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Introduction history and population genetics of intracontinental scotch broom (*Cytisus scoparius*) invasion

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Abstract

Aim: Biological invasions at the intracontinental scale are poorly studied, and intracontinental invasions often remain cryptic. Here, we investigate the recent range expansion of scotch broom (*Cytisus scoparius*) into Norway and clarify whether the genetic patterns indicate natural spread or human introduction. Furthermore, we investigate whether plants were moved within the native range and how this influences invasion success. We also infer the level and structuring of genetic diversity within and between the putative native and introduced range.

Location: Europe.

Methods: We analysed the chloroplast sequence variation in 267 scotch broom samples from its northern expansion front and from its native range across Europe, including herbarium samples dating back to 1835. For 37 populations, we analysed variation in nuclear single-nucleotide polymorphic markers to study gene flow and genetic diversity.

Results: We identified 20 different haplotypes, which lacked spatial and temporal distribution patterns in the recent expansion range in Norway. They also mostly lacked patterns across the native European range of scotch broom. The genetic diversity of nuclear genomic SNP markers across populations in the introduced range was similar to that of populations in the native range, with limited differentiation among populations.

Main conclusions: Scotch broom is alien to Norway and was introduced by humans on multiple occasions from diverse origins over a long period of time. High propagule pressure has probably maintained the high genetic diversity in the novel range through a combination of genetically diverse source populations and high gene flow among them. Within the native European range, our results suggest the presence of cryptic intraspecific admixture, most likely mediated by humans moving genotypes among the regions occupied by distinct native genotypes. Intracontinental invasions may easily go unnoticed and revealing these invasions and the factors driving them may be of great importance for the management of alien species.

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KEYWORDS
cryptic invasion, cryptogenic species, *Cytisus scoparius*, intracontinental plant invasion, introduction history, population genetics, propagule pressure, range expansion, scotch broom

1 | INTRODUCTION

Despite more than 50 years of research on the biological invasion of alien species across continents, studies at the intracontinental scale are rare, because it is difficult to prove whether a species is native or alien (Hulme et al., 2016; Webber & Scott, 2012). Such cases are termed cryptogenic species (Carlton, 1996) or interspecific cryptic invasions. Even when available records indicate a recent colonisation of a species, it should only be classified as alien if it was directly introduced by humans and did not expand its range by natural dispersal from its native range (Gilroy, Avery, & Lockwood, 2017). Throughout this publication, we use the term 'alien' for a species or genotype that occurs at a location as a direct result of human introduction (Hulme et al., 2016; Webber & Scott, 2012) and 'invasive' for an alien species (or genotype) that spreads beyond the location where it was introduced (Cristescu, 2015; Richardson et al., 2000; Wilson, Dormontt, Prentis, Lowe, & Richardson, 2009). When alien genotypes are introduced to a distant location but are within the species' native range, the invasion may be undetected or cryptic (Morais & Reichard, 2018; Saltonstall, 2002). Alien genotypes compete against native genotypes, and if they hybridise, they may genetically contaminate or swamp the native populations, which can lead to the loss of native gene pools and may cause phenotypic changes of the species (Morais & Reichard, 2018; Nielsen, Brandes, Kjær, & Fjellheim, 2016). The admixture of geographically distant genotypes within the native range can also create a source for introductions into the novel range, which will become invasive (Ellstrand & Schierenbeck, 2000). However, while it is generally accepted that the source origin of an introduced organism affects its ability to persist and invade (Henery et al., 2010), the possibility of genetic admixtures of different genotypes prior to their introduction has rarely been considered (Le Roux, Richardson, Wilson, & Ndlovu, 2013).

The introduction history of an alien species is crucial for its establishment and invasiveness (Colautti, Grigorovich, & MacIsaac, 2006; Estoup & Guillemaud, 2010). Of special importance are the quantitative aspects of propagule pressure (Lockwood, Cassey, & Blackburn, 2005; Simberloff, 2009) and the qualitative aspects of propagule origin. Propagule pressure quantifies the total number of introduced propagules, which is affected by the number of introduction events (propagule number) and by the number of individuals introduced in each introduction event (propagule size; Blackburn, Lockwood, & Cassey, 2015; Wittmann, Metzler, Gabriel, & Jeschke, 2014). High propagule pressure not only increases the probability of naturalisation by compensating for stochastic mortalities and increasing the chance of being introduced into favourable habitats, it also shapes the genetic structure of introduced populations (Lockwood et al., 2005). High propagule pressure has been observed in the majority of successful invasions at the intercontinental scale (Blackburn et al., 2015). As the likelihood of multiple introduction events increases over time, propagule pressure may explain the commonly observed lag phases of alien species (Bock et al., 2015; Simberloff, 2009). In addition, once an alien species has become established and reached maturity, its seeds may serve as an additional source of propagules, causing secondary spread in the invaded range. In addition to the number of introduction events, the origins of those introductions affect the invasive potential by increasing genetic diversity and the possibility of preadaptation to the novel environment (Buckley & Catford, 2016). Further, the genetic admixtures resulting from the crossing of plants from geographically distant origins produces novel genotypes that may have stronger invasive potential (Bock et al., 2015; Ellstrand & Schierenbeck, 2000; Morais & Reichard, 2018) and increased ability to adapt to the new environment. Propagules from different origins may have reached the new range by direct introduction, or they may have been moved to another location first where they intermixed with others before moving further into the new range (bridgehead invasion; van Boheemen et al., 2017; Lombaert et al., 2011). Thus, even a limited number of introduction events (of large propagule size) can result in diverse propagule origins (i.e. where they originally evolved) when propagules have already mixed in the source population of the introduction event.

The shrub scotch broom (*Cytisus scoparius* (L.) Link) is native across central Europe and the British Isles but has been classified as an invasive species elsewhere (e.g. in the United States, Chile, Australia, New Zealand (Kang, Buckley, & Lowe, 2007), Brazil (Cordero, Torchelsen, Overbeck, & Anand, 2016), South Africa (Mkhize, Mhlambi, & Nanni, 2013) and India (Srinivasan, Shenoy, & Gleeson, 2007). Since its first record in southern Norway in 1876 (Blytt, 1876), it has spread northwards (Figure 1, see Appendix S1) and has also increased in abundance in threatened habitats, such as endangered coastal heathlands (Lindgaard & Henriksen, 2011). This species was included on the Norwegian Black List in 2007 but was removed in the 2012 revised list, considering that it might be native (Gederaas, Salvesen, & Viken, 2007; Gederaas, Moen, Skjelseth, & Lar sen, 2012). The ongoing range expansion might simply reflect a lag in dispersal to northern habitats that became suitable after the last glacial retreat. This could be due to the species' short distance seed dispersal (Bossard, 1991; Malo, 2004) and geographical barriers (as is proposed for European trees, e.g. *Abies alba* and *Larix decidua* in Svenning & Skov 2004). Alternatively, its recent range expansion can be a result of human introduction. In fact, scotch broom has been planted for soil improvement and stabilisation (Vesthassel, 1926) and as a garden ornamental, and the extended viability of its seeds (Magda, Gleizes, & Jarry, 2013) allows for accidental spread in soil (ballast soil of ships in the past, infrastructure

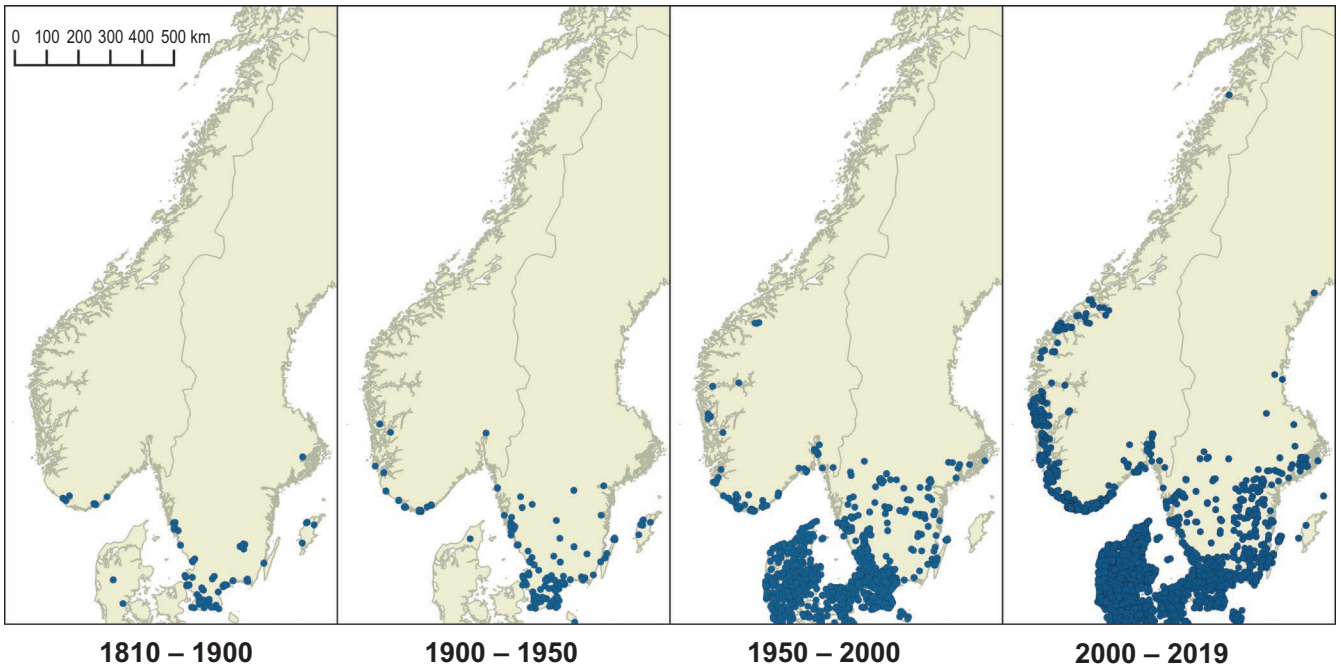

FIGURE 1 Records of *Cytisus scoparius* in Scandinavia across different time periods from the GBIF database (www.gbif.org). Data accessed on 10 May 2019

construction). However, natural expansion and introduction by humans are not mutually exclusive. Both native and introduced populations of scotch broom have been identified in Denmark (Nielsen et al., 2016; Rosenmeier, Kjær, & Nielsen, 2013). With high certainty, a native gene pool exists in Denmark, where it was observed as early as 1648 (Paulli, 1648), and in 1958 this distinct phenotype was described (dwarfed growth form and increased cold hardiness; Böcher & Larsen, 1958; Rosenmeier et al., 2013). More recent studies have revealed that fast spreading, invasive plants in the Danish landscape are likely to constitute an introduced gene pool (Nielsen et al., 2016; Rosenmeier et al., 2013).

This study investigates the range expansion of the intracontinental invader scotch broom in its native distribution range in Europe, with a focus on its northernmost expansion front. More explicitly, we aim to (a) establish whether this range expansion is natural or is caused by human introductions, (b) determine whether the expansion is the result of one or multiple introductions (propagule pressure) and pinpoint the origin of these introductions (propagule origin) to establish the role of propagule pressure and origin in invasion success and (c) quantify the level and geographical structure of genetic diversity to investigate its possible role in invasion success.

2 | METHODS

2.1 | Sampling

We collected leaf samples from a total of 172 populations of scotch broom. We sampled both fresh leaves and leaves from herbarium specimens (see Appendix S2).

The fresh leaf samples were collected in 2012 and 2013, dried on silica gel and stored at 20°C. The sampling focused on the species' northernmost range, which is in Norway, where we sampled 13–26 individuals from each of 25 populations and one individual from each of 11 populations. From the native European range, we sampled 12 populations, each with five to 19 individuals. From seven populations, a single individual sourced from seedlings grown from seed bank samples or from field collections was chosen to represent the population. We also included two individuals of six Danish populations from the study by Rosenmeier et al. (2013). In addition, three individuals of scotch broom were collected in garden centres in eastern Norway (see Appendix 2).

Leaf samples of herbarium specimens were collected from 11 European herbaria (see Acknowledgements). For the herbarium specimens, a single individual represented the population from which the herbarium specimen originated. From the Norwegian herbarium samples, 27 were selected focusing on the four areas representing the species' oldest observations (Figures 1 and 4). They also covered the time span of the Norwegian collections (from 1869 to 1998). For the selection of 81 specimens from other European countries, the aim was to cover an equal number for each country of the oldest (1835–1890) and the most recently (1959–2001) collected specimens (see Appendix S2). As most specimens had no GPS coordinate record, they were estimated based on the specimen location description.

2.2 | DNA extraction

We extracted DNA from up to 10 mg dry tissue, or approximately 50 mg fresh tissue, following the DNeasy Mini Plant Kit (Qiagen)

for most herbarium samples or the DNeasy 96 Plant Kit (Qiagen) for most fresh samples. To obtain a higher yield, we prolonged the chemical cell lysis step (in buffer AP1, at 65°C) from 10 to 30 min. For fresh samples, we implemented the final DNA elution only once, and for herbarium species the first eluate was passed through the filter a second time.

2.3 | Chloroplast sequencing

For the chloroplast genetic analysis, most populations were represented by one individual, but for 17 populations from Norway and the two populations from Denmark we sequenced five to seven individuals to test whether these populations originated from a single or from multiple maternal lines (see Appendix S2). Four chloroplast regions (*trnT-trnL*, *rps4-trnT*, *psbF-psbB* and *psbC-trnS2*; see Appendix S3) of scotch broom were amplified in a polymerase chain reaction (PCR) and sequenced with a similar methodology for fresh samples and herbarium samples. For the fresh samples, the four regions were first PCR amplified with universal primer pairs; the sequencing reaction was then performed with specific internal primers (see Appendix S3). For the herbarium samples with more degraded DNA, five internal primer pairs within these four chloroplast regions were used for both PCR and sequencing reactions (see Appendix S3; Furevik, 2017). The 25 µl PCR mix contained 0.625 units of HotStart *Taq* DNA Polymerase from New England Biolabs (NEB), 1× Standard *Taq* Reaction Buffer (NEB), 50 µM of each dNTP (Invitrogen), 0.4 µM of each primer (Invitrogen) and 1 µl of DNA template. For herbarium samples, the reaction mixture also contained 0.25 µM of additional Mg^{2+} and 0.1% bovine serum albumin. Polymerase chain reactions were performed in a Mastercycler nexus thermocycler (Eppendorf), with an initial polymerase activation of 15 min at 95°C, followed by 30 or 35 cycles (for fresh samples and herbarium samples, respectively) of denaturation for 1 min at 94°C, annealing for 2 min at 52, 53 or 55°C (depending on primers, see Appendix S3), extension for 3 min at 72°C and a final extension of 10 min at 72°C. The PCR products were visualised on 1.5% agarose gels. Herbarium samples that displayed no band on the agarose gel were also PCR amplified with 40 cycles.

The PCR products of most samples were purified and sequenced by Macrogen. Other PCR products were first purified by the Montage PCR_{96} Cleanup Kit (Merck Millipore), then sequencing reactions were performed with a BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) using 1 µM primer and 1 µl of PCR product. This was again purified by the Montage SEQ_{96} Sequencing Reaction Cleanup Kit (Merck Millipore) and sequenced on an ABI 3730 DNA Analyser (Thermo Fisher Scientific).

2.4 | Chloroplast sequence analysis

Sequences were edited and aligned with SEQUENCHER software 4.10.1 (Gene Codes Corporation) and BIOEDIT 7.2.5 (Hall, 1999). First, the fresh samples and the herbarium samples were aligned separately. Unique polymorphisms (present in a single sample only) were verified by resequencing or were otherwise removed. Mononucleotide repeats were excluded from the analysis. To allow the joint analysis of fresh samples and herbarium samples, all sequences were shortened to the herbarium length. Gaps were coded by simple index coding (SIC; Simmons & Ochoterena, 2000). In two cases of complex indels, they were each coded as two separate indels. The phylogeny of all samples, with *Lupinus sp.* as an outgroup (BEAST 1.8.3, Drummond, Suchard, Xie, & Rambaut, 2012); *Lupinus* sequence was obtained from GenBank (accession number: gi_485474291_gb_KC695666.1), was used to verify that all samples represented the species scotch broom, and misidentified samples were removed.

In total, 267 samples were used for the chloroplast analysis (see Appendix S2). To estimate genetic diversity of the haplotypes, samples were grouped by the country of origin. Belgium and the Netherlands were combined due to the small geographical area and the small number of samples from these countries. Two samples from islands (Madeira and Corsica) and the three samples from the garden centre were excluded. Standard genetic diversity indices (number of haplotypes, nucleotide diversity and haplotype diversity) were calculated in DNASP 5 (Librado & Rozas, 2009). To correct for differences in sample size we used the programme CONTRIB 1.4 (Petit, El Mousadik, & Pons, 1998) to apply a rarefaction to a sample size of five and then estimated allelic richness. These calculations of haplotype diversity were also applied to samples grouped by broader geographical areas (i.e. Scandinavia vs. Iberian Peninsula vs. Central Europe) and split by their year of collection (i.e. before 1910 vs. after 1958) to investigate broader geographical patterns and changes over time. Differentiation of haplotype frequencies among the countries was calculated using a pairwise exact test with 100,000 Markov chain Monte Carlo steps in ARLEQUIN 3.5 (Excoffier & Lischer, 2010). The exact test was developed by Raymond and Rousset (1995) based on the Fisher's exact test and has the advantage that it is less affected by the different sample sizes (Goudet, Raymond, deMeeus, & Rousset, 1996; Jogesh, Peery, Downie, & Berenbaum, 2015).

A haplotype network of the 267 samples of *C. scoparius* was constructed using the software PopART v 1.7. (http://popart.otago.ac.nz) and the median joining algorithm (Bandelt, Forster, & Rohl, 1999).

The spatial distribution of the haplotypes was visualised on maps using QGIS 2.14.20 (QGIS Development Team, 2018). To assess the relationship between the genetic distance and the geographical distance of all haplotype samples of scotch broom, we used a Mantel test with 999 permutations in GENALEX 6.5 (Peakall & Smouse, 2006, 2012). Only those 255 samples with a coordinate accuracy of 100 km or better were included in a Mantel test (including multiple samples of some populations). Mantel tests were also performed after splitting the samples into two temporal groups collected either before 1910 or after 1958 and for Norwegian samples only.

We also assessed changes in the abundance of the haplotypes over time by plotting the cumulative count for each haplotype of all herbarium samples in R 3.5.0 (R Core Team, 2018).

2.5 | Nuclear SNPs

The fresh samples were analysed for single-nucleotide polymorphic (SNP) markers (see Appendix S2). This included up to 26 samples for each of the following populations: 20 populations from southern Norway, five from western Norway, two from Denmark, one from France and one from Germany. The seedlings grown from eight seed bank accessions were also included. However, for some of the seed bank populations, only a few individuals were available (see Appendix S2).

2.6 | Nuclear SNP marker development and genotyping

For the nuclear genetic analysis, SNP markers had been developed from double-digest restriction-associated-DNA sequencing (ddRADseq; Peterson, Weber, Kay, Fisher, & Hoekstra, 2012) of 16 Norwegian samples, as described in Nielsen et al. (2016). Two sets of 40-multiplex MassArrays (AGENA Bioscience, San Diego, CA, United States) were designed using the Assay Design Suite 2.0 software (AGENA Bioscience). However, only 36 SNP markers resulted in a suitable quality for this analysis. The MassArrays (AGENA Bioscience) resulted in 36 usable SNP markers from 635 samples with less than 50% missing SNP reads (see Appendix S2).

2.7 | Nuclear SNP data analysis

GenAlEx 6.5 (Peakall & Smouse, 2006, 2012) was used to calculate the proportion of polymorphic loci for each population. Principal component analyses were calculated in R. To study the partitioning of genetic variation within and between groups of populations, we performed three analyses of molecular variance (AMOVA) using Arlequin 3.5. Analysis of the chloroplast DNA variation showed that individuals with haplotype C represented the putatively native genetic lineage; these were treated as a separate group to study the distribution of genetic diversity within and between putatively native and invasive lineages. We first compared individuals with haplotype C versus all others. Next, we compared Norwegian populations to European populations, and finally, we treated the populations of each country as separate groups. We excluded individuals with haplotype C from the two latter analyses to investigate the distribution of genetic variation within and between the putatively invasive

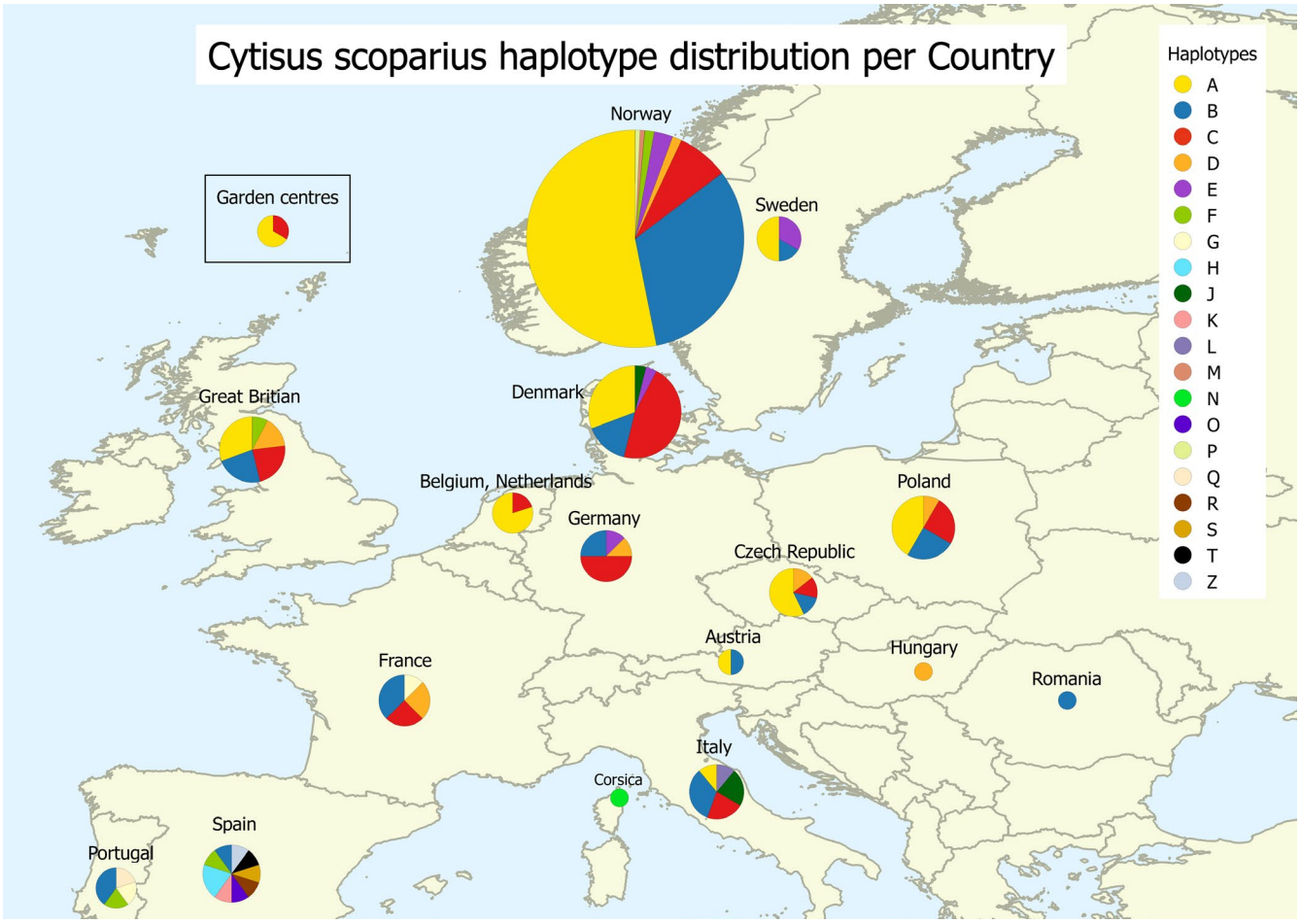

FIGURE 2 Chloroplast haplotypes of *Cytisus scoparius* samples grouped by country of collection. The size of the pie represents the number of samples; each colour represents one haplotype. Haplotypes J to Z were found only once. The haplotype B on Madeira is not shown

TABLE 1 Chloroplast haplotype diversity of samples grouped by country, in order of allelic richness after rarefaction

Country	No. of samples	No. of haplotypes	Haplotype diversity	Nucleotide diversity	Allelic richness after rarefaction to *n* = 5
Belgium and the Netherlands	5	2	0.400	0.00079	1.000
Norway	144	8	0.612	0.00107	1.516
Denmark	28	5	0.624	0.00110	1.556
Sweden	6	3	0.733	0.00112	1.833
Czechia	8	4	0.643	0.00276	1.875
France	10	4	0.733	0.00415	2.052
Poland	12	4	0.758	0.00225	2.072
Germany	8	4	0.750	0.00279	2.143
Great Britain	13	5	0.833	0.00317	2.536
Italy	9	5	0.861	0.00121	2.730
Portugal	5	4	0.900	0.00613	3.000
Spain	10	9	0.978	0.00739	3.778
Austria	2	2	1.000	0.00099	NA
Hungary	1	1	NA	NA	NA
Romania	1	1	NA	NA	NA
Total	262	19	0.667	0.00132	2.052

Note: Note that two samples from small island locations (including one private haplotype) and the three samples from garden centres have been removed.

lineages. Pairwise F_{ST} values among all populations were calculated using Arlequin 3.5. Population structure was calculated with STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) using default settings (admixture model, allele frequencies correlated) and 10,000 burn-in and MCMC repeats. This was run for $K = 2$ to $K = 10$ groups, repeated five times each, and the K with the strongest support was calculated in CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015) based on the Evanno methodology (Evanno, Regnaut, & Goudet, 2005). We also conducted Mantel tests for the nuclear markers with 999 permutations in GenAlEx.

3 | RESULTS

3.1 | Spatial and temporal patterns of chloroplast haplotype diversity

For each of the 267 samples of scotch broom, the four cpDNA regions were concatenated, resulting in sequences that were 1,083 base pairs (bp) in length, including 20 bp of variable length of single-repeating nucleotides and 65 bp of 12 indels. The single-repeating nucleotides were removed for the analyses. The indels were retained as simple index coding (SIC; Simmons & Ochoterena, 2000). The resulting 996 bp sequences and 12 SICs contained 44 mutations at 43 variable sites (one site had three different nucleotide phases).

A total of 20 haplotypes were observed of which 11 were private, that is they were found in only one individual (see Appendix S4). The most common haplotypes (A, B and C) represented 40%, 26% and 16% of all samples, respectively. Seven of the private haplotypes were sampled from the more recent European herbarium specimens (Spain: K, O, R, S and T; Corsica: N; Italy: L), one from an older herbarium specimen (1853 from Portugal: Q) and two from Norway (1928: P; 1977: M). Another private haplotype originated from a Spanish seed bank and was collected in 2003 (Z; see Appendix S2).

Haplotype A dominated the samples from Scandinavia and much of northern Europe (Figure 2). Haplotype A was also identified in two of three ornamental scotch broom plants. Haplotype B was the most widespread and was detected in 13 out of the 15 countries. The third most common haplotype, haplotype C, was of special interest because it is shared with the Danish putative native lineage. We discovered this haplotype widely across Europe, but it was not found in Portugal, Spain or Sweden or in some other countries with very low sample effort. Haplotype C was also present in the garden varieties. Haplotype D was also rather widely spread across Europe, where it was present in seven different countries. Based on our samples, haplotype E was geographically confined to Scandinavia and Germany, quite the opposite of haplotype F, which was detected in the south and west (Spain, Portugal, Great Britain) and in Norway. All remaining haplotypes were private haplotypes or were found in only one country. Most of the private haplotypes were located in southern European countries (Spain, Portugal, Italy and one in Corsica), but two of them were found in Norway.

Haplotype diversity (Hd) per country ranged from 0.4 to 1.00 (Table 1). The lowest Hd was estimated for Belgium and the Netherlands, and the highest Hd values were observed for Spain

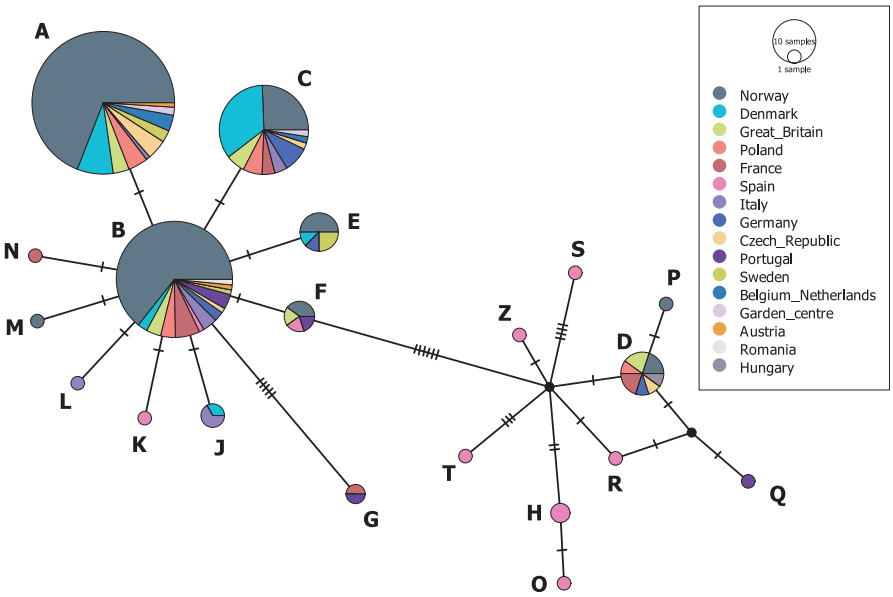

FIGURE 3 Median joining network of chloroplast haplotypes of 267 *Cytisus scoparius* samples. Each circle represents one haplotype. The lines connect the genetically closest haplotypes, with marks representing the number of mutations. Circle size represents the number and colouration represents the origin of samples of each haplotype

and Austria; however, Austria was represented by only two samples. The same general results were obtained after applying rarefaction to a sample size of five (Table 1). Nucleotide diversity (π) of the haplotypes grouped by country ranged from 0.00079 to 0.00739 and showed similar patterns as Hd, with a few exceptions (Table 1).

The exact test resulted in some significant differentiation of haplotype frequencies between countries, most notably between the southwestern (France, Spain and Portugal) and the northeastern countries (see Appendix S5). Furthermore, Norway showed significant differences in haplotype frequencies from seven countries, but this was likely an artefact of the large sampling effort in Norway.

The haplotype network revealed two groups of haplotypes that were separated by six mutations (Figure 3). The three most common haplotypes (A, B and C) were in the same group, and haplotype B was central to the group. The second group mainly consisted of low frequency haplotypes with geographical distributions in southwestern parts of Europe, except for haplotype D, which was central in this group and was found in geographically diverse populations.

The cumulative numbers for each haplotype across the 157 years of sequenced herbarium specimens across Europe showed little change in relative abundance over time (see Appendix S6). Haplotypes A, B and C were the most common haplotypes in the past and in the more recently collected samples (Figure 4). Before 1950, haplotype B was most common. During the years from 1950 to 1980 the number of samples of haplotype A increased disproportionally faster, resulting in haplotype A being most abundant after 1954 (see Appendix S6).

A significant (p = .010) positive correlation between geographical distance and genetic distance was estimated by the Mantel test, indicating some level of geographical structure. Despite this overall correlation, some of the most geographically distant samples contained identical haplotypes. The same level of significance was reached for the subset of samples before 1959 and after 1958. The haplotype analysis of Norwegian populations and herbarium specimens revealed a lack of spatial pattern (Mantel test not significant, Figure 5). Furthermore, there was a lack of temporal pattern in the haplotypes. Haplotypes A, B and C were already present prior to 1900. Haplotype P was identified in a herbarium sample from 1928. The remaining four haplotypes in Norway (D, E, F and M) were identified in more recent material (post 1972). Of the 17 populations for which several individuals were analysed, three populations consisted of two haplotypes, and one had three haplotypes (Figure 5).

3.2 | Genetic diversity and distribution of nuclear SNP markers

Most Norwegian populations had highly polymorphic SNP markers (see Appendix S9), ranging from 72% to 97%, except for population 3 (61%) and population 14 (67%). The polymorphisms of the two Danish populations were in stark contrast, with the putatively introduced type (DI, 94%) at the level of the highest Norwegian values, and the putative native population (DN, 47%) had an SNP diversity that was lower than any other population of this study. Interestingly, the populations from Norway's west coast (BG1 to BG10), at the youngest expansion front and approximately 300 km distant to the other sites, had similarly high levels of polymorphisms as the long-established populations in the south. The European seed bank samples in general had fewer polymorphisms (47%–89%); however, they might have been collected differently. The three populations with the lowest levels of polymorphic nuclear markers (3, DN and 8Wa) all contained chloroplast haplotype C. The PCA of all populations showed a clear separation of these three populations, while the remaining populations greatly overlapped (see Appendix S7). Correlations between SNP genetic and geographical distances were just short of significant (Mantel test, p = .059). The population pairwise genetic distances varied substantially, from 0.02 to 0.52. The most pronounced genetic differentiations of the higher F_{ST} values (F_{ST} > 0.25) were observed for

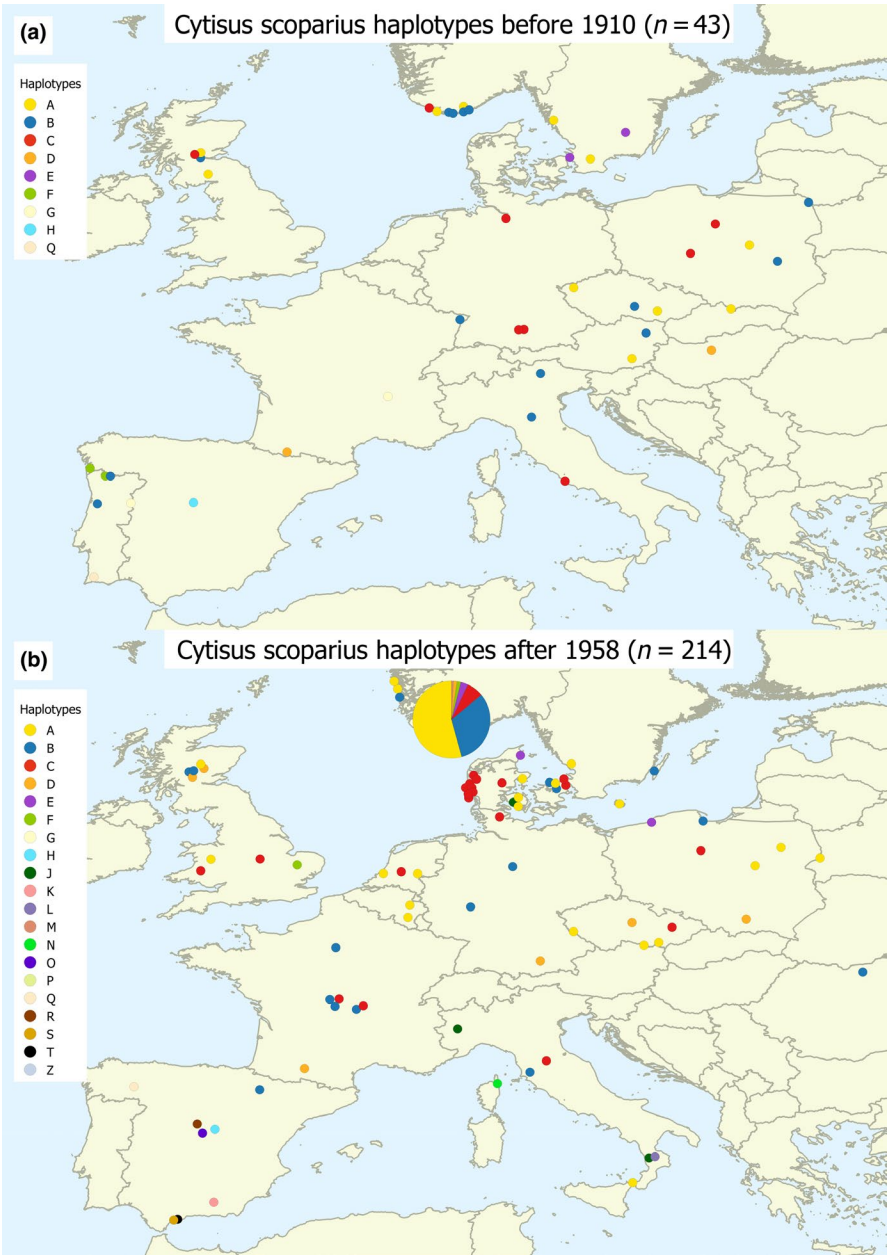

FIGURE 4 Geographical location of *Cytisus scoparius* samples before 1910 (n = 43) and after 1958 (n = 214), coloured by chloroplast haplotype. All samples from southern Norway collected after 1958 are represented as a pie chart. The haplotype B from Madeira is not shown on the map of samples after 1958

comparisons to the three populations with haplotype C (3, DN and 8Wa; see Appendix S10). The genetic differentiation among the remaining populations, those collected in Norway as well as the European collections and seed bank samples, was generally low to moderate (F_{ST} < 0.25).

Most genetic variations were found among samples within populations, with little variation among populations within a geographical area (Table 2). Less variation was found between different levels of geographical scale (e.g. European vs. Norway) or by country. The largest variance at the group scale and the smallest within populations was observed when grouping was based on populations with chloroplast haplotype C compared to all other haplotypes.

The STRUCTURE analysis showed little genetic structure among the populations (Figure 6). Using Evanno's approach (Evanno et al., 2005), the best support was found for four clusters (see Appendix S8). Most noticeable were the distinct ancestry estimates for the populations 3, DN and 8Wa, which contained the chloroplast haplotype C (Figure 6, see Appendix S7). The samples of the remaining haplotypes were highly admixed, and no geographical patterns emerged.

4 | DISCUSSION

4.1 | Northward expansion of scotch broom was driven by human introductions

Our study suggests that the recent range expansion of scotch broom was mainly driven by human introductions; therefore, the species is alien to Norway, and its cryptogenic status is resolved. In Norway, around the year 1900, at least three different haplotypes were

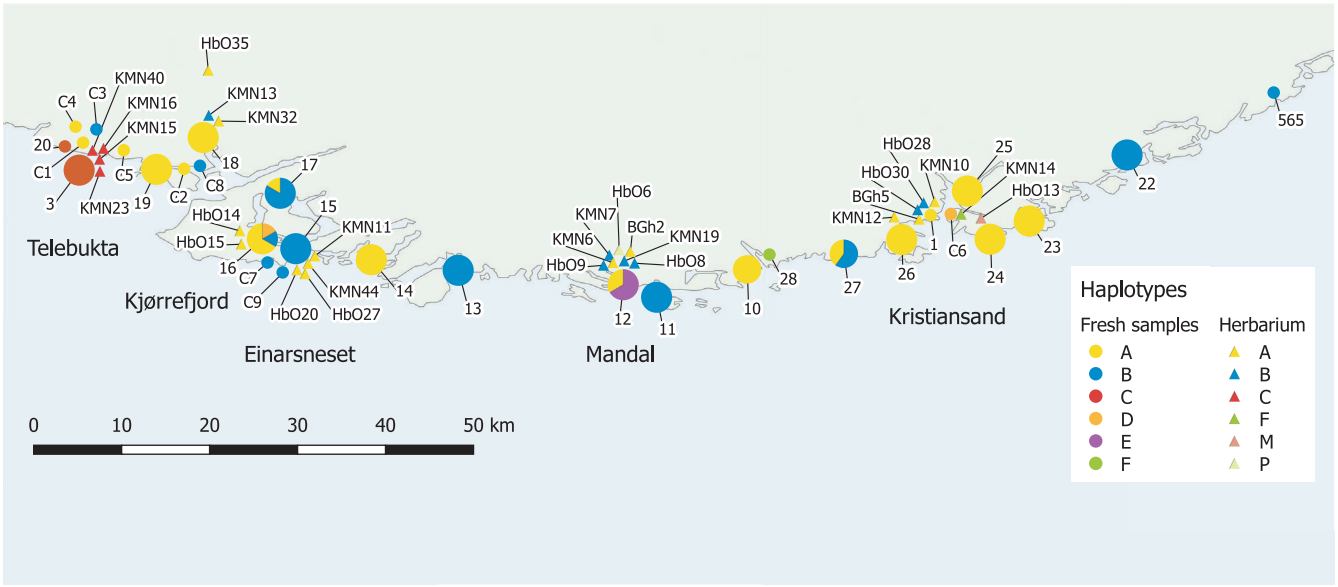

FIGURE 5 Spatial distribution of chloroplast haplotypes along the south coast of Norway. Different colours represent the eight different haplotypes, triangles represent herbarium samples, small circles represent single fresh samples and large circles represent five or six samples from the same population. Some symbols are shifted from their original location to enhance visibility

TABLE 2 AMOVAs of 36 nuclear SNP markers, significant values ($p < .001$) in bold

	Source of variation	*df*	Sum of squares	Variance components	Percentage of variation
a					
Haplotype C versus other haplotypes	Within populations	1,232	5,986.888	4.85949	**76.58**
	Among populations within groups	34	1,168.485	0.84172	**13.26**
	Among groups	1	168.511	0.64472	**10.16**
	Total	1,267	7,323.884	6.34592	
	Fixation indices: FST = 0.23423*, FSC = 0.14764***, FCT = 0.10160***				
b					
Norway versus Europe (haplotype C excluded)	Within populations	1,123	5,685.036	5.06236	**85.52**
	Among populations within groups	31	1,010.700	0.78605	**13.28**
	Among groups	1	58.110	0.07123	**1.20**
	Total	1,155	6,753.846	5.91964	
	Fixation indices: FST = 0.14482*, FSC = 0.13440***, FCT = 0.01203***				
c					
Grouped by countries (haplotype C excluded)	Within populations	1,122	5,674.238	5.05725	**85.95**
	Among populations within groups	26	893.163	0.80757	**13.72**
	Among groups	7	186.445	0.01932	0.33
	Total	1,155	6,753.846	5.91902	
	Fixation indices: FST = 0.14053*, FSC = 0.13770***, FCT = 0.00328***				

Note: (a) samples split by haplotype C and all other haplotypes, (b) grouped by area of origin, native or introduced without haplotype C and (c) grouped by country without haplotype C.
FST: within populations; FSC: among populations within groups; FCT: among groups.
*** indicates significance of p< .001.

already established, and five more haplotypes have since been introduced (Figure 5). The northward expansion during the last century has been extensive (Figure 1) and far exceeds the natural dispersal ability of up to 10 m every three years (Bossard, 1991; Malo, 2004). The eight different haplotypes in this northern expansion range, combined with a lack of temporal and spatial patterns (Figure 5, see

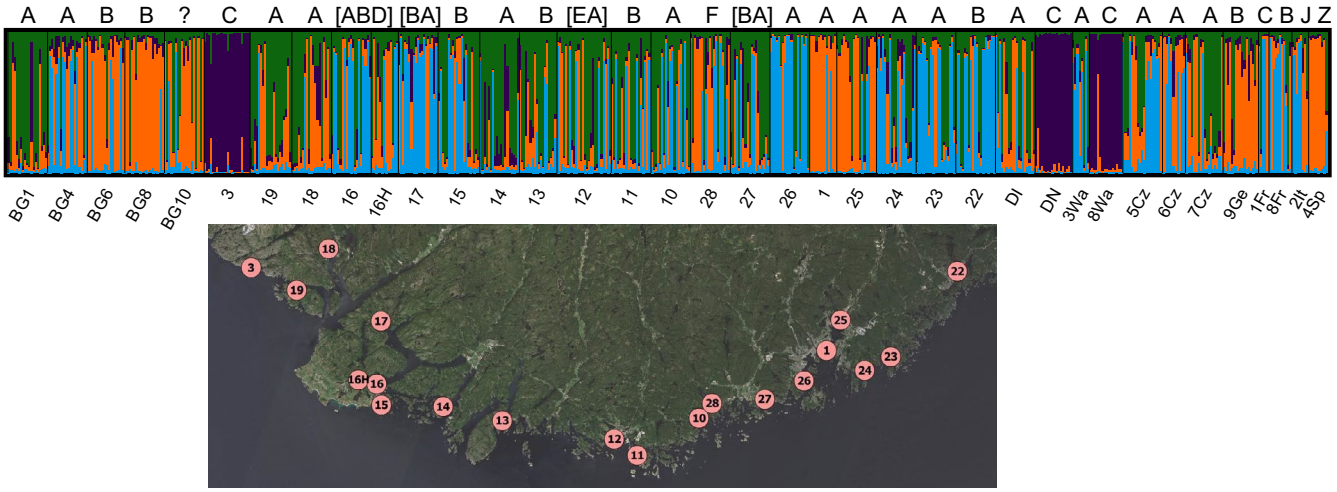

FIGURE 6 STRUCTURE of nuclear SNP data from populations in Norway and Europe. Norwegian samples are roughly sorted by longitude and their location is presented in the inlay of the map Populations BG1 to BG 10 are not included on the map and are located a further 300 km to the northwest. European seed bank samples are marked with the first two letters of their origin country (Wa: Wales, Cz: Czechia, Ge: Germany, Fr: France, It: Italy, Sp: Spain). Capital letters above the STRUCTURE plot indicate the single or multiple haplotypes observed in each population

Appendix S2), suggest different introduction events directly to these locations, possibly followed by occurrences of secondary spread, which is common for introduced species (Kelager, Pedersen, & Bruun, 2013; Wilson et al., 2009). These patterns are opposite of what we would expect from natural colonisation, where a single or very few haplotypes would establish and occupy all suitable habitat, thereby blocking the establishment of other dispersing haplotypes (Harter, Jentsch, & Durka, 2015; Waters, Fraser, & Hewitt, 2013). In support of the human-driven expansion of scotch broom are the observed signs of long-distance dispersal, which must be facilitated by humans, as natural long-distance dispersal is not known for scotch broom. One haplotype (F), of low frequency, occurs in Norway and again (at great distances) in Great Britain and in the Iberian Peninsula, where the latter is a common glacial refugium. Another Norwegian haplotype (D) was not found in neighbouring countries but was present in most other countries (Figure 2).

Nevertheless, we cannot completely exclude the possibility that some of the earliest records in Norway arose from a natural northwards expansion. Previous publications show that natural recolonisation reached as far north as Denmark by 1648 (Paulli, 1648) and maintained a specific phenotype there (Böcher & Larsen, 1958; Nielsen et al., 2016). These native Danish populations belong to chloroplast haplotype C. This haplotype is also present in one remote location in Norway, where it has been present since at least 1896 (see Appendix S2, Figure 5; Agder Museum of Natural History and Botanical Garden; GBIF.org, 2017). This population has not spread beyond its initial location and thus does not contribute to the recent invasion of scotch broom across Norway. This is in line with observations in Denmark, where the low-growing, putative native type is apparently not involved in the spread of the species across the Danish landscape.

Our findings suggest likely introduction routes as stowaways on ships in the past and as escapees from gardens and arboriculture (classification by Hulme et al., 2008). The three locations of the oldest introduction records, which also have the highest haplotype diversity, include an international trading port, a prestigious British-inspired ornamental garden, and a nursery that propagated scotch broom for soil stabilisation (see Kristiansand, Mandal and Kjørrefjord in Figure 5; Lundberg & Rydgren, 1994; Vesthassel, 1926). Another location with an old record contained only a single haplotype (see Telebukta in Figure 5), but this can be explained by the remote and isolated location, which is less frequented by humans. The samples from the Norwegian garden centres revealed the first and third most common chloroplast haplotypes observed in Norway and northern Europe (haplotypes A and C in Figure 2). The one haplotype (A) that increased its relative abundance the most around the years 1960–1980 (Appendix S6) was also the most common haplotype among the garden cultivars (Figure 2). This haplotype may have been preferred for propagation and trade by the horticulture industry. In fact, many invasive plants have been introduced as garden ornamentals (Milbau & Stout, 2008). Given that the three most common haplotypes are genetically quite similar and, at the same time, distant from the group of haplotypes that dominate the Iberian Peninsula (Figure 3), this indicates that geographical regions other than the southwestern part of Europe have been sourced for plant material to be used in the plant industry. For instance, is it known that Italian genotypes have been extensively used for soil improvement in Denmark (Rosenmeier et al., 2013).

4.2 | In the native range: Cryptic invasion by alien genotypes

In an attempt to determine whether human-caused spread occurred only in the species' recent expansion range or also within the species' native range, we sequenced European herbarium specimens

that predated the introduction of scotch broom to Norway and compared their haplotype distribution patterns to those of the present day. The analysis of the oldest available herbarium samples (1835–1910) showed no clear spatial pattern of chloroplast haplotypes and did not differ from present-day distribution (Figure 4). This indicates that human-caused dispersal was already occurring before the early 19th century.

We did, however, also observe some remnants of haplotype distribution patterns that we would have expected to see with the natural postglacial recolonization of scotch broom in its native European range. Most of the private haplotypes were found in former glacial refugial areas (Figures 2 and 3), especially in Spain and Portugal (seven out of 11). The haplotype diversity and genetic diversity calculated for each country also decreased from south-west to north-east (Table 1, Figure 2). This agrees with other studies observing genetic loss during the natural recolonization of Europe from southern refugia after the last glacial maximum (Petit et al., 2003). It must be noted that old herbarium material may be prone to degradation and post-mortem DNA modifications (Staats et al., 2011). We cannot completely exclude that this phenomenon occurred in some of the older herbarium specimens we used. However, only two of the private haplotypes from herbarium material were more than 50 years old, and the rate of post-mortem DNA damage modification was found to be very low (Staats et al., 2011). We thus think it unlikely that post-mortem DNA damage modification had a large impact on our results. Of the four most common and widespread haplotypes, only one (B) was sampled in Spain or Portugal; the other three (A, C and D) were observed in northern countries only but might be present in low abundancies on the Iberian Peninsula or originate from a different glacial refugium. We thus conclude that scotch broom distribution patterns throughout its entire European distribution area are strongly impacted by human dispersal over long distances. Hence, while the species is native in central Europe, there may be genotypes of scotch broom within this native range that are not native to particular regions.

4.3 | Diverse introduction history and high propagule pressure

We suggest that frequent and diverse introductions were crucial for the invasiveness of scotch broom in its northern expansion front. In Norway, scotch broom became established approximately 150 years ago, but only in the last decades was it recognised as invasive (i.e. spreading and increasing in density; Figure 1, see Appendix S1). This is in line with Aikio, Duncan, and Hulme (2010) who calculated a relatively long lag phase of 97 years from introduction to rapid expansion of scotch broom in New Zealand.

Our results support that introductions have occurred repeatedly from a range of origins. This high propagule pressure (quantity of introductions) likely contributes to its invasion in the novel range (Blackburn et al., 2015; Colautti et al., 2006), similar to what has been suggested for many other invasive species (Jogesh et al., 2015; Kelager et al., 2013). Further supporting the high propagule pressure is the fact that the species is widely used as a garden ornamental and is commonly sold in garden centres in Norway. The quality of the propagules (i.e. place of origin) also influences a plant's ability to establish itself and become invasive, as a plant should be suitable to the novel habitat or have a high genetic potential to adapt. We could not locate the geographical origins of the haplotypes because of the lack of spatial patterns across the native range (Figure 4). The possibility that haplotypes from different origins are already mixed across the native range (i.e. intraspecific cryptic invasion) increases the likelihood of introducing haplotypes of different origins, even from a small source range. A mixed ancestry of introduced populations has also been shown for *Spartina alterniflora* during intentional introduction China (Bernik, Li, & Blum, 2016).

4.4 | A uniform invasion front with high genetic diversity

We observed strong genetic admixture of the nuclear markers (Figure 6) following the dispersal and mixing of the different source populations (i.e. different haplotypes). This may have increased the adaptability to novel habitat (Ellstrand & Schierenbeck, 2000; Verhoeven, Macel, Wolfe, & Biere, 2011) and promoted range expansion (Rius & Darling, 2014). High gene flow has minimised any differentiation between the native and introduced range (Figure 6, Table 2, see Appendix S7) and contributed to a high level of polymorphism in the northernmost expansion front (see Appendix S9). Nearly all of the genetic variation was found within the populations, and much less was found among populations (Table 2), a pattern that has been observed in other invasive alien species (Ellstrand & Schierenbeck, 2000; Kelager et al., 2013). Multiple introduction events from different origins can also facilitate biological invasions when these diverse sources hybridise (Ellstrand & Schierenbeck, 2000; Rius & Darling, 2014), resulting in increased genetic diversity at the population and individual level (Colautti & Lau, 2015; Dlugosch & Parker, 2008; Fennell, Gallagher, Vintro, & Osborne, 2014) and possibly creating novel invasive genotypes (Bock et al., 2015).

There is also the possibility of gene flow between a genotype that is adapted to northern climates (i.e. native Danish populations) and introduced genotypes. In Demark, putative native and introduced genotypes readily cross, causing the genetically polluted native plants to change in phenotype (Nielsen et al., 2016). In our study, however, three populations that shared the haplotype of the Danish native population (3, DN, 8Wa) showed a distinct genetic structure and remarkably low nuclear genetic diversity. It has been predicted that a low capacity to tolerate the strong winter conditions in northern temperate areas restricts the ability of invasive species to invade northern temperate areas (L. Rosef & E. Heegaard personal communication). Surprisingly, the identified haplotype of the putative native Danish populations, which is adapted to a northern climate (Böcher & Larsen, 1958), appears to play a minor role in the northern expansion process.

5 | CONCLUSION

This study represents one of the very few examples of intracontinental plant invasion, indicating the complex aspects of the introduction history. The rapid northern range expansion of scotch broom in the recent century was driven by human introductions, thus scotch broom can be classified as alien in Norway. Our results indicate the importance of a diverse introduction history for invasion success, with high propagule pressure and genetic admixture resulting in high genetic diversity. Further, we point out the value of extending the genetic analyses of biological invasions to a species' native range. We found indications that the different genotypes of scotch broom have been moved and combined within the native range already. This is likely to lead to intraspecific admixture and high genetic diversity in the source populations, and it might affect the establishment and invasion at the onset of introduction into a novel range.

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DATA AVAILABILITY STATEMENT

Nuclear SNP data and chloroplast sequences are deposited in the Dryad repository with the https://doi.org/10.5061/dryad.33bp0m3. All chloroplast sequences have been submitted to GenBank under the accession numbers: MN126599-MN127775.

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BIOSKETCH

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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