which is a crucial stage in the process to successful biological invasion, is anticipated to profoundly affect the magnitude and spatial distribution of intraspecific diversity in novel colonized areas (Excoffier et al. 2009). This is because genetic drift at the expansion front can increase at each subsequent founding event, leading to serial founder effects (Le Corre & Kremer 1998, Slatkin & Excoffier 2012). The spatial spread of successfully introduced and established

neobiota can therefore be expected to result in strong genetic clines, to progressively reduce mean within-population genetic diversity along the expansion axis, and to promote the fixation of mostly deleterious alleles towards the expansion front ('allele surfing'; Hallatschek & Nelson 2008, Slatkin & Excoffier 2012, Peischl et al. 2013, Peischl & Excoffier 2015). This can be expected to inflate genetic load at the expanding range margin ('expansion load') and eventually deplete adaptive potential, which seems to contradict the massive proliferation of some introduced species.

Yet, where theory on the evolutionary genetic consequences of spatial expansion is extensive, empirical data, certainly with regard to spreading non-native species, are far more scant. Population genetic research has thus far dealt primarily with the influence of genetic bottlenecks and founder diversity during the initial introduction (reviewed in Dlugosch & Parker 2008), while the influence of subsequent spatial expansion has largely been ignored (but see e.g. Bronnenhuber et al. 2011, Berthouly-Salazar et al. 2013, White et al. 2013). More importantly, and in addition to serial founder effects, also additional introductions after initial establishment, gene flow among populations from different points of entry, and rapid local adaptation may influence the development of population genetic structure in spreading neobiota (Rosenthal et al. 2008, Lachmuth et al. 2010, Bronnenhuber et al. 2011). Invasion histories are however typically difficult to reconstruct with confidence, certainly when historical information is poor and genetic data of the initial stages of the invasion process are not available (Möst et al. 2015). As a consequence, we still know relatively little of the processes shaping the spatial distribution of genetic diversity in spreading neobiota.

Here, we investigated the influence of the introduction and spread of the herbaceous plant species *Sisymbrium austriacum* subsp. *chrysanthum* (Rouy et Fouc) in the Meuse river basin (Belgium and The Netherlands) on the development of population genetic structure. This diploid ($2n=14x$) member of the *Brassicaceae* family is native to the mountainous regions in the south of France and the north of Spain (mainly the Pyrenees; Tutin 1983). The species' self-compatible flowers are pollinated by insects (bees and syrphid flies). In Belgium, Pyrenean rocket was first recorded by A.S.L. Lejeune, who

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surveyed the Vesdre, a tributary to the Meuse, during the early 19th century (Van Landuyt et al. 2006). *S. austriacum* most likely hitched along with massive wool transport from Southwest Europe to the wool industry area near the city of Verviers (Verloove 2006), upstream the river Vesdre during the 18th or early 19th century (area near the most upstream existent introduced populations In1 and In2 in Fig. 1). Prior to processing, wool was washed in the river Vesdre and tributary canals near the city of Verviers, which likely provided plenty of opportunities for the introduction of light and tiny *S. austriacum* seeds contaminating the wool. From there on, the species probably colonized river borders in the valleys of the Meuse basin in downstream direction. Introduced populations outside of the Meuse river basin are very rare and most likely hitchhiked along with sand or gravel excavations for building purposes. Seeds dispersal over longer distances seems primarily facilitated by water, possibly through the downstream movement of fruiting branches, as highlighted by a previous genetic assignment analysis based on AFLP markers across a short transect along the Meuse (area between In10 and In12 in Fig. 1; Jacquemyn et al. 2010).

We previously developed a panel of single nucleotide polymorphisms (SNPs) to screen annotated sequence variation (Vandepitte et al. 2013), which we recently employed to identify the genomic signature of selection between the introduced and native ranges by analyzing gene functions and temporal changes in the most divergent 'outlier' SNPs (Vandepitte et al. 2014). In the present study, we focus on the processes shaping the genome-wide magnitude and spatial distribution of extant genetic diversity in the new range. We first infer the introduction history by comparing genotypes of historical herbarium specimens collected in the introduced range (Vesdre and Meuse) between 1830 and 1955 with extant native and introduced populations. We then explore the current spatial distribution of genetic variation in the introduced range. Finally, we evaluate the genetic consequences of spatial expansion, hypothesizing that serial founder effects and allele surfing would lead to strong genetic clines, progressive loss of gene diversity and the fixation of alleles with increasing distances along the river course from the site of initial colonization.

Materials and methods

Data generation

Individuals were collected and genotyped as previously described in Vandepitte et al. (2014). In short, populations were identified during several field surveys in the Meuse river basin and the Pyrenees in 2009 and 2010. Leaf samples were collected from 11 native and 12 introduced populations spanning the species geographic range. All populations identified along the river Vesdre (In1 to In8 in Fig. 1) consisting of more than 10 individuals were sampled, in addition to four randomly chosen populations along the river Meuse (In9 to In12), where the species is more abundant. The three most downstream located Meuse populations are most likely founded after 1980 (In10 to In12 in Fig. 1; Jacquemyn et al. 2006, 2010). We did not observe the study species upstream of In1 on the Vesdre river (Fig. 1; personal observation K. Van Acker), nor upstream of the intersection of Vesdre and Meuse. Some established populations occur downstream of In12 along the Meuse river (Fig. 1; personal observation K. Vandepitte). We sampled all individuals of populations smaller than 20 individuals, and 20 to 25 individuals in larger populations for genotyping (Table 1). Leaf material of 50 dated and two undated historical specimens collected by Barthélemy CJ Dumortier (°1797-1878) was obtained from the herbaria of the National Botanic Garden of Belgium and the University of Liège (for more details on the extant and historical individuals collected see Vandepitte et al. 2014).

DNA was extracted using the Nucleospin DNA-extraction kit (Macherey Nhgel, Germany). To increase the yield of DNA extracted from herbarium specimens the cell lysis phase was extended to 16h at 65°C. Aerosol resistant pipet tips were used to eliminate cross contamination and ten negative (water) controls were ran along with the samples. Individuals were genotyped on 243 SNPs, previously developed from Paired End RAD (Restriction site associated DNA) sequences, using KASPar technology (Genotyping by Allele-Specific Amplification; Cuppen 2007) as detailed in Vandepitte et al. (2013).

Data trimming

SNPs and individuals with excess missing calls (>10% for SNPs and >20% for individuals) were removed. Analysis of linkage disequilibrium in POPGENE 1.32 (Yeh et al. 1999) showed that none of the remaining SNP pairs were significantly linked across all populations (all $P > 0.01$), as a result of which 206 SNPs were retained in 198 native, 47 historical and 188 current introduced individuals (433 out of 437 attempted individuals). Next, to assure selective neutrality in our dataset, we performed an F_{ST} outlier analysis, using the simulation method of Beaumont & Nichols (1996) extended to the case of a hierarchical population structure in ARLEQUIN version 3.5 (Excoffier & Lischer 2010). Null distributions were generated based on all extant native and introduced individuals applying 1×10^5 simulations across SNPs. All 26 SNPs exceeding the 99% upper or lower F_{ST} confidence intervals were excluded from further analyses. Most positive outlier SNPs were divergent between the two ranges as previously discussed in Vandepitte et al. (2014).

Data analysis

Comparison of native and introduced (historical and extant) genotypes

The genetic structure of native, historical non-native, and extant non-native individuals was compared performing Principal Coordinate Analysis (PCoA) on the standardized covariance matrix of pairwise between-individual genotypic distances as calculated in GenAlEx 6.5 (Peakall & Smouse 2012). Differences in genetic structure between native, historical non-native, and extant non-native individuals were then further examined applying a MANOVA followed by a Tukey LSD Post-hoc comparison on the first ten PCoA axes. The overall distribution of SNP diversity within and among individuals and populations of both native and non-native origin was quantified according to Nei (1973) using GenAlEx 6.5 (Peakall & Smouse 2012), applying 10^4 permutations to evaluate significance.

Next, the genetic structure of native, historical non-native, and extant non-native individuals was further explored applying a model-based clustering method using the software STRUCTURE. This Bayesian method clusters individuals as to minimize Hardy-Weinberg disequilibrium and linkage disequilibrium among individuals (Pritchard et al. 2000). Portions of the genome of individuals are then probabilistically assigned to the inferred genetic clusters for a given value of K, the optimal number of clusters present in the data. We first applied the admixture model with 10^5 burn-ins, 10^5 repetitions and 15 iterations for each value of K, from K=1 to 23, the total number of sampling locations. This identified K=2 as the optimal number of clusters in our data based on second rate order of the evolution of the likelihood of the data $\Pr(X|K)$ vs. K (Evanno et al. 2005). We then reran analysis for this optimal value of K applying 10^6 burn-ins and 10^6 repetitions.

Distribution of SNP diversity within the introduced range

To explore the spatial distribution of SNP diversity within the introduced range, we applied the same procedure as described above. Here we ran the STRUCTURE admixture model on all extant introduced individuals for K=1 to 12, the number of extant introduced populations (Fig. S1), identifying K=5 as the most likely number of overall clusters in the introduced range.

Next, to evaluate the genomic signature of spatial expansion, population genetic diversity measures [allele number (N_a), and expected heterozygosity (H_e , Nei 1973)] were calculated using GenAlEx 6.5 (Peakall & Smouse 2012) and Pearson correlated to a population's distance by water to the putative initial introduction site. These distances were calculated twice using either population In1 or population In2 as putative source (both are situated near the historical wool industry area); with QGIS 1.8.0 (Quantum GIS Development Team 2010). All PCoA scores derived from the standardized covariance matrix of mean between-population genotypic distances were entered as dependents into a multivariate General Linear Model with distance by water to In1 or In2 as a covariate. In addition, Mantel tests were performed correlating interpopulation genetic distances with both

nearest interpopulation distances along the watercourse and Euclidean interpopulation distances. All statistical analyses were conducted in SPSS Statistics 20 (IBM).

Results

Comparison of native and introduced (extant and historical) genotypes

Out of the 160 putatively neutral SNPs further analyzed, 95.0% were polymorphic within the native gene pool (n=198 individuals), 88.7% were polymorphic within the extant introduced gene pool (n=188) and 91.8% within the set of historical herbarium specimens collected in the introduced range (n=47). No SNPs were fixed across the set of historical specimens while being polymorphic within the extant introduced gene pool. In further summaries and visual representation, herbarium specimens were split into two equal age groups: collected between 1829 and 1892 (n=22) and between 1893 and 1955 (n=25).

PCoA on native, introduced ancient and introduced extant genotypes showed that the vast majority of herbarium specimens collected in the introduced range clearly overlapped with present-day introduced genotypes (Fig. 2). Only the oldest available genotypes, most likely collected before 1860, deviated from this group and were situated in between the genotypes of extant introduced individuals and different native sources. The most divergent genotypes were a specimen collected in 1829 by Alexandre LS Lejeune (°1779-1838), who is alleged the first written record of Pyrenean rocket in Belgium, two undated specimens collected by Barthélemy CJ Dumortier, who surveyed the Vesdre valley together with Lejeune around 1820 (Lawalrée 1980), a specimen collected in 1830 in Verviers and, a specimen collected in 1858 in Nessonveaux (area near In3 in Fig. 1). The collectors of the latter two specimens are unknown. MANOVA-based comparison of individual PCoA scores by the four groups (extant native individuals, extant introduced individual, introduced individuals collected between 1829-1892 and introduced individuals collected between 1892-1955) confirmed significant

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differences in genotypic composition (Wilk's Lambda $F=101.956$, $P<1\times 10^{-6}$). A Tukey HSD post-hoc test suggested significant differences between all groups ($P<0.05$), except between present-day introduced genotypes and the more recently collected herbarium specimens (1893-1955).

The PCoA further demonstrates that the current introduced gene pool is a homogeneous genetic group relative to the variation present in the native gene pool. The first PCoA axis, which accounted for 19.0% of the variance, primarily discriminated between the extant native and introduced gene pool while the second (Fig. 2) and further axes (not shown) ordered native populations according to their geographic origin. This is also exemplified by the much stronger genetic differentiation among native than among introduced populations (see also Vandepitte et al. 2014). Pairwise G_{ST} values varied between 0.016 and 0.073 in the introduced range (overall $G_{ST}=0.063 \pm 0.004$ SE; $P<0.0001$) while between 0.046 and 0.27 in the native range (overall $G_{ST}=0.260 \pm 0.012$ SE; $P<0.0001$). Between-range comparisons ranged from 0.10 to 0.31. Gene diversity ($H_{E_native}=0.22$, SE=0.006; $H_{E_introduced}=0.29$, SE=0.004) and allele number ($N_{A_native}=1.651$, SE=0.018; $N_{A_introduced}=1.811$, SE=0.009) were not reduced in extant introduced relative to native populations, indicating there was no lasting genetic bottleneck associated with colonization, as is also supported by the relatively high allelic diversity present in herbarium specimens (Table 1). Inbreeding coefficients (F_{IS}) averaged 0.054 and did not significantly differ between extant native and introduced populations according to a 2-sample t-test ($F_{IS_native}=0.052$, SE=0.014; $F_{IS_introduced}=0.056$, SE=0.011; $t=0.216$, $P=0.831$).

In agreement with the PCoA, STRUCTURE assigned extant native and non-native individuals to two major genetic clusters while several of the oldest herbarium specimens had intermediate genotypes ($K=2$ in Fig. 3). STRUCTURE runs for higher numbers of K demonstrated substructuring by geographic location within the native gene pool while extant introduced genotypes were assigned to a single major genetic cluster ($K=3$ and $K=4$ in Fig. 3).

STRUCTURE indicated the presence of five genetic groups in the introduced range ($K=5$; Fig. 4). Individuals from the known to be relatively recently founded populations downstream the Meuse river (In10 to In12) displayed admixed genetic profiles with substantial portions of four to all five genetic clusters while individuals from the Meuse population In9 were predominantly assigned to a single distinct cluster. Likewise, most populations located upstream along the Vesdre were assigned to one out of four genetic clusters, prevailing in one or more sampling locations along the Vesdre (but see In8). In agreement, population In1, In2, In7 and In9 lied at the four corners of a PCoA plot, while populations located downstream the Vesdre and downstream the Meuse had rather intermediate PCoA scores on the first two axes (Fig. 5), accounting for 22.58% and 18.15% of the variance.

Distances along the water course to the most upstream located population In1 did neither explain the magnitude of within-population genetic diversity nor the spatial distribution of among-population genetic differentiation within the introduced range. Mean within-population genetic diversity parameters (N_A , N_e and H_e) did not decrease with increasing distance from In1 (Fig. 6, Table 1). Furthermore, although STRUCTURE indicated that some nearby populations shared similar genomic profiles (e.g. In10, 11 and 12 in Fig. 4), a multivariate GLM showed no significant effect of distance by water from In1 on population genetic structure (Wilk's Lambda $F=0.54$; $P=0.79$). In accord, Mantel tests rendered no significant overall correlation between distances along the watercourse or Euclidean interpopulation distances and interpopulation mean genotypic distances (both $P>0.05$). Analyses with distances from In2 instead of In1 were not significant either (not shown).

Discussion

Contrasting common expectations regarding the genetic consequences of serial population founding during spatial expansion (e.g. Nei et al. 1975, Le Corre & Kremer 1998, Slatkin & Excoffier 2012), no evidence was found for progressive loss of genetic diversity or fixation of alleles with increasing distance from the site of initial colonization, nor did allele frequencies mirror between-population distances along the predominant expansion axis. The magnitude of genetic differentiation among introduced populations was modest but significant (overall $G_{ST_introduced}=0.063$ vs. $G_{ST_native}=0.26$) and rather patchily than gradually distributed throughout the novel range.

The genetic composition of the historical introduced gene pool suggested no significant influence of additional introductions much after initial establishment, or additional points of entry, on the extant distribution of genetic variation among non-native populations. Given extensive diversity and genetic differentiation within the native gene pool ($G_{ST}=0.26$), an additionally introduced lineage would most likely appear as a distinct genetic group within the non-native (past) gene pool (Fig. 2). Nonetheless, the genetic composition of almost all individuals collected in the second half of the 19th and early 20th century clearly overlapped with the extant, rather homogeneous, introduced gene pool (Fig. 2), which renders additional introductions after the first half of the 19th century improbable. The extant introduced gene pool thus most likely stems from one main lineage, derived through the admixture of multiple genotypes during the species establishment upstream the Vesdre in the 18th or early 19th century (Fig. 2 and Fig 3). The overall genetic divergence between the native and introduced gene pool may indicate environmental selection (Vandepitte et al. 2014), severe genetic drift following initial introduction and/or admixture with an uncharacterized source; yet, is possibly somewhat inflated by ascertainment bias. The genetic material of approximately equal small numbers of native and introduced individuals was pooled in SNP discovery (Vandepitte et al. 2013), which may have favored SNPs differentiating between the two ranges.

Historical records of wool import strengthen these inferences. From 1672 onwards, manufacturers in Verviers started to import fine merino wool from Southwest Europe (mainly Spain), which increasingly became indispensable during the 18th up to the early 19th century because the local wool was too coarse to sustain the high product quality needed for the mounting export (Dechesne 1926, Lebrun 1948). Then, during the 19th century, wool import to Verviers increasingly shifted from import from Southwest Europe to import from overseas (Lebrun 1948) until the collapse of local wool industry in the 20th century, effectively drying up the source of additional introductions from the species' native range.

The maintenance of genetic diversity and the absence of significant spatial genetic structure in the introduced range most probably stems from a non-serial pattern of population founding during expansion. Most theoretical work on the genetic consequences of range expansion thus far assumed diffuse, continuous dispersal patterns (Le Corre & Kremer 1998, Slatkin & Excoffier 2012). However, the few available simulation studies on the influence of dispersal modes during spatial expansion indicate that the combination of short distance dispersal with occasional long leaps forward, unless very rare, preserve genetic diversity better than pure diffusion-based dispersal models (Bialozyt et al. 2006, Fayard et al. 2009). In introduced *S. austriacum*, river dynamics and flooding events likely occasionally transfer propagules over long distances (see Jacquemyn et al. 2006; Jacquemyn et al. 2010), which brings diversity to and ahead of the expansion front, allows recombination of upstream sources (Fig. 4) and so retains gene diversity during spatial expansion (e.g. In 12 in Fig. 6). The presence of modest albeit significant among-population genetic differentiation may then seem counterintuitive yet fits early simulation models by Ibrahim et al. (1996) showing that stratified dispersal, i.e. involving both short and long distance dispersal, can generate a patchy genetic structure by the establishment of 'pocket populations' ahead of the expansion front. In this context, it is perhaps noteworthy that the genetically distinct population In9 was sampled in an area along the river Meuse where the species was already abundant by the late 19th century (based on notes written on herbarium material), while *S. austriacum* remained long absent from adjacent places.

Furthermore, in addition to facilitating occasional long leaps forward, the predominant downstream movement of seeds by water, no longer compensated by additional introductions upstream (see above), may have drained alleles from upstream located populations. This possibly enhanced genetic divergence and the depletion of alleles from upstream located populations (cfr. 'the unidirectional dispersal paradox'; Honnay et al. 2010).

Along with river dynamics, the species' predominant outcrossing mating system, as indicated by the low overall estimate of inbreeding (mean $F_{IS}=0.05$; Hamrick & Godt 1996), may have assisted in retaining gene diversity. The maintenance of outcrossing during establishment, as suggested by the comparable degree of homozygosity in extant and historical individuals (H_O in Table S1), possibly delayed the rapid proliferation of *S. austriacum* through Allee effects (reduced population growth at low densities). This could have allowed the arrival and intermingling of distinct genotypes and so boosted genetic diversity before the species actually set off along the river course. Likewise, outcrossing may have mitigated the rapid proliferation of (self-pollinating) individuals far ahead of the expansion front and so promoted the maintenance of population genetic diversity throughout spatial expansion (cfr. pushed waves; Hallatschek & Nelson 2008). Cross-pollination between nearby populations (e.g. In5 and In6; Fig. 1) may also have assisted in restoring the genetic diversity lost through population founding. Nonetheless, the influence of interpopulation pollen exchange is likely limited as most populations, certainly those upstream the Vesdre, are relatively spatially isolated from one another. Finally, the long-time storage of genotypes by means of a persistent seed bank does likely not assist in maintaining genetic diversity in this study system (germination after 4 years of storage at room temperature is <3 %; K. Vandepitte, unpublished data).

The case of *S. austriacum* hence suggests that spreading introduced species can avert the expected progressive loss of genetic diversity during spatial expansion and maintain high adaptive potential through a discontinuous pattern of dispersal. Several previous investigations, finding no evidence of serial population bottlenecks during the spread of exotic species do exist. Population genetic studies

of the Japanese rose (*Rosa rugosa*) in Europe (Kelager et al. 2013) the South American weed *Flaveria bidentis* in Northern China (Ma et al. 2011), the Eurasian round Goby (*Neogobius melanostomus*) in the North-American Great Lakes (Bronnenhuber et al. 2011), and an earlier investigation of *Sisymbrium austriacum* across a short transect downstream the river Meuse (mean $\Theta_{ST}=0.083$; Jacquemyn et al. 2006, 2010), failed to detect loss of genetic variation during spatial expansion. This maintenance of genetic diversity was in all cases attributed to admixture through long-distance gene flow. However, these studies were typically based on less accurate anonymous genetic markers (SSRs, ISSRs or AFLPs), did not rule out the potential influence of complex introduction scenarios nor covered the entire range. In contrast, a recent genome-wide survey of non-native populations of the very slowly dispersing introduced bank vole in Ireland showed a significant reduction in genomic diversity along the species' expansion axis (White et al. 2013), which again underscores the influence of dispersal ability and characteristics on the distribution of genetic diversity in spreading non-native species.

In conclusion, the results of this study deviate from the expectations laid out in the model of serial founding events in population expansion. This is most likely because expansion did not occur as a series of consecutive founding events. As such, it does not provide counter evidence to the serial founding events model (e.g. Le Corre & Kremer 1998), in which populations expand gradually outward from an initial source, but instead shows that expansion may also occur from multiple dispersal events and admixture from the same founding populations. Along with similar findings in different organisms (e.g. Bronnenhuber et al. 2011, Berthouly-Salazar et al. 2013), this work therefore underscores the need to integrate more complex, discontinuous dispersal modes in simulation and analytical studies on the evolutionary genetic consequences of range expansion as to generate theory apt to more dynamic systems. In the context of biological invasions, our findings in *S. austriacum* indicate that dispersal of non-native plant propagules by the dynamics of rivers and streams can assist in upholding evolutionary potential during non-native spread, potentially

stimulating further expansion. Finally, our research highlights the value of a strong historical framework in interpreting extant patterns of genetic diversity in spreading introduced species.

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Data accessibility

See <http://dx.doi.org/10.5061/dryad.hm74t> for the SNP dataset used in this manuscript and Vandepitte et al. (2014) for additional information on SNPs, populations and for the untrimmed genotypes.

References

- Beaumont MA, Nichols RA (1996). Evaluating loci for use in the genetic analysis of population structure. *Proc R Soc Lond B* 263: 1619–1626.
- Berthouly-Salazar C, Hui C, Blackburn T, Gaboriaud C, van Rensburg B, van Vuuren B, Le Roux J (2013) Long-distance dispersal maximizes evolutionary potential during rapid geographic range expansion. *Molecular Ecology* 22: 5793-5804.
- Bialozyt R, Ziegenhagen B, Petit RJ (2006) Contrasting effects of long distance seed dispersal on genetic diversity during range expansion. *Journal of Evolutionary Biology* 19: 12-20.
- Bronnenhuber JE, Dufour BA, Higgs DM, Heath DD (2011) Dispersal strategies, secondary range expansion and invasion genetics of the nonindigenous round goby, *Neogobius melanostomus*, in Great Lakes tributaries. *Molecular Ecology* 20: 1845-1859.
- Cuppen E (2007) Genotyping by Allele-Specific Amplification (KASPar). *Cold Spring Harb Protoc.* doi:10.1101/pdb.prot4841.

- Dechesne L (1926) Industrie drapière de la Vesdre avant 1800. J. Wykmans, Liège, MCMXXVI.
- Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* 17: 431-449.
- Excoffier L, Foll M, Petit RJ (2009) Genetic Consequences of Range Expansions. *Annual Review in Ecology, Evolution, and Systematics* 40: 481-501.
- Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564-567.
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611-2620.
- Fayard J, Klein EK, Lefevre F (2009) Long distance dispersal and the fate of a gene from the colonization front. *Journal of Evolutionary Biology* 22: 2171.
- Hallatschek O, Nelson DR (2008) Gene surfing in expanding populations. *Theoretical Population Biology* 73: 158-170.
- Honnay O, Jacquemyn H, Breyne P, Nackaerts K, Van Looy K (2010). Patterns of population genetic diversity in riparian and aquatic plant species along rivers. *Journal of Biogeography* 37: 1730-1739
- Ibrahim KM, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity* 77:282–291.
- Jacquemyn H, Honnay O, Van Looy K, Breyne P (2006). Spatiotemporal structure of genetic variation of a spreading plant metapopulation on dynamic riverbanks along the Meuse River. *Heredity* 96: 471-478.
- Jacquemyn H, Van Looy K, Breyne P, Honnay O (2010) The Meuse river as a corridor for range expansion of the exotic plant species *Sisymbrium austriacum*: evidence for long-distance seed dispersal. *Biological Invasions* 12: 553-561.
- Kelager A, Pedersen JS, Bruun HH (2013) Multiple introductions and no loss of genetic diversity: invasion history of Japanese Rose, *Rosa rugosa*, in Europe. *Biological Invasions* 15: 1125-1141.
- Lachmuth S, Durka W, Schurr FM (2010) The making of a rapid plant invader: genetic diversity and differentiation in the native and invaded range of *Senecio inaequidens*. *Molecular Ecology* 19: 3952-3967.
- Lawalrée A (1980) "Barthélemy Dumortier 1797-1878", in *Florilège des Sciences en Belgique*, vol. II, p. 545-546.
- Lebrun P (1948) L'industrie de la laine à Verviers pendant le XVIII^e et le début du XIX^e siècle. Contribution à l'étude des origines de la révolution industrielle. *Faculté de Philosophie et Lettres, Liège*.
- Le Corre V, Kremer A (1998) Cumulative effects of founding events during colonisation on genetic diversity and differentiation in an island and stepping-stone model. *Journal of Evolutionary Biology* 11: 495-512.
- Ma JW, Geng SL, Wang SB, et al. (2011) Genetic diversity of the newly invasive weed *Flaveria bidentis* (Asteraceae) reveals consequences of its rapid range expansion in northern China. *Weed Research* 51: 363-372.
- Möst M, Oexle S, Marková S, Aidukaite D, Baumgartner L, Stich HB, Wessels M, Martin-Creuzburg D, Spaak P (2015) Population genetic dynamics of an invasion reconstructed from the sediment egg bank. *Molecular Ecology*, 24: 4074-4093.

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences* 70: 3321-3323.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution* 29:1-10.

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537-2539.

Peischl S, Dupanloup I, Kirkpatrick M & Excoffier L (2013) On the accumulation of deleterious mutations during range expansions. *Molecular Ecology* 22: 5972-5982.

Peischl S & Excoffier L (2015) Expansion load: recessive mutations and the role of standing genetic variation. *Molecular Ecology* 24: 2084-2094.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Rosenthal DM, Ramakrishnan A, Cruzan MB (2008) Evidence for multiple sources of invasion and intraspecific hybridization in *Brachypodium sylvaticum* (Hudson) Beauv. in North America. *Molecular Ecology*, 17, 4657–4669.

Slatkin M, Excoffier L (2012) Serial Founder Effects During Range Expansion: A Spatial Analog of Genetic Drift. *Genetics* 191: 171-181.

Tutin, TG (1983) *Flora Europaea: Psilotaceae to Plantaceae* (Cambridge University Press, Cambridge) 1, 325 pp.

Vandepitte K, Honnay O, Mergeay J, et al. (2013) SNP discovery using Paired-End RAD-tag sequencing on pooled genomic DNA of *Sisymbrium austriacum* (Brassicaceae). *Molecular Ecology Resources* 13: 269-275.

Vandepitte, K., De Meyer, T., Helsen, K., Van Acker, K., Roldan-Ruiz, I., Mergeay, J., Honnay, O. (2014) Rapid genetic adaptation precedes the spread of an exotic plant species. *Molecular Ecology* 23: 2157-2164.

Van Landuyt, W., Hoste, I., Vanhecke, L., Van Den Bremt, P., Vercruysse, W., de Beer, D. (2006) *Atlas van de flora van Vlaanderen en het Brussels Gewest. Flo.Wer/Instituut voor Natuur- en Bosonderzoek/Nationale Plantentuin van België: Brussel. ISBN 90-726-1968-4. 1007 pp.*

White TA, Perkins SE, Heckel G, Searle JB (2013) Adaptive evolution during an ongoing range expansion: the invasive bank vole (*Myodes glareolus*) in Ireland. *Molecular Ecology* 22: 2971-2985.

Figure legends

Fig 1. Map of the extant sampled populations across the native and introduced range of *Sisymbrium austriacum* (adapted from Vandepitte et al. 2014). In the introduced range, the tributary Vesdre and main river Meuse flow from In1 to In12.

Fig 2. Principal Coordinates Analysis (PCoA) plot based on the individual scores of native (grey symbol), historical introduced (red and green) and extant introduced (blue) *Sisymbrium austriacum* genotypes. For visual representation, herbarium specimens were assigned to two age classes: collected before (red) and after (green) 1892.

Fig 3. STRUCTURE barplot of individual assignment results of all native, historical non-native, and extant non-native *Sisymbrium austriacum* individuals for K=2 to 4. The method of Evanno et al. (2005) indicated K=2 as the most likely number of major clusters. The two leftmost groups are non-native herbarium specimens collected before (1) and after (2) 1892. Numbers 3 to 14 in the graph represent individuals from introduced populations (In1 to In12 in consecutive order); number 15 to 25 the eleven sampled native populations (Na1 to Na11). From K=5 on, results were inconsistent among iterations and are therefore not displayed.

Fig. 4. STRUCTURE barplot of individual assignment results for K=5, of extant non-native *Sisymbrium austriacum* populations. Individuals are sorted from the left to the right by sampling location along the river course. Numbers correspond to the population codes In_ in Fig. 1.

Fig. 5. Principal Coordinates Analysis (PCoA) plot based on mean genotypic distances between extant introduced populations only.

Fig 6. Population genetic diversity (gene diversity, H_E) versus distance by water to the introduction site (approximated as In1). Error bars represent the standard error.

Table 1. Mean population genetic parameters of *S. austriacum* based on 160 SNPs as calculated in GenAlEx 6.5 (Peakall & Smouse 2012).

PopID	n	N _A	SE(N _A)	Ho	SE(Ho)	He	SE(He)	F _{IS}	SE(F _{IS})
Herb ₁₈₂₉₋₁₉₉₂	22	1.888	0.025	0.244	0.014	NA	NA	NA	NA
Herb ₁₈₉₃₋₁₉₅₅	25	1.900	0.024	0.271	0.015	NA	NA	NA	NA
In1	30	1.825	0.030	0.250	0.016	0.285	0.015	0.121	0.027
In2	11*	1.781	0.033	0.261	0.018	0.280	0.015	0.064	0.033
In3	19	1.838	0.029	0.285	0.017	0.300	0.014	0.071	0.029
In4	18	1.856	0.028	0.292	0.017	0.307	0.014	0.062	0.029
In5	12*	1.813	0.031	0.289	0.019	0.295	0.014	0.024	0.033
In6	11*	1.788	0.032	0.290	0.020	0.291	0.015	0.020	0.033
In7	16*	1.788	0.032	0.258	0.017	0.271	0.015	0.047	0.027
In8	15*	1.825	0.030	0.279	0.018	0.308	0.014	0.105	0.032
In9	17*	1.781	0.033	0.275	0.019	0.276	0.015	0.030	0.032
In10	7*	1.769	0.033	0.275	0.019	0.274	0.015	-0.005	0.035
In11	10*	1.806	0.031	0.283	0.018	0.293	0.015	0.026	0.028
In12	22	1.869	0.027	0.295	0.017	0.324	0.014	0.100	0.031

Na1	14*	1.588	0.039	0.196	0.018	0.205	0.016	0.048	0.034
Na2	19	1.681	0.037	0.213	0.018	0.218	0.015	0.032	0.029
Na3	20	1.613	0.039	0.202	0.019	0.206	0.016	0.049	0.031
Na4	20	1.719	0.036	0.242	0.018	0.239	0.015	-0.002	0.026
Na5	18	1.563	0.039	0.208	0.019	0.195	0.016	-0.040	0.028
Na6	20	1.600	0.039	0.196	0.018	0.212	0.016	0.072	0.030
Na7	14*	1.606	0.039	0.214	0.019	0.221	0.017	0.052	0.031
Na8	15*	1.694	0.037	0.200	0.016	0.229	0.016	0.131	0.029
Na9	20	1.738	0.035	0.210	0.016	0.226	0.015	0.063	0.024
Na10	19	1.650	0.038	0.208	0.018	0.225	0.016	0.089	0.031
Na11	19	1.713	0.036	0.214	0.016	0.224	0.015	0.074	0.027

n=number of individuals genotyped, N_A =number of alleles, H_o = observed heterozygosity, H_e =expected heterozygosity, F_{IS} =inbreeding coefficient, SE=standard error. *: all individuals present at this location were sampled.