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Range expansion of a selfing polyploid plant despite widespread genetic uniformity

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• *Background and Aims* Ongoing and previous range expansions have a strong influence on population genetic structure of plants. In turn, genetic variation in the new range may affect the population dynamics and the expansion process. The annual *Ceratocapnos claviculata* (Papaveraceae) has expanded its Atlantic European range in recent decades towards the north and east. Patterns of genetic diversity were investigated across the native range to assess current population structure and phylogeographical patterns. A test was then made as to whether genetic diversity is reduced in the neophytic range and an attempt was made to identify source regions of the expansion.
• *Methods* Samples were taken from 55 populations in the native and 34 populations in the neophytic range (Sweden, north-east Germany). Using amplified fragment length polymorphism markers an analysis was made of genetic variation and population structure (Bayesian statistical modelling) and population differentiation was quantified. Pollen/ovule ratio was analysed as a proxy for the breeding system.
• *Key Results* Genetic diversity at population level was very low (mean $H_e = 0{\cdot}004$) and two multilocus genotypes dominated large parts of the new range. Population differentiation was strong ($F_{ST} = 0{\cdot}812$). These results and a low pollen/ovule ratio are consistent with an autogamous breeding system. Genetic variation decreased from the native to the neophytic range. Within the native range, H_e decreased towards the north-east, whereas population size increased. According to the Bayesian cluster analysis, the putative source regions of the neophytic range are situated in north-west Germany and adjacent regions.
• *Conclusions Ceratocapnos claviculata* shows a cline of genetic variation due to postglacial recolonization from putative Pleistocene refugia in south-west Europe. Nevertheless, the species has expanded successfully during the past 40 years to southern Sweden and north-east Germany where it occurs as an opportunistic neophyte. Recent expansion was mainly human-mediated by single long-distance diaspore transport and was facilitated by habitat modification.

Key words: AFLP, anthropogenic dispersal, autogamous, *Ceratocapnos claviculata*, founder effects, genetic differentiation, genetic diversity, global change, neophyte, postglacial colonization, range expansion, therophyte.

INTRODUCTION

Processes of global change such as climate warming, eutrophication, human-mediated long-distance transport and habitat modification foster invasions and range expansions of plant species (Walther *et al.*, 2005; Maskell *et al.*, 2006; Thuiller *et al.*, 2006; Wilson *et al.*, 2009). Both invasions and range expansions may impact not only the distribution of species but also the intraspecific patterns of genetic variation (Olivieri, 2009; Gurevitch *et al.*, 2011). In turn, genetic variation in the new range may affect or constrain the population dynamics and the expansion process (Olivieri, 2009; Lachmuth *et al.*, 2010).

Current patterns of genetic diversity and variation in European plant species are the result of both past phylogeographical processes and more recent anthropogenic influences. After the climatic oscillations of the Quaternary ice ages, previous glacial and periglacial areas were recolonized. Across their ranges, many species show a 'southern richness – northern purity syndrome', characterized by a gradient of high genetic diversity of populations at low latitudes and low diversity at higher latitudes (Hewitt, 2000). Populations at the rear, southern edge were often able to persist during Quaternary oscillations through relatively small altitudinal shifts, while following matching suitable climatic conditions. Such relict populations may feature high levels of differentiation among populations and low levels of within-population diversity, indicating local adaptation (Hampe and Petit, 2005). In contrast, at the northern range margin, repeated range contractions have probably eliminated various genotypes. During postglacial recolonization, rapid expansion by populations from the leading edge and from a limited number of single refugial populations was accompanied by successive losses of alleles along the colonization pathway through repeated

bottleneck effects and founder events (Hewitt, 1999; Wilson *et al.*, 2009). This pattern of 'rear vs. leading edge' contrasts with a general 'centre-periphery' pattern predicting that marginal populations are genetically less diverse than those from the centre of a species distribution range (Sagarin and Gaines, 2002).

Natural and anthropogenic dispersal processes have differently affected patterns of genetic diversity, which may influence later establishment success. During natural migrations from the leading edge, species expand first by short-distance dispersal according to the typical dispersal distance of their propagules via diffusion or via corridors, which link previously separated suitable areas (Hewitt, 2000). Second, rare long-distance dispersal events enable colonization of potentially suitable areas distant from the current range (Arrigo *et al.*, 2010; Hampe, 2011). Due to the rarity and stochasticity of successful long-distance dispersal, populations in the new range are expected to show strong founder effects and may diverge genetically from source populations (Ibrahim *et al.*, 1996). Typically, clines of genetic diversity towards the northern range edge are explained by such sampling effects during range expansion (Hewitt, 1999), which in turn allow routes of invasion to be disentangled. In contrast to natural dispersal pathways, human-mediated dispersal processes tend to introduce larger numbers of propagules from more diverse sources over shorter periods of time (Simberloff, 2009; Wilson *et al.*, 2009). Consequently, genetic diversity in introduced populations of invasive species often has been found to be equal to that of native populations so that no pronounced genetic bottlenecks were detectable (Bossdorf *et al.*, 2005). Multiple introductions from different source populations or strong propagule vectors may have compensated for losses of genetic diversity (Roman and Darling, 2007; Lachmuth *et al.*, 2010). In addition, admixture of different source regions in the new range may even lead to higher genetic variation and may result in higher physiological plasticity (Novak and Mack, 2005; Wilson *et al.*, 2009). However, in the case of anthropogenic dispersal, genetic diversity may be reduced in the invasive range because of bottlenecks during the invasion process or as a consequence of genetically impoverished source populations that served as origin for the founder individuals (Durka *et al.*, 2005). Thus, propagule pressure, the number of introduction events and the source regions as well as the genetic structure within the source region are decisive factors for genetic diversity and establishment in the target region and should be taken into consideration when studying range expansions.

Plant breeding systems have a strong influence on both genetic diversity of populations and the population dynamics and thus may have an impact on expansion success (Hao *et al.*, 2011). Self-fertilizing species with short life cycles such as many weeds are often successful and fast colonizers. If there is only a low number of diaspores introduced, self-fertilization is advantageous for fast population growth (Barrett *et al.*, 2008). However, the degree of self-fertilization may vary within and among populations of a species. Because within narrow phylogenetic groups the breeding system may be indicated by pollen to ovule ratios (Cruden, 1977; Michalski and Durka, 2009), changes in the rate of self-pollination during range expansion can be expected to be accompanied by changes in pollen to ovule ratios (e.g. Thomas and Murray, 1981). Self-fertilizing species produce less pollen grains and thus have a lower ratio of pollen grains to ovules per flower than related outcrossing taxa. In contrast, outcrossing plants need compatible partners and insect-pollinated species depend on pollinators. Furthermore, highly selfing species are genetically less diverse at the population level and therefore are expected to show lower evidence of genetic bottlenecks than outcrossing species (Brown and Marshall, 1981). Selfing species also are less likely to suffer from inbreeding depression because deleterious alleles tend to be purged through selection (Barrett and Charlesworth, 1991). Despite this, reduced genetic diversity in selfing species may also have long-term negative impacts due to a reduced evolutionary potential for adaptation to changing environments (Leimu *et al.*, 2006; Olivieri, 2009). However, a number of successful large-scale invasions despite very low genetic variation have been reported (*Bromus tectorum*: Novak *et al.*, 1991; *Reynoutria japonica*: Hollingsworth *et al.*, 1998; *Rubus alceifolius*: Amsellem *et al.*, 2000; *Pennisetum setaceum*: Le Roux *et al.*, 2007; *Ferula loscosii*: Perez-Collazos *et al.*, 2009; Grimsby and Kesseli, 2010; *Eichhornia crassipes*: Zhang *et al.*, 2010; *Rosa rubiginosa*: Zimmermann *et al.*, 2010). The success of populations lacking genetic variation might be based on either niche matching (Pérez *et al.*, 2006) or the presence of general-purpose genotypes, which exhibit high phenotypic plasticity (Richards *et al.*, 2006; Roman and Darling, 2007). Annual selfing plants represent the 'blueprint' of a successful colonizer (Baker, 1967; but see Petit *et al.*, 2004, and references therein). However, recent reviews (Bossdorf *et al.*, 2005; Wilson *et al.*, 2009) on the genetic structure of invasive plant species included only few annual selfing species and are thus biased towards perennial and outcrossing species. Therefore, in the present study we analysed genetic diversity and structure of populations of *Ceratocapnos claviculata* in its entire native and recently colonized neophytic range. This species is a selfing, annual forest herb native to western Europe. During the past 40 years, it has expanded its range further towards the north-east into temperate and sub-continental regions occupying similar habitats as in the native range (Voss *et al.*, 2011). Both climate change and atmospheric nitrogen deposition (Lethmate *et al.*, 2002) have been suggested to facilitate the expansion of *C. claviculata.* Also, anthropogenic transport of seeds as contaminants of pulpwood (Often *et al.*, 2006) may have enhanced dispersal. In addition, disturbance of sites through forest machinery may create safe sites for establishment and colonization (Voss *et al.*, 2011).

We studied population genetic patterns across the entire range of *C. claviculata* and characterized putative dispersal pathways of the rapidly expanding species. We addressed the following questions: (1) How do genetic diversity and differentiation vary across the native range? (2) Is genetic diversity reduced in the neophytic range despite successful expansion? (3) Does *C. claviculata* feature a similar degree of autogamy in populations in the native and neophytic range as reflected by pollen to ovule ratios? (4) What are the potential source regions of the expansion?

MATERIAL AND METHODS

Study species

The biology of *Ceratocapnos claviculata* (L.) Lidén (Papaveraceae; formerly Fumariaceae) has been reviewed recently (Voss *et al.*, 2012). It is a tetraploid ($2n = 4x = 32$) summer and winter annual with a climbing habit. The species has a eu-atlantic distribution and occurs originally in semi-shaded open oak–birch forests or substitute communities such as pine forests or on sun-exposed forest clearings. Furthermore, it is found in fringes, hedgerows or ditches along roads. In the neophytic range it occurs mostly in substitute communities as above. Its small flowers may be pollinated by insects (Apidae, Bombyliidae) but selfing due to autonomous self-pollination often occurs (Lidén, 1986). The main flowering time lasts from June to September. An average individual produces about 300 black, shiny seeds of 1·3 mg. Seeds have an inconspicuous, tiny aril and lack appendages for animal or wind dispersal. Thus, under natural conditions long-distance seed dispersal should be rare.

Study area and sampling

We sampled 89 populations across the entire range of *C. claviculata* (Fig. 1 and Supplementary Data Table S1), 34 of which were from the neophytic range in Sweden ($n = 16$), eastern Germany ($n = 17$) and northern France ($n = 1$). The northern French population was discovered about 15 years ago (Decocq, 2000) in a region where the species had not previously been observed. All other populations ($n = 55$) were situated in the native range. Populations WG 14–18 (cf. Fig. 1) represent old outposts of the native range, where the species had already been found before 1930. In the neophytic range, we visited almost all currently known localities of the species. Preliminary genotyping analyses had shown low genetic variation within populations. We therefore maximized the number of populations and limited the number of samples within populations. In each population we sampled, if possible, four plants randomly along a line transect, collecting young, fresh leaves for genetic analysis, resulting in a total of 342 samples. Leaves were dried and stored in silica gel at room temperature. Additionally, to determine the pollen/ovule ratio, we collected flower buds shortly before anthesis from three of the four sampled individuals and stored them in 70 % ethanol. Finally, we estimated population size on a logarithmic scale (1–100 individuals = 1, 101–1000 = 2, 1001–10 000 = 3, >10 000 = 4) by walking line transects across populations.

Breeding system

The ratio of pollen grains to ovules per flower can be indicative of the mating system within phylogenetic lineages (Cruden, 1977; Michalski and Durka, 2009). For characterization of the breeding system, we estimated the pollen/ovule ratio and related it to values of other Papaveraceae. For 233 flowers we suspended the total amount of pollen in 100 μL water and counted pollen grains in five 2-μL aliquots under a microscope. Ovules were prepared and counted under a dissecting microscope.

Population genetic analysis

Amplified fragment length polymorphisms (AFLP, Vos *et al.*, 1995) were generated following Kloss *et al.* (2011) with four primer combinations (ACT/CTA-FAM, ACA/CTA-VIC, AAG/CAC-NED, AGG/CAT-PET). For genotyping we used peak-height raw data, adjusted an individual peak height-threshold (between 80 and 800 rfu) for each locus and kept only highly reproducible loci. Across all 342 samples of *C. claviculata* this resulted in a total of 108 loci, all of which were used for data analysis and 40 of which (37·0 %) were polymorphic. The error rate was tested on 29 samples that were repeated from the same DNA extract. The error rate was very low (0·25 %; 12 mismatches in 4727 locus × individual pairs). The correlation between fragment size and fragment frequency was $r = -0{\cdot}073$ ($P = 0{\cdot}451$), indicating absence of size homoplasy.

Genetic variation within populations and regions was estimated as the percentage polymorphic loci (PLP) at the 5 % level, i.e. loci with allelic frequencies in the range 0·05–0·95. Assuming fixed homozygosity in this highly selfing species, allele frequency was set equal to AFLP fragment frequency and expected heterozygosity (gene diversity; H_e) was calculated. The calculations were performed in AFLP-SURV v. 1·0 (Vekemans, 2002) following the approach of Lynch and Milligan (1994). The number of private alleles occurring in only one population and the number of rare alleles with a frequency of ≥5 % occurring in ≤50 % of all populations was assessed manually.

Genetic population structure was analysed using a Bayesian statistical model using BAPS 5·2 (Corander *et al.*, 2006). BAPS searches for genetically homogenous groups of individuals. We performed non-spatial clustering with unlinked marker data with a maximum number of clusters set to $K = 15$ and without taking their population affiliation into account. Relationships among clusters were further analysed by neighbour joining of Nei's genetic distance in PHYLIP v. 3·68 (Felsenstein, 2004). The robustness of each node was estimated by bootstrapping distance matrices with 100 replications in AFLP-SURV. Additionally, we conducted a principal coordinates analysis (PCoA) in GeneAlEx 6·41 (Peakall and Smouse, 2006) to visualize the relationships among populations.

Genetic differentiation among populations was quantified with Wright's *F*-statistics in AFLP-SURV and differentiation among populations, native and neophytic range and among BAPS groups was quantified by analyses of molecular variance (AMOVA; Stewart and Excoffier, 1996) with 1000 permutations in GeneAlEx 6·41. We tested whether population structure followed an isolation by distance pattern by correlating pairwise Nei's genetic distance to geographical distance and performing a Mantel test in FSTAT v.2·9.3·2 (Goudet, 2001).

Statistical analysis

ANOVA and Tukey's HSD (honestly significant difference) test were used for unequal sample size as post-hoc tests to compare genetic diversity between regions, between native and neophytic range, and between BAPS groups. For the

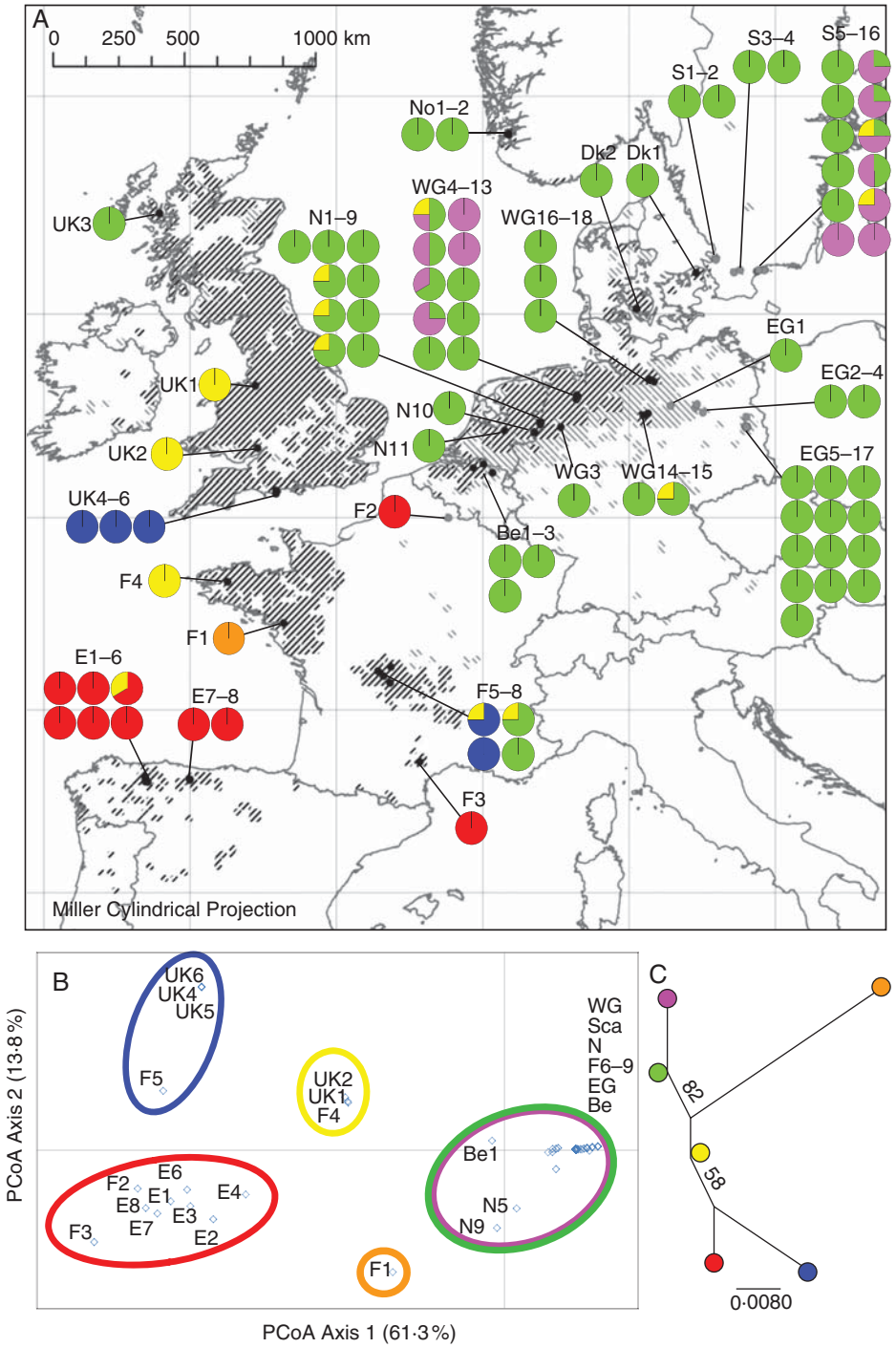

FIG. 1. Distribution of native (black hatched) and neophytic (grey hatched) range (cf. Voss *et al.*, 2012), sampling sites (cf. Supplementary Data Table S1) and genetic structure of *Ceratocapnos claviculata*. (A) BAPS cluster membership (genetic groups denoted by different colours, pie charts show the affiliation of individuals to genetic groups). (B) PCoA of populations based on Nei's genetic distance. Only the first two axes are shown, which accounted for 75·1 % of variation (three axes explained 84·0 %). Colours of the circles indicate the BAPS cluster to which the majority of individuals were affiliated. (C) Neighbour-joining tree of BAPS clusters based on Nei's distance. Numbers at branches show bootstrap support (>50 %) based on 100 replicates.

BAPS analysis, we assigned admixed populations to the prevailing group. Similarly, ANOVAs were performed to compare pollen/ovule ratios between the regions and between native and neophytic range. We used linear regression to test whether diversity could be explained by population size classes or latitude and longitude. In these analyses only populations sampled with at least three individuals were included. To meet assumptions of normality, data were square root transformed before analysis whenever necessary. These calculations were performed in STATISTICA 9·1 (StatSoft Inc., 2010).

RESULTS

Pollen/ovule ratio

Ceratocapnos claviculata produced a mean of 2·19 (± 0·03 s.e., $n = 245$) ovules per flower and a mean of 504 (± 10 s.e., $n = 245$) pollen grains per flower. The mean pollen/ovule ratio of 245 (± 7 s.e.) was ten times lower than most other, yet outcrossing, Papaveraceae species (Supplementary Data Table S2), indicating an autogamous breeding system. The pollen/ovule ratio differed slightly between geographical regions (Supplementary Data Table S2, ANOVA: $F_{5,239} = 3{\cdot}647$, $P < 0{\cdot}01$), but there was no significant difference between native and neophytic range (*t*-test: $P = 0{\cdot}272$).

Genotypic and genetic variation

Across all 342 individuals of *C. claviculata*, only 46 multilocus AFLP genoptypes were detected. This indicates very low levels of genetic variation. In 58 of 89 populations (65 %), only a single multilocus genotype was found (Supplementary Data Table S1). The most abundant multilocus genotype, 'A' (207 individuals, 60·5 %), occurred in 61 populations and was the sole genotype in 25 populations. The second most abundant genotype, 'B' (35 individuals, 10·2 %), was found in 12 populations. In 11 populations a single private allele was found (Be1, WG11, E4, F2, F4, F5, UK1, UK2, EG2, EG8, S8) and two private alleles were found in two Spanish populations each. Thus, estimates of genetic diversity of *C. claviculata* at population level were very low (Table 1): mean PLP = 0·8 % (± 0·15 s.e., $n = 89$), mean gene diversity $H_e = 0{\cdot}004$ (± 0·0008 s.e.) and at the species level $H_T = 0{\cdot}022$.

Genotypic variation was higher in the native than in the neophytic range with 39 and ten genotypes found in the two regions, respectively. Also genetic variation was higher in the native than in the neophytic range, both at population (*t*-test: $P < 0{\cdot}01$) and overall level for gene diversity (Table 1). However, H_e also differed significantly between geographical regions and showed a decline both from south to north and from west to east (Fig. 2), essentially representing a decrease of genetic variation with distance from Spain.

The proportion of large populations (≥1000 individuals) tended to be higher in the neophytic range (41 %) than in the native range (20 %; but χ^2-test: $P > 0{\cdot}05$, $n = 77$) and population size was positively correlated with latitude and longitude ($r^2 = 0{\cdot}1$, $P < 0{\cdot}01$; $r^2 = 0{\cdot}07$, $P < 0{\cdot}05$; $n = 77$). However, genetic variation was not correlated with population size ($r^2_{all} = 0{\cdot}03$, $P > 0{\cdot}05$; $n = 77$).

Population structure and genetic differentiation

Bayesian analysis of population structure revealed six clusters with a clear geographical pattern (Fig. 1). Cluster 1 (red) comprised most of the Spanish and some French samples. Cluster 2 (yellow) consisted of populations in France, the UK and single individuals in other regions. Cluster 3 (blue) was confined to France and the UK while Cluster 4 (orange), consisting of a single genotype, occurred only in one population in France. Cluster 5 (green) included genotype 'A' and was dominant in the coastal lowlands of Belgium, Netherlands, and northern Germany and Scandinavia. Cluster 6 (pink), including genotype 'B', was closely related to Cluster 5 and occurred only in north-west Germany and Sweden. The neighbour-joining tree of clusters indicated a major separation between south-west Europe (clusters 1 + 3) and north-east Europe (clusters 5 + 6) (Fig. 1C). The clusters differed strongly with respect to genotypic and genetic variation. Clusters 1 and 2 harboured by far the largest number of genotypes and showed higher H_e while the more northern clusters 5 and 6 had the lowest H_e (Table 1). Results of the BAPS analysis were largely corroborated by the PCoA (Fig. 1B), which revealed five groups.

Wright's *F*-statistic indicated very strong population differentiation (overall $F_{ST} = 0{\cdot}812$) as expected from the very low levels of within-population variation. Hierarchical AMOVA showed that differentiation between the native and neophytic range accounted for 13 % of variation whereas most variation resided among populations within ranges (65 %, Table 2). We found higher differentiation in the native range ($\Phi_{ST} = 0{\cdot}766$) than in the neophytic range ($\Phi_{ST} = 0{\cdot}455$) due to low levels or lack of variation in the latter. Across the entire range there was a weak pattern of isolation by distance for Nei's genetic distance ($r = 0{\cdot}64$, $P = 0{\cdot}05$, Mantel-test); this was not present

TABLE 1. *Values of genetic variation across populations in the native and neophytic range and in clusters identified in the BAPS analysis: number of populations and individuals (n_p and n_i), expected heterozygosity (H_e), standard error (± s.e.), percentage polymorphic loci at 5 % level (PLP), total number of alleles, rare alleles and private alleles, and number of genotypes*

	n_p	n_i	H_e	± s.e.	PLP	± s.e.	No. of alleles	No. of rare Alleles	No. of private alleles	No. of genotypes
Population means										
Native	55	207	0·0053	0·0012	1·03	0·229	105	19	0	39
Neophytic	34	135	0·0021	0·0007	0·44	0·143	89	3	0	10
Overall	89	342	0·0041	0·0008	0·80	0·154	108	0	0	46
Clusters										
C1-Red		35	0·0272	0·0080	8·3		91	2	5	19
C2-Yellow		22	0·0348	0·0096	6·5		95	2	7	14
C3-Blue		16	0·0113	0·0054	4·6		87	2	2	4
C4-Orange		4	0	0	0		81	0	0	1
C5-Green		229	0·0017	0·0009	0		86	0	4	5
C6-Pink		35	0·0005	0·0005	0		84	0	1	2

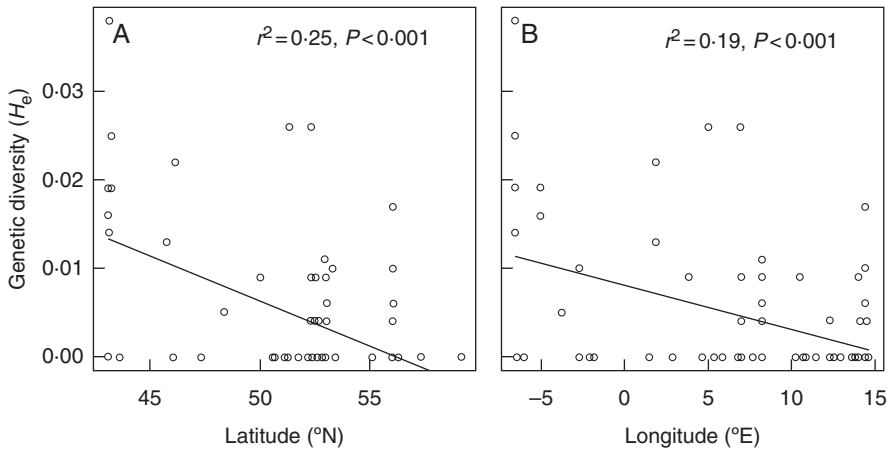

FIG. 2. Population-level genetic diversity H_e in relation to (A) latitude and (B) longitude; only populations with at least three samples are considered, $n = 87$.

TABLE 2. *Summary results of analyses of molecular variance (AMOVA) of* C. claviculata *(all values are significant at* $P < 0{\cdot}001$*)*

	Overall F_{ST}	Percentage variation		
		Among groups	Among populations	Within populations
Range				
Total range	0·760		76	24
Native range	0·766		77	23
Neophytic range	0·455		46	54
Groups				
Native vs. neophytic	0·776	13	65	22
BAPS clusters	0·849	77	7	15

when neophytic or native range were analysed separately ($P > 0{\cdot}225$), indicating a predominant role of genetic drift relative to gene flow.

Putative source regions of the neophytic range were apparent from the cluster memberships. North-west Germany was the likely source region of the Swedish invasive populations as these were the only regions that shared cluster 6 (pink). The eastern German neophytic area was affiliated to cluster 5 (green) and consisted almost exclusively of genotype 'A', suggesting that the adjacent native area with the same genotype was the most likely source region. The single newly established population in northern France was part of cluster 1 (red), suggesting southern French or Spanish populations as the source.

DISCUSSION

Genetic diversity and structure across the native range

Overall, *Ceratocapnos claviculata* showed low levels or total lack of genetic variation within populations across large areas and pronounced population differentiation. This is characteristic of many selfing species (Barrett and Husband, 1990; Nybom, 2004) and thus was expected due to the autogamous breeding system which was also corroborated by the relatively low pollen/ovule ratio compared with other Papaveraceae.

Across the native range, we still found significant differences in genetic diversity between populations in the southern part of the range, namely France and Spain, and the northern part, namely UK, the coastal lowlands of Belgium, Netherlands, and western Germany and Scandinavia. Our findings are thus in line with a general pattern of a latitudinal cline of genetic diversity from southern Europe to northern Europe (Hewitt, 1999; Hampe and Petit, 2005). Lower genetic diversity in the northern parts of the range may be related to post-glacial recolonization processes. Range contractions and expansions during the Quaternary in the course of climatic oscillations may have eliminated genotypes in northern Europe but not those in the refugia. During recolonization of the northern European sites a loss of alleles may be the result of rapid expansion of already impoverished populations from the leading edge or from single refugial populations (Hewitt, 1999). Additionally, founder effects and an autogamous breeding system may have enhanced genetic erosion (e.g. Durka, 1999; Prentis *et al.*, 2008). Thus, patterns of genetic variation in the native range of *C. claviculata* do not follow the central–marginal model but show a rear edge versus leading edge pattern.

The current distribution of *C. claviculata* and models of the distribution of climatic zones during the Pleistocene suggest that the Iberian Peninsula served as a refugium (Voss *et al.*, 2012), which is corroborated by the comparatively high levels of genetic variation found in this region. However, France also had relatively high diversity levels and harboured members of five of the six genetic clusters. Thus, Atlantic regions north of the Pyrenees may have represented additional refugia, as has also been proposed for other species such as the oceanic orchid *Himantoglossum hircinium* (Pfeifer *et al.*, 2009) and the European-wide *Corylus avellana* (Palmé and Vendramin, 2002).

Causes and consequences of range expansion

C. claviculata was first observed in Sweden in 1958 (Bjuv, Skåne; Hylander, 1971). It has been hypothesized that seeds of this and other species may have been introduced with imported uncorticated pulpwood after heavy storms and windthrows

(Oredsson, 2005). Two such occasions when large amounts of timber and pulpwood (>250 000 m^3) were transported from north-west Germany and the Netherlands to southern Swedish saw-mills and paper plants occurred in 1972 and 1976 (Oredsson, 2005). Our results support this hypothesis as the introduced populations in Sweden were probably of north-west German origin. Although it is impossible to identify the precise source region for the eastern German invasive populations, they probably also originated from adjacent areas in north-west Germany. Anthropogenic dispersal probably explains the long distances (>50 km). Interestingly, in northern France a single population, which reportedly was newly established, belonged to the genetic cluster found in Spain and southern France. Thus, the species seems to be strongly dispersal-limited and anthropogenic long-distance seed dispersal is the principle cause for its range expansion. Like other species that are mainly distributed in forests, recent massive human intervention such as clearcuts and transport of wood or saplings and habitat modification facilitated and accelerated expansion (Often *et al.*, 2006; D'Andrea *et al.*, 2009; Wilson *et al.*, 2009).

Northward range expansion as a response to recent climate change has been reported in a number of plant species (Thuiller *et al.*, 2006). The northern range margin of the Atlantic species *Ilex aquifolium* parallels the 0 °C isoline. In recent decades this isoline has shifted northwards, as has the northern margin of *Ilex* (Walther *et al.*, 2005). For *C. claviculata*, which has a similar distribution to *Ilex*, the recent range expansion also has been related to recent milder winters (Lethmate *et al.*, 2002). In fact, the geographical area of the climatic niche of *C. claviculata* has expanded and climatic conditions in the native and neophytic range are very similar with only slightly increased temperature differences between winter and summer as well as the proportion of summer rain towards the recently colonized range (Voss *et al.*, 2012). Similar to other species, the current distribution is not in equilibrium with the climatic niche, which may suggest further range expansion (Magri *et al.*, 2006; D'Andrea *et al.*, 2009). However, both ecologically suitable microsites for germination and human-induced dispersal appear to be prerequisites for the expansion and establishment of the annual *C. claviculata.*

The pollen/ovule ratios were similar in the old and new range, suggesting maintenance of the autogamous breeding system. However, in the new range we observed lower genetic diversity than in the old range and widespread genetic uniformity. In general, genetic impoverishment may be due to founder events and genetic bottlenecks during the invasion process (Barrett and Kohn, 1991; Edwards *et al.*, 2006; Okada *et al.*, 2009). However, it is more likely that for *C. claviculata* pre-existing low levels of genetic diversity in the source populations are responsible. The occurrence of two BAPS cluster groups in the Swedish neophytic range suggests either a single introduction from a source population containing both clusters, or multiple introductions from several source populations from the Netherlands, Belgium or north-west Germany. The new eastern German populations lacked both genetic diversity within and differentiation between populations, suggesting a single introduction and further dispersal within eastern Germany.

Low genetic variability may limit colonization of new habitats due to lack of evolutionary potential under variable or novel environmental conditions (Stebbins, 1957). However, *C. claviculata* is a successful colonizer despite very low levels or lack of genetic diversity. In fact, we observed an increase of population size from south-west to north-east of the range, possibly indicating increased fitness. This paradox may be interpreted against the background of phenotypic plasticity. Despite very low levels of genetic variation, *C. claviculata* shows highly plastic responses, for example of leaf morphology, to changing light conditions, enabling the colonization of various habitats (Voss *et al.*, 2011, 2012). Phenotypic plasticitiy in turn may be enhanced by processes conveying intragenomic variation, such as polyploidy or epigenetic variation, which may be particularly relevant for the success of species during range expansion (Prentis *et al.*, 2008; Pandit *et al.*, 2011). Thus, in the polyploid *C. claviculata* despite the lack of genotypic variability, intragenomic diversity and fixed heterozygosity may account for phenotypic plasticity.

The role of phenotypic plasticity for the success of colonizing species has been classified as either a 'Jack-of-all-trades' strategy (Richards *et al.*, 2006) of a robust general-purpose genotype that maintains high fitness also in unfavourable environments or an opportunistic 'Master-of-some-situation' strategy, in which the colonizer is better able to increase fitness than resident species under particularly favourable conditions (Baker, 1967; Richards *et al.*, 2006). *C. claviculata* is able to colonize a wide range of habitats on non-calcareous soils such as open forests, fringes, hedgerows, ditches and disturbed bogs. However, data both from common garden experiments and field sites suggest that *C. claviculata* attains higher fitness (higher individual biomass) under particular conditions (Voss *et al.*, 2011, 2012). For example, all these habitats are semi-shaded and have humic soils of intermediate moisture and some open sites in the herb layer. The latter conditions facilitate seedling establishment, which is the most critical stage of annual species (Voss *et al.*, 2012). Thus, the species may be assigned as a 'Master-of-some-situation' type, similar to other successful colonizers (Richards *et al.*, 2006).

Finally, two methodological shortcomings should be considered related to the partitioning of genetic variation and that we sampled a large number of populations across the entire range of the species with only moderate sample sizes per population. First, the finding of private alleles in Sweden and eastern Germany might indicate that new mutations have occurred in recent decades or that we missed to detect the respective alleles in the native range. Secondly, the extremely low level of variation present in the species precluded a more stringent identification of both the genetic relationships and the demographic and historical processes in the native and neophytic range. Many of the multilocus genotypes differed only by the presence or absence of a few AFLP bands, making it impossible to assess their phylogenetic relationships. The low sample size per population may have limited the detection of shared AFLP bands and thus the amount of gene flow among populations, but this was partly compensated for by a high number of populations. Still, in certain regions, such as France, which harboured five of the six clusters, a denser sampling could advance our understanding of the history of *C. claviculata*.

Conclusions

Ceratocapnos claviculata is an example of a successful autogamous, therophytic neophyte with extremely low genetic diversity. Our analyses suggest Pleistocene refugia in southwest Europe (Spain, France). During the process of postglacial recolonization, the species lost a large part of its genetic variability, which is illustrated by a north-eastward decline of genetic diversity with increasing distance from Spain. Our data corroborate the proposed colonization of southern Sweden from sites in the Netherlands and north-west Germany, which was mediated through anthropogenic dispersal. Low genetic diversity in the newly colonized range is related to genetically impoverished source populations rather than to founder effects. Polyploidy may alleviate the low level of genetic diversity, which does not compromise range expansion. We suggest that a combination of increased opportunities for long-distance dispersal through anthropogenic activities, an extension of the climatic niche owing to recent climate change and suitable habitat quality through land-use changes may contribute to range expansion of many species.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: details of regions sampled, populations, geographical coordinates, number of samples, invasion status, population size, gene diversity and number of genotypes. Table S2: pollen/ovule ratio in *C. claviculata* in different regions and the pollen/ovule ratio in related Papaveraceae species.

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SUPPLEMENTARY DATA

TABLE S1. Regions, population codes, number of samples, locations of sampled populations (in WGS84 geo-coordinates in decimal degree east, E, and north, N), invasion status, population size class (1 = 1–100; 2 = 101–1000; 3 = 1001–10 000; 4 >10 000 individuals), percentage polymorphic markers (PLP), gene diversity (H_e) and number of multilocus genotypes (#Genotypes)

Region	Population code	No. of samples	E	N	Invasion status	Size class	PLP	H_e	#Genotypes
Coastal Lowlands									
Belgium	Be1	4	5.0197	51.3409	native	n.a.	5.6	0.026	2
	Be2	4	4.6729	51.2433	native	n.a.	0	0	1
	Be3	4	5.3332	51.1119	native	n.a.	0	0	1
Netherlands	N1	4	6.9618	52.3301	native	1	5.6	0.026	2
	N2	3	6.9645	52.3355	native	1	0	0	1
	N3	4	6.9698	52.3873	native	1	0.9	0.004	2
	N4	4	6.9475	52.3845	native	1	0	0	1
	N5	4	6.9607	52.3794	native	2	0.9	0.004	2
	N6	4	6.9893	52.3858	native	2	0.9	0.004	2
	N7	4	6.9893	52.3766	native	2	1.9	0.009	2
	N8	3	6.9656	52.3792	native	1	0	0	1
	N9	3	6.9574	52.3758	native	1	0	0	1
	N10	4	6.7501	52.1392	native	2	0	0	1
	N11	4	5.7461	52.1739	native	3	0	0	1
W. Germany	WG3	4	7.6650	52.2636	native	4	0	0	1
	WG4	4	8.1934	53.0305	native	2	0.9	0.004	2
	WG5	3	8.1942	52.9433	native	2	1.9	0.011	2
	WG6	4	8.2025	53.0363	native	2	0	0	1
	WG7	4	8.2099	53.0375	native	1	0.9	0.006	2
	WG8	4	8.2178	53.046	native	1	0	0	1
	WG9	4	8.2197	53.001	native	3	0	0	1
	WG10	4	8.2224	53.0051	native	2	0	0	1
	WG11	4	8.2347	52.993	native	2	1.9	0.009	3
	WG12	4	8.2401	53.0012	native	2	0	0	1
	WG13	4	8.2501	53.0079	native	2	0	0	1
	WG14	4	10.4793	52.554	native	n.a.	1.9	0.009	2
	WG15	4	10.647	52.5986	native	n.a.	0	0	1
	WG16	4	10.663	53.4325	native	2	0	0	1
	WG17	4	10.7022	53.4262	native	3	0	0	1
	WG18	4	10.8498	53.3739	native	3	0	0	1
E. Germany									
	EG1	4	11.4111	52.7855	invaded	3	0	0	1
	EG2	4	12.2485	52.733	invaded	3	0.9	0.004	2
	EG3	4	12.3138	52.8153	invaded	2	0	0	1
	EG4	4	12.5165	52.6222	invaded	n.a.	0	0	1
	EG5	4	14.0095	52.5145	invaded	4	0.9	0.004	2
	EG6	4	13.9697	52.2817	invaded	3	0	0	1
	EG7	4	13.9608	52.2723	invaded	3	0	0	1
	EG8	4	13.9757	52.2953	invaded	2	1.9	0.009	3
	EG9	4	14.0159	52.2634	invaded	2	0	0	1
	EG10	4	13.9709	52.2645	invaded	2	0	0	1
	EG11	4	14.0066	52.2808	invaded	3	0	0	1

	EG12	4	13.9768	52.2638	invaded	3	0	0	1
	EG13	4	14.034	52.2723	invaded	1	0.9	0.004	2
	EG14	4	13.9948	52.2422	invaded	1	0	0	1
	EG15	4	14.0301	52.2523	invaded	1	0	0	1
	EG16	4	14.0372	52.2678	invaded	1	0	0	1
	EG17	4	14.036	52.2601	invaded	1	0	0	1
France									
	F1	4	–1.8073	47.2947	native	3	0	0	1
	F2	4	3.8309	49.9976	invaded	2	1.9	0.009	3
	F3	4	2.8442	43.6	native	1	0	0	1
	F4	3	–3.7218	48.3602	native	1	0.9	0.005	2
	F5	4	1.8604	46.143	native	n.a.	3.7	0.022	3
	F6	1	1.6	45.9	native	n.a.	0	0	1
	F7	3	1.4187	46.0295	native	n.a.	0	0	1
	F8	4	1.8302	45.7194	native	n.a.	2.8	0.013	2
Scandinavia									
Denmark	Dk1	4	12.2857	55.9756	native	2	0	0	1
	Dk2	4	10.2457	55.1274	native	3	0	0	1
Norway	No1	4	5.8728	59.1648	native	n.a.	0	0	1
	No2	4	5.845	59.153	native	n.a.	0	0	1
Sweden	S1	4	12.9343	56.3002	invaded	1	0	0	1
	S2	4	12.9631	56.2822	invaded	1	0	0	1
	S3	4	13.5764	55.9863	invaded	2	0	0	1
	S4	4	13.796	56.0358	invaded	1	0	0	1
	S5	4	14.3619	56.0041	invaded	3	0	0	1
	S6	4	14.3857	56.0115	invaded	2	0	0	1
	S7	4	14.4001	56.0257	invaded	4	0.9	0.006	2
	S8	4	14.4188	56.0324	invaded	3	3.7	0.017	2
	S9	4	14.4307	56.0371	invaded	2	1.9	0.010	3
	S10	4	14.4381	56.0345	invaded	4	0.9	0.004	2
	S11	4	14.4518	56.0361	invaded	4	0.9	0.004	2
	S12	3	14.5922	56.0429	invaded	1	0	0	1
	S13	4	14.6050	56.0502	invaded	2	0	0	1
	S14	4	14.6162	56.0446	invaded	3	0	0	1
	S15	4	14.6221	56.0464	invaded	1	0	0	1
	S16	4	14.6255	56.0342	invaded	2	0	0	1
Spain									
	E1	4	–6.54037	43.1949	native	1	4.6	0.025	4
	E2	4	–6.54099	43.1955	native	2	3.7	0.019	3
	E3	4	–6.52793	43.1185	native	2	2.8	0.014	4
	E4	3	–6.53376	43.0969	native	1	6.5	0.038	3
	E5	1	–6.53927	43.0677	native	1	0	0	1
	E6	4	–6.45162	43.0579	native	1	0	0	1
	E7	4	–5.00835	43.1235	native	1	3.7	0.019	4
	E8	4	–5.01647	43.1094	native	1	2.8	0.016	3
United Kingdom									
	UK1	4	–2.76947	53.2725	native	3	1.9	0.010	3
	UK2	4	–2.70313	51.7446	native	3	0	0	1

UK3	4	–6.04550	57.3529	native	2	0	0	1
UK4	4	–2.04167	50.6583	native	2	0	0	1
UK5	4	–2.07333	50.6017	native	2	0	0	1
UK6	4	–2.06081	50.6817	native	2	0	0	1

TABLE S2. Number of samples (*n*), and means (± s.e.) of ovules and pollen per flower and pollen/ovule ratio in *C. claviculata* in different regions. Different letters indicate significant differences according to HSD test, ($P < 0.05$). 'Coastal Lowlands' include Belgium, Netherlands and N. Germany. Pollen/ovule ratio in related Papaveraceae species are also given

	n	Ovules		Pollen		Pollen/Ovule ratio	
Ceratocapnos claviculata							
Spain	9	2.11	(0.20)	440[ab]	(33)	233[ab]	(37)
France	38	2.08	(0.06)	537[b]	(17)	265[a]	(11)
UK	16	2.06	(0.06)	479[ab]	(44)	234[ab]	(22)
Coastal Lowlands	80	2.28	(0.05)	513[ab]	(17)	237[ab]	(11)
E Germany	28	2.25	(0.12)	414[a]	(46)	195[b]	(25)
Scandinavia	74	2.16	(0.06)	525[ab]	(14)	265[a]	(15)
Native	165	2.21	(0.04)	517	(11)	249	(8)
Invasive	80	2.15	(0.06)	477	(20)	237	(13)
Total	245	2.19	(0.03)	504	(10)	245	(7)
Related Papaveraceae species							
Corydalis orthoceras[1, *]						1717–4169	
Corydalis lineariloba[1, *]						2152–3720	
Corydalis fumariifolia[2, *]						3000	
Corydalis fumariifolia[3, *]						1400	
Corydalis cava[4, *]						3244	
Hypecoum procumbens ssp. *fragrantissimum*[5, *]						4934	
Sarcocapnos pulcherrima[6, †]						2780	
Hypecoum procumbens ssp. *procumbens*[5, †]						465–1293	

* Self-incompatible, † Selfing
References: [1] Fukuhara (2000), [2] Sunaga (1988), [3] Ohara and Higashi (1994), [4] Christ *et al.* (2001), [5] Dahl (1989), [6] Salinas and Suarez (2003).

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