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Cytisus scoparius - a genetic and historical analysis of introduction history in Norway

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Content

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Abstract

Cytisus scoparius is an invasive species threatening the local flora where the shrub is introduced. Studies show that the shrub spreads rapidly and has damaging effects in areas where the plant is considered invasive. *Cytisus scoparius* has been considered native in Norway but may threaten vulnerable habitats such as coastal heathlands, calling for a regulation of the shrub's expansion. This study uses molecular and historical analysis to investigate if in fact *C. scoparius* is native to the Norwegian flora or if it is introduced through human actions. 28 Norwegian *C. scoparius* populations were sampled and compared with 27 non-Norwegian samples and 22 Norwegian herbaria samples. The samples were grouped into nine haplotypes and analyzed to find if there is a genetically distinct variation of *C. scoparius* in Norway, and to decide whether there has been a single or multiple introductions to Norway, and if there is a traceable introduction path. My results reveal high genetic variation among all samples. The nine haplotypes identified are scattered over a large area, which indicates multiple introductions to Norway and makes it difficult to trace possible introduction routes.

1. Introduction

On a global scale, alien species are one of the biggest threats to biological diversity (Parker 2000; Richardson et al. 2000; Bohn et al. 2004). Though not all alien species are harmful, introduction of new species may have negative effects on native species. By outnumbering already existing species, alien species may change functions and structures in the ecosystem in which they are introduced, or change the genetic composition of native species by hybridizing with these (Parker et al. 1997; Bond et al. 2002; Guo 2006). Globally, 10% of alien species are able to establish stable populations, and 10% of these become invasive (Williamson & Brown 1986). In the Nordic climate of Norway with long, cold winters and short growth periods, even less species will be able to establish stable populations. According to the Norwegian Environment Agency (Fremstad et al. 2005-6), an estimated 3-5% of the introduced species of vascular plants will successfully develop stable populations in Norway (Fremstad & Elven 1997).

Biology of Invasive Species

Earlier comparative studies on invasiveness in alien species, such as studies by Pappert et al. (2000) and Genton et al. (2005), have shown that high genetic variation in successful invaders is common. This variation is largely due to multiple introductions of the invasive species. It commonly leads to genetic changes in the exotic populations. These changes may facilitate the establishment of the population in their new habitat (Maron et al. 2004; Guo 2006). Multiple introductions enable the invasive species to combine a number of genetic variations, which is part of the reason for the positive correlations seen between multiple introductions and success in invasiveness (Dlugosch & Parker 2008). Phenotypic plasticity, such as reallocation of resources, change in plant size and changes in tolerance level are also significant characteristics when it comes to determining the success of invaders (Bohn et al. 2004; Maron et al. 2004; Guo 2006). Example of studies done by Sexton et al. (2002); Parker et al. (2003) and Maron et al. (2004) show how introduced plants use reallocated resources against competitors and herbivores in their new environments to enhance reproduction and growth, and this facilitates establishment of populations in the introduced plants' new environments. Many of the world's invasive plants are nitrogen-fixing legumes, though the

impact of this trait is not well studied with regards to invasive success (Richardson et al. 2000). *Cytisus scoparius* is an example of a successful invader in which nitrogen-fixing bacteria facilitate growth. This mutualistic symbiosis gives *C. scoparius* the ability to establish populations in low nutrient soils and may, therefore, give the species higher likelihood of successfully establishing populations in new habitats (Richardson et al. 2000).

Cytisus scoparius in Norway

Cytisus scoparius is native to most parts of Europe, though it has been introduced to other parts of the world such as Australia, Africa and North America (Parker 1997; Potter et al. 2009). On these continents the shrub seems to spread rapidly, taking over open field landscape and threatening native species (Parker et al. 1997; Potter et al. 2009). Studies from areas where the shrub is considered invasive show that *C. scoparius* does have damaging effects on the native species already growing in those areas (Parker 1997; Potter et al. 2009). In Norway, however, the species is characterized as native to the Norwegian flora and we find populations of *C. scoparius* along the south coast from Grimstad to Stavanger (Fig. 3). The first record of *C. scoparius* is from Kristiansand in 1875 (Gederaas 2012; Elven 2016) and there are a few more records of populations in Flekkefjord, Mandal and Grimstad before 1900 (GBIF-Norway 2007-2016). According to the Species Map Service (GBIF-Norway 2007-2016) and herbarium records, *C. scoparius* spread more rapidly after 1950 (Fig. 1), and is currently believed to be spreading further north along the west coast of Norway (Elven 2016). Invasion of vulnerable habitats such as costal heathlands at Jæren can be expected (Nilsen et al. 2009; Gederaas 2012), and brings up questions of whether or not the shrub should be controlled. *Cytisus scoparius* has been used as an ornamental plant in gardens and parks for years (Lagerber et al. 1955; Engelskjøn et al. 1997; Elven 2015). Since plants normally need time to adjust to new environments, they can be held as ornamental plants for several years before they start spreading. This may be the case for *C. scoparius*, which has a lag time of up to 97 years (Aikio et al. 2010). The shrub is still commonly used as such and is sold in garden centers and nurseries around the country, thus spreading further with human help (Elven 2015).

Because of its rapid expansion, *C. scoparius* is considered to be a problematic species, in Norway (Nilsen et al. 2009). The current status of the species is a topic of debate. The shrub was eliminated from the black list of invasive species in 2012 because it is believed to be native to the Norwegian flora (Gederaas 2012). The Norwegian Black List 2012 base its

definition of alien species on the International Union for Conservation of Natures (IUCN) definition of alien species, which is as follows:

”"**Alien species**" (non-native, non-indigenous, foreign, exotic) means a species, subspecies, or lower taxon occurring outside of its natural range (past or present) and dispersal potential (i.e. outside the range it occupies naturally or could not occupy without direct or indirect introduction or care by humans) and includes any part, gametes or propagule of such species that might survive and subsequently reproduce.” (ISSG 2008)

The Norwegian Black List 2012 considers all species with established populations prior to 1800 to be native (Gederaas 2012). Since the first population of *C. scoparius* was recorded in 1875, it is questionable as to whether or not *C. scoparius* is native to the Norway. Even as late as 2012, it was suggested that certain areas in Norway are unique because of the appearance of *C. scoparius* and that these areas should be conserved on the basis of, among others, the presence of the species (Haraldstad & Gunnarsli 2012). This suggestion is based upon the assumption that *C. scoparius* is a native Norwegian plant. Although the shrub is considered problematic it will not, due to the absence from the invasive species list, be assessed on the basis of invasiveness and threat to native flora, and hence, it is not regulated (Gederaas 2012). There is, thus, a need to establish whether or not *C. scoparius* is indeed native or if it has been introduced to these particular areas and Norway as a whole.

Although the ballistic seed dispersal of *C. scoparius* is sufficient enough for expansion, colonization at greater distance is most likely caused by human activity (Malo 2004). It is unlikely that the specie has expanded to Norway without the help of humans because the nearest population of *C. scoparius* is found outside of Norway is located in Jutland, Denmark (Elven 2016). The scattered distribution seen according to the Species Map Service (GBIF-Norway 2007-2016) in the first half of the 1900s (Fig. 1) gives reason to believe that there have been multiple introductions of *C. scoparius* to Norway.

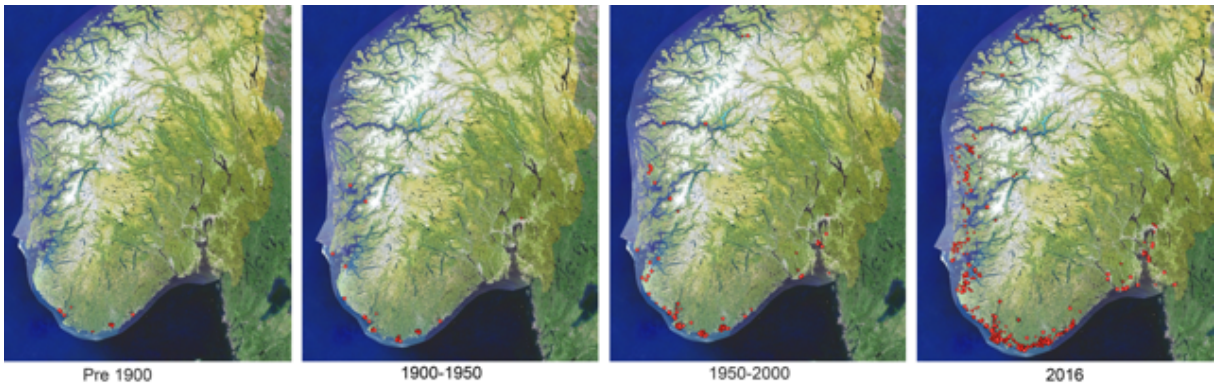


Figure 1. Distribution map of *C. scoparius* from before 1900 and until 2016.

The Danish Paradox

According to a study conducted in Denmark, the Danish population of *C. scoparius* consists of two different gene pools: one invasive and one non-invasive (Rosenmeier et al. 2013). The non-invasive part of the population has shown to comprise the oldest Danish populations and has been present in the Danish flora since the 17th century, thus this population is believed to be native (Rosenmeier et al. 2013). Since the nearest population of *C. scoparius* found outside of Norway is located in Denmark, this study, together with the fact that the Norwegian distribution of *C. scoparius* was very low until the 1950s, may indicate that we also have two genetically distinct populations in Norway. If there are, in fact, two genetically distinct types of *C. scoparius*, as seen in Denmark, this should be investigated because *C. scoparius* is currently considered to be a native plant that is expanding rapidly and with expectations of further expansion (Elven 2016). It is necessary to establish this before a possible control plan is prepared and set in action.

Use of Molecular Methods

Investigating introduction history of invasive species will answer questions about species origin and introductions routes. In order to determine introduction history, the use of sub-fossil pollen, seeds and other parts of plant has typically been used. The limitations with this is the difficulty of accurately identifying species or subspecies with this sort of material (Buchwald 2008). The use of molecular techniques and DNA sequencing in studies of non-native species where similarities in genetic structure are used to identify relationships between populations (Bond et al. 2002; Saltonstall 2002) could reveal more precise knowledge of introduction history and geographical origin of a species. Such information could be difficult to determine with other methods or by considering historical events only (Guo 2006;

Fitzpatrick et al. 2012). Chloroplast DNA (cpDNA) has a higher degree of conservation than nuclear DNA. The substitution rate for this organelle genome is also lower, with an estimated rate of 1.0-3.0x10^{-9} substitutions per site per year (Wolfe et al. 1987; Diekmann et al. 2009). CpDNA is usually inherited maternally, which provides information about seed dispersal and specie origin (Balfourier et al. 2000; Diekmann et al. 2009). Intraspecific variation has been detected even with the high degree of conservation of cpDNA (Saltonstall 2002). It is, thus, possible to compare present day samples with cpDNA taken from herbarium records to better understand introduction history and to predict possible introduction routes. Molecular techniques together with herbarium and museum records will give a well-rounded picture of specie origin and introduction history and can be used to predict further spread to new locations (Guo 2006). This information may again be helpful in the process of finding suitable control agents for that particular species if invasiveness is a problem. Also, further import of the species can be prevented when the country of origin and manner of introductions have been established (Bond et al. 2002).

In this study, fresh plant material of *C. scoparius* from Norway has been collected and sequenced. This material has been compared with plant material from the Danish study of Rosenmeier et al. (2013) and various places throughout Europe in order to find possible introduction routes to Norway. Herbarium specimens dating back to 1889 have been sequenced to decide if there was one specific haplotype typical to Norway before the expansion was seen. Herbarium records were also used to establish which population in Europe, if any, was related to the oldest populations found in Norway.

Objectives

The objectives of this thesis are to investigate Norwegian introduction history of *C. scoparius* through genetic analysis of chloroplast DNA sequences and to determine if a native population exists. By investigating chloroplast DNA from Norwegian populations and comparing these with the two different genotypes found in the Danish study and various European samples, I seek to answer the questions (i) Is there a genetically distinct population of *C. scoparius* in Norway that could be native (ii) Have there been single or multiple introductions of *C. scoparius* to Norway (iii) Which is the most likely introduction path of *C. scoparius*? Definitive answers to these questions will help shape regulation or preservation plans of *C. scoparius* and determine the plant's future impact on Norwegian flora.

2. Method

Study Species

Cytisus scoparius is a large shrub in the Fabacea family (Fig. 2), which may grow to more than two meters in height. It has green twigs without thorns and small yellow flowers that usually flower between May and June. Its leaves are small and trifoliate, some with tiny hair (Rosenmeier et al. 2013). *Cytisus scoparius* shows ballistic primary seed dispersal with legumes that discharge explosively when they dry. Seeds that are spread by this manner have an average distance of spread between 2 and 5 meters. Elaiosomes are responsible for secondary dispersal by ants (myrmecochory) (Parker 1997; Malo 2004; Kang et al. 2007). Seeds have high viability and can be transported longer distances by water (Turner 1933; Kang et al. 2007). The roots have nitrogen-binding bacteria, which facilitate growth in areas with low nutrient soil (Wheeler et al. 1987; Parker et al. 1997; Fogarty & Facelli 1999). In its native environment the shrub is typically found growing in dry, sandy soil in moorland and on cliffs. It has a tendency to spread rapidly in abandoned pastures and meadows and in fire-prone scrublands, especially in areas where it is introduced (Malo 2004). This is the type of environment where Norway has seen the expansion in the most recent years (Elven 2016). In Europe, *C. scoparius* is found in much the same growth conditions as in Norway sunny with dry, sandy soil. As for countries where is it introduced and considered invasive, it is also abundant along roadsides (Parker 2000).

Figure 2. *Cytisus scoparius*. Photo: Beate Beatriz Furevik and Siri Fjellheim

Data Collection

The sample area for this study was the south coast of Norway, from Lillesand in the East, to Flekkefjord in the West (Fig. 3A). Leaf samples were collected from 28 *C. scoparius* populations across the sampling area. Samples were stored in small bags with silica gel until they could be stored at -20°C. All Samples from Norway were collected by Siri Fjellheim and Line Rosef in June 2012.

16 samples from non-Norwegian locations were obtained from seed banks or collected by collaborators (E1-E15, 8-1-2, Fig. 3B). The seeds were raised in greenhouse and leaves were harvested for DNA extractions. All leafs were collected in Eppendorf tubes in liquid nitrogen and extracted for DNA immediately after collection. All Danish samples were provided from Denmark by Rosenmeier et al. (2013) as DNA extracts.

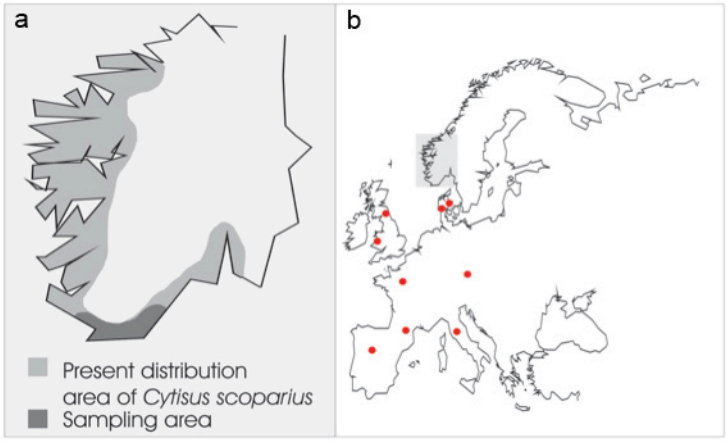


Figure 3. Map A showing the distribution area for *C. scoparius* in Norway in light gray with the sampling area in darker gray. Map B over Europe shows sampling locations in red.

22 herbariums samples were collected from Oslo herbarium (HbO), Kristiansand herbarium (KMN) and Bergen herbarium (BG). A small leaf sample was taken, with permission, from the specimen that was in good condition. All samples were stored in Eppendorf tubes at room temperature until DNA extraction could be performed. Table 1 gives a complete overview of all samples with locations.

Table 1. Overview of all *C. scoparius* samples with sample number, coordinates, locality and country of origin.

Sample	Coordinates		Locality	Land	Year of collection	Herbarium number
	North	East				
1	58,354444	8,065556	Kristiansand	Norway	2012	
3	58,284167	6,683889	Telleviken	Norway	2012	
11	57,99317	7,50697	Skjonøy	Norway	2012	
12	57,99316	7,50703	Risøbank	Norway	2012	
13	58,04424	7,13093	Spangereid	Norway	2012	
14	58,06457	6,96603	Ytre Skarstein	Norway	2012	
15	58,671	6,79453	Einarsneset	Norway	2012	
16	58,10389	6,75994	Kjørrefjord	Norway	2012	
17	58,18984	6,79378	Åpta	Norway	2012	
18	58,2955	6,6477	Flekkefjord	Norway	2012	
19	58,23511	6,55948	Hidra	Norway	2012	
20	58,28628	6,92772	Åna-Sira	Norway	2012	
22	58,26269	8,3976	Lillesand	Norway	2012	
23	58,13805	8,21196	Høvåg	Norway	2012	
24	58,11764	8,14004	Sodefjed	Norway	2012	
25	58,19126	8,07322	Hamresanden	Norway	2012	
26	58,10251	7,97079	Møvik	Norway	2012	
28			Kallåsen	Norway	2012	
565			Fevik	Norway	2012	
C 1	58,28271	6,43151	Hidra	Norway	2012	
C 2	58,23666	6,61195	Hidra	Norway	2012	
C 3	58,2901	6,44097	Midtbø	Norway	2012	
C 4	58,29377	6,43939	Åna-Sira	Norway	2012	
C 5	58,27908	6,48042	Berrefjord	Norway	2012	
C 6	58,15559	8,05381	Kristiansand	Norway	2012	
C 7	58,316111	6,951667	Einarsneset	Norway	2012	
C 8	58,14313	6,375587	Kvellandsnes	Norway	2012	
C 9	58,239722	6,888333	Einarsneset	Norway	2012	
E 1			Unknown	France	2012	
E 2			Unknown	Italy	2012	
E 3			Wales	UK	2012	
E 4			Unknown	Spain	2012	
E 5			Unknown	Czech Republic	2012	
E 6			Unknown	Czech Republic	2012	
E 7			Unknown	Czech Republic	2012	
E 8			Wales	UK	2012	
E 9			Unknown	Scotland	2012	
E 10			Unknown	Scotland	2012	
E 11			Unknown	Scotland	2012	
E 12			Unknown	Czech Republic	2012	
E 13			Unknown	France	2012	

E 14	Ben Vrackie	Scotland	2012	
E 15	Pitlochry	Scotland	2012	
8-1-2	Unknown	France	2012	
Hen 14	Henne Strand	Denmark	2009	
Hen 18	Henne Strand	Denmark	2009	
LB 3	Lystbæk	Denmark	2009	
LB 15	Lystbæk	Denmark	2009	
Nb 2	Nr. Nebel	Denmark	2009	
Nb 4	Nr. Nebel	Denmark	2009	
ViH 18	Villingerød	Denmark	2009	
ViH 19	Villingerød	Denmark	2009	
Mel 2	Melby	Denmark	2009	
Mel 19	Melby	Denmark	2009	
Sb 13	Svanning Bjerge	Denmark	2009	
HbO 28	Roligheten	Norway	1889	HbO 975
HbO 30	Roligheten	Norway	1903	HbO 973
KMN 10	Oddersnes	Norway	1945	21933
KMN 12	Oddersnes	Norway	1966	21935
KMN 13	Randesund	Norway	1977	21936
HbO 9	Risøbank	Norway	1904	HbO 983
KMN 6	Risøbank	Norway	1911	21929
HbO 6	Risøbank	Norway	1928	HbO 980
KMN 19	Risøbank	Norway	1936	1585
BG 2	Risøbank	Norway	1947	S-18715
HbO 11	Risøbank	Norway	1958	HbO 988
HbO 8	Risøbank	Norway	1975	HbO 982
KMN 40	Hidra	Norway	1913	47554
KMN 32	Flekkefjord	Norway	1962	65888
KMN 15	Hidra, Telleviken	Norway	1977	21938
KMN 23	Hidra, Telleviken	Norway	1996	23777
HbO 35	Sira	Norway	1998	HbO 107304
HbO 15	Kjørrefjord	Norway	1909	HbO 992
HBO 14	Lista	Norway	1954	HbO 991
KMN 11	Lista	Norway	1966	21934
HbO 20	Einarsneset	Norway	1973	HbO 5016
HbO 27	Lista/Einarsneset	Norway	1977	HbO 21254

DNA Extraction

All leaf samples were prepared for DNA extraction by being transferred to Eppendorf Tubes and kept in liquid nitrogen. Plant tissue was disrupted using a TissueLyser (QIAGEN®, Valencia, CA, USA) at 30 Hz for 2x1 minute. Samples were extracted using DNeasy Qiagen kit (QIAGEN®) according to manufacturer's protocol. DNA quality was measured both by

using a spectrophotometer (NanoDrop8000, Thermo Fisher Scientific, Waltham, MA, USA) and by gel electrophoresis run on 1% agarose gel.

DNA extractions from herbariums samples were performed using DNeasy Qiagen kit (QIAGEN®) modified according to a protocol from M. Bendiksby at the Natural History museum. To obtain maximum quality DNA, two replicate tubes of each sample were used, each containing a smaller amount of leaf tissue. Elution was performed twice, reusing the first elute for the second elution step. Finally, the two replicate tubes were pooled together. DNA quality was measured with spectrophotometer and gel electrophoresis with 2% agarose gel. All DNA extractions were stored at -18°C. For complete extraction protocol see appendix II.

Polymerase Chain Reaction and Sequencing

Six samples, four Norwegian and two Danish, were screened for chloroplast DNA variation in non-coding regions using eleven primer combinations chosen from literature (Demesure et al. 1995; Hamilton 1999; Shaw et al. 2005; Haider 2011). For complete primer information see appendix I. The samples selected for screening included the Danish samples of different haplotypes, and the sample from the population predicted to be a native Norwegian population. Products were sent to GATC Biotech: Sequencing and Bioinformatics AG (www.gatc-biotech.com) for sequencing. Four primer combinations, which showed variation between the six screening samples, were chosen for further work. All primers used were universal chloroplast primers for non-coding regions. Further details of primers are given in Table 2.

Polymerase Chain Reactions (PCR) amplification was performed with reaction volumes of 25 µl, containing 1 µl DNA template, 0.4 µM primers (Invitrogen, Carlsbad, CA, USA), 0.2 mM of each nucleotide dNTP (Invitrogen), 0.75 U HotStarTaq polymerase (Invitrogen), 1x PCR buffer (Invitrogen) and H_2O to make the final volume of 25 µl. PCR amplifications were preformed in a Mastercycler (Eppendorf, Hamburg, Germany) thermocycler with the following program: initial denaturation for 10 minutes at 95°C, 30 cycles of denaturation for 1 minute at 94°C, annealing for 2 minutes at 55°C (53°C for rps4R2-trnTR), extension for 3 minutes at 72°C and completed with 5 minutes at 72°C and stored at 8°C until collected.

PCR amplification conducted on herbarium material was done with slight differences from fresh material. $MgCl_2$ 2.5mM and 2.5µl biovine serum albumin (BSA) 1% was added to the

reaction mix to improve DNA quality. The H_2O amount was adjusted to make the final reaction volume 25 µl. PCR program was performed as with fresh material, except with 35 cycles and annealing temperature would vary according to primer melting temperature (Table 2).

To optimize the quality of the DNA sequences of the herbaria samples I tested two different polymerases; AmpliTaq Gold® DNA polymerase (Termo Fisher, Waltham, MA, USA) and HotStarTaq polymerase (Invitrogen). Two different PCR programs were tested for each of the two polymerases. The programs for AmpliTaq Gold®DNA polymerase (Termo Fisher) were as follows: Program 1: initial denaturation for 10 minutes at 95^{o}C, 30 cycles of denaturation for 30 seconds at 95^{o}C, annealing for 30 seconds at 55^{o}C, extension for 1 minute at 72^{o}C and completed with 5 minutes at 72^{o}C and stored at 8^{o}C. Program 2: initial denaturation for 10 minutes at 95^{o}C, 35 cycles of denaturation for 45 seconds at 94^{o}C, annealing for 45 seconds at 55^{o}C, extension for 1 minute at 72^{o}C and completed with 10 minutes at 72^{o}C and stored at 8^{o}C. The programs for HotStarTaq polymerase (Invitrogen) were as follows: Program 1: initial denaturation for 10 minutes at 95^{o}C, 35 cycles of denaturation for 45 seconds at 95^{o}C, annealing for 30 seconds at 56^{o}C, extension for 45 seconds at 72^{o}C and completed with 5 minutes at 72^{o}C and stored at 8^{o}C. Program 2: initial denaturation for 10 minutes at 95^{o}C, 35 cycles of denaturation for 1 minute at 94^{o}C, annealing for 2 minutes at 52^{o}C, extension for 3 minute at 72^{o}C and completed with 5 minutes at 72^{o}C and stored at 8^{o}C. HotStarTaq polymerase (Invitrogen) was selected for further work and program 2 was used. This program was also tested with and without the addition of $MgCl_2$ and BSA 1%.

Because herbarium DNA is expected to be degraded, nested primers with shorter product length (200-300 base pairs) were designed to make sure regions of variations would amplify well (Table 2). The program used for designing nested primers was Primer3 (Untergasser et al. 2012).

Table 2. Overview of primers used for PCR amplification of fresh and herbarium material and sequencing, with PCR temperature used and expected product length.

Location	Forward Primer sequence	Location	Reverse primer sequence	Temperatur for PCR program	Expected product size	Author
trnT(UGU)	CATTACAAATGCGATGCTCT	trnL(UAA)	TCTACCGATTTCGCCATATC	55	752	Taberlet, Pierre et al. (1991)
rpS4R2	CTGTNAGWCCRTAATGAAAACG	trnT(UGU)	AGGTTAGAGCATCGCATTTG	53	402	Taberlet, Pierre et al. (1991)
psbF	CGCAGTTCGTCTTGGACCAG	psbB	GTTTACTTTTGGGCATGCTTCG	55	853	Hamilton (1999)
psbC	GGTCGTGACCAAGAAACCAC	trnS2	GGTTCGAATCCCTCTCTCTC	55	1680	Demesure et al. (1995)
Herbarium primers						
trnT2F	TGCCAGAACTGTTGAATTGAT	trnLR	ATCGACCGTTCGAGTATTCC	53	229	
trnT2F herb1	TCGAACGGTCGATTCTTTTT	trnLR herb1	CCGGGATCTTAGTTAGTTACGG	53	298	
rps4R2	CGTGACATAAAAACTCCTTTTGG	trnTR	CGATAGCCGGCTTTTCTCTA	53	300	
psbF herb1	CCACGATCAAATTTATGGAAGC	psbB herb1	ATTCGAAGAACATGGGGACT	52	208	
psbC	CTACGCCACCCACTGAATTT	trnS2	CGGATCTGCTCAAGGACCTA	52	248	
psbC herb1	AATGGAACCGAGCCATACAT	trnS2 herb1	TCTGGGACCTGAGACTCTTGA	53	328	
Sequencing primers						
trnT2F int	TGCCAGAACTGTTGAATTGAT	trnLR int	CCGGGATCTTAGTTAGTTACGG			
rps4 int	CCTGGTAAAACTCCCAGACG	trnT int	CGATAGCCGGCTTTTCTCTA			
psbF int	GACAAGCAGTCGGATAGACCA	psbB int	GGGCAACCCTCTCAACAACT			
psbC int	CGTTCTTGCCAAGGCTGTAT	trnS2 int	TTGGGATTTGGCGGTATTTA			

PCR products were inspected with gel electrophoresis before being purified for sequencing reactions. Clean up of both PCR product and sequencing product was done by using Montage® $PCR_{\mu 96}$ Plate and Montage®$SEQ_{\mu 96}$Plate from Millipore (EMD Millipore, Billerica, Massachusetts, USA), according to manufacturer's protocol.

Cycle sequencing was performed (in both directions) with reaction volume of 10 µl (1 µl BigDye® Terminator, 0.5µl 5x sequencing buffer, 0.5 µM primer, 1 µl PCR product and H_2O to make a total volume of 10 µl) in a Mastercycler (Eppendorf, Hamburg, Germany) thermocycler with the following cycle-sequencing program: 25 cycles with 96°C for 10 seconds, 50°C for 5 seconds, 60°C for 4 minutes and stored at 4°C. Nested primers were also designed for the sequencing reactions (Table 2) with Primer3 (Untergasser et al. 2012). The same protocol was used to sequence herbaria samples. Herbarium PCR products were diluted 1:1 before purification and sequencing. The same nested primers designed for herbarium PCR amplification were used for sequencing herbarium samples. ABI 3730 automated sequencer (ABI-3100, applied Biosystems, USA) was used for reading sequence signals.

Sequences were assembled and edited using Sequencher 4.0.5 (Gene Codes Corporation, AnnArbor, MI, USA). The four primer regions were assembled into one alignment and gaps were coded using simple indel coding by Simmons and Ochoterena (2000).

Phylogenetic Analysis

A haplotype network was generated using TCS: phylogenetic network estimation using statistical parsimony version 1.21 (Clement et al., 2000). Maximum parsimony tree with *Lupinus luteus* (accession nr 485474291) as outgroup and 500 bootstrap replications was constructed using MEGA version 6 (Tamura et al., 2013). The phylogenetic analyses were done on a selection of nine samples representing each one of the haplotype groups detected (marked with bold in Table 1 and 4).

Maps

Maps showing the sample collection were downloaded from CartoDB and edited in Adobe Photoshop. Maps showing the Norwegian *C. scoparius* distribution over time were downloaded from Species Map Service 1.6 (GBIF-Norway 2007-2016).

3. Results

Sequencing Data

The four chloroplast-DNA regions sequenced gave a length of 1403 base pairs (bp) after trimming off unreadable ends. After removing one mononucleotide microsatellite, 16 polymorphic SNP markers, two insertion/deletions (indel) and two microsatellites, were identified. The remaining microsatellites included one di-nucleotide and one penta-nucleotide microsatellite. After editing, the length of each region is as follows: psbB-psbF was 377bp long, with three SNPs, two indels and one microsatellites, psbC-trnS2 was 545bp long, with two SNPs, rps4-trnTR was 216bp long with three SNPs and one microsatellite and trnT2F-trnLR was 262bp long with 8 SNPs (Table 3). The two indels and two microsatellites were coded by simple indel coding (Simmons & Ochoterena 2000). The whole data set of 77 samples was divided into nine haplotype groups (A-I) based on their variation (Table 4).

Sequence Variation

Table 3 shows the variation found in each cp DNA region for the nine haplotypes. Haplotype B does not show any variation. Haplotypes A, D and G only show one position with variation position 294, 390 and 1355 respectively. Haplotypes C show two positions with variation (positions 221 and 1115) and haplotype H show two positions with variation (position 222 and 1284). Haplotype I show five positions with variation (positions 372, 924, 1286, 1323, 1370), haplotype F has 7 (positions 28, 329, 372, 723, 924, 1046, 1139), and haplotype E show nine positions with variation (positions 28, 372, 723, 924, 1046, 1070, 1139, 1237, 1285).

Table 3. Table showing variation of SNP markers, microsatellites and indels with chloroplast DNA region and base pair position for each haplotype group.

Haplotype	psbB-psbF						psbC-trnS2		rps4-trnTR				trnT2F-trnLR							
	28	221	222	294	329	372	390	723	924	1046	1070	1115	1139	1237	1284	1285	1286	1323	1355	1370
A	-	T	G	A	-	G	C	A	T	-	A	A	T	A	A	A	A	T	T	T
B	-	T	G	-	-	G	C	A	T	-	A	A	T	A	A	A	A	T	T	T
C	-	G	G	-	-	G	C	A	T	-	A	G	T	A	A	A	A	NA	NA	NA
D	-	T	G	-	-	G	T	A	T	-	A	A	T	A	A	A	A	T	T	T
E	+	T	G	-	-	A	C	G	C	+	G	A	G	G	A	T	A	T	T	T
F	+	T	G	-	+	A	C	G	C	+	A	A	G	NA	NA	NA	NA	NA	NA	NA
G	-	T	G	-	-	G	C	A	T	-	A	A	T	A	A	A	A	T	A	T
H	-	T	T	-	-	G	C	A	T	-	A	A	T	A	T	A	A	T	T	T
I	NA	NA	NA	-	-	A	C	A	C	-	A	A	T	A	A	A	T	G	T	G

Haplotype Groups

Table 4 shows the total of 77 samples divided into groups A-I based on their haplotypes. The samples form three larger groups A, B and D, and six smaller groups C, E, F, G, H and I. Four haplotype groups (F, G, H and I) contain one sample each. The Norwegian samples are represented in all groups except C and F, with sample 12, 28 and HbO 11 making up individual groups (G, H and I). The Danish samples are represented in three groups (A, B and C). The samples considered to be native to Denmark are all grouped in group A, while the samples considered to be introduced to Denmark are grouped into haplotypes A, B and C, with one sample in each group. The non-Norwegian samples are represented in all the larger groups (A, B and D) with multiple samples, only sample E 2 from Italy form a smaller group with one sample from Denmark (C), and E4 from Spain make up an individual group (F).

Table 4. *C. scoparius* populations, Norwegian, European and herbarium populations, with location and year of collection, divided into haplotype groups.

Sample	Locality	Land	Year of collection
Haplotype A			
3	Televiken	Norway	2012
20	Åna-Sira	Norway	2012
22	Lillesand	Norway	2012
E 1	Unknown	France	2012
E 8	Wales	UK	2012
Hen 14	Henne Strand	Denmark	2009
Hen 18	Henne Strand	Denmark	2009
LB 3	Lystbæk	Denmark	2009
LB 15	Lystbæk	Denmark	2009
Nb 2	Nr. Nebel	Denmark	2009
Nb 4	Nr. Nebel	Denmark	2009
ViH 18	Villingerød	Denmark	2009
ViH 19	Villingerød	Denmark	2009
KMN 15	Flekkefjord	Norway	1977
KMN 23	Televiken	Norway	1996
KMN 40	Midbøheien	Norway	1913
Haplotype B			
11	Skjonøy	Norway	2012
13	Spangereid	Norway	2012
15	Einarsneset	Norway	2012
17	Åpta	Norway	2012
C 3	Midtbø	Norway	2012
C 7	Einarsneset	Norway	2012
C 8	Kvellandsnes	Norway	2012
C 9	Einarsneset	Norway	2012
565	Fevik	Norway	2012
E 9	Unknown	Scotland	2012
E 11	Unknown	Scotland	2012
E 13	Unknown	France	2012
8-1-2	Unknown	France	2012
Mel 2	Melby	Denmark	2009
Mel 19	Melby	Denmark	2009
HbO 8	Risøbank	Norway	1975
HbO 9	Risøbank	Norway	1904
HbO 28	Roligheten	Norway	1889
HbO 30	Roligheten	Norway	1903
KMN 13	Randesund	Norway	1977
KMN 19	Risøbank	Norway	1936
Haplotype C			
E 2	Unknown	Italy	2012
Sb 13	Svanninge Bjrge	Denmark	2009
Haplotype D			
1	Roligheten	Norway	2012
14	Ytre Skarstein	Norway	2012

16	Kjørrefjord	Norway	2012
18	Flekkefjord	Norway	2012
19	Hidra	Norway	2012
23	Høvåg	Norway	2012
24	Sodefjed	Norway	2012
25	Hamresanden	Norway	2012
26	Møvik	Norway	2012
C 1	Hidra	Norway	2012
C 2	Hidra ferjeleiet	Norway	2012
C 4	Åna-Sira	Norway	2012
C 5	Berrefjord	Norway	2012
E 3	Wales	UK	2012
E 5	Unknown	Czech Republic	2012
E 6	Unknown	Czech Republic	2012
E 7	Unknown	Czech Republic	2012
E 15	Pitlochry	Scotland	2012
BG 2	Risøbank	Norway	1947
HbO 14	Lista	Norway	1954
HbO 15	Kjørrefjord	Norway	1909
HbO 20	Einarsneset	Norway	1973
HbO 27	Lista/Einarsneset	Norway	1977
HbO 35	Sira	Norway	1998
KMN 6	Risøbank	Norway	1911
KMN 10	Oddersnes	Norway	1945
KMN 11	Lista	Norway	1966
KMN 12	Oddernes	Norway	1966
KMN 32	Flekkefjord	Norway	1962
Haplotype E			
C 6	Kristiansand	Norway	2012
E 10	Unknown	Scotland	2012
E 12	Unknown	Czech Republic	2012
E 14	Ben Vrackie	Scotland	2012
HbO 6	Risøbank	Norway	1928
Haplotype F			
E 4	Unknown	Spain	2012
Haplotype G			
12	Risøbank	Norway	2012
Haplotype H			
28	Kallåsen	Norway	2012
Haplotype I			
HbO 11	Risøbank	Norway	1958

Sample Locations

All but one of the Norwegian samples with haplotype A are found in the Flekkefjord area, only sample 22 is found in Lillesand (Fig. 4). The two groups containing the majority of the

Norwegian samples, haplotypes B and D, are scattered along the sampling area, with largest number of haplotype D samples around Flekkefjord and Kristiansand, and the largest number of haplotype B samples found in the Farsund area. The three haplotypes with only one single Norwegian sample, haplotypes G, H and I, are found in Søgne (H) and Mandal (G, I; I shown in Fig. 5).

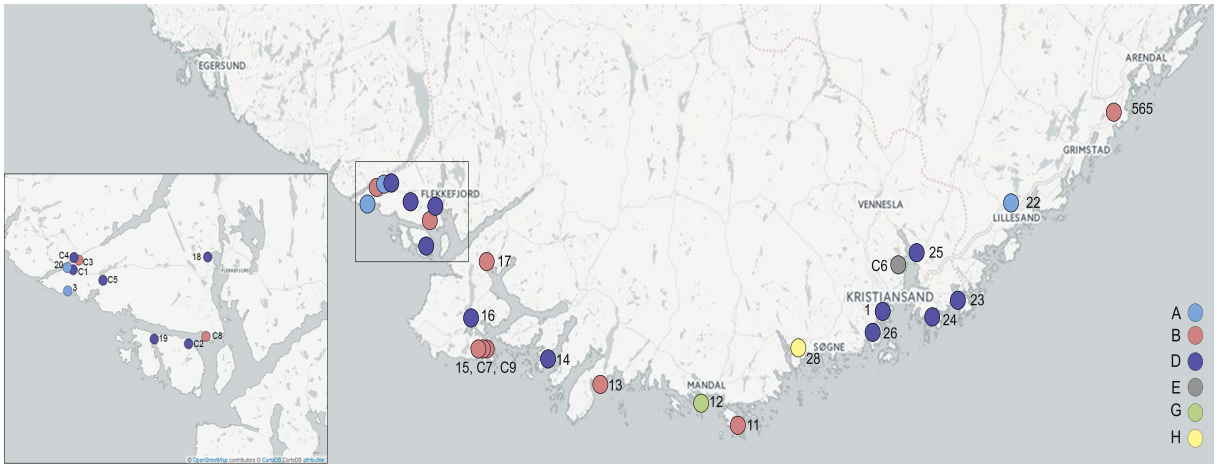


Figure 4. Map over southern Norway showing the Norwegian samples of *C. scoparius* (sampled June 2012) and their sampling location. Samples are numbered according to sample name and color-coded according to haplotype group. Locations are only indicative.

The herbaria samples are found in Hidra, Kjørrefjord (Farsund), Risøbank (Mandal) and Roligheten (Kristiansand) (Fig. 5), with the oldest sample (HbO 28) from 1889 collected in Roligheten. Among the herbaria samples, four different haplotypes are found in Mandal, one exclusively (haplotype I). Three of these haplotypes were represented in the area even before 1950 (haplotypes B, D and E; see Table 4). The herbaria map (Fig. 5) shows that haplotype A is not found in anywhere other than Televiken, though during field season 2012, one sample with haplotype A was identified at Lillesand. In Fig. 5 it is apparent that the few populations of *C. scoparius*, which were found in Norway before the expansion, were more grouped together and isolated from each other than what is found today. Figure 6 shows the same map as Fig. 4 and Fig. 5 with all samples collected in Norway combined. The most variation is found at Risøbank with five different haplotypes (B, D, E. G and I). Two of these (haplotypes G and I) are found exclusively in Risøbank. Mandal and Flekkefjord also show much variation, each with three different haplotypes represented (B, D and E in Mandal, A, B and D in Flekkefjord).

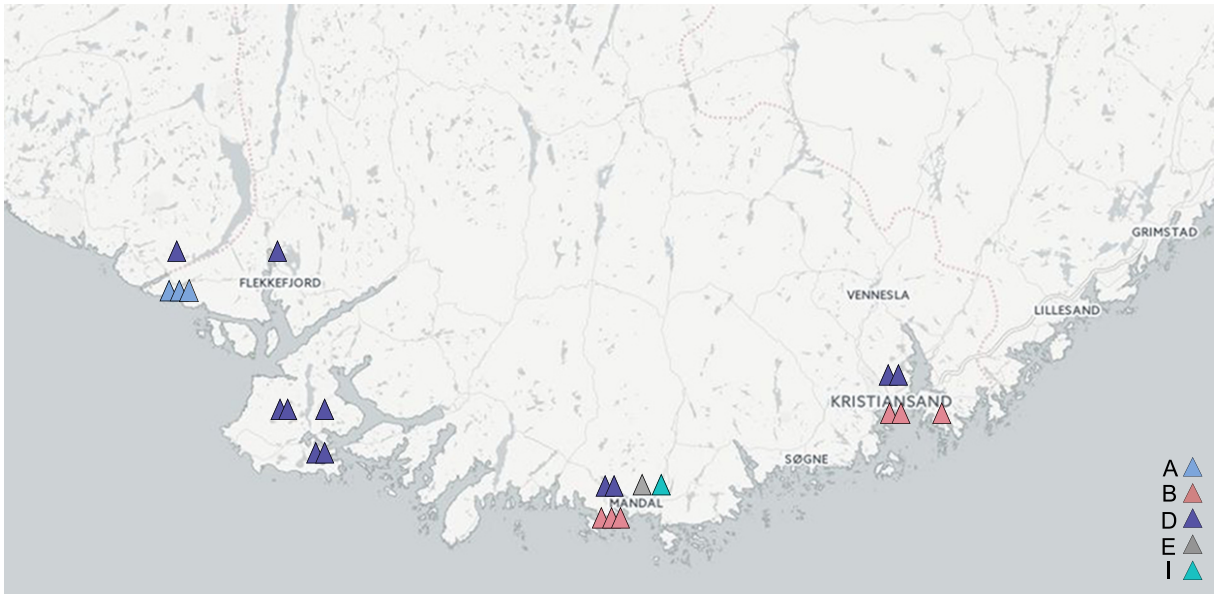


Figure 5. Map over southern Norway showing the herbaria samples of *C. scoparius* and their locations. Samples are color-coded according to haplotype group. Locations are only indicative.

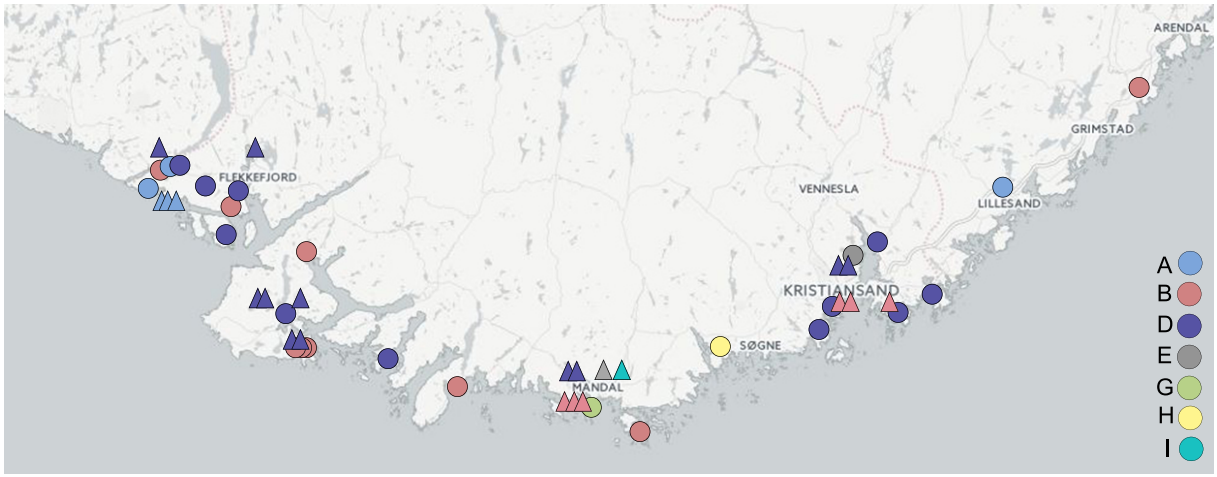


Figure 6. Map over southern Norway showing both the Norwegian samples (circles) and the herbaria samples (triangles) of *C. scoparius* and their locations. Samples are color-coded according to haplotype group. Locations are only indicative.

The map showing haplotypes found in Europe (Fig. 7) reveals two haplotype groups, C and F that are found in Denmark, Italy and Spain but not in Norway. Haplotype A is found in Denmark, Wales and France as well as Norway. Haplotype E include samples from Scotland and Czech Republic, together with two Norwegian samples. Haplotype B and D are, as with the Norwegian samples, scattered over larger areas of Europe. Haplotype A is found in three other countries (Denmark, France and UK) besides Norway, and is as abundant in Europe as haplotypes B and D, though with only one sample from each France and UK.

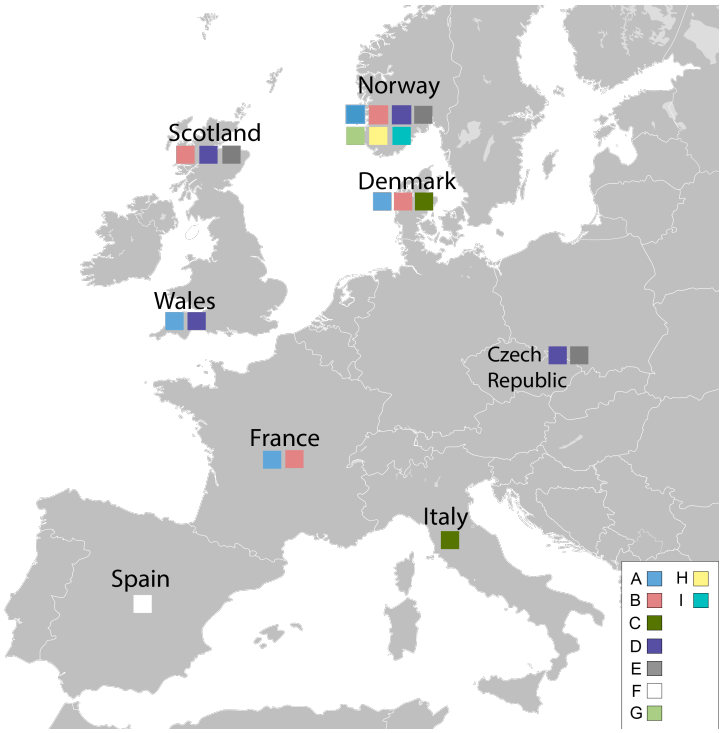


Figure 7. Map over Europe showing the European samples of *C. scoparius* and their country of origin. Samples are color-coded according to haplotype group.

None of the above maps show any specific pattern in the location of the haplotypes. The map showing Norwegian herbarium samples (Fig. 5) shows somewhat of a pattern. In this map haplotype A and B are the haplotypes with multiple samples appearing in only one or two localities, while haplotype D appears in multiple locations. All maps show that haplotypes B and D are the most abundant and numerous.

Phylogenetic Tree and Haplotype Network

The phylogenetic tree (Fig. 8) is a maximum parsimony tree showing the haplotype groups with bootstrap values. The branches of the clade within the third node all have weak branch support while the third node has stronger support with a bootstrap value of 88. This indicates that this clade, containing haplotypes A, B, H, C, D and G, have uncertain positions in the tree. Within the remaining clade of F, E, I and the out-group *Lupinus luteus*, haplotype E and F has a very strong support value of 96. The node separating E and F from I and the out-group has weak support with a bootstrap value of 55, which means that these positions also are relatively uncertain.

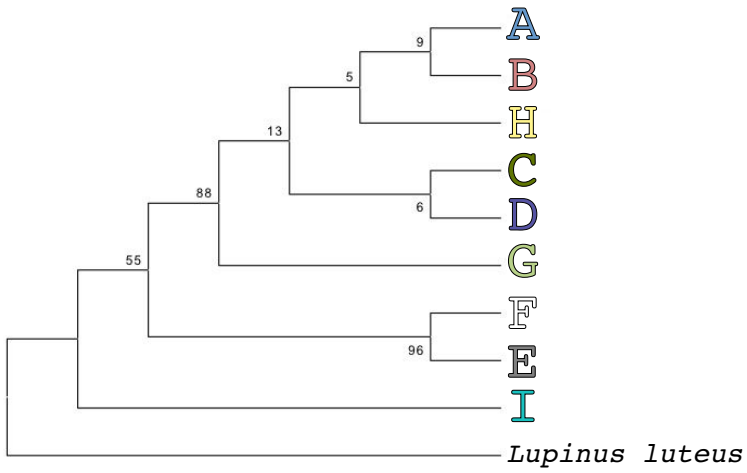


Figure 8. A maximum parsimony tree with 500 bootstrap replications of the nine haplotype groups and with *Lupinus luteus* as an outgroup.

The phylogenetic network (Fig. 9) indicates that haplotype A, D and G relate to haplotype B in the same way. As do haplotype C and H. The small circles indicate the variation between each haplotype, B to A, C, D, G and H; C to I; I to F and F to E. The circles correspond to the number of variation from Table 3 above. The network supports (Fig. 8) the dividing of the branches in the phylogenetic tree, where the node with bootstrap value of 88 separates haplotype A, B, H, C, D and G from F, E and I, but where the order of the first clade (A, B, C, D, G and H) is uncertain.

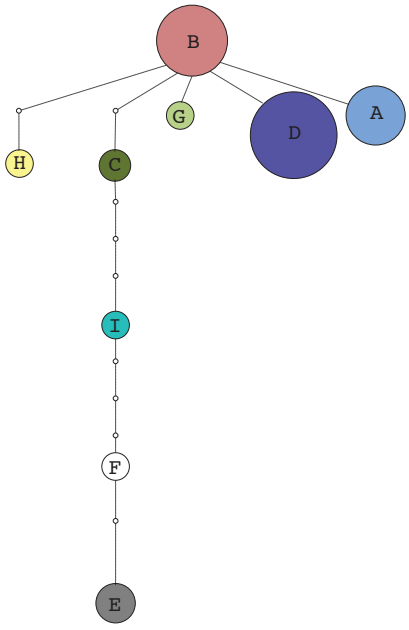


Figure 9. Phylogenetic network showing the nine haplotypes. The different circle sizes illustrates the sizes of the groups and the small circles indicate the number of variations between the different haplotype groups.

4. Discussion

By sequencing chloroplast DNA I investigated the genetic variation among the *C. scoparius* populations found in Norway and I attempted to detect a distinct genotype that is likely to be native to Norway. I also wanted to follow introduction pathways from Europe to Norway with intentions of better understanding where the *C. scoparius* population in Norway originated. My results revealed high degree of genetic variation among the Norwegian samples (Table 3). All together the 77 samples sequenced in this study were grouped into nine different haplotype groups (Table. 4). Even herbaria samples from before 1950 revealed greater variation than expected, with four haplotypes present, compared to the small number of populations found in Norway at this time (Fig. 1, Table 4). The non-Norwegian samples sequenced also show much genetic variation (Fig. 7). There is no clear distribution pattern of the haplotypes in Norway or in Europe (Fig. 6 and 7). Among both the Norwegian samples and the non-Norwegian samples the most common haplotypes (B and D) are scattered over a wide geographic area (Fig. 6 and 7). The genetic variation and lack of pattern indicate several independent introductions of *C. scoparius* to Norway.

This great variation and lack of pattern makes it difficult to detect any introduction pathway. It is apparent that the haplotypes found in Norway are also found in most of Europe. Though the sampling size of non-Norwegian plants is quite small, my results reveal that most haplotypes are common throughout Europe. With a greater sample size I predict that I would find even the haplotypes represented by only one sample to be more abundant. The hypothesis of multiple introductions is supported by the large variation found. Even when studying the herbaria samples the genetic variation seen early on and variation found today that is not found in the herbaria samples supports this hypothesis.

My results do not reveal a distinct haplotype that might be native to the Norwegian flora. Televiken is one location where there is a probability of finding what could be a population with such native haplotype. The first description of *C. scoparius* found in this area is from 1892 with two descriptions of the plant (GBIF-Norway 2007-2016). Descriptions from the Species Map Service (GBIF-Norway 2007-2016) and local history books (*bygdebøker*) such as Engelskjøn et al. (1997) indicates that *C. scoparius* was quite abundant in the area Televiken/Åna-Sira. My one herbaria sample from that area dating from before 1950 is

haplotype A (Table 4). Recent samples from the Flekkefjord area show three different haplotypes (A, B and D). I found haplotype A in only one other location in Norway (Lillesand). I would expect to find a native haplotype in more than two locations, both among the herbaria samples and recent samples. Today the populations found in Televiken are small. From the descriptions in Species Map Service (GBIF-Norway 2007-2016) it seems like the populations have decreased, in contrast to what is happening throughout the rest of the sample area. The strongest reason to believe that haplotype A is a native haplotype is because the native samples from Denmark all have haplotype A. One problem with assuming that this is a native variant would be that these populations are not the earliest described populations in Norway.

The earliest described populations were found in Mandal and Kristiansand. Unfortunately these samples did not amplify well, probably because the DNA was too degraded; hence I do not know the haplotype of these populations. The oldest sample in this study dates from 1889 and was found at Rogligheten, Kristiansand. This sample has haplotype B, which is a different haplotype than what I found the herbarium sample from Televiken to be. Even though the Species Map Service (GBIF-Norway 2007-2016) shows new populations at Roligheten between 1875 and 1889, there is a possibility that my sample from 1889 is the same population as the earliest described in this region. Hence the earliest population described could very well have haplotype B.

I find haplotype B to be one of the most common haplotypes both in and outside Norway. Even though the map of herbaria samples only show two locations for haplotype B, both the map of Norway and the map of Europe show that haplotype B are found in locations spread over a large area. In total haplotype B is found in 15 different locations (Fig 6 and 7). Again native haplotype would be expected to be found in many locations over large areas in its native country and it would be expected to be found in the majority of the older herbaria samples (Saltonstall 2002). This is somewhat the case with haplotype B. Despite representation in the oldest herbaria samples and being the most abundant haplotype in Norway today, by looking at historical events and with the fact that haplotype B is the most abundant haplotype in Europe too, I would argue that haplotype B is most likely introduced to the locations where it is found today.

When *C. scoparius* is at its northernmost limit in Norway (Engelskjøn et al. 1997) a native Norwegian haplotype could be one that is also exclusive to Norway. The herbarium sample (HbO 11) is a sample with a unique haplotype (I) and dates from 1958. It is found at Risøbank, which already in early times harbored a variety of haplotypes. This sample is one of three Norwegian samples with haplotypes unique to Norway in this study, two of which are from Risøbank. If any of these three haplotypes had been native to Norway, the expectations would be to find this haplotype in at least some of the few populations described from the beginning of 1900s, which is not the case in this study. My sampling of herbaria records could have missed populations with these haplotypes but the herbaria samples were selected based upon the few locations shown by the Species Map Service (GBIF-Norway 2007-2016). The intention of sampling was to determine haplotypes of the earliest populations in order to investigate if these were identical and if this haplotype is the most abundant haplotype in Norway today. I believe broader sampling of European populations would reveal more locations with these particular haplotypes, even though in this study they are only present in one sample each.

Already present in a flora Europea from 1968 (Tutin et al. 1968) *C. scoparius* was described to have a distinct type in Denmark that should probably be recognized as a further subspecies. It is also mentioned that hybridization have occurred between the two variants, which has been proven by Rostgaard Nielsen et al. (2016). This Danish variant of *C. scoparius* is phenotypically quite different from the *C. scoparius* found other places in Europe (Rosenmeier et al. 2013). Analyzing the morphological measurements of the Norwegian populations and comparing these with morphological measurements of the Danish populations could further support the genetic findings. If plants with haplotype A are also smaller in growth this will support the predictions that the populations in Televiken/Åna-Sira area could be of a native type.

Historical Events at Sampling Locations

Most locations along the sampling area have shown great increase in *C. scoparius* populations and the samples analyzed show high genetic variety among populations. Some locations show particularly high variation and one location shows a decrease of populations. It is interesting to look more closely at the local history of these locations to see if historical events around the time of the earliest descriptions, or events around time of expansion, could explain some of the variety or loss of variety seen in these areas.

Mandal is the location in Norway where I found the most diversity in haplotypes (Fig. 6). Haplotypes found here are found in all but two of the sampled locations in Europe. Mandal also has two haplotypes that, according to this study, are found exclusively here. By comparing different time ranges, it seems likely that *C. scoparius* was introduced anthropogenically to this location. There was only one observation in this particular location before 1900, but after the turn of the century, the observations of *C. scoparius* increased (GBIF-Norway 2007-2016). According to historical records, Scottish workers were brought to Risøbank in the mid 1800s to plant a pine forest. The Scottish workers brought thousands of plants of pine and larch for planting the forest, and *C. scoparius* was planted to prevent sand drift (Salvesen 2004). In 1901 the Scottish Lord Salvesen built a mansion at Risøbank. An English garden was established around the mansion and gardeners were brought to Mandal from England (Gulbrandsen 2011). Local stories says that it was common practice in the area to visit the English garden in order to collect flowers and plants for use in private gardens (pers. Com. locals).

Later, during World War II, the Germans had barracks and bunkers at Risøbank. Roughly 2000 German soldiers were placed at this location and the area was closed off to the public (Salvesen 2004). In "Våre ville planter" of Lagerber et al. (1955) both Risøbank and Sjøsanden are mentioned as places were *C. scoparius* is most likely not growing wild, because the findings of *C. scoparius* populations in these areas are too close to parks and planted areas. Historical events like these support hypotheses about introduction as well as explanation why there can be found such genetic variation among the *C. scoparius* populations in the area.

Other locations where large variation is found also have a history that leads us to believe that *C. scoparius* may have been introduced. Oddernes in the Kristiansand area is the location of the earliest described *C. scoparius*. This description from 1875 is the very first description of the plant in Norway. The location is described to be "Oddernes sogn" (GBIF-Norway 2007-2016). In old times a *sogn* is an area where people visited the same church (Rasmussen 2016). Torfinn Skard describes in his book about gardening and nurseries "Hagebruk og gartneri i Norge" (1950) how in the late 1700s and 1800s, gardens at rectories and churches were important in teaching locals about gardening. Locals would come to learn how to make private gardens and could also get seeds and ornamental plants from the church garden to

plant in their private gardens. This may very well explain how the first occurrence of *C. scoparius* have expanded to a larger area in Kristiansand. According to local history books, *bygdebøker* (Rudjord 1974) Oddernes was a place where there has long been industrial activity, with the first larger factory established by an Irish. The establishment of parks in connection with factories was at this time very common (Rudjord 1974). This shows how Oddernes have had connections to various countries in Europe through labor and the import of commodities and labor, since many of the factory owners were of foreign origin (Rudjord 1974). Haplotypes found in samples from Kristiansand are also found in samples from Denmark, Scotland and France. These connections between Kristiansand and the rest of Europe could explain how *C. scoparius* may have been introduced to the Kristiansand area.

In Kjørrefjord at Farsund, there has been a plant school since 1880, which was established to help the replantation of forest at Lista (Øyen 2006). Again, according to the Species Map Service (GBIF-Norway 2007-2016), there are no observations of *C. scoparius* in this area before 1900, though an observation from 1916 is described as "abundantly growing wild in the planted forest" implying that *C. scoparius* by this time is expanding in the forest. As was the case in Risøbank, *C. scoparius* was also here imported here and planted on the sand dunes of Kjørrefjord to prevent sand drift (Berge 1926) and has been expanding from this. From the time when the plant school was established, the culture has been to import plants to grow around the school's area. Lagerber et al. (1955) describes in "Våre ville planter" how *C. scoparius* seeds were imported from Western Europe.

In the Flekkefjord area there seem to have been a decrease in population growth rather than expansion. This is one of the areas along the coast with some of the earliest registered observations of *C. scoparius* in Norway. In the book, "Flora på Hidra, Vest- Agder" (Engelskjøn et al. 1997) in which several descriptions of findings of plants are collected, *C. scoparius* is described in 1914 to be "abundant along the coast from Sieråsen to Berrefjord, especially at Televiken". In "Våre ville planter" (Lagerber et al. 1955) this area is described as a possible location for native populations, since populations found here are growing in heathlands and located far from people and gardens. There have only been six observations of *C. scoparius* in this area after 2000 (GBIF-Norway 2007-2016). When the area was searched during field season 2012, one population was found at Televiken, and only one individual plant was found in the village of Sira. Based on the old descriptions from floras and

bygdebøker (Lagerber et al. 1955), one would expect more populations to be established here today.

In the Flekkerfjord area *C. scoparius* is commonly called Tyberis or Tiberis. Such local names, which have little association with common name in other languages, could indicate that the plant has been growing in these areas for a very long time, and is commonly found here (Lagerber et al. 1955; Engelskjøn et al. 1997). This supports the argument that the populations in the Flekkefjord area may be native populations of *C. scoparius*. These populations have certainly not expanded and seem to have a hard time surviving. Not even populations with abundant haplotypes have managed to expand. It would be interesting to have older descriptions of the phenotypes of these populations to compare them to the native Danish populations. Investigation of resemblance between the phenotypes could support the predictions that the populations here may be native.

With the major increase in expansion in mind, studying land use and gardening habits in Norway in the first half of 1900s could further reveal how use of land in Norway has changed during the years. Termination of grazing in uncultivated areas is an example of changes that facilitates the expansion of *C. scoparius* (Elven 2015). Gardening habits could also play a part in the expansion history. Information about where people get their gardening inspiration, where people cultivated plants and what people did with old plants they no longer wanted in their garden would inform the ways in which humans might be responsible for the spread of *C. scoparius*. Old records of plants sold from garden nurseries may show which plants were popular at certain times. Together with information about land use, this could provide insight into how the shrub has spread so rapidly in certain areas and may give clues about the origin of *C. scoparius*.

5. Conclusion

My findings of high genetic variety among the *C. scoparius* population in Norway and the scattered distribution of haplotypes found both in and outside Norway supports the hypothesis of that there have been multiple introductions of *C. scoparius* in Norway. Old records describing how *C. scoparius* was brought to and planted in Norway to improve soil quality, to

prevent sand drift and to add ornamental character to gardens also imply that the majority of the *C. scoparius* populations in Norway were introduced through human actions. There could be value in preserve the few populations in the Televiken/Åna-Sira that could be native because they do not show signs of aggressive behavior and may have historical and cultural value for the area. An experiment similar to what Rostgaard Nielsen et al. (2016) did on hybridization in the Danish populations, could be interesting to do on these populations in order to investigate if hybridization have occurred in the same way as it did in Denmark. In conclusion it is very unlikely that *C. scoparius* is native to the Norwegian flora. On that note the further expansion of *C. scoparius* should be closely observed, and actions should be taken if expansion to unwanted or vulnerable areas are detected. It is important to define control options for the most vulnerable areas because of the likely spread.

6. References

Aikio, S., Duncan, R. P. & Hulme, P. E. (2010). Lag‐phases in alien plant invasions: separating the facts from the artefacts. *Oikos*, 119 (2): 370-378.

Balfourier, F., Imbert, C. & Charmet, G. (2000). Evidence for phylogeographic structure in Lolium species related to the spread of agriculture in Europe. A cpDNA study. *Theoretical and Applied Genetics*, 101 (1-2): 131-138.

Bendiksby, M., Thorebek, L. & Halvorsen, R. (s. a.). Simple procedures for obtaining DNA sequences from old herbarium material.

Berge, A. (1926). *Lista- en bygdebok*: Tønsberg Aktietrykkeri.

Bohn, T., Sandlund, O. T., Amundsen, P. A. & Primicerio, R. (2004). Rapidly changing life history during invasion. *Oikos*, 106 (1): 138-150.

Bond, J. M., Veenendaal, E. M., Hornby, D. D. & Gray, A. J. (2002). Looking for Progenitors: A Molecular Approach to Finding the Origins of An Invasive Weed. *Biological Invasions*, 4 (4): 349-357.

Buchwald, E. (2008). Gyvel- dansk, italiensk eller invasiv? *Urt*, 32: 88-95.

Demesure, B., Sodzi N Fau - Petit, R. J. & Petit, R. J. (1995). A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology*, 4 (0962-1083 (Print)): 129-131.

Diekmann, K., Hodkinson, T. R., Wolfe, K. H., van den Bekerom, R., Dix, P. J. & Barth, S. (2009). Complete Chloroplast Genome Sequence of a Major Allogamous Forage Species, Perennial Ryegrass (*Lolium perenne L.*). *DNA Research: An International Journal for Rapid Publication of Reports on Genes and Genomes*, 16 (3): 165-176.

Dlugosch, K. M. & Parker, I. M. (2008). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, 17 (1): 431-449.

Elven, R. (2015). *Norsk rødliste for arter 2015*: Artsdatabanken. Available at: http://data.artsdatabanken.no/Rodliste2015/rodliste2015/Norge/101925 (accessed: 20. July).

Elven, R. (2016). *Cytisus scoparius* gyvel. *Artsdatabanken*.

Engelskjøn, T., Åsen, P. A., Larsen, O., Lie, A. & Vågen, I. (1997). *Floraen i Hidra, Vest-Agder*: Fagenhet for Botanikk.

Fitzpatrick, B., Fordyce, J., Niemiller, M. & Reynolds, R. G. (2012). What can DNA tell us about biological invasions? *Biological Invasions*, 14 (2): 245-253.

Fogarty, G. & Facelli, J. (1999). Growth and competition of *Cytisus scoparius*, an invasive shrub, and Australian native shrubs. *Plant Ecology*, 144 (1): 27-35.

Fremstad, E. & Elven, R. (1997). Alien plants in Norway and dynamics in the flora: a review. *Norsk Geografisk Tidsskrift - Norwegian Journal of Geography*, 51 (4): 199-218.

Fremstad, E., Norderhaug, A. & Myking, T. (2005-6). Endring i norsk flora. *Direktoratet for natruforvaltning*.

GBIF-Norway, N. B. I. C. a. (2007-2016). *Species Map Service 1.6*. Available at: http://artskart.artsdatabanken.no/FaneKart.aspx?Date=0,0&GPND=True&DT=11111&BBOX=-552812,6424201,1590979,7966051&Height=511&Width=724.

Gederaas, L., Moen, T.L., Skjelseth, S. & Larsen, L.-K. (eds.). (2012). *Alien species in Norway – with the Norwegian Black List 2012.* The Norwegian Biodiversity Information Centre, Norway. (accessed: 28.03.2014).

Genton, B. J., Shykoff, J. A. & Giraud, T. (2005). High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction. *Molecular Ecology*, 14 (14): 4275-4285.
Gulbrandsen, T. B. r. (2011). Mandal Kommune: Risøbank.
Guo, Q. F. (2006). Intercontinental biotic invasions: what can we learn from native populations and habitats? *Biological Invasions*, 8 (7): 1451-1459.
Haider, N. (2011). Chloroplast-specific universal primers and their uses in plant studies. *Biologia Plantarum*, 55 (2): 225-236.
Hamilton, M. B. (1999). Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology*, 8 (1365-294X (Electronic)): 521-523.
Haraldstad, Ø. & Gunnarsli, K. S. (2012). Uttalelse til høring- Forslag til forskrift og faggrunnlag for kystlynghei som utvalgte naturtype.
ISSG, I. (2008). The International Union for
Conservation of Nature - Invasive Species Specialist
Group.
Kang, M., Buckley Ym Fau - Lowe, A. J. & Lowe, A. J. (2007). Testing the role of genetic factors across multiple independent invasions of the shrub Scotch broom (*Cytisus scoparius*). *Molecular Ecology*, 16 (0962-1083 (Print)): 4662-4673.
Lagerber, T., Holmsboe, J. & Nordhaugen, R. (1955). Våre ville planter. Bind IV.
Malo, J. E. (2004). Potential ballistic dispersal of *Cytisus scoparius* (Fabaceae) seeds. *Australian Journal of Botany*, 52 (5): 653-658.
Maron, J. L., Vila, M., Bommarco, R., Elmendorf, S. & Beardsley, P. (2004). Rapid evolution of an invasive plant. *Ecological Monographs*, 74 (2): 261-280.
Nilsen, L. S., Måren, I. E. & Pedersen, O. (2009). *Fargen er purpur*
– kystlyngheivegetasjon
i Norge. Naturen. Available at: http://www.miljodirektoratet.no/Global/dokumenter/tema/kulturlandskap/Naturen 0902.pdf (accessed: June 26.).
Pappert, R. A., Hamrick, J. L. & Donovan, L. A. (2000). Genetic Variation in *Pueraria lobata* (Fabaceae), An Introduced, Clonal, Invasive Plant of the Southeastern United States. *American Journal of Botany*, 87 (9): 1240-1245.
Parker, I., Harpole, W. & Dionne, D. (1997). Plant Community Diversity and Invasion of the Exotic Shrub *Cytisus Scoparius*:Testing Hypotesis of Invasibility and Impact. *Ecology and Conservation of the South Puget Sound Prairie Landscape*: 149-163.
Parker, I. M. (1997). Pollinator limitation of *Cytisus scoparius* (Scotch Broom), an invasive exotic shrub. *Ecology*, 78 (5): 1457-1470.
Parker, I. M. (2000). Invasion dynamics of *Cytisus scoparius*: A matrix model approach. *Ecological Applications*, 10 (3): 726-743.
Parker, I. M., Rodriguez, J. & Loik, M. E. (2003). An Evolutionary Approach to Understanding the Biology of Invasions: Local Adaptation and General‐Purpose Genotypes in the Weed *Verbascum thapsus*. *Conservation Biology*, 17 (1): 59-72.
Potter, K. J. B., Kriticos, D. J., Watt, M. S. & Leriche, A. (2009). The current and future potential distribution of *Cytisus scoparius*: a weed of pastoral systems, natural ecosystems and plantation forestry. *Weed Research*, 49 (3): 271-282.
Rasmussen, T. (2016). *Store Norske Leksikon: Historisk betydning av Sokn*. Store Norske Leksikon: Foreningen Store norske leksikon.
Richardson, D. M., Allsopp, N., D'Antonio, C. M., Milton, S. J. & Rejmanek, M. (2000). Plant invasions - the role of mutualisms. *Biological Reviews*, 75 (1): 65-93.

Rosenmeier, L., Kjær, E. D. & Nielsen, L. R. (2013). The Scotch broom, *Cytisus scoparius* (Fabaceae), a paradox in Denmark – an invasive plant or endangered native species? *Botanical Journal of the Linnean Society*, 171 (2): 429-440.
Rostgaard Nielsen, L., Brandes, U., Dahl Kjær, E. & Fjellheim, S. (2016). Introduced Scotch broom (*Cytisus scoparius*) invades the genome of native populations in vulnerable heathland habitats. *Molecular Ecology*, 25 (12): 2790-2804.
Rudjord, K. (1974). *Oddernes bydebok bygde historie*: Kristiansand Kommune.
Saltonstall, K. (2002). Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences*, 99 (4): 2445-2449.
Salvesen, T. (2004). Historisk rundtur i Mandal.
Sexton, J. P., McKay, J. K. & Sala, A. (2002). PLASTICITY AND GENETIC DIVERSITY MAY ALLOW SALTCEDAR TO INVADE COLD CLIMATES IN NORTH AMERICA. *Ecological Applications*, 12 (6): 1652-1660.
Shaw, J., Lickey Eb Fau - Beck, J. T., Beck Jt Fau - Farmer, S. B., Farmer Sb Fau - Liu, W., Liu W Fau - Miller, J., Miller J Fau - Siripun, K. C., Siripun Kc Fau - Winder, C. T., Winder Ct Fau - Schilling, E. E., Schilling Ee Fau - Small, R. L. & Small, R. L. (2005). The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany*, 92 (0002-9122 (Print)): 142-166.
Simmons, M. P. & Ochoterena, H. (2000). Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology*, 49 (1063-5157 (Print)): 369-381.
Skard, T. (1950). Hagebruk og gartneri i Norge.
Taberlet, P., Gielly L Fau - Pautou, G., Pautou G Fau - Bouvet, J. & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, 17 (0167-4412 (Print)): 1105-1109.
Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, 17 (5): 1105-1109.
Turner, J. H. (1933). The Viability of Seeds. *Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew)*, 1933 (6): 257-269.
Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. & Webb, D. A. (1968). *Flora Europea*: Cambridge The university press.
Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B., Remm, M. & Rozen, S. (2012). *Primer3- new capabilities and interfaces*: Nucleic Acids Research. p. Enhancements and modifications of primer design program Primer3.
Wheeler, C. T., Helgerson, O. T., Perry, D. A. & Gordon, J. C. (1987). Nitrogen Fixation and Biomass Accumulation in Plant Communities Dominated by *Cytisus scoparius* L. in Oregon and Scotland. *Journal of Applied Ecology*, 24 (1): 231-237.
Williamson, M. H. & Brown, K. C. (1986). The analysis and modeling of British invasions *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 314 (1167): 505-522.
Wolfe, K. H., Li, W. H. & Sharp, P. M. (1987). Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of the National Academy of Sciences of the United States of America*, 84 (24): 9054-9058.
Øyen, B.-H. (2006). *Lerk (Larix) i Norge- del I. Dyrningshistorie*: Skogforskningen.

Appendix

Appendix I: Complete list of primer pairs used for screening

Table 1 shows the primer used for screening variations in *C. scoparius*.

Table 1. Over view of primers used for screening variation in *C. scoparius.* The table show primer name, sequence, expected primer length and melting temperature.

Location	Primer sequence	Location	Primer sequence	Temperature for PCR program	Product size	Authur
trnT(UGU)2F	CAA ATG CGA TGC TCT AAC CT	5'trnL(UAA)R	TCT ACC GAT TTC GCC ATA TC	55	752	Taberlet, P. et al. (1991)
rpS4R2	CTG TNA GWC CRT AAT GAA AAC G	trnT(UGU)R	AGG TTA GAG CAT CGC ATT TG	53	402	Taberlet, P. et al. (1991)
psbF	CGC AGT TCG TCT TGG ACC AG	psbB	GTT TAC TTT TGG GCA TGC TTC G	55	853	Hamilton (1999)
psbC	GGT CGT GAC CAA GAA ACC AC	trnS2	GGT TCG AAT CCC TCT CTC TC	55	1680	Demesure et al. (1995)
rpoB	CKA CAA AAY CCY TCR AAT TG	trnCR	CAC CCR GAT TYG AAC TGG GG	55	1174	Shaw et al. (2005)
trnDF	ACC AAT TGA ACT ACA ATC CC	trnT	CTA CCA CTG AGT TAA AAG GG	51	1066	Demesure et al. (1995)
trnCF	CCA GTT CRA ATC YGG GTG	ycf6R	GCC CAA GCR AGA CTT ACT ATA TCC AT	56	690	Shaw et al. (2005)
ycf6F	ATG GAT ATA GTA AGT CTY GCT TGG GC	psbMR	ATG GAA GTA AAT ATT CTY GCA TTT ATT GCT	56	825	Shaw et al. (2005)
psbMF	AGC AAT AAA TGC RAG AAT ATT TAC TTC CAT	trnDR	GGG ATT GTA GYT CAA TTG GT	54	965	Shaw et al. (2005)
trnCF	CCA GTT CRA ATC YGG GTG	psbMR	ATG GAA GTA AAT ATT CTY GCA TTT ATT GCT	55	-	Shaw et al. (2005)
ycf6F	ATG GAT ATA GTA AGT CTY GCT TGG GC	trnDR	GGG ATT GTA GYT CAA TTG GT	55	-	Shaw et al. (2005)

Appendix II: Protocol for DNA extraction and PCR amplification of herbaria material

DNA extractions and PCR amplifications for herbaria specimen was done according to a protocol developed by M. Bendiksby at Natural History museum of Oslo (Bendiksby et al. s. a.).

Protocols are as follows:

Box 1.

The regular procedure --- DNA extraction: We crushed 10 to 30 mg of leaf tissue in a 2 mL plastic tube with two tungsten carbide beads for 2 x 1 minute at 30 Hz on a mixer mill (MM301, Retsch GmbH & Co., Haan, Germany). We extracted total DNA from the crushed samples using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) or the E.N.Z.ATM SP Plant DNA Mini Kit (Omega Bio-tek, Inc., Norcross, GA, USA) according to the manufacturers' manuals.

PCR amplification: We amplified DNA in 25 µL reactions using the AmpliTaq DNA polymerase buffer II kit (Applied Biosystems, Foster City, California, USA) and 0.2 mM of each dNTP, 0.04% bovine serum albumen (BSA), 0.01 mM tetramethylammonium chloride (TMACl), 0.4 µM of each primer, and 2 µL unquantified genomic DNA. Amplifications were performed in a GeneAmp PCR System 9700 (Applied Biosystems). We performed all PCR amplifications under the following cycling conditions: 95°C for 10', 31 cycles of 95°C for 30'', 60°C for 30'', 72°C for 1', followed by 72°C for 10' and a final hold at 10°C. AmpliTaqGold® DNA Polymerase (Applied Biosystems) was used for amplifying DNA obtained from old herbarium specimens or DNA extracts of reduced quality, whereas AmpliTaq® DNA Polymerase (Applied Biosystems) was used for all high-quality DNA extracts.

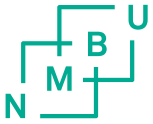
PCR purification and sequencing: PCR products were purified using 2 µL 10 times diluted ExoSAP-IT (USB Corporation, Cleveland, Ohio, USA) to 8 µL PCR product, incubated at 37°C for 45 minutes followed by 15 minutes at 80°C. Prepared amplicons for sequencing contained: 9 µL 0-30x diluted purified PCR product (depending on product strength) and 1 µL of 10 µM primer (the same primers as used in the PCR). Cycle sequencing was performed by the ABI laboratory staff at the Centre for Ecological and Evolutionary Synthesis, Department of Biology, University of Oslo. The ABI BigDye Terminator sequencing buffer and v3.1 Cycle Sequencing kit (Applied Biosystems) were used for the cycle sequencing reaction, and sequences were processed on an ABI 3730 DNA analyser (Applied Biosystems).

Box 2.

Nested PCR --- In this procedure a second set of amplification cycles are performed using a pair of 'nested' primers sited within the DNA sequence defined by the original primers (Barbara & Garson, 1993). We performed the pre-nested PCR (i.e., the first set of amplification cycles) as described in the regular procedure (Box. 1), but with only 25 amplification cycles. As template for the nested PCR (i.e., the second set of amplification cycles), we used a dsH_20-diluted (100x) product from the pre-nested PCR, and otherwise identical conditions as described in the regular procedure (Box. 1). Optimizations to improve sequence quality included: (1) adjusting the number of amplification cycles in the two separate runs; (2) testing various dilutions (10x-1000x) of the PCR product used as template for the second run.

The replicate PCR procedure --- We added template DNA to multiple identical PCR reactions (8-16 tubes) and performed the PCR amplification using the same PCR mix and cycling conditions as described in Box 1, but with 34 cycles. For purification of the PCR products, we added five times the PCR volume of PBI-buffer from the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) to each replicated PCR product before applying all to the same QIAquick DNA-binding column (Qiagen). For the remaining of the procedure, the columns were treated as described in the manufacturer's manual.

The replicate DNA extraction procedure --- We re-extracted DNA as described in Box 1, but in 2-4 replicate tubes that each included smaller amounts (< 10 mg) of leaf tissue. We performed the DNA elution twice in the same tube using the first eluate in the second elution step. Finally, we pooled DNA extracts from replicate tubes prior to use.



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