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Ursula Brandes

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Summary

The number of alien invasive species is continually increasing, threatening the environment and economy and their management is challenging. For cases of cryptic invasions, it is not even known if a species is native or alien in the considered range. The aspects and processes of biological invasions are complex. Recent research has focussed on the introduction history of alien species and its effect on invasiveness. The introduction history affects the genetic structure of alien populations, which may play an important role in biological invasions.

In this PhD project, we focus on the shrub *Cytisus scoparius* ((L.) Link) and its northernmost range expansion. We genetically analysed its populations at the expansion front, in Norway, to distinguish between natural expansion and human introductions. We further described its introduction history and suggested how that may affect its invasiveness. Further, we explored genetic patterns within its native range in Europe, and the interaction between native populations and alien populations in the native range. Finally, we observed the impact of *C. scoparius* on the vegetation composition of a threatened heathland ecosystem in the invaded range.

We found evidence that *C. scoparius* has been introduced to Norway on multiple occasions and from a range of different origins, and that there are high levels of genetic admixture. This high propagule pressure and high genetic diversity probably increases invasion success. Further, the genetic patterns in the native range revealed that the different populations have been moved across the native range. This can cause admixture of the populations of different origins already in the species' native range and serve as source for further introductions into the new range. Previous studies on *C. scoparius* in Denmark have suggested that a native and an introduced lineage are present. We further observed that these distinct populations hybridise and that the alien populations threaten the native population through genetic swamping. This will lead to the extinction of the native type and its phenotype of dwarfed growth.

Our vegetation survey of a heathland in Norway revealed some changes in the presence of *C. scoparius.* In the presence of the invader, the cover of dwarf shrubs was reduced and the level of nitrogen availability was increased. Combining our results that *C.*

scoparius is alien in Norway and that it impacts protected ecosystems, validates the need for its management.

Sammendrag

Antall fremmede invaderende arter øker kontinuerlig. De truer miljøet, og skjøtselen av dem er utfordrende og ressurskrevende. Biologiske invasjoner er komplekse. Ved kryptiske invasjoner, er det usikkert om en art er stedegen eller fremmed i det undersøkte området. Nyere forskning har fokusert på introduksjonshistorien til fremmede arter og hvordan dette påvirker invasjonen. Introduksjonshistorien påvirker den genetiske strukturen til fremmede populasjoner, og dette kan være en viktig faktor ved biologiske invasjoner.

I dette PhD-prosjektet, fokuserer jeg på den nordligste spredningen av busken *C. scoparius*. Ved å undersøke de nordligste populasjonene genetisk forsøker vi å skille mellom naturlig ekspansjon og menneskelige introduksjoner. Jeg beskriver hvordan introduksjonshistorien kan påvirke invasjonen av gyvel (*Cytisus scoparius)*. Videre ble genetiske mønstre undersøkt innenfor det naturlige utbredelsområdet i Europa, og samspillet mellom stedegne populasjoner og innførte populasjoner i det naturlige utbredelsområdet ble undersøkt. Til slutt undersøkte vi effekten av *C. scoparius* på vegetasjonssammensetningen i et truet lynghei-økosystem i det invaderte området.

Vi fant ut at *C. scoparius* har blitt introdusert til Norge ved flere anledninger og fra flere forskjellige områder, med høye nivåer av genetisk blanding. Det høye propagultrykket og det store genetiske mangfoldet øker trolig invasjonssuksessen. Genetiske mønstre i det naturlige utbredelsesområdet viste at de ulike populasjonene faktisk har blitt transportert rundt her. Dette kan føre til blanding av populasjoner av ulikt opphav allerede i artenes opprinnelige utbredelsesområde, og videre være kilde til videre introduksjoner i det nye området. Tidligere studier av *C. scoparius* i Danmark har antydet at vi både finner en stedegen og en introdusert populasjon. Vi fant ut at de ulike populasjonene hybridiserer og at den stedegne populasjonene er truet av genetisk innblanding fra den fremmede populasjonen. Dette fører til at den stedegne populasjonens fenotype med dvergformet vekst er truet av utryddelse.

Vår undersøkelse av en lynghei i Norge viste at *C. scoparius* hadde en negativ påvirkning på vegetasjonen. Dekningen av dvergbusker ble redusert og tilgangen på nitrogen økte. Resultatene viser at *C. scoparius* er fremmed i Norge, den påvirker truede økosystemer, og det er tydelig behov for skjøtsel av disse områdene.

List of papers

1) **Introduction history and population genetics of intra-continental plant invasion by scotch broom (*Cytisus scoparius*).**
   Brandes U, Furevik BB, Nielsen LR, Kjær ED, Rosef L, Fjellheim S (under review)

2) **Introduced Scotch broom (*Cytisus scoparius*) invades the genome of native populations in vulnerable heathland habitats.**
   Nielsen LR, Brandes U, Kjær ED, Fjellheim S
   Molecular Ecology, 25(12), 2790-2804. doi:10.1111/mec.13666

3) **Impact of *Cytisus scoparius* on coastal heathland vegetation.**
   Brandes U, Heegaard E, Rosef L (Manuscript)

1. Introduction

1.1. Biological invasions

Biological invasions are continuing to increase in number and in the impact they cause to the environment and human society (Early et al., 2016; Seebens et al., 2017). Several decades of research have looked at a range of aspects, such as the traits and the processes that allow alien species to survive and thrive in a new environment, genetic characteristics of and processes during invasion and the impact on ecosystems and ecosystem services.

1.2. Use of terms ‘alien’ and ‘invasive’

There has been much discussion about different uses and definitions of the terms ‘alien’ and ‘invasive’. I am using the term ‘alien’ for a species or other taxa that occurs at a location as the direct result of transport by human (Philip E. Hulme et al., 2016; Webber & Scott, 2012). Thus, this term always has to be used in relation to some geographic entity. A species that colonises new regions by natural spread and without human transport should not be termed ‘alien’, even though its range expansion may be the indirect result of human activity such as global warming or land use change (Gilroy, Avery, & Lockwood, 2017). For the term ‘invasive’ I follow the definition by Colautti and MacIsaac (2004) for alien taxa that are either widespread or locally dominant or both in their introduced range. This includes the definition for invasive by Richardson et al (2000) as species which spread across a far distance from the location they were first introduced to (Heger, Saul, & Trepl, 2013; Wilson, Dormontt, Prentis, Lowe, & Richardson, 2009), but it also includes alien taxa that have not spread far but have become highly abundant in local distribution. Both of these definitions allow us to use the term ‘invasive’ without evidence that an invasive species causes impact, which is often complex and delayed, thus can be difficult to determine. Yet, an alien species that has become invasive is more likely to cause impact and it would be more severe. Both definitions for ‘alien’ and ‘invasive’ can be used at the taxonomic scale of species, but also at smaller scales of populations or genotypes. As an example, within the species region, a population is alien when it differentiated elsewhere and was introduced by human.

1.3. The introduction-naturalisation-invasion continuum ('the continuum')

For a species to become invasive, it has to pass several stages, which are separated by specific barriers that need to be overcome, one after another (Figure 1) (T. M. Blackburn et al., 2011; Colautti & MacIsaac, 2004; Richardson & Pyšek, 2006; Richardson et al., 2000). If one barrier is not passed, the advanced stages of invasive will not be reached. A species, or more precisely the populations of the species, needs certain characteristics to pass each barrier and these characteristics may either be pre-adapted (inherited before it was introduced) or evolves before it can pass the barrier. Earlier barriers can shape the population and thus affect how it will succeed through the subsequent barriers. Despite this overarching effect of earlier stages they have only recently become the focus of biological invasion research, as new methods emerge, especially genetic analyses. This thesis focusses on these early stages.

In the traditionally first step of biological invasions, individuals of a species need to succeed the transport by humans beyond its natural distribution range, and across natural geographical barriers to natural dispersal. This includes being either selectively or accidentally picked up by human and then surviving the conditions and time period of the transportation (Briski et al., 2018). These processes can be described as a part of the introduction history. On arrival, the individuals of the alien species need to be able to survive under the new environmental conditions, such as climate and resource availability (Higgins & Richardson, 2014). In many cases of deliberate introduction the survival of the introduced species is fostered by human husbandry (Mack et al., 2000; Richardson & Rejmánek, 2011; Wilson et al., 2009) or limited to human disturbed sites. At this stage, an alien plant species is casual in the novel range and may perish once it reaches senescence or mortality exceeds population growth. The species becomes naturalised, when it has overcome reproductive barriers, such as isolation from mating partners if self-incompatible or lack of vectors for pollination. Naturalised aliens maintains a viable population size that is safe from adverse environmental stochasticity and does not depend on human nurture. Naturalised alien species are likely to persist but unlikely to have any perceptible impact as they occur in low abundance. In order to shift into the next stage and becoming invasive, a species needs to overcome barriers of secondary dispersal within the novel range (Richardson et al., 2000), or if using the definition by Colautti and MacIsaac (2004), it needs to become dominant in the introduced ecosystem. It has often been observed that naturalised species remain restricted to their range of initial introduction for many decades or even over a century and then rapidly increase their range within a short time period (Bock et al., 2015; Williamson, Pyšek, Jarošík, & Prach, 2005). This time of little population growth has been termed as a lag phase. A lag phase can last a few years or over a century, which poses the question if there are any naturalised species that do not become invasive or if they are just undergoing a very long lasting lag phase and may become invasive in the future. During the lag phase, a species may overcome demographic barriers or it requires subtle environmental changes (increase in efficiency of pollinators, environmental changes) or evolutionary changes (D. Simberloff, 2009). The lag phase might also be an artefact (Aikio, Duncan, & Hulme, 2010; Pyšek & Hulme, 2005).

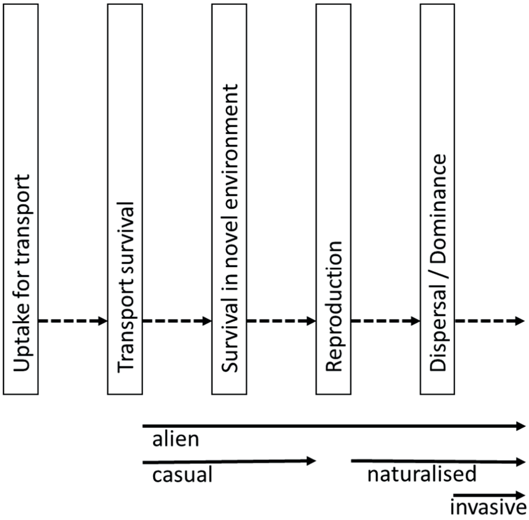

Figure 1 The introduction-naturalisation-invasion continuum of biological invasions. The populations of a not native species need to overcome different barriers to enter the next level of the continuum. Figure inspired by Richardson (2000), Lockwood, Cassey, and Blackburn (2005), Colautti, Grigorovich, and MacIsaac (2006), T. M. Blackburn et al. (2011) and (Zenni et al., 2017).

At each transition from one stage to the next, the alien species might require a different set of characteristics and adaptations (Kolař & Lodge, 2001; Richardson & Pyšek, 2012; Theoharides & Dukes, 2007; van Kleunen, Dawson, & Maurel, 2015). Each barrier might also shape the population that advances to the next stage. Alternatively, a species might overcome barriers with human assistance (Jane A. Catford et al., 2012). This is especially apparent for the large number of ornamental species that become invasive (Foxcroft, Richardson, & Wilson, 2008; Pergl et al., 2016). While past research has mostly focussed on the traits that allow alien species to become invasive or naturalised, in recent years the importance of introduction history of alien species has been acknowledged. Aspects of an alien species' introduction may not only shape its further progress along the invasion continuum, but the introduction of alien species is also the most efficient target for management (P. E. Hulme et al., 2018; Pyšek & Richardson, 2010).

1.4. What makes an alien species invasive?

1.4.1. Invasive traits

It appears that some species are more successful invaders than others (Petr Pyšek et al., 2014; Rejmánek, 1996; Williamson & Fitter, 1996). Yet, the ongoing search for invasive traits has given little success in better understanding and predicting biological invasions (Kueffer, Pyšek, & Richardson, 2013). Instead, it has shown the complexity of biological invasion events (e.g. Gurevitch, Fox, Wardle, Inderjit, & Taub, 2011; van Kleunen et al., 2015). Invasion success depends not only on the alien species, but also on the abiotic and biotic aspects of the invaded environment and other influences, especially from human activity (Kueffer et al., 2013; Perkins & Nowak, 2013). Some species are adapted to thrive in human modified ecosystems, such as arable fields or railroads, in their native range. Once introduced to a new range, they are pre-adapted to grow in human modified environments there. But, in many cases alien species thrive in new natural habitats that differ to some degree to their native natural habitats (Hejda, Chytrý, Pergl, & Pyšek, 2015). This may be explained by phenotypic plasticity, also a pre-adapted trait. Larger phenotypic plasticity increases the alien species' ability to inhabit an empty niche in the new environment. While individuals of a species can succeed in changing, heterogeneous and fluctuating environmental conditions through phenotypic plastic traits, the same can be reached from high genetic diversity (or epigenetic diversity) at the population level (Liao, D'Antonio, Chen, Huang, & Peng, 2016).

1.4.2. Genetic diversity

It is expected that an introduced population is more likely to establish and become invasive in a novel range if it is genetically diverse. A genetically diverse population is more likely to contain a least a few individuals that can survive and produce offspring in the new environment, thus it aids the naturalisation of an alien species. If genetic diversity is maintained (interbreeding and population growth) selective pressure can lead to the rapid evolution towards better adaptations to the new environment (Prentis, Wilson, Dormontt, Richardson, & Lowe, 2008). Rapid evolutionary adaptation have been observed in several cases of invasive species (Marchini, Arredondo, & Cruzan, 2018; Sultan, Horgan-Kobelski, Nichols, Riggs, & Waples, 2013; Vandepitte et al., 2014). In small populations, genetic diversity is also important to prevent effects of inbreeding depression. Several studies have compared genetic diversity between a species in its native range and in its invaded range, resulting in all possible outcomes of increased, decreased and similar genetic diversity (Dlugosch, Anderson, Braasch, Cang, & Gillette, 2015; Rollins et al., 2013; Schrieber & Lachmuth, 2017). It appears that genetic diversity is not a general prerequisite for invasion success. If an alien species is introduced to a environment that is similar to its native origin (i.e. similar abiotic conditions, functional biotic conditions or human modification) low genetic diversity gives the best advantage (i.e. highest fitness), as the whole population would be pre-adapted to the introduced environment. If the introduced environment is very different to the native environment or fluctuates strongly, the alien species requires high genetic diversity to ensure its survival and evolve better adaptation. In this case, the disadvantage of producing some badly adapted offspring may pay off with the increased chances of some well-adapted offspring.

1.4.3. Introduction history

The genetic diversity of an introduced population depends on its introduction history, the quantity and quality of the introductions, and also on the levels of gene flow among the different introduced populations (resulting in genetic admixture). The characteristics of the introduction history of an invasive species has been considered highly influential on its invasion success. However, there are few cases where the introduction effort has actually been recorded, and these are obviously restricted to cases of intentional introductions. Using genetic markers can reveal the extent of introductions and the source locations and characterise the quantity and quality of genetic diversity in the invaded range (Cristescu, 2015; Estoup & Guillemaud, 2010; Ray & Quader, 2014; Shirk, Hamrick, Zhang, & Qiang, 2014). Again, the results of such empirical studies gave all possible outcomes, and the effect of introduction history on the invasion success is context dependent.

The quantity of introductions can be described as the propagule pressure, which combines propagule frequency (number of introduction events) and propagule size (number of individuals per introduction event) (Tim M. Blackburn, Lockwood, & Cassey, 2015; Lockwood et al., 2005). The quality depends on the origins of the introductions and to what extend this source range is genetically differentiated. It has turned out that most invasive alien species have been introduced multiple times and in large numbers, often from different origins, but not always (Bossdorf et al., 2005; Wilson et al., 2009). Genetic diversity is reduced in the invaded range when introduction events are few and small or the sources of introductions are low in genetic differentiation. If these introduction events are not followed by genetic admixture, evolutionary processes such as genetic drift, inbreeding or directional selection would lower genetic diversity. On the other hand, genetic diversity increases in the introduced range if propagules are sourced from multiple and genetically distinct origins, and they admix. Genetically distinct sources may be due to a wide range of sources of the introduction, but may also be due to the mixture of previously differentiated populations within the source range. This may occur when the introductions cover a large range of original locations and habitats, are spaced out over time or when the source population is already composed of several divergent origins. Any intermediate or combination between these two extreme scenarios will result in positive or negative shift in genetic diversity. As it can influence all further processes of the invasion continuum, it is essential to characterise the introduction history of an alien species for studying all aspects of invasion biology (Colautti et al., 2006). In this study, we go even one step ahead of the introduction history of an alien species and study processes that shape the possible native sources for the introductions into novel ranges (Paper 2). Other cases have associated hybridisation and increased genetic diversity with invasive success, but they usually focus on the introduced range, not the source of the introductions.

1.5. Cryptogenic species and cryptic invasion

A particular group of alien species has often been excluded from research and management, because they are cryptogenic (not known if they are native or alien; Carlton, 1996; Morais & Reichard, 2018). There are two types of cryptic invasions, intra-specific and inter-specific (Morais & Reichard, 2018). In cases of inter-specific cryptic invasions, an alien species was not identified as such because it either is visually indistinguishable from native or other alien species, i.e. cryptic species (Morais & Reichard, 2018), or because it is not clear if it has colonised an area by natural means of by human introduction, i.e. cryptogenic species (Camarda, Cossu, Carta, Brunu, & Brundu, 2016; Geller, Darling, & Carlton, 2010). The distinction between natural range expansion and human introduction can be specifically challenging in cases of intra-continental range expansion, where no clear dispersal barriers are present. Species that were introduced from a different continent and across a wide geographic barrier can usually easily be identified as alien. It is not even necessary to know the native range of such species. However, in cases of species range expansion at close vicinity to its more or less clearly defined native range, it can be challenging or even impossible to prove if it is native or alien. Genetic analyses can aid the distinction between natural range expansion and human introduction in cases of cryptogenic species. Natural species range expansion is characterised by reduced genetic diversity towards the expansion front and by a patchy distribution of the different haplotypes. On the other side, human introductions is likely to lack these patterns and genetic diversity may reflect the level of human movement and trade (Figure 2). Because of this additional step to verify a species as alien and the remaining uncertainties of this, studies at the intra-continental scale are scarce and the majority of invasion research has been at the inter-continental scale (i.e. alien species originating from a different continent). This poses a considerable knowledge gap on biological invasions. There may be differences between the introduction history of inter- or intra-continental introductions, such as a higher propagule pressure, additional types of introduction pathways and less propagule loss during lengthy introductions. There might also be less requirement to adapt to the new environment, as they should be more similar.

In the other case of a cryptic invasion is at the intra-specific scale, when a species' invasion is caused by the introduction of an alien lineage to a region where the species is native but represented by a different and distinct native lineage (Morais & Reichard, 2018; Saltonstall, 2002). The invasion usually follows a hybridisation between the native and alien lineage, which may results in an invasive hybrid population. Alternatively, one of the distinct lineages (either native or alien) may become invasive due to genetic introgression from the other (Ellstrand & Schierenbeck, 2000). This hybrid population would not exist without human dispersal of one or several lineages of the species, which then may become invasive in its native range. Some cases show that alien invasive lineages per se can replace the native lineage (Saltonstall, 2002; Tano, Halling, Lind, Buriyo, & Wikström, 2015). Some such cases may have been recorded as examples for native species becoming invasive.

Both types of cryptic invasion can pose significant issues to nature managers, when they do not know if a species is alien and native and if eradication can be justified (Morais & Reichard, 2018; P. Pyšek et al., 2013). In the case of intra-specific cryptic invasion a paradox of invasive and threatened populations of the same species in the same region may occur (Rosenmeier, Kjaer, & Nielsen, 2013).

1.6. Impact of invasive plant species or populations

A multitude of case studies has confirmed a range of specific impacts, by many alien species, in a range of different invaded ecosystems (Ehrenfeld, 2010). As so often for biological invasions, no generalities could be drawn. Instead one species may have a different suit of impacts on the different ecosystems, and these may change during the different stages of invasion and with increasing abundance, and those impacts are further influenced by other environmental changes such as land use change and global warming (A. Ricciardi, Hoopes, Marchetti, & Lockwood, 2013; Vilà et al., 2011). Invasive species may alter the environmental conditions, interrupt the circulation of nutrients, water and energy, change the interaction between species within or across trophic levels, and affect biodiversity (e.g. Ehrenfeld, 2010). In some cases, alien species have cause major changes in an ecosystem, therefore can be called ecosystem engineers or ecosystem transformers

(Fei, Phillips, & Shouse, 2014; Jones, Lawton, & Shachak, 1997; Richardson et al., 2000; Wright & Jones, 2006).

Some of the most substantial impact from alien species can be associated with invaders introducing novel traits to the ecosystem. Of major concern are alien species that can fixate atmospheric nitrogen , especially when they invade nitrogen poor habitats to which this type of nitrogen fixation is novel (Castro-Díez & Alonso, 2017; Lazzaro et al., 2018; Vetter et al., 2018). The invasion of nitrogen fixing plants can increase the nitrogen availability in an ecosystem or change the circulation/flux of nitrogen, but not always (Ehrenfeld, 2010; Lee et al., 2017; Rice, Westerman, & Federici, 2004). Especially in nitrogen poor habitats, where it might be the reducing factor for plant growth, nitrogen increase can increase primary production and therefore the uptake of other soil nutrients.

Alien species have been listed as one of the largest threat to biodiversity globally (Bellard, Cassey, & Blackburn, 2016; Mollot, Pantel, & Romanuk, 2017; Murphy & Romanuk, 2014; Pereira, Navarro, & Martins, 2012; Pyšek, Blackburn, García-Berthou, Perglová, & Rabitsch, 2017). It is often stated that invasive alien species are threatening native biodiversity but few studies can clearly confirm this, while other cases show an increase or no change in biodiversity (Gaertner, Breeyen, Hui, & Richardson, 2009; Mollot et al., 2017; Powell, Chase, & Knight, 2011; P. Pyšek et al., 2012). Biodiversity loss occurs at several scales: At the landscape level with the loss and degradation of ecosystems, at the species level with the loss and reduction in abundance of species, and at the genetic level with the loss of genetic diversity and of genetic distinct populations. The effects of alien species on biodiversity might change over time, with different invader densities or depend on the invaded habitats.

While most studies focus on alien species causing decline in species richness and to some extend also ecosystem degradation, we also look at the loss of genetic diversity of the native species, in our case the native population of the same species as the invading population. We expect a loss of genetic diversity when species cover declines, as less individuals usually represent less genotypes and less genetic variation. Another direct and irreversible impact from alien plant species or alien genotypes is genetic contamination by hybridisation and genetic admixture (T. M. Blackburn et al., 2014; Pyšek et al., 2017;

Todesco et al., 2016). In plants, hybridisation can occur between species that are closely related (inter-specific) and also between different populations of the same species that had existed in separation (intra-specific). When native and alien plant populations hybridise successfully, the native genome is likely to be irreversibly contaminated (Todesco et al., 2016). It can be debated how much of the genome of a native species/ population can be replaced by alien genes until it should not be categorised as a native species/ population any more. This becomes important when considering the benefit of introducing alien genotypes into threatened small, inbred populations to genetically rescue them from succumbing inbreeding depression (Frankham, 2015).

Evaluating and predicting the impact caused by alien species and genotypes is complex, context dependent and highly uncertain. Yet, it is fundamental to have proof of negative impact from alien species for their management, to claim funding and to ensure public support and possibly even public involvement. The majority of scientists and nature conservation managers agree that the harmful impact from alien species needs to be prevented, minimalised, mitigated or at least contained (Daniel Simberloff, 2014). Alien species management is time consuming and costly and has to be ongoing over long time to succeed. Nature conservation managers have to justify these efforts. With the large and steadily increasing numbers of alien species establishing worldwide (Seebens et al., 2017) the limited resources need to be focussed on managing the most harmful alien species (and also on feasible management options; McGeoch et al., 2016). Therefore, many attempts have been made to rank alien species according to their impact (Kriticos, Beautrais, & Dodd, 2018; Tanner et al., 2017; Vilà et al., 2018). Information on the negative impact of an alien species in one country is also valuable for other countries where this species is not found yet, so they can focus on preventing this species to enter their country. Further, information about the impact of some alien invasive species may help to estimate the impact of other similar alien species that have not yet become invasive (invasion debt; Rouget et al., 2016).

It should not be ignored that alien species deliver many benefits to human societies (large amounts of human food production is based on alien species, however most of them are not invasive) and there are examples of alien species values for nature conservation (e.g. *Tamarix* for roosting birds in the US; M. A. Schlaepfer, Sax, & Olden, 2011). This paradox of our dependence on alien species on one hand and the threat on our wellbeing on the other hand emphasizes the importance to evaluate all the impacts of alien species. Yet, whenever new alien species enter a country, it is fundamental to make fast decisions, as only quick response can be efficient to remove such species (D. Simberloff et al., 2013).

1.7. *Cytisus scoparius*

The shrub *Cytisus scoparius* is native to central and southern Europe and the British Isles. It has been introduced across the world and is considered invasive in Australasia, America, Africa and India (Downey & Brown, 2000; Mkhize, Mhlambi, & Nanni, 2013; Paynter, Downey, & Sheppard, 2003; Peterson & Prasad, 1998; Potter, Kriticos, Watt, & Leriche, 2009; Shaben & Myers, 2010; Srinivasan et al., 2012; Srinivasan, Shenoy, & Gleeson, 2007; Wearne & Morgan, 2004). *C. scoparius* has also been considered unwanted within its native range, as it colonizes open areas with conservation value. It has been debated if the species is native or alien to Norway, while it is high certain that it is native as far north as Denmark (Rosenmeier et al., 2013). Its current northern distribution extends into Sweden and Norway, as far north as Bodø, 67.2804° N, 14.4049° E (GBIF) In recent decades, this species has rapidly expanded in distribution and can reach high densities in Norway. In a Danish study, it was shown that at least two different types of *C. scoparius* grow in Denmark, one native and one alien (Rosenmeier et al., 2013). It was shown that the Danish native populations are phenotypically distinct, as they have evolved particular adaptation to the habitat and climate in Denmark. However, if these native and alien types hybridise and potentially create a new population with high genetic diversity and high adaptive potential is unknown. Furthermore, the alien populations may displace the native population in the heathland, or if they hybridise, the native population may be genetically contaminated. Displacement and hybridisation may change the physiology of *C. scoparius* and its interactions with the environment.

The life cycle and ecology of *C. scoparius* has previously been studied in its native and introduced range. The shrub prefers open and disturbed habitat and often establishes on abandoned pasture in its native range (Prevosto, Dambrine, Coquillard, & Robert, 2006). In its introduced range it also invades native grassland and native as well as non-native forests (Haubensak & Parker, 2004; Wearne & Morgan, 2004), and causes significant losses to exotic pine plantations (Watt & Rolando, 2014). *C. scoparius* can reach a life span of around ten years in Europe and up to 23 years in Australia (Prevosto, Robert, & Coquillard, 2004). At an age of two to five years each shrub can produce large amount of hard coated seeds (Sheppard, Hodge, Paynter, & Rees, 2002). The seed pods open explosively, and seeds can be spread for up to seven meters and then be dispersed by ants for up to a further five meters (Bossard, 1991; Malo, 2004). Seeds have been germinated after 80 years of dry storage (Turner, 1933), however seed viability under natural conditions averages only five years (Magda, Gleizes, & Jarry, 2013). Seed development increases through cross-pollination by honeybees and bumblebees (Paynter et al., 2010). In Norway, *C. scoparius* is most rapidly spreading along roadsides, and other human made habitats, but it also thrives in protected natural and semi-natural ecosystems, such as heathlands and sand dunes.

2. The thesis

2.1. Aims and objectives

This PhD project focusses on the intra-continental invasion of *Cytisus scoparius*, at its northern most range expansion. Very few studies focus on intra-continental invasions while most of our knowledge of biological invasions derives from inter-continental scale studies. In order to identify if *C. scoparius* should be classified as native or alien in Norway, it needs to be established if it migrated to Norway by natural processes of range expansion or if it was introduced by human (Paper 1). This thesis focuses on the early stages of the invasion process, especially on the introduction history (Paper 1). This includes estimating the propagule pressure, finding introduction origin, and describing the genetic diversity within and admixture among the introduced populations. These aspects have been considered as major predictors for invasion success but empirical studies and experiments vary in their results. We aimed to consider all these aspects in one case study. Little research has considered processes, which may already take place in a species' native range and affect the species' invasive potential after its introduction into a new area. We study the hybridisation between native and alien genepools in the species' native range, which may create a new and even more invasive genotype that may subsequently be introduced into the novel range and enhance the alien species' abilities to survive, naturalise and become invasive there. The native range and introduction history of an alien species may also effect its impact and we studied both in the same case. The impact of alien species is highly case specific and needs to be assessed specifically for each alien species, in each invaded habitat and under each set of other circumstances. Information of the impact of *C. scoparius* on Scandinavian heathlands is also needed for the implementation of its management. *C. scoparius'* ability to fix nitrogen is of special concern in low nutrient ecosystems. *C. scoparius* may affect the abiotic environmental conditions and to the vegetation composition of the heathland and we investigate if ecosystems are degraded by the invasion of alien *C. scoparius*. This study is particularly informative, as we study a wide range of aspects (as mentioned above) on the same case, the invasion of *C. scoparius* in northern heathlands.

Each of the three papers were conducted with their specific aims:

Paper 1) Resolve if *C. scoparius* was introduced to Norway (is alien) and reconstruct its introduction history to determine if high propagule pressure, diverse introduction origins and high gene flow may have facilitated this invasion. Reveal if human long distance dispersal also took place within the native range, as the likely source for introductions.

Paper 2) Characterise the gene flow between native and alien populations in Denmark and uncover the resulting consequences for the native population (genotype and phenotype).

Paper 3) Document the ecological impact of *C. scoparius* on coastal heathland in Norway.

3. Summary of methods

3.1. Genetic methods

We used genetic analyses of a spatial and temporal diverse set of *C. scoparius* populations to reconstruct the introduction history and estimate gene flow in the introduced range (Paper 1) but also in the native range (Papers 1 & 2). Samples were collected from 36 populations in Norway, 17 populations in Europe, 8 populations in

Denmark and 108 herbarium specimens from across Europe and dating back to 1835. We analysed chloroplast markers (haplotypes) and nuclear markers. The haplotypes allow us to reconstruct the introduction history into the northern range expansion and movement of different haplotypes within the native range (Papers 1 & 2). Nuclear markers reveal gene flow within and among different populations and their genetic diversity (Papers 1 & 2).

3.2. Ecological methods

To study the impact of *C. scoparius* on heathland, we compared the vegetation composition in the presence and absence of this shrub (Paper 3). For the impact on the vegetation, we compared abundance of the dominant heathland plant species and for impact on the environmental conditions, we compared the weighted averages of the indicator values for the species present in a survey plot (Ellenberg indicator values and Grime's CSR values).

4. Main results and discussion

4.1. *Cytisus scoparius* is alien to Norway (with one possible exception)

This study reveals that *C.* scoparius is mostly alien to Norway, because the geographical distribution of the haplotypes (i.e. chloroplast markers) does not follow the pattern we would expect from natural range expansion (Paper 1; Figure 2). In the case of a natural range expansion, we would have expected a single haplotype or very few haplotypes dominating in different regions each and they would usually persist over time (Eckert, Samis, & Lougheed, 2008; Harter, Jentsch, & Durka, 2015; Waters, Fraser, & Hewitt, 2013). We observed numerous different haplotypes in Norway and no general distribution pattern in space or time.

One exemption can be made for the population at Telebukta. This population has a 100 year history of one and the same haplotype, at a currently isolated location, remote from human infrastructure (haplotype C in Paper 1). The population share the chloroplast haplotype with the native Danish population and it is also distinct from other Norwegian populations in nuclear markers. This could be taken as indicative of natural establishment in Norway. However, the natural harbour at this site may have been used for trading in historical times and current well-maintained cabins suggest regular recreational use (personal observation). Natural migration directly from Denmark is unlikely, as *C. scoparius* does not float on water (Watterson & Jones, 2006). Furthermore, the population in Norway does not resemble the typical growth habit of the putative native Danish populations (personal observation). Both populations in Norway and Denmark show no sign of invasive behaviour. It is even uncertain if the Norwegian population will persist in the long term because of its very low fecundity and very few mature plants (personal observation). Based on these results, we cannot resolve the status of this population as either native or alien.

Based on our extensive genetic analysis we could mostly resolve this particular case of a cryptogenic species (uncertain if it is alien or native; Carlton, 1996). Cryptogenic species are common and widespread, but very few cases have been resolved (Camarda et al., 2016; Geller et al., 2010; Haydar, 2012; Lambdon et al., 2008; Morais & Reichard, 2018). We showed how genetic analysis can uncover cryptic species, but also reveal limits of this approach, as we could not clearly classify one of the population (Paper 1). For all other populations, we have justified that they are alien and our study is valid to fill the knowledge gap of alien species invasion at the intra-continental scale (Avery, Fonseca, Campagne, & Lockwood, 2013; Carlton, 1996; Morais & Reichard, 2018). Only as this species in Norway is now classified as alien (with one possible exception), it can be included in alien species management plans (Gilroy et al., 2017). Based our results, *C. scoparius* is now included in the Norwegian List of alien species (Artsdatabanken, 2018).

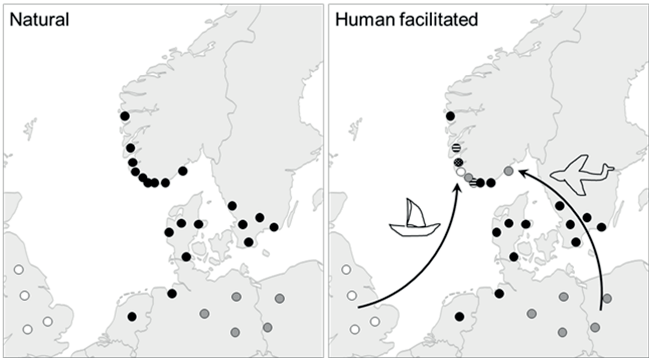

Figure 2 Conceptional map of the spatial distribution patterns expected from either natural or human facilitated range expansion. The different patterns of the dots represent different haplotypes.

4.2. Frequent and diverse introduction history into northern range expansion

4.2.1. High propagule pressure (quantity)

Our results of the chloroplast markers of *C. scoparius* in Norway, suggest high propagule pressure, especially in the form of multiple introductions. Eight different haplotypes in the Norwegian range indicates that at least as many introduction events have taken place (high propagule number (Colautti et al., 2006); Paper 1; but see below). A higher number of introduction events is likely, as each haplotype may be introduced repeatedly. We also found indication for multiple introduction to the same locations, as haplotypes differed within locations and over time (Paper 1). High propagule pressure (i.e. high propagule number and/ or high propagule size) enables the introduced population to overcome stochastic mortalities (Tim M. Blackburn et al., 2015; Lockwood et al., 2005). It also increases the probability that some individuals that prosper in the new environment are included. Multiple introduction events have often been associated with invasion success (Bossdorf et al., 2005; Rius & Darling, 2014; Wilson et al., 2009). High propagule pressure (i.e. larger number of introduced individuals) should also reduce the effects of genetic bottlenecks during the introduction and founder effects in the new range (Bock et al., 2015; D. Simberloff, 2009). Based on the fast range expansion of *C. scoparius* in Norway (GBIF, 2017) and on our results that the two most common haplotypes are widely spread across the novel range, we conclude that either, multiple introductions of the same haplotype to different locations in Norway took place, or secondary spread by human from the first site of introduction, or both. Secondary spread is also a commonly observed in alien species and in fact can be seen as the fundamental aspect for the alien species to become invasive (Kowarik, 2003). Based on the definition of invasive species by Richardson et al. (2000), spread to distant locations must take place, and this often occurs by human secondary spread. Our study on the introduction history of *C. scoparius* further discovered multiple different haplotypes at some locations, particularly in places with a history of gardening culture (e.g. plant nursery, English style gardens, townships; Paper 1). We suspect that the escape of *C. scoparius* plants from gardens has contributed to its invasion, which is supported by our findings that the most common haplotype in Norway matched with plants sold in garden centres (Paper 1). In fact, ornamental trade has been addressed as the most common type of intentional introduction of plant species that later became invasive (Dehnen-Schmutz, Touza, Perrings, & Williamson, 2007; P. E. Hulme et al., 2018; Lambdon et al., 2008). Overall, we found indications that high propagule pressure may play an important role in the establishment and invasiveness of *C. scoparius* in its new range expansion. This matches with the recent focus in invasion biology on reconstructing introduction histories of alien species, which has been described as a fundamental determinant for the introduced species' establishment and invasiveness in the new range (Tim M. Blackburn et al., 2015; Cristescu, 2015; Estoup & Guillemaud, 2010; Wilson et al., 2009).

4.2.2. Several origins of introductions (quality)

As chloroplast haplotypes are genetically conserved, we assume eight different origins of the introductions into Norway, one for each haplotype (Paper 1). The source of an introduced population is affecting its success in naturalisation and invasiveness. It is often assumed that alien populations are more likely to succeed in a new environment when they originate from a native environment with similar conditions (e.g. similar climate or land use), and thus are pre-adapted to the new environment (Bock et al., 2015; D. R. Schlaepfer, Glättli, Fischer, & van Kleunen, 2010) (Rius & Darling, 2014). On the opposite side, an alien species from a different environment and a different phylogenetic history, may have obtained distinct traits, which may give it a competitive advantage in the new ecosystem (e.g. increased resource availability, novel weapons, enemy release, empty niche; (Jane A. Catford, Jansson, & Nilsson, 2009; Le Roux et al., 2011).

However, based on our analyses of chloroplast markers across the native range in Europe, we were not able to define the origins of the eight haplotypes. The reason for this is that we did not observe the expected patterns of haplotype distribution after natural expansion in these European samples (Paper 1). We expect that after the glacial retreats in Europe, *C. scoparius* would have recolonised from southern refugia populations, as has been shown for other plant species (Petit et al., 2003). Based on our findings, this natural re-colonisation pattern was dissolved by human spread over wide distances. This mixing of the different haplotypes, has taken place for a long time, even before they were introduced into the new northern-most range. The introduction of alien haplotypes (i.e. not naturally present at the location) within the species' native range, presents an intra-specific cryptic invasion (Morais & Reichard, 2018). The accumulation of different haplotypes in the native range may be the source range for the introductions into the alien range. It makes common sense that if a species has been moved by human into a novel range, it may have been moved within its native range as well, but many other case studies have neglected this aspect.

Against our expectations, the putative native Danish haplotype (in Telebukta, Norway), which has been described as adapted to northern climate, does not appear to be invasive in the northern most novel range (Paper 1). Neither does it show 'invasive' behaviour in Denmark (Rosenmeier et al., 2013). We found that in Denmark these native shrubs are confined to the heathland where it did not grow very tall and produced relatively few flowers (Paper 2). The invasion of *C. scoparius* in Denmark appears to derive entirely from alien populations, or the admixture of their alien genes. These alien populations inhabit mostly agricultural habitats and grow tall with profound flowering and seeding (Paper 2). There might be several reasons why the northern most native populations (in Denmark) do not cause the species rapid expansion further north (in Norway). It is possible that the Danish native populations lack evolutional potential because of their reduced genetic diversity and that they lack 'invasive' traits and the ability to evolve them (Paper1) (Rosenmeier et al., 2013). Low genetic diversity is common for populations at the vicinity of a species' natural distribution range (Eckert et al., 2008; Excoffier, Foll, & Petit, 2009). The southern lineages of *C. scoparius* grow taller and appear to produce more flowers and seeds compared to the Danish lineage and this might be fundamental for its invasion success. Overall, a wider range of different origins of introductions to Norway enhances the chance of establishment, as some plants may be pre-adapted to the new habitat. If the different haplotypes also hybridise and produce fertile offspring with admixed genotypes, they can rapidly evolve and adapt to the new environment. While migration (introductions) of propagules can be inferred by analysing the haplotype structure (chloroplast markers), the genetic admixture can be implied from the nuclear markers.

4.3. Hybridisation and high gene flow among populations

Our study indicates that high gene flow occurs among the different populations in the introduced range of *C. scoparius* in Norway (migration by introduction is followed by genetic admixture; Paper 1). The lack of genetic differentiation among the populations, the much higher genetic variation within the populations than among them, and the high proportion of polymorphism (Paper 1) show this. Some of the *C. scoparius* populations in Norway contain two or three different haplotypes, thus they probably originate from several source locations and are able to hybridise. These populations originating from distinct haplotype lines, may not only compensate for genetic bottlenecks during an introduction, but may even increase genetic diversity compared to the native range (Dlugosch & Parker, 2008; Ellstrand & Schierenbeck, 2000). When the populations from different origins hybridise and produce fertile offspring, the resulting population may increase in genetic diversity, which allows for rapid evolution, and may also produce novel genotypes with advantageous combinations of their parents' genes (Estoup et al., 2016; Prentis et al., 2008). Another, short term advantage is temporary heterosis (hybrid vigour) (Keller, Fields, Berardi, & Taylor, 2014; Li, Stift, & van Kleunen, 2018).

Our observations that high genetic diversity is maintained in the invasive populations, indicates that in this study, the evolution of invasiveness may play a more important role than pre-adaptation: if the invasive traits were pre-adapted we would expect that the best pre-adapted population would dominate in the invaded range, indicated by low genetic diversity and low heterozygosity in the invaded range (Rius & Darling, 2014). This hybridisation may occur at several different stages of the introduction process, after the differentiated populations/ haplotypes have been moved together.

We also observed that in the species' native range, the native and alien haplotypes hybridise and this seems to play a role in the 'invasive' behaviour there (Paper 2). Very few individuals of the alien populations established in the Danish heathland, which is the habitat of native populations. Instead, their genes were introgressed into the native populations by pollen flow, and thus into the heathland. This could indicate that the alien populations are not well adapted to grow in the heathland, though it may be more likely due to the lack of seed dispersal into the heathland. In any case, hybridisation of two clearly differentiated and even phenotypically distinct populations produces vital offspring and provides a possible source of further introductions into the new range.

The one population in Telebukta, in Norway, which shares the haplotype with the native Danish haplotype differs yet again. Despite our knowledge that this haplotype is genetically compatible to hybridise with other haplotypes (Paper 2) we found no evidence that it does so, as it is genetically rather distinct (Paper 1). Mechanisms that are preventing these hybrids between native and alien plants might be either geographical isolation or a lower fitness of their offspring. Considering the low fecundity of this particular haplotype and its small stature, which is adapted to low nutrient habitats, it is likely that any hybrids of this ancestry may succumb competition by other more vigorous plants. However, we did not find individuals with other than the native Danish haplotype in the Telebukta population, thus geographic isolation is the most likely explanation for the lack of hybridisation.

4.4. Expanding the introduction history

The history of research on biological invasion has repeatedly shown how concepts, which originally appeared to be simple and straightforward, developed into much more complex and multifaceted processes, such as the continuum of how alien species become invasive, aspects of genetic diversity and evolution of invasiveness or propagule pressure. I argue for distinguishing three parts of the introduction history of alien species.

In this thesis, we gathered evidence that the genetic population structure in the invaded range has been shaped at three distinct stages of the introduction history (Figure 3). Genetic structure was shaped not only from how the populations were introduced into the new range (Figure 3A), but also by secondary introductions within the introduced range (Figure 3B) and by the movement of the distinct populations within the native range, thus in the source for introductions into the novel range (Figure 3C). This project indicates that in the search for the processes that enable an alien species to become invasive, it might be useful to include not only the actual introduction into the novel range, but also events that have occurred previously and within the native range, shaping the source of the introductions (Le Roux, Richardson, Wilson, & Ndlovu, 2013; Zenni et al., 2017).

Combining our observations from Paper 1 and Paper 2, indicates that in the case of the invasive range expansion of *C. scoparius* into Norway, the hybridisation of distinct lineages (probably multiple) already occurred in the native range. This may have produced admixed populations with high invasive potential, which may act as the source for introductions into novel ranges (van Boheemen et al., 2017). However, the data available for this study does not provide clear evidence for admixed source populations and their possible involvement in the invasion in the species' alien range. Yet, we are confident that gene flow (propagule movement followed by hybridisation of distinct lineages) between distinct lineages of separate origins has occurred.

By distinguishing between three stages of the introduction history of an alien species, we acknowledge that gene flow among distinct population may also occur for up to three times during the species introduction history continuum (Figure 3). A) Numerous introductions from distinct populations of different origins into the same invaded location takes place. B) Numerous introductions from distinct populations occur first to different locations across the invaded range and are then spread further into one location by secondary spread. C) Distinct populations are mixed together already in the source range (native range or different invaded range) and this mixed populations serves as the source for the introduction into the invaded range (i.e. intra-specific cryptic invasion or bridgehead for invasion). In the cases A) and B), high propagule numbers that are also sourced from differentiated origins result in high haplotype diversity, while in case C), a low propagule number of a large propagule size may be sufficient, if the source of the propagules already contains multiple different haplotypes.

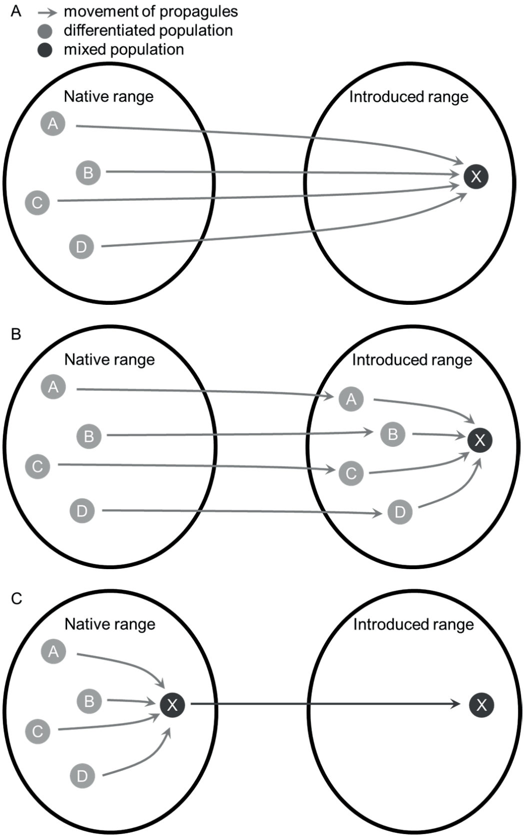

Figure 3 Three stages of an introduction that may increase gene flow among distinct populations and may increase genetic diversity and adaptive potential of alien populations in the introduced range. The letters A to D may represent distinct populations but also units at different scales, such as different species, different haplotypes, different individuals, different genomes, etc.

4.5. Alien *C. scoparius* populations cause ecological impact on heathland (in both the invaded and native range of the species)

4.5.1. In the invaded range, *C. scoparius* changes heathland vegetation and ecosystem properties

Proof of negative impact of an alien species is necessary to justify and prioritise the management of alien species, such as eradication or ban from sale. While alien *C. scoparius* spreads predominantly into human made habitats (transport ways, construction sites and urban areas), its establishment in natural dune ecosystem and semi-natural heathland has been of recent concern for biodiversity protection.

Our survey showed that the plant composition of coastal heathland changed in the presence of *C. scoparius* shrubs (Paper 3). It also alters environmental conditions of the habitat, such as the increase of nitrogen in the ecosystem. The observed displacement of dwarf shrub cover causes a threat to the heathland. Yet if this was due to direct impact from *C. scoparius,* by competition or allelopathy, we would expect that the dwarf shrubs will recover after the removal of *C. scoparius*. However, we also observed environmental change in the presence of *C. scoparius* shrubs, in particular an increase in soil nitrogen. Nitrogen increase is a common threat to heathland conservation (Borchard et al., 2017; Lindgaard & Henriksen, 2011; Press, Potter, Burke, Callaghan, & Lee, 1998). It is likely to leave a lasting legacy, even when *C. scoparius* is removed. The increase in soil nitrogen may also continue to rise over the time that *C. scoparius* is present, therefore the early removal may keep its impact low. For management consideration, the expected legacy of raised soil nitrogen also might require additional management such as soil removal, in order to restore the heathland before invasion of *C. scoparius* (Borchard et al., 2017; Broadbent et al., 2017; Grove, Parker, & Haubensak, 2015).

We found no evidence for the loss of any vascular plant species by *C. scoparius* invasion. However, we cannot exclude that extinctions may occur after a longer lasting invasion. It has previously been observed that plant species can persist against extinction threats for an extended period of time causing an extinction debt.

It is interesting that *C. scoparius* causes the reduction of all native shrub species but only some of the gramineae species. The dwarf shrubs would be the native species most similar to the invasive shrub. If the impact from *C. scoparius* on the heathland species is driven by competitive forces, we expect that native plants that are the most ecologically similar are affected the strongest (Jane A Catford, Bode, & Tilman, 2018). Two of the dwarf shrubs, *C. vulgaris* and *E. nigrum* are also adapted to habitat with very low nitrogen (Elleneberg EIV for nitrogen of 2 and 1 respectively), thus with the increase of nitrogen they lose competitive advantage against any species that is adapted to higher nitrogen level (Paper 3).

4.5.2. In the native range of *C. scoparius*, alien populations swamp native populations and likely cause invasiveness

For the heathland habitats in the native range of *C. scoparius*, the invasion of alien con-specific populations may cause an additional different type of impact: Genetic contamination of the native populations (T. M. Blackburn et al., 2014; Pyšek et al., 2017; Todesco et al., 2016). The loss of locally distinct genetic variation leads to an overall loss of genetic biodiversity of this species. In our study on the alien population invasion in Denmark (Paper 2), we could show that gene flow by pollen from the invasive to native gene pool was stronger than the opposite direction. This leads to the swamping and over time to the extinction of the distinct Danish gene pool. With the loss of genetic diversity, adaptive traits may also get lost, as does the potential to adapt to future environmental changes. We found indications that the replacement of the Danish genome causes phenotypic changes of the plants of *C. scoparius* in the heathland, such as taller growth. Similar changes in phenotypes due to alien populations have been observed for *Phragmites australis*, (Price et al 2014) and *Phalaris arundinace*ae (Spyreas et al. 2010). This change in height may change the conditions for other heathland species, and there are likely to be other phenotypic changes that we did not measure.

4.5.3. Introduction history and impact

In the case of *C. scoparius* in Norway, we showed that multiple introductions from a diverse range of origin occurred (Paper 1). This enhances the likelihood that the introductions include individuals that can cause impact and can cause greater impact. The wide range of origins also increases the range of possible impact (Pergl et al., 2017), as the origin of an alien species may also influence the impact it causes on the recipient environment (Buckley & Catford, 2016; Pergl et al., 2017; Anthony Ricciardi, Jones, Kestrup, & Ward, 2010).

Further, we have indications that introductions occurred on the pathway of the trade of garden ornamentals. Such intentional human introduction usually correlates with high propagule pressure, thus high abundance and most impact in proximity of human infrastructure (Buckley & Catford, 2016). We also expect that garden plants would be selected for traits to grow under the environmental conditions, such as cold hardiness. This suggests that the plants of *C. scoparius* on sale in Norway were selected from locations of colder climates. The two haplotypes that we found in Norwegian garden shops (however our samples were not exhaustive) were also included in samples from the northern parts of the species natural distribution (Haplotype A and C in Paper 1) and may trace back to an eastern refuge (data not shown). Further, horticulturists would chose plants that are easy to propagate, that grow fast and look beautiful. *C. scoparius* is admired for its abundant flowering, so plants with high fecundity would be preferred. One of the haplotypes sold as garden ornamental fits these requirements, and has established well across Norway and grows into dense and tall thickets in Norway and Denmark (own observations). The other haplotype appears to be related to the Danish populations, does not behave invasive and might have been selected for its compressed growth. There is also anecdotal information that *C. scoparius* has been grown in an nursery for soil stabilisation of sandy soils and possibly as fertiliser for plantations, which suggests that it might have been selected to grow particularly well in sandy and nutrient poor soils and to increases soil fertility.

When studying the impact of an alien invader, it might be revealing to consider its introduction pathway. This includes the source ranges of introductions, the modes of introductions and releases (intentional or accidental), as well as the quantities of these processes. When introductions of alien organisms are intentional, it can be expected that individuals are selected from source locations that promise the greatest pre-adaptations to establish in the new range (e.g. similar climate) and for traits allowing for high fitness (i.e. access and efficiency of resource use, fast growth, high fecundity); sampling effect in (Zenni et al., 2017). These traits may also lead to stronger impact on the invaded habitats. Accidental introductions are usually sourced from places of high human impact and introduced to places with high human impact, so the pre-adaptation to these types of habitats might be of importance.

5. Conclusions

We studied the introduction history, population genetics and ecological impact of *C. scoparius* in its northern range expansion, combining our findings on a range of different aspects of biological invasions in one case study.

Our study of *C. scoparius* covered two different types of cryptic invasions: inter-specific cryptic invasion in the recent range expansion and intra-specific cryptic invasion in the species' native range. Although the issue of cryptic invasions has long been acknowledged (Carlton, 1996; Morais & Reichard, 2018), very few of such cases have been studied, especially for plant species and at the intra-continental scale, and for both types in the same case. In our pursuit for understanding of the complex aspects and mechanisms that enable alien populations to become invasive, we cannot effort to exclude or even misclassify the large number of cases of cryptogenic species, cryptic invasions and intra-continental invaders in general.

We present a case where a species, which has been excessively moved into new ranges by human transport, has also been transported within its native range. In hindsight, this seems logical, as the reasons why this species is moved should be the same within and beyond its native range. Yet, not many case studies focussing on the introduction history of alien invasive species consider the propagule movement within the species native range and conduct an extensive sampling to cover the species' native range. In our example, it appears that *C. scoparius* is moved by the ornamental plant trade, and this pathway probably occurs both, within and beyond its native range. Other introduction pathways, for other uses or by accidental transportation might also be indiscriminate between native and invaded range. Thus, I argue that species which are introduced into novel ranges are highly likely to be moved within their native ranges as well. The mixing of different lineages of a species within the native range, diversifies the populations in the native range. Theses populations may serve as the source for further introductions into novel ranges, and their diversification is likely to increase the first stages of establishment and then the invasion success of the species in its introduced range. Thus, high population genetic diversity, which is often assumed to increase invasion success of alien species, may already develop before a population is introduced to a novel location. In this case multiple introduction are not necessary, as long as the source range is highly diverse and the propagule size is also large. As recent research effort on explaining how introduced species become invasive in the novel ranges, has moved towards the early stages of the introduction history, our research suggest that it might be useful to even include the source range of the introductions. In fact, current research turns to focus more on the source of invasions (Grosholz, 2018). Ground breaking is the results by Seebens et al. (2018) that the number of 'emerging alien species', that is species that have not been found as alien elsewhere, is continuously high. They conclude that this can only be explained by an increase of the source of species for introductions. Maybe this can also be explained by the conditioning of the populations of a species that has previously not been able to establish in a novel range. Based on the results of this thesis, the cryptic intra-specific admixture of distinct populations may create new 'invasive' populations. While this species has previously not been able to establish in a novel range, the admixed 'invasive' populations provide a new pool for successful establishment in novel ranges. Genetic changes of plant populations due to horticultural use have been suggested to increase their invasiveness (Le Roux et al., 2013; Thomson et al., 2012; Zenni et al., 2017)

Some of the results of this thesis might be useful for the management of *C. scoparius* in its introduced range and possibly also in its native range. We could show that the presence of *C. scoparius* in Norway is predominantly caused by human introductions. For all but one populations, we are certain that they are alien to Norway. For one population in Telebukta, further analysis is required to determine if it may be a native population or if it was also introduced and also to determine this population contains conservation value. Pairing this research with historical data on *C. scoparius* in Norway and information on the horticultural use and trait could also add additional information on our overall understanding of this plant invasion. Within the native range of *C. scoparius*, we revealed the threat to protected ecosystems from invasions of introduced populations of the same species, especially to the native populations.

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Paper I

Introduction history and population genetics of intra-continental plant invasion by scotch broom (*Cytisus scoparius*)

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Abstract

Aim Biological invasions at the intra-continental scale are poorly studied, and intra-continental invasions often remain cryptic. Here, we study the recent range expansion of scotch broom (*Cytisus scoparius*) into Norway and investigate if the genetic patterns support a natural spread or human introductions. We also investigate if plants were moved within the native source region, and link it to Norway. We also infer the level of gene flow and genetic diversity within and between the putative native and introduced range.

Location Europe

Methods We analysed 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 further generated 36 nuclear markers, to observe gene flow and genetic diversity.

Results We identified 20 different haplotypes, which lack spatial and temporal distribution patterns in the recent expansion range in Norway and mostly lack patterns across its native European range. Genetic diversity in populations in the introduced range was similar to populations in the native range with limited differentiation among the populations or locations.

Main conclusions Scotch broom is most likely alien to Norway, introduced by human on multiple occasions from diverse origins over a long time period. This high propagule pressure has probably maintained 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 a cryptic intra-specific invasion, most likely mediated by humans moving genotypes among regions occupied by distinct native genotypes. Intra-continental invasions may easily go unnoticed, and revealing them and the factors driving them may be of great important for their management and nature conservation.

Introduction

Despite more than 50 years of a growing research on biological invasion of alien species across continents, studies at the intra-continental scale are rare. Intra-continental introductions can cause the same threat to native environment and ecosystem services as those from a different continent (Mueller & Hellmann, 2008) and therefore need to be managed (Buckley & Catford, 2016; Hughes & Convey, 2010). Yet, to justify control efforts of an invasive species it is imperial to know if it is alien or native (Philip E. Hulme et al., 2016; Webber & Scott, 2012). Throughout this publication we use the term alien for a species or genotype which occurs at a location as a direct result of human introduction (Webber and Scott 2012, Hulme et al. 2016), and invasive for an alien species (or genotype), which spreads beyond the location where it was introduced to (Cristescu, 2015; Heger, Saul, & Trepl, 2013; Richardson et al., 2000; Wilson, Dormontt, Prentis, Lowe, & Richardson, 2009). Alien invasion studies at intra-continental level are rare, because at this scale it is difficult to prove if a species is native or alien (Philip E. Hulme et al., 2016; Webber & Scott, 2012), even when available records indicate a recent colonisation of a species, it should only be classified as alien if it was directly introduced by human and did not expand its range by natural dispersal from its native range (Gilroy, Avery, & Lockwood, 2017; Statham, Sacks, Aubry, Perrine, & Wisely, 2012). Twenty years ago, Carlton (1996) criticized that species were often classified as native when their introduction could not be confirmed, even if they were likely to be alien. To address this issue of undetected, hence cryptic invasions, he established the term of cryptogenic species for those ambiguous cases between native and alien (Geller, Darling, & Carlton, 2010; Lambdon et al., 2008).

A different type of cryptic invasions occurs within a species' native range, at the population scale, when alien genotypes are introduced to a distant location but still within the species' native range (Morais & Reichard, 2018; O'Donnell, Drost, & Mock, 2017; Saltonstall, 2002). In such cases of intra-specific introductions, we refer to alien genotypes throughout this publication. Alien genotypes compete against native genotypes and if they hybridise they may genetically contaminate or swamp the native populations, which again 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; Price, Fant, & Larkin, 2014). 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). Yet, while it is generally accepted that the source origin of an introduced organism is affecting its ability to persist and invade (Henery et al., 2010), the possibility of genetic admixture of different genotypes previous 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 (Brockerhoff, Kimberley, Liebhold, Haack, & Cavey, 2014; Colautti, Grigorovich, & MacIsaac, 2006; Erfmeier, Hantsch, & Bruelheide, 2013; Estoup & Guillemaud, 2010). Of special importance are the quantitative aspects of propagule pressure (Williamson & Fitter, 1996; Wittmann, Metzler, Gabriel, & Jeschke, 2014) 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 the number of individuals introduced in each introduction event (propagule size; Blackburn, Lockwood, & Cassey, 2015; Colautti et al., 2006; Lockwood, Cassey, & Blackburn, 2005; Simberloff, 2009). High propagule pressure does not only increase the probabilities of naturalisation by compensating for stochastic mortalities and increasing the chance of being introduced into favourable habitat, 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 inter-continental scale (Blackburn et al., 2015; Bossdorf et al., 2005; Erfmeier et al., 2013; Wilson et al., 2009). As propagule pressure usually increases over time, this may be the cause for the commonly observed lag phases (Simberloff, 2009; Williamson, Pysek, Jarosik, & Prach, 2005). Besides the quantity of a species' introduction, the origins of the introductions affect its invasive potential by affecting genetic diversity and adaptability (Buckley & Catford, 2016; Fridley & Sax, 2014). A range of different propagule origins increases the possibility of pre-adaptation to the novel environment (Buckley & Catford, 2016). Further, the genetic admixture 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) as well as 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, and consequently moved further into the new range (bridgehead invasion; Lombaert et al., 2011; van Boheemen et al., 2017). Thus, few introduction events (of large propagule size) can result in diverse propagule origins (i.e. where they originally evolved) when those have mixed already in the source populations of this introduction event.

The shrub scotch broom (*Cytisus scoparius*, (L.) Link) is native across central Europe and the British Isles, but and has been classified as invasive across the globe (Cordero, Torchelsen, Overbeck, & Anand, 2016; Downey & Brown, 2000; Kang, Buckley, & Lowe, 2007; Mkhize, Mhlambi, & Nanni, 2013; Neubert & Parker, 2004; Srinivasan, Shenoy, & Gleeson, 2007). Since its first record in southern Norway in 1876 (Blytt, 1876) it has spread northwards (Figure 1, see Appendix S1 in Supporting Information) and also increases its abundance in threatened habitats, such as endangered coastal heathlands (Lindgaard & Henriksen, 2011; Nilsen, Måren, & Pedersen, 2009). The ability to fix atmospheric nitrogen by association with microorganisms raises conservation concerns (Broadbent et al., 2017; Haubensak & Parker, 2004). This species has been included in the Norwegian Black list in 2007 but was removed in the 2012 reviewed list, considering that it might be native (L. M. Gederaas, T. L.; Skjelseth, S.; Hansen, L.-K., 2013; L. S. Gederaas, I.; Viken, Å., 2007). The ongoing range expansion might simply reflect lag in dispersal to northern habitats, which have become 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 proposed for European trees; e.g. *Abies alba*, *Larix decidua* in Svenning & Skov, 2004). Alternatively, the recent range expansion can mainly be a result of human introduction. In fact, scotch broom has been planted for soil improvement and stabilization (Vesthassel, 1926) and as a garden ornamental, and the extended viability of seeds (Magda, Gleizes, & Jarry, 2013) allows for accidental spread in soil (ballast soil of ships in the past, infrastructure construction). Yet, natural expansion or introduction by human 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 its distinct phenotype was described (dwarfed growth form and increased cold hardiness) (Böcher & Larsen, 1958; Rosenmeier et al., 2013). More recent studies revealed that fast spreading, invasive plants in the Danish landscape are genetically separable from plants with dwarf morphology and are therefore likely to present an introduced gene pool (Nielsen et al., 2016; Rosenmeier et al., 2013).

This study investigates the distribution and range expansion history of the intra-continental invader scotch broom with focus on its northern most expansion front, but also including the native source range. More explicitly, we aim at 1) establishing if this range expansion is natural or caused by human introductions 2) characterising the introduction history from spatiotemporal analysis of chloroplast and nuclear markers in the introduced and native range to gain insight into the role of propagule pressure and propagule origin for invasion success 3) quantifying gene flow from the native range to the expansion front, as well as between populations in the expansion front to investigate the possible role of genetic diversity in invasion success.

Methods

Sampling

We collected scotch broom tissue samples, from across Europe and from herbarium specimen dating back to 1835 (see Appendix S2 in Supporting Information). Fresh samples were collected in 2012 and 2014, dried on silica gel and stored at -20^{0}C. Sampling focussed on the species' northern most range in Norway represented by 25 populations with 20 individuals and 11 populations with single samples. The native European range was represented by 12 populations with five to 20 individuals and seven populations with one individual, sourced from seedlings grown from seed bank samples or from field collections. Three individuals of scotch broom, were collected in garden centres in eastern Norway.

Herbarium specimens were sampled from 11 herbaria across Europe. As most specimen had no record of GPS coordinates, those were estimated based on the specimens' location description. From the Norwegian herbarium samples, 27 were selected focussing on the four areas representing the species' oldest observations (Figure 1 and 5). They also cover the timespan of Norwegian collections (from 1869 to 1998). For the selection of 95 specimens from other European countries the aim was to cover an equal number for each country of the oldest (1835 to 1909) and the most recently (1959 to 2001) collected specimens (see Appendix S2).

DNA extraction

We extracted DNA from up to 10 mg of dry tissue or about 50 mg of fresh tissue following the DNeasy Mini Plant Kit (Qiagen, Hilden, Germany) for most herbarium samples or the DNeasy 96 Plant Kit (Quiagen) for most fresh samples. For higher yield, we prolonged the chemical cell lysis step (in buffer AP1, at 65^{0}C) from 10 to 30 minutes. For fresh samples, we implemented the final DNA elution only once, and for herbarium species used the first elute to run through the filter a second time.

Chloroplast sequencing

For the chloroplast analysis, most populations were represented by one individual, but for 17 populations from Norway and the two populations from Denmark we sequenced five or six individuals, in order to test if these populations originate from a single or from multiple maternal lines (see Appendix S2). Four chloroplast regions of scotch broom were amplified in a polymerase chain reaction (PCR) and sequenced with similar methodology for fresh samples and herbarium samples. For the fresh samples, the four regions were first PCR amplified with universal primer pairs and then the sequencing reaction was performed with specific internal primers (see Appendix S3 in Supporting Information; Furevik 2017. For the herbarium samples, with more degraded DNA, five internal primer pairs within these four chloroplast regions were used for both PCR and sequencing reaction (see Appendix S3; Furevik 2017). The 25 µL PCR reaction mix contained 0.625 units HotStart *Taq* DNA Polymerase from New England Biolabs (NEB, Ipswitch MA, United States), 1x Standard *Taq* Reaction Buffer (NEB), 50uM of each dNTP (Invitrogen, Carlsbad CA, United States), 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 % of bovine serum albumin. PCR reactions were performed in a Mastercycler nexus thermocycler (Eppendorf, Hamburg, Germany) with an initial polymerase activation of 15 min at 95^0C, followed by 30 or 35 cycles (for fresh samples and herbarium samples, respectively) of denaturation for 1 min at 94^0C, annealing for 2 min at 52, 53 or 55^0C (depending on primers, see Appendix S3) and extension for 3 min at 72^0C, and a final extension of 10 min at 72^0C. PCR product were visualised on 1.5 % agarose gels. Herbarium samples that developed 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 (Seoul, South Korea). Other PCR products were first purified by the Montage PCR_{96} Cleanup Kit (Merck Millipore, Billerica MA, United States), then sequencing reaction was performed with BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham MA, United States), using 1 µM of the primer and 1 µL of the 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).

Chloroplast sequence analysis

Sequences were edited and aligned with SEQUENCHER software 4.10.1 (Gene Codes Corporation, Ann Arbor, MI, USA) and BIOEDIT 7.2.5 (Hall, 1999). At first, fresh samples and herbarium samples were aligned separately. Unique polymorphisms (present in a single sample only) were verified by re-sequencing or were otherwise removed. Mononucleotide repeats were excluded from the analysis. In order 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 and 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, Suchard et al. 2018; *Lupinus* sequence was obtained from GenBank, accession numbers: gi_485474291_gb_KC695666.1) was used to verify that all samples represent 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 geographic area and the small number of samples from these countries. Two samples from islands (Madeira, Corsica) and the three samples from the garden centre were excluded. Standard genetic diversity indices (number of haplotypes, nucleotide diversity, haplotype diversity) were calculated in DNASP 5 (Librado & Rozas, 2009). To correct for differences in sample sizes we used the programme CONTRIB 1.4 (Rémy J. Petit, El Mousadik, & Pons, 1998) to apply a rarefaction to a sample size of five and then estimate allelic richness. These calculations of haplotype diversity were also applied on the 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), in order 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).

The spatial distribution of the haplotypes was visualised on maps using QGIS 2.14.20 (QGIS Development Team, 2018). To assess the relationship between 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 (Rod Peakall & Smouse, 2012; R. Peakall & Smouse, 2006). 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 also for Norwegian samples only.

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

Nuclear SNPs

The fresh samples were analysed for single nucleotide polymorphic (SNP) markers (see Appendix S2). This included up to 20 samples for the 20 population in southern Norway, five populations in western Norway, and two populations in Denmark. The seedlings grown from seed bank samples were also included. However, for some of those populations only few individuals were available (5 to 20; see Appendix S2).

Nuclear SNP marker development and genotyping

For the nuclear genetic analysis, SNPs markers had been developed from double-digest restriction-associated-DNA sequencing (ddRADseq; Peterson et al. 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 suitable quality for this analysis.

Nuclear SNP data analysis

The MassArrays (AGENA Bioscience) resulted in 36 usable SNP markers and 587 samples with less than 50% missing SNP reads. These samples covered 25 Norwegian populations and two Danish populations, which were mostly represented by 19 individuals (13 to 26; see Appendix S2). Samples from ten accessions from European seed banks were included in some of the analyses but interpretation of these data is limited due to the low sample sizes of some populations 5 to 17) and different sampling.

GENALEX 6.5 (2012; R. Peakall & Smouse, 2006) was used to calculate the proportion of polymorphic loci for each population. Principal Component Analyses were calculated in R. AMOVAs comparing three different groupings of the populations (haplotype C versus all others, Norway versus Europe, one group for each country excluding haplotype C) were calculated in ARLEQUIN 3.5 and complemented with the corresponding pairwise F_{ST}'s among all populations. Population structure was calculated with STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000), with default settings 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.

Results

Spatial and temporal patterns of chloroplast haplotype diversity

For each of the 267 samples of scotch broom, the four cpDNA regions were concatenated, which resulted in 1083 basepairs (bp) long sequences, including 20 bp of variable length of single-repeating nucleotides and 65 bp of 12 indels, which all were removed for the analyses. The indels were retained as simple index coding (SIC; Simmons and Ochoterena 2000). The resulting sequences of 996 bp and 12 SIC, contained 44 mutations at 43 variable sites (one site with 3 different nucleotide phases).

A total of 20 haplotypes were observed of which 11 were private, i.e. found in only one individual (see Appendix S4 in Supporting Information). 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 herbariums specimens (5 x Spain: K, O, R, S, T; Corsica in France: N; Italy: L), one from the older herbarium specimens (1853 from Portugal: Q), and two from Norway (1928: P; 1977: M). Another private haplotype originates from a Spanish seed bank, collected in 2003 (Z).

All samples were grouped by the country of collection, resulting in groups of one (Hungary and Romania) to 144 (Norway) samples (Figure 2). Two samples from islands (Corsica and Madeira) were included as separate entities. The most common haplotype A dominated the samples from Norway, Sweden, Belgium and the Netherlands, Czechia, Poland and to a lesser extend Great Britain. 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 genotype. We discovered this haplotype widely across Europe, but not in Portugal and Spain, nor in Sweden and some 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), but also in Norway. All remaining haplotypes were private haplotypes or found in only one country. Most of those private haplotypes were located in southern European countries Spain, Portugal, Italy and one on 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, where all but one of the five samples sequenced to the same haplotype. Slightly lower than average Hd values, were estimated for Norway, Denmark and Czechia. Highest Hd values were observed for (in order of increasing value) Great Britain, Italy, Portugal, Spain and Austria. Yet, Austria was represented by only two samples. After applying rarefaction to a sample size of five, this order remained, apart from a slight decrease of Hd below the average for Sweden. 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 differentiations, mostly between the south-western and the north-eastern countries (see Appendix S5 in Supporting Information).

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

A significant ($p = 0.010$) positive correlation between geographic distance and genetic distance was estimated by the Mantel test, indicating some level of geographic 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 or after 1958. The analysis of haplotypes of Norwegian populations and herbarium specimens revealed neither spatial (Mantel test not significant) nor temporal patterns (Figure 5). Of the 17 populations for which several individuals were analysed, five populations consist of more than one different haplotypes, one of these even of three (Figure 5).

Genetic diversity and distribution of nuclear SNP markers

Most Norwegian populations had highly polymorphic SNP markers (Table 2) ranging from 72 to 97 percent, except for population 3 (61%) and population 14 (67%). The polymorphism 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 lower SNP diversity than any other population of this study. Interestingly, the populations from Norwegians west coast (BG1 to BG10), at the youngest expansion front and about 300 km distant to the other sites, have similarly high levels of polymorphism as those long established populations in the south. The European seed bank samples in general have a little lower polymorphism (47 to 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 the haplotype C. The PCA of all populations showed a clear separation of these three populations (3, DN and 8Wa, which all have the chloroplast haplotype C) while the remaining populations greatly overlapped (see Appendix S6 in Supporting Information). Correlation between SNP genetic and geographic distances were just short of significant (Mantel test, $p = 0.059$). The population pairwise genetic distances varied substantially from 0.02 to 0.52. The most pronounced genetic differentiation of the higher F_{ST} values ($F_{ST} > 0.25$) were observed for comparisons to the three populations with haplotype C (3, DN and 8Wa) (see Appendix S7 in Supporting

Information). The genetic differentiation among the remaining populations, those collected in Norway as well as the European collections and seed bank samples, is generally low to moderate ($F_{ST} < 0.25$).

Most genetic variance was found among samples within the populations (77 to 86%), little variation among populations within a geographic area and diminishing small variation between different levels of geographical scale, such as native or introduced area (i.e., European vs Norway) or country (Table 3). The largest variance at the group scale and the smallest within populations was observed when grouping was based on populations with the haplotype C versus all other haplotypes.

The structure analysis showed little genetic structure among the populations (Figure 6a). Using Evanno's approach (Evanno et al., 2005) there was best support for four clusters (Figure 6c). Most noticeable are the distinct ancestry estimates for the populations 3, DN and 8Wa, which contained the chloroplast haplotype C (Fig. 6a, see Appendix S6). The samples of the remaining haplotypes were highly admixed.

Discussion

Northward expansion of scotch broom is driven by human introductions

Our study suggests that the recent range expansion of scotch broom is mainly driven by human introductions, therefore the species is alien to Norway and its cryptogenic status is resolved. The first botanical record of scotch broom in Norway was as late as 1869 (Blytt, 1876; GBIF, 2017; Figure 1), while in Denmark it was included in the native flora already in 1648 (Paulli, 1648). In Norway and by 1900, there were already at least three different haplotypes established and five more haplotypes have been introduced since (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 lack of temporal and spatial patterns (Figure 5), suggest different introduction events directly to these locations, possibly followed by occurences of secondary spread, which is common in introduced species (Foxcroft, Richardson, & Wilson, 2008; 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 to occupy all suitable habitat and block the establishment of other dispersing haplotypes (Eckert, Samis, & Lougheed, 2008; Harter, Jentsch, & Durka, 2015; Waters, Fraser, & Hewitt, 2013).

There are also indications of long distance dispersal, which must be facilitated by humans as natural long-distance dispersal is not known for scotch broom. One less frequent haplotype (F) occurs in Norway and then at a great distance in Great Britain and on the Iberian Peninsula, the latter a common glacial refugium. Another Norwegian haplotype (D) was not found in neighbouring countries but was present in most other countries.

Yet, we cannot completely exclude natural northwards expansion into Norway. Previous publications show that natural recolonization has reached as far north as Denmark by 1648 (Paulli 1648) and maintained a specific phenotype there (Böcher and Larsen 1958; Nielsen et al. 2016). Haplotype C in this study matches this Danish native phenotype and is also present at one remote location in Norway since 1896 (see Appendix S2, Figure 5; Agder Museum of Natural History and Botanical Garden; GBIF, 2017). Nevertheless, 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 the obervations in Denmark, where the low growing, putative native types are apparently not involved in the spread of the species across the Danish landscape.

Our findings suggest introduction routes as stowaways on ships in the past and as escapes from gardens and arboriculture (classification by P. E. Hulme et al., 2008). The three locations of the oldest introduction records, which also exibit the highest haplotypic diversity, have a history of an international trading port, a prestigious British inspired ornamental garden or a nursery propagating scotch broom for soil stabilisation (see Kristiansand, Mandal and Kjørrefjord in Figure 5; Lundberg & Rydgren, 1994; Vesthassel, 1926). The fourth location of the oldest records of scotch broom in Norway (see Telebukta in Figure 5) is the only one that contains a single haplotype, but this can be explained by the remote and isolated location and less frequent visits by human. The samples from the Norwegian garden centres sequenced to the first and third most common haplotypes observed in Norway and northern Europe (haplotypes A and C in Figure 2). The one haplotype that increased its relative abundance around the years 1960 to 1980 (Figure 4) 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), including closely related *Genista* species (A. Kleist, Herrera-Reddy, Sforza, & Jasieniuk, 2014; Annabelle Kleist & Jasieniuk, 2011).

In the native range: Cryptic invasion by alien genotypes

In an attempt to determine if human spread occurred only in the species' recent expansion range or also within the species' native range, we sequenced European herbarium specimens predating the introduction to Norway and compared their distribution patterns to present day distribution pattern of haplotypes. Even the analysis of the oldest available herbariums samples (1835 to 1910) showed no clear spatial pattern of chloroplast haplotypes and did not differ from present day distribution. This indicates that human dispersal was most likely already occurring before the early 19th century (Figure 3). Furthermore, over the last 150 years most haplotypes increased at similar rates (Figure 4).

We also observed some remnants of haplotype distribution patterns we would have expected to see with natural postglacial recolonization of scotch broom in its native European range. Most of the private haplotypes were found in former glacial refugial areas (Figure 2), especially in Spain and Portugal (seven out of 11). The haplotype diversity and genetic diversity calculated for each country also decrease 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 (Eckert et al., 2008; Petit et al. 2003; but Havrdova et al., 2015). Of the four most common and widespread haplotypes, only one (B) was sampled in Spain or Portugal the other three (A, C, 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, genotypes may be not.

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 was established for about 150 years, but only in the last decades was it recognised as invasive (i.e. spreading and increasing in density; Figure 1, see Appendix S1). This matches with Aikio, Duncan, and Hulme (2010) who calculated a relatively long lag phase of 97 years for 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), as it has been suggested for many other invasive species (e.g. Jogesh et al., 2015; Kelager et al., 2013; Ray & Quader, 2014; Shirk, Hamrick, Zhang, & Qiang, 2014). The quality of the propagules, i.e. place of origin, also influences a plant's ability to establish and to 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 haplotypes origins, because of the lack of spatial patterns across the native range (Figure 3). This situation, that haplotypes from different origins are mixed already across the native range (i.e. intra-specific cryptic invasion), increases the likelihood of introducing haplotypes of different origins even from a small source range.

A uniform invasion front with high genetic diversity

We observed strong genetic admixture in the nuclear markers (Figure 6) following the dispersal and mixing of the different source populations (i.e. different haplotypes). This may have contributed to increase 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 3, see Appendix S6) and contributed to a high level of polymorphism in the northern most expansion front (Table 2). Nearly all of the genetic variation was found within the populations, even within individuals, much less among populations (Table 3), 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; Hufbauer, Rutschmann, Serrate, Vermeil de Conchard, & Facon, 2013; Rius & Darling, 2014) to result in increased genetic diversity at the population and individual level (Colautti & Lau, 2015; Dlugosch & Parker, 2008; Fennell, Gallagher, Vintro, & Osborne, 2014) and possibly create novel invasive genotypes (Bock et al., 2015).

There is also a possibility for gene flow between a genotype that is adapted to northern climate (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 share 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 invasive species' ability for invading northern temperate areas (L Rosef and E Heegaard unpublished). Surprisingly, the identified haplotype of the putative native Danish populations, which is adapted to northern climate (Böcher & Larsen, 1958), appears to play a minor role in the northern expansion process.

Conclusion

This study represents one of very few examples of intra-continental plant invasions, indicating the complex aspects of the introduction history. The rapid northern range expansion of scotch broom in the recent century was most likely driven by human introductions, thus it can be classified as alien in Norway. Our results indicate the importance of a diverse introduction history, with high propagule pressure and genetic admixture resulting in high genetic diversity, for invasion success. Further, we point out the value of extending the genetic analyses of biological invasions to the 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 intra-specific admixture and high genetic diversity in the source populations, and might affect the establishment and invasion right at the start of the introductions into a novel range.

Biosketch

UB has a long interest in biological invasions, especially the processes facilitating invasions, the impact on the invaded ecosystems and alien species management. This publication is a major part of UB's PhD thesis under close supervision of SF on this publication and PhD supervision of LR.

Author contributions: SF, EDK, LRN and LR conceived the project idea, wrote the application and designed the project and the experiments, as well as discussed initial results and now final results, and commented on the paper. SF, LR, UB and BBF collected samples; BBF designed chloroplast markers and contributed to the lab work; UB implemented the project, including lab work and data analysis, and wrote the manuscript.

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Tables

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 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	265	19	0.727	0.00215	2.052

Table 2 Percentage of Polymorphic loci based on SNP data for 25 Norwegian populations and 2 Danish populations (DI: Danish introduced, DN: Danish native).

Population	sample size	**Percentage Polymorphic Loci**
BG1	19	72.22
BG4	19	83.33
BG6	18	86.11
BG8	19	94.44
BG10	19	97.22
3	22	61.11
19	20	91.67
18	19	97.22
17	19	94.44
16	19	97.22
16H	14	88.89
15	20	94.44
14	19	66.67
13	18	97.22
12	26	97.22
11	19	91.67
10	19	86.11
28	19	80.56
27	19	86.11
26	19	97.22
1	13	75.00
25	19	86.11
24	19	83.33
23	19	86.11
22	19	86.11
DI	19	94.44
DN	18	47.22

Table 3 AMOVAs of 36 nuclear SNP markers, significant values in bold. a) Samples split by haplotype C and all other haplotypes b) grouped by area of origin, such as native or introduced and c) by country.

	Source of variation	Among groups	Among populations within groups	Within populations
a	Haplotype C versus other haplotypes	**10.16**	**13.26**	**76.58**
b	Norway versus EU (haplotype C excluded)	**1.20**	**13.28**	**85.52**
c	Grouped per country (haplotype C excluded)	0.33	**13.72**	**85.95**

Figures

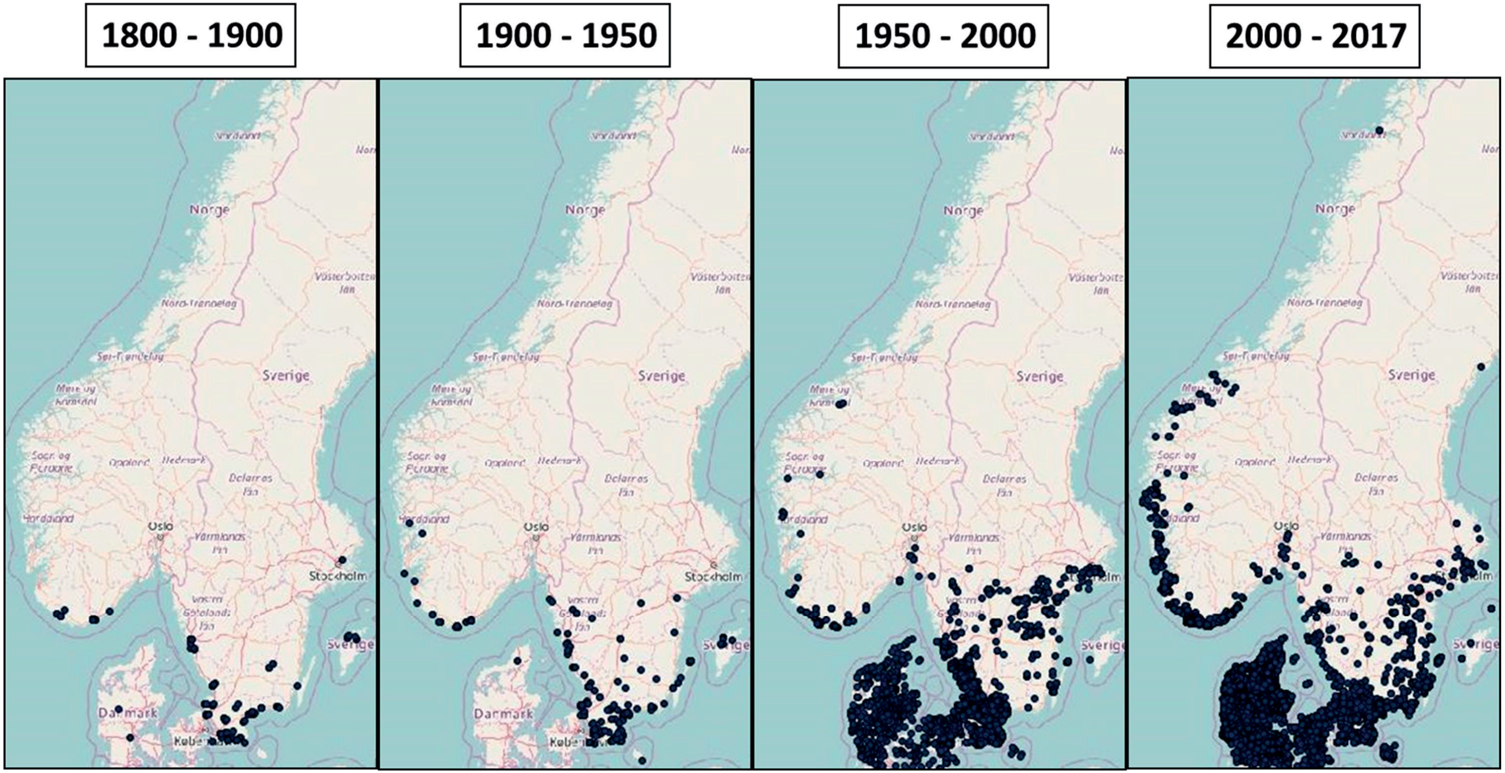


Figure 1 Records of GBIF database on Cytisus scoparius *in Scandinavia across different time periods Data access on 19 May 2017.*

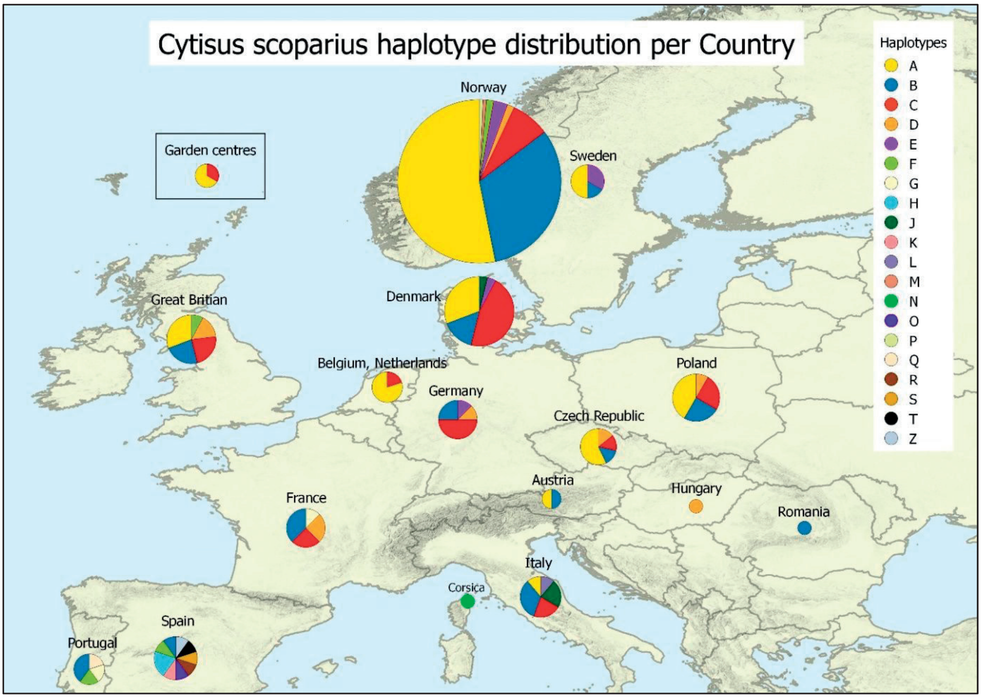


Figure 2. Chloroplast haplotypes of C. scoparius *samples grouped by country of collection. The size of the pie represents the number of samples, each colours represents one haplotype. Haplotypes J to Z were found only once. The haplotype B on Madeira is not shown.*

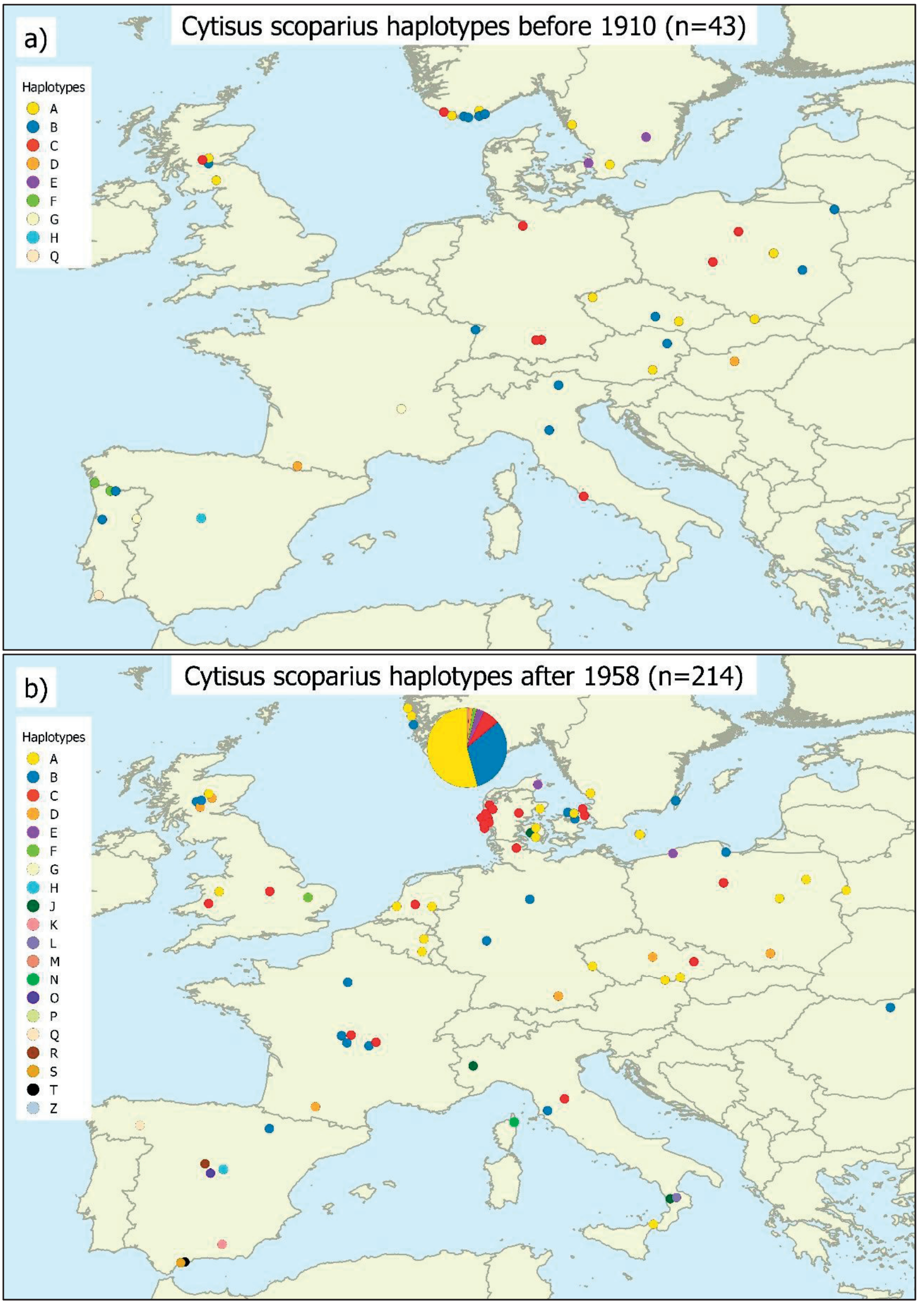


Figure 3 Geographic location of C. scoparius samples before 1910 (n= 43) or after 1958 (n= 214), coloured by chloroplast haplotype. All samples from southern Norway collected after 1958 are represented as a pie chart. The haplotype B on Madeira is not shown in the map of samples after 1958.

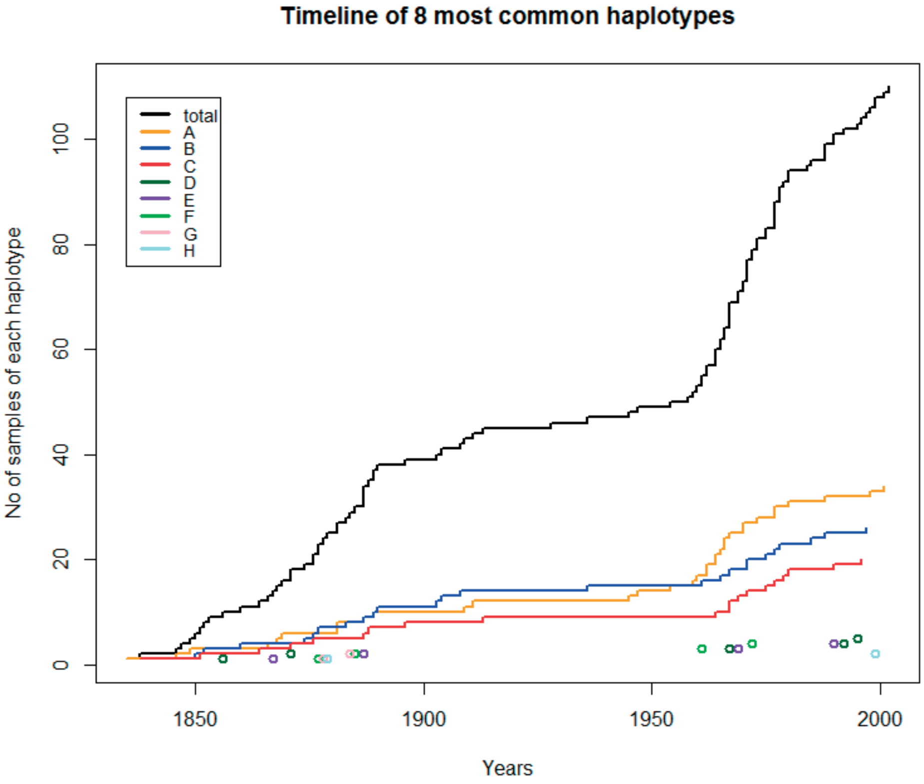


Figure 4 Time line for the accumulative number of chloroplast haplotypes identified among the herbarium samples. The black line represent the accumulative sum for all haplotypes, the coloured lines represent one of the eight haplotypes represented by at least two samples. Note that sampling was focussed on the oldest and most recent herbarium specimens from Europe, and across the complete sampling time period for Norwegian specimens.

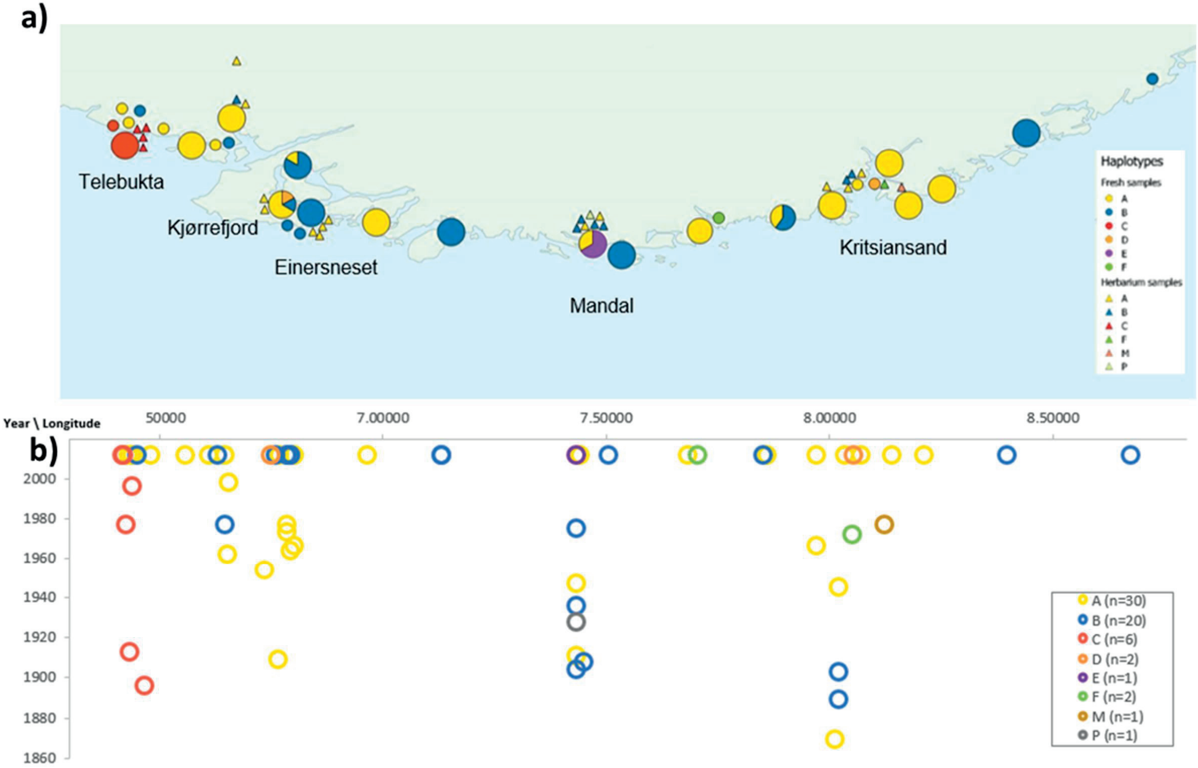


Figure 5 a) Spatial distribution of chloroplast haplotypes along Norwegians south coast. Different colours represent the eight different haplotypes, triangles represent herbarium samples, small circles single fresh samples and large circles five or six samples from the same population. b) Haplotype distribution over time of collection, along the longitudinal location.

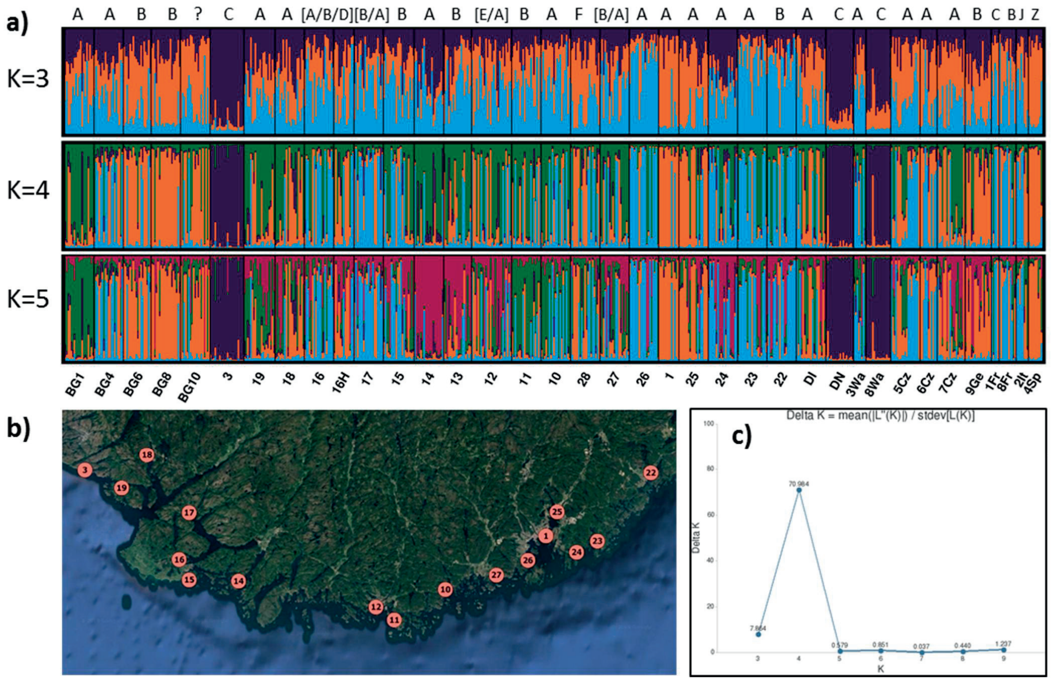
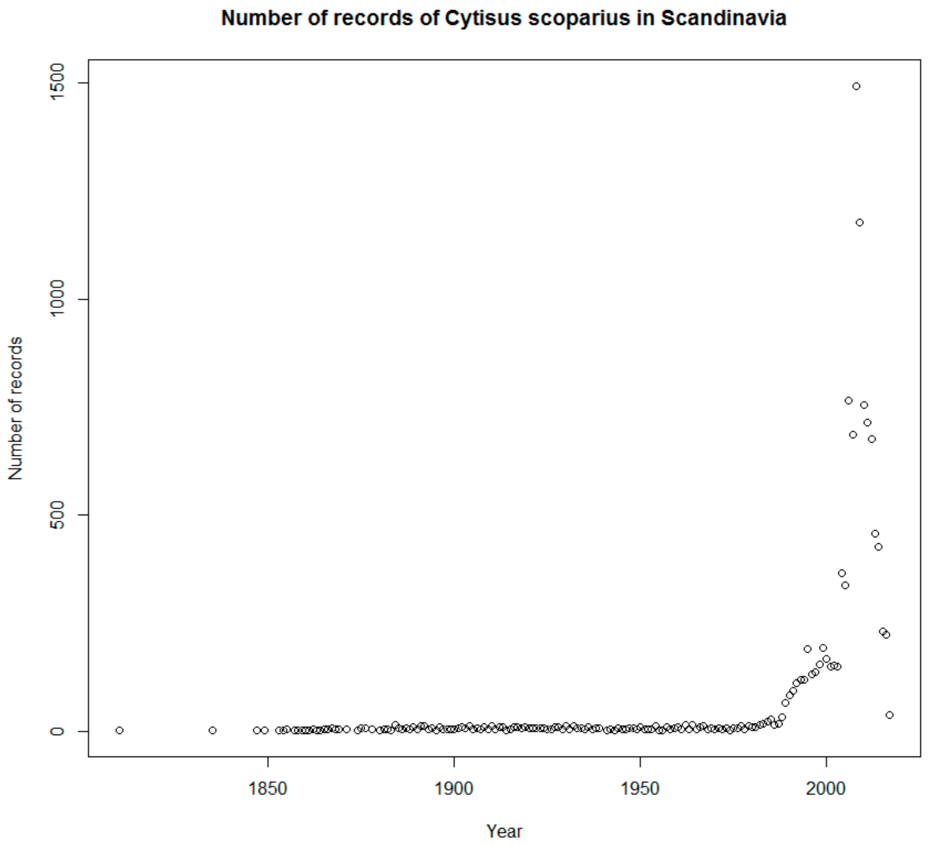


Figure 6 a) Structure of nuclear SNP data from populations in Norway and Europe. Norwegian samples are roughly sorted by longitude and located on the inlay map b). Populations BG1 to BG 10 are not included in the map and are located a further 300 km to the north-west. European seed bank samples are marked 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. c) Evanno shows best support for K= 4.

Supplementary material



S1 Records of GBIF database on Cytisus scoparius *in Scandinavia over the years. Data access on 19 May 2017.*

S 2 List of all used samples used for analyses

Sample Type	ID	Samples for chloro-types	Samples for SNPs	Latitude	Longitude	Accuracy	Year	Country/ Island	Haplotype
Herbarium old	WAG11	1	0	37.31667	-8.55000	0	1853	Portugal	Q
Herbarium old	LISU20	1	0	40.59028	-8.39694	3	1877	Portugal	B
Herbarium old	LISU17	1	0	41.82361	-8.04056	15	1877	Portugal	F
Herbarium old	FI16	1	0	41.82056	-8.04056	15	1883	Portugal	B
Herbarium old	LISU19	1	0	40.62083	-6.90139	5	1884	Portugal	G
Herbarium recent	W2	1	0	32.73556	-16.99944	0	1997	Madeira	B
Herbarium old	GB24	1	0	40.72944	-4.02000	3	1879	Spain	H
Herbarium old	LISU9	1	0	42.17500	-8.72056	10	1885	Spain	F
Herbarium recent	GB4	1	0	36.57000	-4.81250	10	1971	Spain	T
Herbarium recent	GB5	1	0	36.53444	-4.98778	5	1972	Spain	S
Herbarium recent	GB43	1	0	37.33028	-3.19944	100	1973	Spain	K
Herbarium recent	AA29	1	0	40.82472	-3.93889	70	1978	Spain	R
Herbarium recent	FI10	1	0	40.41667	-3.70361	60	1984	Spain	O
Herbarium recent	AA30	1	0	42.35167	-1.15056	4	1985	Spain	B
Herbarium recent	AA31	1	0	40.58611	-3.14806	10	1999	Spain	H
Seed bank	4Sp	1	0	42.49139	-6.76306	0	2003	Spain	Z
Herbarium old	FI19	1	0	46.40833	11.38917	100	1874	Italy	B
Herbarium old	GB23	1	0	41.59278	12.48250	20	1888	Italy	C
Herbarium old	WA25	1	0	44.45889	10.98722	4	1890	Italy	B
Herbarium recent	AA26	1	0	38.20583	15.50472	5	1977	Italy	A
Seed bank	2It	1	0	39.30877	16.34638	125	1977	Italy	J
Herbarium recent	WAG12	1	0	43.13333	10.91667	0	1988	Italy	B
Herbarium recent	GB29	1	0	45.07083	7.68611	70	1988	Italy	J

Herbarium recent	FI7	1	0	43.64361	11.65000	10	1990	Italy	C
Herbarium recent	WAG10	1	0	39.36667	16.50000	0	1999	Italy	L
Herbarium old	WAG15	1	0	48.81306	7.79111	20	1850	France	B
Herbarium old	GB27	1	0	42.90306	0.07139	80	1856	France	D
Herbarium old	LISU4	1	0	45.38194	4.57417	5	1878	France	G
Herbarium recent	AA27	1	0	45.94278	3.16778	40	1971	France	B
Herbarium recent	AA22	1	0	48.69778	2.24889	4	1971	France	B
Herbarium recent	AA21	1	0	46.10000	3.47583	2	1971	France	C
Herbarium recent	AA23	1	0	42.63111	9.46444	3	1978	Corsica	N
Seed bank	1Fr	1	5			country	1982	France	C
Herbarium recent	WAG6	1	0	43.30000	0.85000	0	1992	France	D
European	13Fr	1	0			country	2012	France	B
European	8Fr	1	11			country	2012	France	B
Herbarium old	EB12	1	0	56.11389	-3.79167	2	1847	United Kingdom	B
Herbarium old	EB7	1	0	56.26222	-3.77833	5	1866	United Kingdom	A
Herbarium old	EB21	1	0	56.18500	-4.05000	0	1876	United Kingdom	C
Herbarium old	EB6	1	0	55.30083	-3.45194	2	1881	United Kingdom	A
Herbarium recent	AA10	1	0	52.39306	0.52389	4	1961	United Kingdom	F
Herbarium recent	AA20	1	0	52.65694	-1.13333	50	1964	United Kingdom	C
Seed bank	3WA	1	8	52.64643	-3.32609	country	1988	United Kingdom	A
Seed bank	8Wa	1	16			country	2008	United Kingdom	C
European	15Sc	1	0	56.70436	-3.72971	2	2012	United Kingdom	A
European	9Sc	1	0			country	2012	United Kingdom	B
European	11Sc	1	0			country	2012	United Kingdom	B
European	10Sc	1	0			country	2012	United Kingdom	D
European	14Sc	1	0	56.74897	-3.71728	2	2012	United Kingdom	D
Herbarium recent	AA19	1	0	50.04694	5.46861	50	1970	Belgium	A
Herbarium recent	AA18	1	0	50.59472	5.55361	70	1970	Belgium	A
Herbarium recent	WAG5	1	0	52.00887	4.37114	1	1980	Netherlands	A
Herbarium recent	WAG3	1	0	52.08797	5.17048	0	1980	Netherlands	C

Herbarium recent	WAG7	1	0	52.00167	5.90222	0	2001	Netherlands	A
Herbarium old	AA5	1	0	53.32803	9.84400	30	1838	Germany	C
Herbarium old	FI13	1	0	48.34444	10.59167	20	1871	Germany	C
Herbarium old	FI15	1	0	48.32917	10.52528	1	1887	Germany	C
Herbarium recent	AA17	1	0	54.53444	9.56111	10	1967	Germany	C
Herbarium recent	AA13	1	0	54.29611	9.6375	20	1969	Germany	E
Herbarium recent	GB2	1	0	50.51806	8.26667	0	1978	Germany	B
Herbarium recent	W3	1	0	48.09913	11.37958	4	1995	Germany	D
European	9Ge	1	17	52.31386	10.14544	1	2014	Germany	B
Herbarium old	W9	1	0	48.21215	16.10010	10	1860	Austria	B
Herbarium old	GB15	1	0	47.06889	15.47111	2	1889	Austria	A
Herbarium old	FI4	1	0	49.19500	16.60667	355	1835	Czechia	A
Herbarium old	AA7	1	0	49.39833	15.58694	10	1852	Czechia	B
Herbarium old	GB6	1	0	50.23139	12.87167	10	1868	Czechia	A
Herbarium recent	AA11	1	0	49.61000	17.26056	40	1969	Czechia	C
Seed bank	Cz5	1	19	48.92754	16.66691	1	1994	Czechia	A
Seed bank	6Cz	1	11	48.80425	16.00909	1	1996	Czechia	A
Seed bank	7Cz	1	19	49.41360	12.86310	0	2010	Czechia	A
European	12Cz	1	0			country	2012	Czechia	D
Herbarium old	WA45	1	0	51.76722	18.08528	10	1851	Poland	C
Herbarium old	WA30	1	0	53.08306	19.19389	2	1864	Poland	C
Herbarium old	WA34	1	0	54.04222	23.35583	20	1876	Poland	B
Herbarium old	WA9	1	0	49.29000	19.88972	2	1881	Poland	A
Herbarium old	WA8	1	0	52.14250	20.71750	2	1887	Poland	A
Herbarium old	WA42	1	0	51.41639	21.96917	10	1887	Poland	B
Herbarium recent	WA46	1	0	52.70056	23.86778	15	1960	Poland	A
Herbarium recent	WA37	1	0	52.35361	20.97000	3	1964	Poland	A
Herbarium recent	WA35	1	0	53.17194	22.12556	2	1965	Poland	A
Herbarium recent	WA47	1	0	54.35194	18.64639	20	1967	Poland	B
Herbarium recent	GB34	1	0	49.96861	20.57250	2	1967	Poland	D

Herbarium recent	AA28	1	0	53.03000	18.54639	1	1975	Poland	C
Herbarium old	AA3	1	0	47.44351	19.01932	500	1871	Hungary	D
Herbarium recent	AA14	1	0	47.60806	25.77694	100	1965	Romania	B
Herbarium recent	O22	1	0	56.03361	12.05472	5	1962	Denmark	A
Herbarium recent	AA58	1	0	55.40361	10.40222	1	1967	Denmark	A
Herbarium recent	AA66	1	0	55.11667	14.91833	20	1988	Denmark	A
Denmark	SB16	1	0	55.12698	10.26123	0	2009	Denmark	A
Denmark	DI	5	19	56.23485	10.58280	0	2014	Denmark	A
Denmark	Mel2	2	0	56.01858	11.98982	0	2009	Denmark	B
Herbarium recent	AA56	1	0	55.55472	8.15806	0	1967	Denmark	C
Herbarium recent	AA65	1	0	56.05389	9.66667	1	1979	Denmark	C
Denmark	Hen14	2	0	55.74770	8.17947	0	2009	Denmark	C
Denmark	ViH18	2	0	56.08722	12.40427	0	2009	Denmark	C
Denmark	LB3	2	0	56.25555	8.40995	0	2009	Denmark	C
Denmark	DN	7	18	55.47464	8.10444	0	2014	Denmark	C
Herbarium recent	AA67	1	0	57.28056	10.49139	15	1990	Denmark	E
Denmark	SB13	1	0	55.12698	10.26123	0	2009	Denmark	J
Herbarium old	O17	1	0	57.70861	11.97444	15	1846	Sweden	A
Herbarium old	O14	1	0	55.98278	13.61556	2	1849	Sweden	A
Herbarium old	O11	1	0	57.17333	15.18500	120	1867	Sweden	E
Herbarium old	O7	1	0	56.04639	12.69444	15	1887	Sweden	E
Herbarium recent	AA68	1	0	56.90722	12.76583	2	1959	Sweden	A
Herbarium recent	O21	1	0	56.58333	16.46667	5	1961	Sweden	B
Herbarium Norway	BGh5	1	0	58.15953	8.01314	1	1869	Norway	A
Herbarium Norway	HbO28	1	0	58.14969	8.02172	0	1889	Norway	B
Herbarium Norway	KMN16	1	0	58.26756	6.46702	1	1896	Norway	C
Herbarium Norway	HbO30	1	0	58.14969	8.02172	0	1903	Norway	B
Herbarium Norway	HbO9	1	0	58.01679	7.43513	0	1904	Norway	B
Herbarium Norway	KMN7	1	0	58.01966	7.44939	0	1908	Norway	B
Herbarium Norway	HbO15	1	0	58.10245	6.76728	0	1909	Norway	A

Herbarium Norway	KMN6	1	0	58.01679	7.43513	0	1911	Norway	A
Herbarium Norway	KMN40	1	0	58.28671	6.43293	0	1913	Norway	C
Herbarium Norway	HbO6	1	0	58.01679	7.43513	0	1928	Norway	P
Herbarium Norway	KMN19	1	0	58.01679	7.43513	0	1936	Norway	B
Herbarium Norway	KMN10	1	0	58.16316	8.02135	0	1945	Norway	A
Herbarium Norway	BGh2	1	0	58.01679	7.43513	0	1947	Norway	A
Herbarium Norway	HbO14	1	0	58.11140	6.73671	0	1954	Norway	A
Herbarium Norway	KMN32	1	0	58.29806	6.65257	0	1962	Norway	A
Herbarium Norway	KMN44	1	0	58.06925	6.79563	0	1964	Norway	A
Herbarium Norway	KMN11	1	0	58.06714	6.80339	0	1966	Norway	A
Herbarium Norway	KMN12	1	0	58.13582	7.97115	0	1966	Norway	A
Herbarium Norway	HbO20	1	0	58.06684	6.78646	0	1973	Norway	A
Herbarium Norway	HbO27	1	0	58.05787	6.78701	0	1977	Norway	A
Herbarium Norway	HbO35	1	0	58.42348	6.65707	1	1998	Norway	A
Norway	1	1	13	58.14708	8.03370	0	2012	Norway	A
Norway	10	5	19	58.04913	7.68440	0	2012	Norway	A
Norway	14	6	19	58.06457	6.96603	0	2012	Norway	A
Norway	18	6	19	58.29550	6.64770	0	2012	Norway	A
Norway	19	6	20	58.23511	6.55948	0	2012	Norway	A
Norway	23	6	19	58.13805	8.21190	0	2012	Norway	A
Norway	24	6	19	58.11764	8.14004	0	2012	Norway	A
Norway	25	6	19	58.19125	8.07215	0	2012	Norway	A
Norway	26	6	19	58.10251	7.97079	0	2012	Norway	A
Norway	16	6	19 + 14	58.10389	6.75994	0	2012	Norway	A,B,D(x4,x1,x1)
Norway	C1	1	0	58.28271	6.43151	0	2012	Norway	A
Norway	C2	1	0	58.23666	6.61195	0	2012	Norway	A
Norway	C4	1	0	58.29377	6.43939	0	2012	Norway	A
Norway	C5	1	0	58.27908	6.48042	0	2012	Norway	A
Norway	BG1	1	19	60.24978	5.01008	0	2013	Norway	A
Norway	BG4	1	19	60.58903	4.85235	0	2013	Norway	A

Herbarium Norway	HbO8	1	0	58.01589	7.43517	0	1975	Norway	B
Herbarium Norway	KMN13	1	0	58.29262	6.64666	0	1977	Norway	B
Norway	11	6	19	57.99317	7.50697	0	2012	Norway	B
Norway	15	6	20	58.06710	6.79453	0	2012	Norway	B
Norway	22	6	19	58.26269	8.39760	0	2012	Norway	B
Norway	565	1	0	58.38085	8.67507	0	2012	Norway	B
Norway	13a	6	18	58.04424	7.13093	0	2012	Norway	B
Norway	17	6	19	58.18984	6.79378	0	2012	Norway	B, A (x5, x1)
Norway	27	5	19	58.07589	7.86111	0	2012	Norway	B, A (x3, x2)
Norway	C3	1	0	58.29010	6.44097	0	2012	Norway	B
Norway	C7	1	0	58.06101	6.78679	0	2012	Norway	B
Norway	C8	1	0	58.24203	6.63219	0	2012	Norway	B
Norway	C9	1	0	58.06099	6.78926	0	2012	Norway	B
Norway	BG6	1	18	59.87546	5.09828	0	2013	Norway	B
Norway	BG8	1	19	59.23276	5.43316	0	2013	Norway	B
Herbarium Norway	KMN15	1	0	58.26811	6.42690	0	1977	Norway	C
Herbarium Norway	KMN23	1	0	58.26659	6.44066	0	1996	Norway	C
Norway	3	6	22	58.26782	6.43182	0	2012	Norway	C
Norway	20	1	0	58.28628	6.42772	0	2012	Norway	C
Norway	C6	1	0	58.15559	8.05381	0	2012	Norway	D
Norway	12	6	26	58.01715	7.44309	0	2012	Norway	E, A (x4,x2)
Herbarium Norway	KMN14	1	0	58.15088	8.05197	0	1972	Norway	F
Norway	28	1	19	58.06455	7.70557	0	2012	Norway	F
Herbarium Norway	HbO13	1	0	58.13696	8.12397	0	1977	Norway	M
Garden Centre	GC2	1	1			NA	2014	Garden Centre	A
Garden Centre	GC7	1	1			NA	2014	Garden Centre	A
Garden Centre	GC6	1	1			NA	2014	Garden Centre	C
Norway	BG10	0	19	60.55823	4.9701389	0	2013	Norway	unknown

S 3 PCR and sequencing primers used within four chloroplast regions.

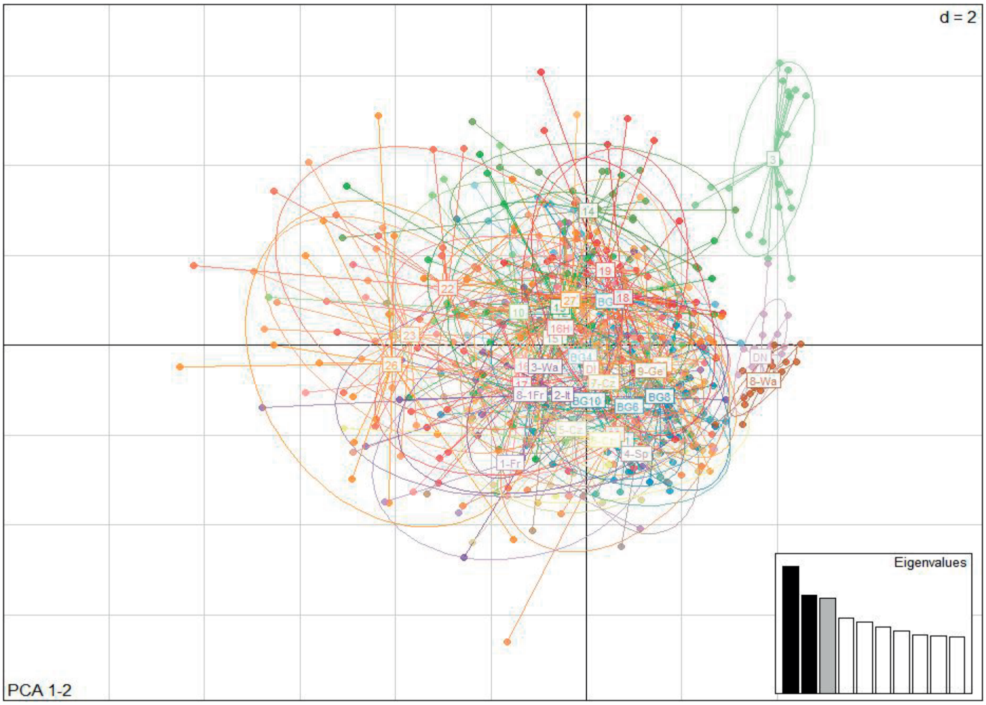
Chloroplast region	Primer	Primer sequence	Internal primer	Internal primer sequence	Herbarium primer	Herbarium primer sequence	Reference
trnT - trnL	trnT$^{(UGU)}$2F	CAAATGCGATGGCTCTAACCT	trnT2F int	TGC CAG AAC TGT TGA ATT GAT	trnT2F herb int	TGCCAGAACTGTTGAATTGAT	Taberlet et al. (1991)
	5'trnL$^{(UAA)}$R	TCTACCGATTTCGCCATATC	trnLR int	CCGGGATCTTAGTTAGTTACGG	trnLR herb int	ATCGACCGTTCGAGTATTCC	
					trnT2F herb1	TCGAACGGTCGATTCTTTTT	
					trnLR herb1	CCGGGATCTTAGTTAGTTACGG	
rps4 - TrnT	rpS4R2	CTGTNAGWCCRTAATGAAAACG	rps4 int	CCTGGTAAAACTCCCAGACG	rps4R2 herb	CGTGACATAAAAACTCCTTTTGG	Taberlet et al. (1991)
	trnT$^{(UGU)}$R	AGGTTAGAGCATCGCATTTG	trnT int	CGATAGCCGGCTTTTCTCTA	trnTR herb int	CGATAGCCGGCTTTTCTCTA	
psbF - psbB	psbF	CGCAGTTCGTCTTGGACCAG	psbF int	GACAAGCAGTCGGATAGACCA	psbB herb2	GGGTCATCTCCCGGATAAAG	Hamilton (1999)
	psbB	GTTTACTTTTGGGCATGCTTCG	psbB int	GGGCAACCCTCTCAACAACT	psbF herb2	GCTCAAGTAGAATTTGGAGCA	
psbC - trnS2	psbC	GGTCGTGACCAAGAAACCAC	psbC int	CGTTCTTGCCAAGGCTGTAT	psbC herb int	CTACGCCACCCACTGAATTT	Demesure et al. (1995)
	trnS2	GGTTCGAATCCCTCTCTCTC	trnS2 int	TTGGGATTTGGCGGTATTTA	trnS2 herb int	CGGATCTTGCTCAAGGACCTA	

S4 Numbers of each chloroplast haplotype of different sample types

Haplotype	Herbarium total	EU Herbarium old	EU Herbarium recent	Herbarium Norway	fresh Norway	fresh Denmark	EU fresh	fresh total	Garden centre	total
A	34	9	12	13	63	6	5	74	2	110
B	26	10	9	7	40	2	5	47		73
C	20	7	9	4	7	13	2	22	1	43
D	5	2	3		2		3	5		10
E	4	2	2		4			4		8
F	4	2	1	1	1			1		5
G	2	2								2
H	2	1	1							2
J	1		1			1	1	2		3
K	1		1							1
L	1		1							1
M	1			1						1
N	1		1							1
O	1		1							1
P	1			1						1
Q	1	1								1
R	1		1							1
S	1		1							1
T	1		1							1
Z							1	1		1
total	108	36	45	27	117	22	17	156	3	267

S 5 Results from exact test of chloroplast haplotype frequencies per country.

	n	Sweden	Denmark	Poland	Czech Rep	Austria	Germany	Belgium + Netherlands	Great Britain	Italy	France	Spain	Portugal	signif. diff.
Norway	**144**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	**+**	**+**	**+**	**+**	**+**	**7**
Sweden	**6**		**+**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**-**	**2**
Denmark	**28**			**-**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**6**
Poland	**12**				**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**2**
Czech Rep	**8**					**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**3**
Austria	**2**						**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0**
Germany	**8**							**+**	**-**	**-**	**-**	**-**	**-**	**2**
Belgium + Netherlands	**5**								**-**	**-**	**+**	**-**	**+**	**3**
Great Britain	**13**									**-**	**-**	**+**	**-**	**2**
Italy	**9**										**-**	**-**	**-**	**2**
France	**10**											**+**	**-**	**6**
Spain	**10**												**-**	**6**
Portugal	**5**													**5**



S6 PCA on 36 nuclear SNPs of all populations: 25 from Norway (BG1 – BG10, 1 – 28), 2 from Denmark (DN, DI) and 10 from European seed banks (1Fr - 9Ge). Eigenvalue: 5.56

S7 *Table of population pairwise FST values. Values in red print are not significant (p >0.05). Cells filled in red contain values over 0.25, cells filled in blue contain values under 0.25. Analysis was done in Arlequin based on 36 nuclear SNP markers.*

	10	11	12	13	14	15	16	16H	17	18	19	22	23	24	25	26	27	28	1	BG10	BG1	BG8	BG6	BG4	DI	1_Fr	8_1Fr	2_It	3_Wa	4_Sp	5_Cz	6_Cz	7_Cz	9_Ge	3	8_Wa	DN
10	0																																				
11	0.065	0																																			
12	0.052	0.090	0																																		
13	0.113	0.093	0.033	0																																	
14	0.191	0.211	0.129	0.135	0																																
15	0.091	0.127	0.063	0.066	0.159	0																															
16	0.060	0.053	0.024	0.052	0.141	0.048	0																														
16H	0.121	0.146	0.030	0.050	0.133	0.081	0.028	0																													
17	0.129	0.125	0.086	0.077	0.159	0.087	0.046	0.070	0																												
18	0.076	0.086	0.042	0.082	0.152	0.099	0.060	0.073	0.124	0																											
19	0.075	0.132	0.039	0.101	0.158	0.095	0.087	0.097	0.151	0.025	0																										
22	0.187	0.225	0.143	0.180	0.234	0.187	0.122	0.121	0.144	0.165	0.187	0																									
23	0.126	0.170	0.139	0.144	0.260	0.138	0.125	0.172	0.119	0.179	0.184	0.176	0																								
24	0.161	0.189	0.111	0.104	0.164	0.08	0.108	0.136	0.104	0.134	0.151	0.180	0.221	0																							
25	0.164	0.171	0.128	0.131	0.202	0.094	0.104	0.146	0.173	0.101	0.115	0.250	0.251	0.185	0																						
26	0.147	0.154	0.116	0.112	0.222	0.130	0.089	0.111	0.112	0.142	0.158	0.161	0.176	0.160	0.202	0																					
27	0.074	0.094	0.062	0.087	0.125	0.080	0.042	0.038	0.087	0.050	0.090	0.131	0.107	0.158	0.114	0.145	0																				
28	0.154	0.092	0.111	0.131	0.254	0.135	0.057	0.149	0.146	0.135	0.160	0.267	0.246	0.205	0.143	0.160	0.125	0																			
1	0.176	0.213	0.146	0.168	0.312	0.159	0.124	0.156	0.189	0.169	0.177	0.265	0.228	0.264	0.152	0.222	0.130	0.156	0																		
BG10	0.139	0.174	0.108	0.136	0.267	0.108	0.110	0.169	0.143	0.131	0.152	0.192	0.131	0.209	0.146	0.216	0.117	0.203	0.184	0																	
BG1	0.161	0.125	0.125	0.185	0.297	0.176	0.100	0.196	0.204	0.126	0.150	0.224	0.202	0.295	0.220	0.266	0.125	0.204	0.233	0.134	0																
BG8	0.170	0.165	0.071	0.088	0.224	0.112	0.085	0.134	0.093	0.094	0.128	0.241	0.174	0.169	0.142	0.209	0.108	0.148	0.166	0.083	0.188	0															
BG6	0.179	0.167	0.078	0.110	0.260	0.096	0.087	0.101	0.124	0.110	0.151	0.174	0.175	0.190	0.163	0.185	0.111	0.170	0.139	0.096	0.158	0.090	0														
BG4	0.124	0.141	0.083	0.117	0.251	0.121	0.096	0.145	0.121	0.075	0.112	0.169	0.155	0.132	0.173	0.163	0.128	0.191	0.230	0.115	0.191	0.124	0.091	0													
DI	0.086	0.069	0.055	0.071	0.181	0.069	0.030	0.079	0.106	0.060	0.077	0.161	0.140	0.153	0.091	0.135	0.059	0.078	0.100	0.068	0.086	0.082	0.076	0.079	0												
1_Fr	0.098	0.102	0.080	0.134	0.294	0.121	0.019	0.112	0.074	0.115	0.155	0.191	0.174	0.194	0.205	0.105	0.109	0.063	0.192	0.145	0.218	0.140	0.151	0.108	0.034	0											
8_1Fr	0.091	0.100	0.064	0.085	0.248	0.102	0.031	0.119	0.085	0.093	0.133	0.157	0.152	0.125	0.184	0.081	0.123	0.099	0.167	0.161	0.220	0.150	0.127	0.069	0.064	0.044	0										
2_It	0.076	0.107	0.069	0.125	0.224	0.066	0.024	0.080	0.076	0.101	0.107	0.110	0.130	0.156	0.099	0.144	0.057	0.144	0.130	0.084	0.126	0.133	0.091	0.111	0.036	0.099	0.106	0									
3_Wa	0.079	0.101	0.052	0.094	0.228	0.054	0.030	0.095	0.098	0.100	0.094	0.198	0.133	0.152	0.139	0.149	0.102	0.111	0.182	0.116	0.145	0.145	0.108	0.090	0.052	0.054	0.077	0.063	0								
4_Sp	0.200	0.180	0.173	0.203	0.402	0.197	0.150	0.258	0.208	0.195	0.268	0.344	0.265	0.228	0.280	0.231	0.227	0.194	0.278	0.236	0.348	0.219	0.198	0.167	0.161	0.195	0.126	0.255	0.215	0							
5_Cz	0.099	0.192	0.108	0.162	0.288	0.155	0.106	0.173	0.138	0.131	0.132	0.221	0.182	0.205	0.202	0.178	0.140	0.162	0.144	0.134	0.246	0.142	0.170	0.122	0.100	0.092	0.080	0.140	0.156	0.203	0						
6_Cz	0.149	0.193	0.115	0.156	0.290	0.140	0.081	0.153	0.129	0.134	0.155	0.167	0.167	0.175	0.175	0.182	0.091	0.126	0.120	0.127	0.241	0.117	0.135	0.136	0.061	0.101	0.127	0.093	0.156	0.256	0.060	0					
7_Cz	0.118	0.118	0.072	0.078	0.194	0.109	0.053	0.109	0.099	0.079	0.112	0.178	0.151	0.169	0.136	0.147	0.065	0.102	0.089	0.117	0.156	0.079	0.110	0.148	0.051	0.127	0.070	0.098	0.125	0.197	0.080	0.091	0				
9_Ge	0.154	0.174	0.096	0.141	0.265	0.174	0.122	0.150	0.130	0.107	0.155	0.214	0.217	0.169	0.252	0.225	0.144	0.186	0.215	0.198	0.270	0.098	0.135	0.146	0.135	0.147	0.131	0.185	0.189	0.193	0.152	0.126	0.116	0			
3	0.323	0.336	0.260	0.307	0.364	0.301	0.300	0.342	0.332	0.237	0.245	0.381	0.407	0.289	0.318	0.367	0.282	0.368	0.438	0.387	0.391	0.364	0.349	0.270	0.290	0.468	0.401	0.370	0.345	0.522	0.418	0.395	0.388	0.374	0		
8_Wa	0.268	0.241	0.218	0.248	0.355	0.205	0.200	0.282	0.216	0.212	0.242	0.369	0.304	0.304	0.266	0.336	0.198	0.201	0.280	0.246	0.261	0.231	0.193	0.246	0.168	0.286	0.286	0.257	0.181	0.369	0.269	0.265	0.198	0.259	0.377	0	
DN	0.295	0.244	0.237	0.264	0.372	0.235	0.212	0.312	0.216	0.206	0.248	0.351	0.328	0.270	0.287	0.336	0.230	0.215	0.341	0.299	0.285	0.239	0.243	0.263	0.212	0.329	0.318	0.305	0.264	0.400	0.320	0.289	0.243	0.219	0.360	0.137	0

Paper II

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Introduced Scotch broom (*Cytisus scoparius*) invades the genome of native populations in vulnerable heathland habitats

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Abstract

***Cytisus scoparius* is a global invasive species that affects local flora and fauna at the intercontinental level. Its natural distribution spans across Europe, but seeds have also been moved among countries, mixing plants of native and non-native genetic origins. Hybridization between the introduced and native gene pool is likely to threaten both the native gene pool and the local flora. In this study, we address the potential threat of invasive *C. scoparius* to local gene pools in vulnerable heathlands. We used nuclear single nucleotide polymorphic (SNP) and simple sequence repeat (SSR) markers together with plastid SSR and indel markers to investigate the level and direction of gene flow between invasive and native heathland *C. scoparius*. Analyses of population structures confirmed the presence of two gene pools: one native and the other invasive. The nuclear genome of the native types was highly introgressed with the invasive genome, and we observed advanced-generation hybrids, suggesting that hybridization has been occurring for several generations. There is asymmetrical gene flow from the invasive to the native gene pool, which can be attributed to higher fecundity in the invasive individuals, measured by the number of flowers and seed pods. Strong spatial genetic structure in plastid markers and weaker structure in nuclear markers suggest that seeds spread over relatively short distances and that gene flow over longer distances is mainly facilitated by pollen dispersal. We further show that the growth habits of heathland plants become more vigorous with increased introgression from the invaders. Implications of the findings are discussed in relation to future management of invading *C. scoparius*.**

Keywords: asymmetric gene flow, *Cytisus scoparius*, fecundity, hybridization, introgression, invasive species

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Introduction

Introductions of exotic species to new territories are considered major threats to native species and ecosystems (Vitousek *et al.* 1996; Parker 2000). Negative impacts of such introductions include a decrease in species richness at the local scale (Powell *et al.* 2011; McKinney *et al.* 2012) and changes in ecosystem function by altering abiotic environmental variables (light, soil moisture and soil characteristics) (Diquelou & Roze 1999; Fogarty & Facelli 1999; Vilà *et al.* 2000). Introduced plants meet and often interact with the native flora, creating a risk of hybridization if the species are closely related. New lineages can arise if strong reproductive barriers are present between homoploid recombinant hybrids and their parental taxa, or if hybridization is followed by chromosome doubling (allopolyploidy) (Lowe & Abbott 2004). In cases of no or only weak reproductive barriers, different scenarios are expected depending on the fitness of the hybrids. If the fitness of hybrids is lower than that of their parents, then the rarest taxon may become replaced by the superior

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taxon (i.e. demographic swamping or pollen swamping; Wolf *et al.* 2001; Buggs & Pannell 2006). In case of highly fit hybrid recombinants, one or both parental taxa may become genetically swamped (Wolf *et al.* 2001). If ongoing hybridization asymmetrically favours one of the two hybridizing taxa as the mother (Tiffin *et al.* 2001), and if backcrossing is mainly towards one taxon, introgression may become unidirectional (Field *et al.* 2011). Unidirectional backcrossing towards a numerically inferior, vulnerable taxon could potentially lead to the loss of this rare taxon. While asymmetrical gene flow and introgression are not uncommon in plants, their role in invasion biology is less investigated and little is known about the underlying mechanisms (Todesco *et al.* 2016). Given the potentially devastating outcome of swamping of native species by invasive ones, more research is needed.

Scotch broom, *Cytisus scoparius* (L.) Link ($2n = 2x = 46$), is a highly successful invasive species of the Fabaceae family and poses problems at the global level. It is outcrossing and depends on insects, mainly bees, for pollination (Parker 1997). The species is native to Europe (Potter *et al.* 2009) but has been introduced to all continents except Antarctica (Kang *et al.* 2007). Its massive expansion is closely linked to life history traits that enable the species to rapidly colonize new areas, such as abundant seed production, long-lasting seed banks (Turner 1933; Smith & Harlen 1991; but see also Magda *et al.* 2013), ease of establishing in newly disturbed areas and its ability to grow rapidly (Fogarty & Facelli 1999). In addition, as a nitrogen-fixing species, *C. scoparius* increases the nutrients in soils, which can inhibit the growth of coexisting native species (Fogarty & Facelli 1999).

In Denmark, where this study took place, *C. scoparius* is considered a native species. It is mentioned in some of the oldest systematic botanical records dating back to 1648 (Paulli 1648). However, *C. scoparius* is a highly variable species where genetic origin is decisive for its phenotype. A transplanting study comparing 19 origins across Europe revealed large differentiation among populations within *C. scoparius* and identified nine highly distinct ecotypes according to pronounced differences in growth form, frost hardiness and flowering intensity (Böcher & Larsen 1958). Several populations (including origins from France, Spain, the Netherlands, Luxemburg and Hungary) exhibited erect growth, 150–250 cm height, and possessed only medium to low frost hardiness, while other populations were less erect, 50–150 cm high, and had high frost hardiness (called B2 in Böcher & Larsen 1958). The latter group includes the population considered native to Denmark. Low types with relatively poor frost hardiness that originated from northern Germany were also identified, and plants from a single origin on the island of Jersey were completely prostrate. Collections from Danish sites where *C. scoparius* was not considered by the authors to be native belonged to the tall types with low frost hardiness and high mortality. Böcher & Larsen (1958) suggested that these tall types were introduced from non-Danish sources, while native types were characterized by their low height, high survival rates and abundant flowering. A recent study based on microsatellite [simple sequence repeat (SSR)] markers confirmed the presence of two gene pools of Danish *C. scoparius,* of which one is considered to be invasive (Rosenmeier *et al.* 2013). This study revealed that native types of *C. scoparius* growing in the vulnerable sandy heathlands in the western part of Denmark were genetically distinct from the vigorous invasive plants of *C. scoparius* (Rosenmeier *et al.* 2013). The types differed in growth habit corresponding to the findings of Böcher & Larsen (1958), but despite the genetic and morphological differences, the native form has never been given formal taxonomic status. Rosenmeier *et al.* (2013) raised the concern that the invasive type of *C. scoparius* could pose a threat to native gene pools of *C. scoparius* if the range of the invaders expanded further and the two forms come into contact. As the introduced *C. scoparius* is an effective colonizer with vigorous growth once introduced, massive hybridization and introgression into the native population could be expected, although the opposite scenario with asymmetric gene flow from the native form into invading *C. scoparius* is also possible (Currat *et al.* 2008). Differences in soil preferences, with the invading *C. scoparius* tending to grow on more fertile soils, may reduce the risk of the invasive type establishing populations that intermix with the native type on the poorest soils, but pollen dispersal may provide a genetic bridge between geographically close populations growing on different soil types. However, it is unknown whether the two types are reproductively compatible, whether F1 hybrids are fertile or whether prezygotic reproductive barriers that may prevent hybridization between the two types exist.

In the present study, we address the hypothesis that the local *C. scoparius* from vulnerable heathlands is being genetically swamped by an introduced invasive type. We specifically ask (i) whether *C. scoparius* from surrounding areas of the heathland is spreading into the heathland by pollen flow, seed flow or by both means, (ii) whether the native gene pool of *C. scoparius* is at risk of being swamped by invasive *C. scoparius* due to asymmetric gene flow and subsequent backcrossing and (iii) whether continuous hybridization can cause phenotypic changes to the growth form of *C. scoparius* in heathlands. Our study site covers a native heathland surrounded by agricultural land with farms and roads. While the heathland is dominated by the low growing native phenotype, the adjacent areas are dominated by tall and vigorous plants. Both phenotypes grow sufficiently close to allow pollen dispersal by insects between them. The patterns of hybridization and asymmetric gene flow were investigated using both nuclear markers (biparentally inherited) and plastid markers (maternally inherited). The implications of the findings are discussed in relation to the future management of invading *C. scoparius*.

Material and methods

Study site

The study site is located in western Jutland, near Holstebro in Denmark. The site was selected because tall plants from potentially introduced seed sources of *C. scoparius* grow close to a native heathland population. The habitat (Ulfborg-Stråsø plantage GPS 56°15′20.0″N 8°24′35.8″E) is characterized as hilly heathland on old sand dunes with typical heathland flora comprised of, *for example*, common heather (*Calluna vulgaris*), common juniper (*Juniperus communis*) and red crowberry (*Empetrum rubrum*). Conifers have previously been planted in the southeastern corner, but most of these have subsequently been removed. The surrounding areas are agricultural land with farms and houses.

Sampling

Sampling within and outside the heathland took place on 14 and 15 January 2015. Within the heathland, sprigs were collected from 183 randomly chosen individuals growing in patches in the area. Outside the heathland, sprigs were collected from 66 plants in the bordering areas along road sides and pastures on private farms and houses where tall *C. scoparius* has been observed to spread and increase in density in the preceding decades (see Fig. 1). GPS coordinates and morphological measurements (height, basal diameter, widest width and perpendicular width) were recorded for each individual we sampled.

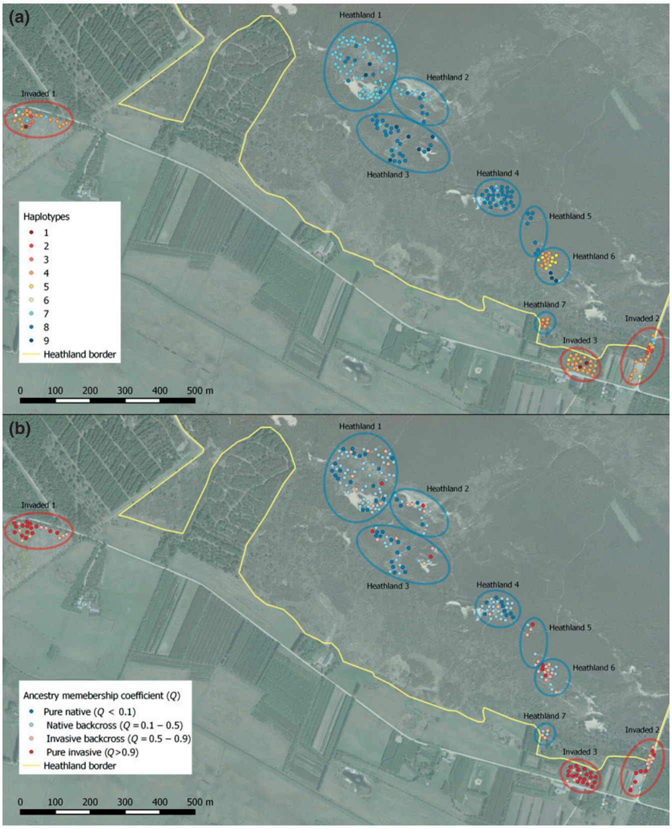


Fig. 1 Distribution of 249 individuals in the sampling area. (a) Distribution of the 6 invasive (1 to 6) and 3 native (7 to 9) haplotypes based on one chloroplast indel marker and one microsatellite marker. (b) Distribution of individuals with ancestry membership defined by the software STRUCTURE with two groups ($K = 2$). The analysis is based on 36 nuclear single nucleotide polymorphic markers and 5 microsatellite markers. Note the different class ranges. The basemap is sourced from Google, Aerodata International Surveys, DigitalGlobe, Scankort. The points for some individuals were slightly moved from their exact location to increase visibility.

The area was revisited on 29 June 2015, when the relationship between plant size and fecundity was assessed during peak flowering of both types to observe whether plants with erect phenotypes had higher fecundity compared to shorter plants. For this study, a total of 100 plants were selected to represent the range in size variation among plants of the two types. Forty plants were selected in the invaded area and 60 plants from the heathland. Plant heights were measured, and the number of flowers and pods per plant was counted if <50 and estimated to the hundred for plants with more than 50 flowers/pods.

DNA extraction

Genomic DNA was isolated from ten milligrams of dry tissue using the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany) protocol with the following adjustments. To increase DNA yield, the chemical cell lysis step (in buffer AP1, at 65 °C) was extended from 10 to 30 minutes. Additionally, the final DNA elution step was conducted only once and with water. Samples were normalized to a concentration of 10 ng/μl, except where initial concentration was lower.

Plastid DNA sequencing

Two plastid DNA haplotypes were distinguished based on one chloroplast marker (indel), which previously has been found to differentiate between the native and introduced type of *C. scoparius* in Denmark (Brandes *et al.*, unpublished). The DNA region between the two universal plastid primers *psb*B and *psb*F (length of 778 bp in *Medicago* sp.; Hamilton 1999) was amplified using the polymerase chain reaction (PCR). The 25 μl PCRs contained 1× Standard *Taq* Reaction Buffer (New England Biolabs (NEB), Ipswich, MA, USA), 0.625 units HotStart *Taq* DNA polymerase (NEB), 50 μM of each dNTP (Invitrogen, Carlsbad, CA, USA), 0.4 μM of each primer and 1 μl of DNA template. PCR was performed using a Mastercycler nexus thermocycler (Eppendorf, Hamburg, Germany) with an initial 15 min at 95 °C, followed by 30 cycles of 1 min at 94 °C, 2 min at 55 °C and 3 min at 72 °C, with a final extension step of 5 min at 72 °C. PCR products were purified and sequenced by Macrogen (Seoul, South Korea), using the specifically designed internal primers psbF_int (GACAAG CAGTCGGATAGACCA; Invitrogen) and psbB_int (GGGCAACCCTCTCAACAACT; Invitrogen).

SNP marker development and genotyping

We generated 80 novel single nucleotide polymorphic (SNP) markers for *C. scoparius* from a reduced representation library, following the method of double-digest restriction-associated DNA sequencing (ddRAD-seq) as described by Peterson *et al.* (2012). The library was constructed based on 16 leaf tissue samples from different populations outside Denmark. In accordance with the ddRAD protocol (Peterson *et al.* 2012), DNA samples were normalized to 500 ng, digested using the restriction enzymes *Msp*I and *Eco*RI^{HF} (NEB) and purified with AMPure XP Beads (Beckman Coulter, Brea, CA, USA). Each sample was ligated with one common adapter and one uniquely bar-coded adapter (differing by at least two nucleotides) and again purified to remove unligated and incorrectly ligated fragments. After pooling all samples, DNA fragments were size-selected to approximately 400 bp (including adapters and barcodes) using a Pippin Prep (Sage Scientific, Beverly, MA, USA) with a 2% agarose gel. The remaining DNA fragments were PCR-amplified for 12 cycles and sequenced using an Illumina Miseq (Illumina, San Diego, CA, USA) platform with the V2 sequencing kit (2 × 251 nucleotides).

Forward sequence reads were analysed for SNPs using STACKS (v1.18) (Catchen *et al.* 2011). As reads were of different lengths, they were shortened to 240 nucleotides and then analysed in STACKS S with a minimum stack depth of 5 (-m), minimum distance allowed between stacks of 4 (-M) and otherwise default values. Of the resulting 7395 polymorphic stacks, only those with one or two SNPs (1992 and 1625 stacks, respectively) were selected. These 3617 stacks were further scrutinized based on the following four criteria: we only kept stacks that were generated for at least 10 of the 16 samples, had no more than two alleles in any sample, reached a minimum allele frequency of 0.1 across all samples and included at least one sample that was homozygous for each of the two alleles. For stacks containing two SNPs, only one SNP was selected.

Based on the 116 best-suited stacks, two sets of 40-multiplex MASSARRAY (AGENA Bioscience, San Diego, CA, USA) were designed using the ASSAY DESIGN SUITE 2.0 software (AGENA Bioscience). Only 36 of these 80 SNP markers were included in further data analysis because 30 markers failed, two were monomorphic and 12 markers had more than 10% missing values.

Microsatellite genotyping

Five primer pairs for nuclear simple sequence repeat (SSR) loci (Cs03, Cs18, Cs34, Cs39 and Cs65) and one primer pair for a plastid SSR locus (Ccmp5) previously used for analysing *C. scoparius* in Denmark (Rosenmeier *et al.* 2013) were used. PCR amplification was performed with Multiplex PCR Mastermix (Qiagen) following the procedure in the Qiagen Multiplex PCR handbook (version from 2010) but with the reaction volume scaled down to 10 μl. PCR was performed in a Bio-Rad C1000 Thermo cycler (Bio-Rad Laboratories, Hercules, CA, USA) with an initial denaturation step at 95 °C for 15 min, followed by 30 cycles of 30 s at 94 °C, 1 min 30 s at 57 °C and 60 s at 72 °C, followed by a final 30-min extension step at 60 °C and storage at 5 °C. GeneScan 500 LIZ was used as an internal size standard and separation of diluted PCR products (1:30) was performed on ABI 3130XL Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA).

Statistical analyses

For statistical analyses, we combined the nuclear SNP and SSR markers and analysed them together, except for diversity measures which were calculated for each marker type and approximate Bayesian computation (ABC) for which only nuclear SNP markers were used. The plastid indel and mononucleotide SSR markers were combined into unique haplotypes.

We used two approaches to verify that there were two different genetic groups in the study system. First, we used principal components analysis (PCoA) as implemented in GENALEX 6.501 (Peakall & Smouse 2006, 2012). Second, we used a model-based Bayesian nonhierarchical clustering method that clusters individuals into K groups according to where maximum linkage disequilibrium between groups is present. For this analysis, we used the program STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000; Falush *et al.* 2003), and we assessed 10 replicates of each K from 1 to 10 using a burn-in length of 500 000 and 1 000 000 MCMC repeats after burn-in. The program CLUMPAK (Kopelman *et al.* 2015) was used to combine the results of the ten replicates. We evaluated likelihood plots and ΔKs (Evanno *et al.* 2005) to identify the most likely number of groups using STRUCTURE HARVESTER (Earl & von Holdt 2012).

To investigate the gene flow between the heathland and invaded areas, we used results from the STRUCTURE analysis of two groups ($K = 2$), assuming that one group corresponded to the invasive and the other to the native genome. For subsequent analyses and discussions of results of the STRUCTURE analysis with two groups, we defined a high ancestry membership coefficient (Q) as representing the invasive genome and a low Q as representing the native genome. Individuals with $Q > 0.9$ were defined as pure invasive, and individuals with $Q < 0.1$ were defined as pure native.

To examine hybridization and introgression between native and invasive genotypes, we compared the level of invasive genome content in heathland individuals with the native genome content in invaded area individuals. We did this by testing whether the distribution of the ancestry membership coefficients Q in the heathland population differed from the distribution of Q-1 values in the invaded area using the nonparametric Kolmogorov–Smirnov test in the R package 'STATS' (R core team 2015).

We used the program NEWHYBRIDS (Anderson & Thompson 2002) to investigate whether potential hybrids were first generation, or whether there were more complex hybridization and introgression patterns, including backcrossing and advanced-generation hybrids. NEWHYBRIDS is a model-based method that calculates the posterior probability that an individual is assigned to different hybrid categories. Individuals with $Q > 0.9$ were defined as pure invasive and individuals with $Q < 0.1$ as pure native. We used the genealogical categories suggested in the software (pure native, pure invasive, F1, F2, F1 backcrossed to pure native and F1 backcrossed to pure invasive). We used 500 000 sweeps for burn-in and 1 000 000 sweeps after burn-in.

Furthermore, we used approximate Bayesian computation as implemented in the program DIYABC version 2.1 (Cornuet *et al.* 2014) to estimate the onset of admixture and admixture rate between native and invasive gene pools based on the 36 nuclear SNP markers. Individuals with $Q > 0.9$ were defined as pure invasive and individuals with $Q < 0.1$ as pure natives. 10^6 data sets were simulated with parameter values drawn from prior distributions as shown in Table S1 (Supporting information). We used the proportion of zero values and mean nonzero values of F_{ST} and Nei's distances and admixture estimates as our 14 summary statistics. We simulated 1000 data sets from the posterior distribution of parameters and compared estimated and observed summary statistics to estimate the goodness of fit of the model. To evaluate the model, we chose summary statistics not used previously to inform the model (variance of nonzero values and mean of total distribution for F_{ST} and Nei's distances and admixture estimates).

Genetic characteristics (average number of alleles, N_A; effective number of alleles, N_e; observed and expected heterozygosity, H_o and H_e; and fixation index, F_{IS}) were calculated separately for each marker type for individuals in the heathland and invaded area, as implemented in GENALEX 6.501. To establish how variation was divided between and among plants sampled in the heathland and invaded area, we ran an analysis of molecular variance (AMOVA) and calculated F_{ST} using GENALEX 6.501.

To visualize the distribution of genetic diversity across the sample site, we plotted both plastid haplotypes and ancestry membership coefficients for each individual on a map using QGIS v2.6.1-Brighton (QGIS Development Team 2015).

To assess the contribution of pollen relative to seed dispersal on a local scale, genetic structure among plants in the heathland was quantified using the *Sp* statistic (Vekemans & Hardy 2004). We calculated geographic distances among all plants in the heathland from GPS coordinates and compared them to genetic distances for the nuclear markers and plastid haplotypes, respectively. As the chloroplast is maternally inherited while nuclear markers are biparentally inherited, gene flow facilitated by pollen and seeds can be separated. *Sp* statistics were calculated using the regression slope of pairwise kinship coefficients between individuals from the heathland on the logarithm of spatial distance (Vekemans & Hardy 2004). Seed dispersal relative to overall gene flow (σ_s/σ_g) was calculated as $[2Sp(\text{nuclear})/Sp(\text{plastid})]^{1/2}$, while the ratio between pollen and seed dispersal (σ_p/σ_s) was calculated as $[Sp(\text{plastid})/Sp(\text{nuclear}))-2]^{1/2}$, as described in Budde *et al.* (2013). Pairwise kinship coefficients (Loiselle *et al.* 1995) and regression slopes were estimated using SPAGEDI (Hardy & Vekemans 2002) with calculations based on allele identity/nonidentity.

The impacts of hybridization on the phenotypes of plants were assessed using linear regression of the height/width ratio on the corresponding ancestry membership coefficient (Q) as estimated from the STRUCTURE analysis with $K = 2$. This analysis was restricted to heathland plants to compare plants growing under similar conditions. To further reduce any bias from spatial variation across the heathland, we separated the sampled part of the heathland into blocks of each 50 m × 50 m and included block effects in the regression analysis.

The sum of flowers and pods per plant was used as a proxy for fecundity (*Fec*). The relationship between height and fecundity of the plants was quantified and tested based on a simple linear regression model between height and log-transformed fecundity: $L(Fec) = \log(Fec+1)$ on height. The analysis was based on the 60 plants measured in the heathland to estimate how size relates to reproductive energy in the native habitat. Block effects were again included to reduce any effects of spatial variation across the heathland.

Results

We scored one polymorphic plastid indel and 36 polymorphic nuclear SNP markers, as well as one plastid and five nuclear microsatellite markers.

The individuals formed two groups along the first PCoA axis corresponding to individuals sampled inside or outside the heathland (Fig. 2; PCoA axes 1 and 2 explained 9.6 and 4.8% of the variation, respectively). There is, however, some overlap between the two groups. Likelihood scores and ΔK (Fig. S1, Supporting information) from STRUCTURE analyses also indicated clustering of two genetic groups mainly corresponding to individuals sampled inside or outside the heathland (Fig. 3b). Ancestry membership coefficients (Q) showed a gradual transition between the two groups, indicating hybridization and introgression between the two gene pools (Fig. 3b). In total, 44 individuals had Q-values below 0.1 (indicating pure native genotypes), all of them within the heathland. Forty-nine individuals had a Q-value above 0.9 (indicating pure invasive genotypes), and of these, eight were inside the heathland. Forty-one individuals in the heathland area had Q-values above 0.5 (indicating high contribution from the invasive gene pool), whereas only one individual in the invaded area had a Q-value below 0.5 (indicating high contribution from the native gene pool; Figs 1b and 3b). Individuals with a high contribution from the invasive

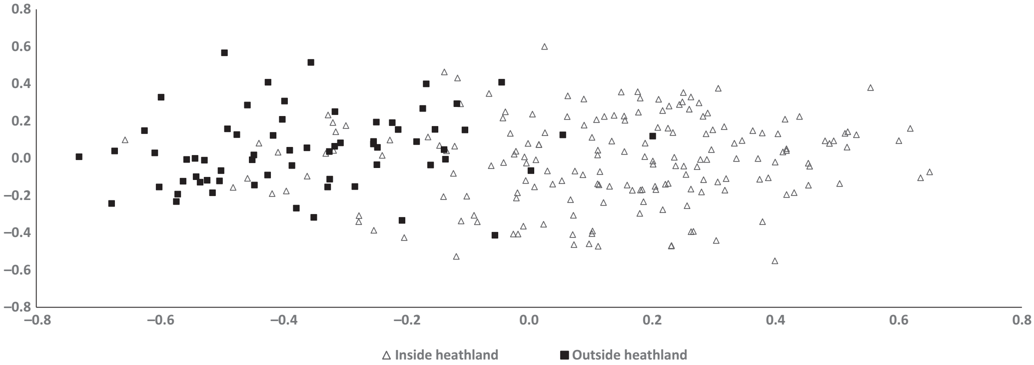


Fig. 2 Principal components analysis of 249 *Cytisus scoparius* individuals. The analysis is based on 36 nuclear single nucleotide polymorphic and five microsatellite markers. The first and second axes explain 9.6 and 4.8% of the variation, respectively.

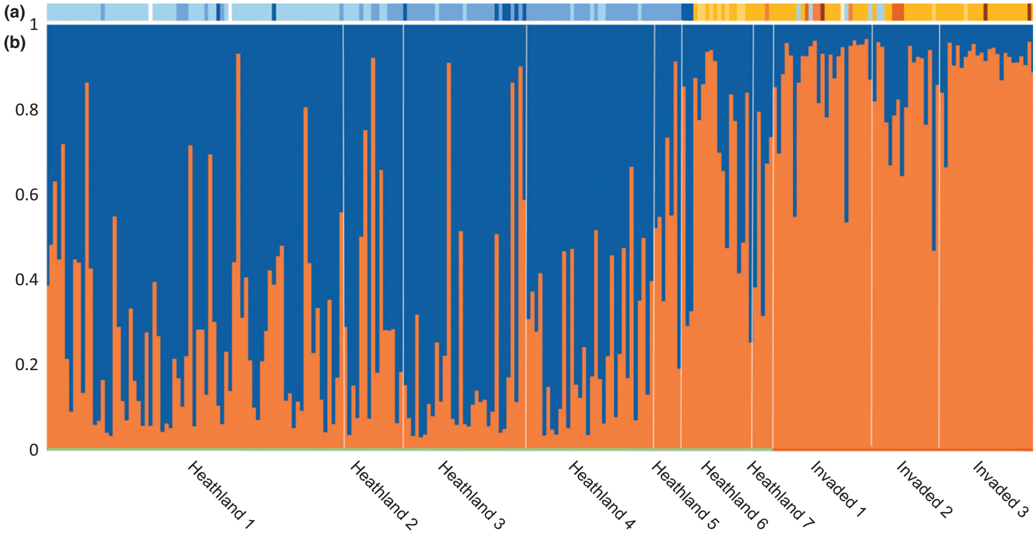


Fig. 3 (a) Plastid haplotype of 247 *Cytisus scoparius* individuals (two individuals with missing data are shown in white). The analysis is based on one indel and one microsatellite marker. Colours refer to haplotypes in Fig. 1. Blue and orange colours represent the two groups defined by the diagnostic indel, and shades within each group represent variation in the mononucleotide SSR marker. (b) Population structure of 249 *C. scoparius* individuals with two groups ($K = 2$) from the software STRUCTURE. The analysis is based on 36 nuclear single nucleotide polymorphic and five microsatellite markers. Each bar represents one individual, and each colour represents the fraction of the genome assigned to each group (Blue = native, red = invasive). The green and red bars below the plot represent individuals sampled in the heathland and invaded area, respectively. The invaded and heathland subgroups indicated under the plot are as described in Fig. 1.

gene pool were found in all parts of the heathland but dominated the southeast part of the heathland (heathland 5, 6 and 7; Fig. 1b). These areas are the closest to the invaded area.

The Kolmogorov–Smirnov test showed that the distribution of invasive and native genome content in the heathland and native areas, respectively, differed ($D = 0.4062$, $P = 0.000$; Fig. 4). We interpret this as a reflection of asymmetric gene flow between the individuals in the heathland and the invaded area, with higher gene flow from the invaded area into the heathland than vice versa.

The results from the NEWHYBRIDS simulations showed that most individuals fell into three genealogical categories: pure native, pure invasive or F2 hybrids (Fig. 5). We interpret the latter category as including both F2 and advanced-generation hybrids. None of the individuals were classified as F1 hybrids with a likelihood higher than 0.07. Where there were signs of F1 backcrossing to pure native or invasive gene pools, there was a tendency for backcrossing to the gene pool representing the area where the individual was growing.

Timing of the onset of admixture of the native and invasive gene pool was estimated with approximate Bayesian computation in DIYABC to be approximately 10 years ago (0.62–32.1 years ago, 95% CI) using a generation time of three years (Parker 1997; Table 1). Admixture towards the total hybrid population (both heathland and invaded area) was approximately equal from the native and invasive gene pools (Table 1). The model showed a relatively good fit, and none of the 14 summary statistics used were outside the 95% CI.

The level of genetic diversity was similar between the heathland and invaded area individuals (*e.g.* microsatellites: $N_e = 5.5 \pm 1.96$ and 5.41 ± 1.83, respectively, and $H_e = 0.74 \pm 0.6$ and 0.75 ± 0.6, respectively; Table 2), while F_{IS} was approximately twice as high for invasive individuals than for native ones. AMOVA revealed that 11% of the variation was between the heathland and invaded area groups, while the remaining 89% was within. The level of genetic differentiation was significant ($F_{\mathrm{ST}} = 0.11$, $p = 0.001$).

The diagnostic indel in the plastid region mainly divided the samples into two groups corresponding to the heathland and invaded area (Figs 1a and 3a). There were, however, 20 individuals in the heathland that had the invasive plastid indel haplotype and six individuals

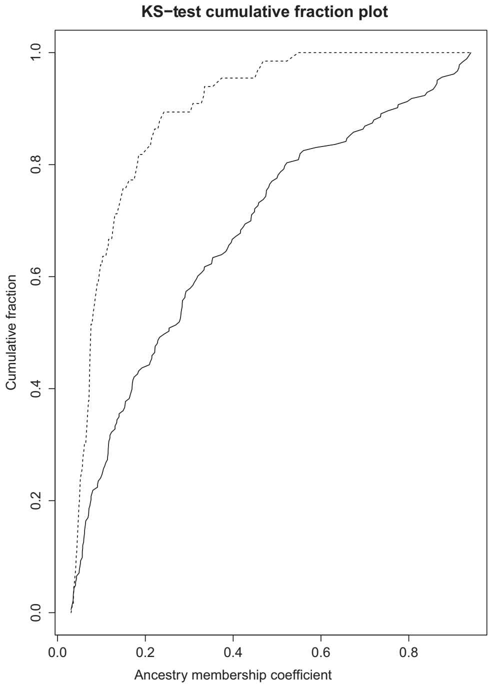


Fig. 4 Cumulative fraction plot of ancestry membership coefficients from the Structure analysis with $K = 2$ (i.e. the fraction of individuals (y) having a value of x or smaller). The dotted line shows the distribution of native ancestry membership coefficients (1-*Q*) in the invaded area, and the solid line shows the distribution of invasive ancestry membership coefficients (*Q*) in the heathland area.

in the invaded area that had the native plastid indel haplotype. We identified six different alleles in the mononucleotide plastid microsatellite locus. We believe that there is homoplasy among the plastid microsatellite alleles because we found the same SSR alleles in the two groups defined by the indel marker; hence, they should be interpreted with caution. Plastid mononucleotide microsatellites mutate with a manifold higher rate than indels (Provan *et al.* 2001; Yamane *et al.* 2006; Jakobsson *et al.* 2007). Based on this, we chose to score haplotypes based on the combination of the plastid indel and the plastid microsatellite marker, where the indel represents an older mutation event that reflects the introduction history to the area and the plastid microsatellite variation represents more recent mutations within the two haplogroups defined by the plastid indel. There is likely no homoplasy among the plastid microsatellite alleles within each of the plastid indel haplogroups. PCR artefacts are known to be a problem in mononucleotide microsatellites, but even if PCR artefacts are present, they will not impact the main conclusions drawn from the data. By combining the plastid indel and the microsatellite marker, we identified nine haplotypes: six within the invasive plastid indel haplotype and three within the native plastid indel haplotype (Figs 1a and 3a). Within the heathland, one plastid haplotype dominated the northwestern part of the sampled heathland area (haplotype 7; Fig. 1a) and another the southeastern (haplotype 8, Fig. 1a), with some mixing and with the third native plastid haplotype present in both parts (haplotype 9). In the furthest southeastern part of the heathland sample area, a patch of 15 plants with two invasive haplotypes were observed (haplotypes 4 and 5; Fig. 1a in 'Heathland 6'). These plants were growing within 250 m of another group of five invasive *C. scoparius* individuals, located just inside the heathland boundary (haplotypes 3 and 4; Fig. 1a 'Heathland 7'). The *C. scoparius* plants outside the heathland were dominated by one plastid haplotype (haplotype 4 Fig. 1a), and the five other haplotypes were present in minor frequencies throughout the area (there were only one to four samples with the invasive haplotypes 1, 2, 3, 5 and 6).

The degree of spatial genetic structure was significant for both nuclear and plastid markers ($Sp = 0.0095$ and 0.4892, respectively). Pairwise kinship coefficients were significant up to 30 m for nuclear SNP and microsatellite markers (Fig. 6a), while significance based on plastid haplotype similarity extended almost 200 m (Fig. 6b). The ratio of seed dispersal to overall geneflow distance (σ_s/σ_g) was 0.20, while the pollen to seed dispersal ratio (σ_p/σ_s) was 7.04, implying that pollen-mediated gene flow is much stronger than gene flow via seeds.

Plants predicted to be of pure native type based on their low ancestry membership coefficients (*Q*) were in general less erect compared to introgressed individuals with an approximate linear relationship between height/width ratio and *Q*-value ($t = 4.08$; $P < 0.001$; Fig. 7a). Fecundity observations revealed a positive relationship between height and log-transformed fecundity (flowers + pods) among the 60 heathland plants ($t = 2.82$; $P < 0.01$; Fig. 7b). The approximately log-linear relationship suggests that fecundity within the sampled height classes increases almost exponentially with plant height, where an average plant of 120 cm in the heathland is predicted to have 10 times more flower/pods than an average 20 cm plant in the same area. Fecundity observations also revealed that plants growing outside the heathland developed more flowers and seed pods than plants within the heathland (Fig. 7b).

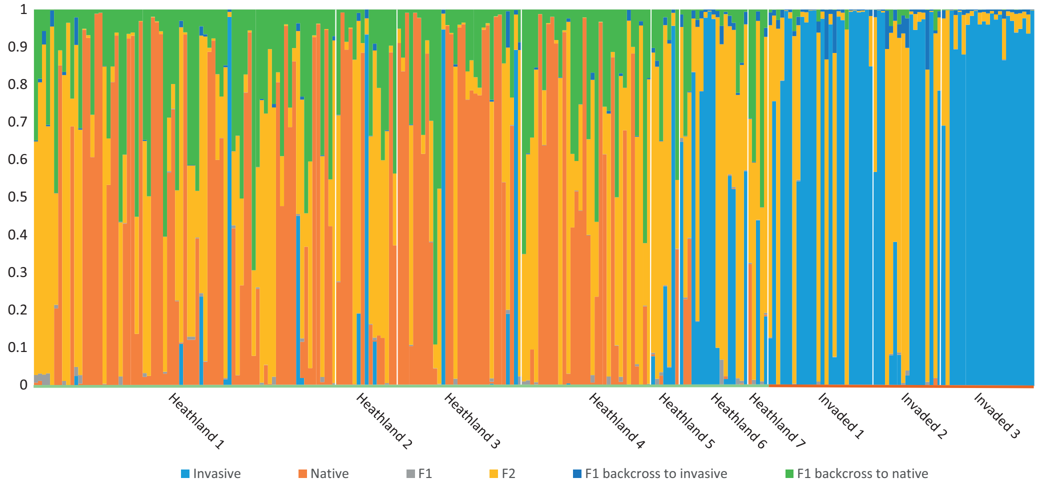
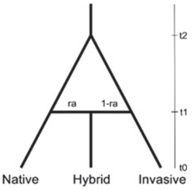


Fig. 5 Assignment of genotypes to six genealogical classes from the software NEWHYBRIDS based on 36 nuclear single nucleotide polymorphic and five microsatellite markers. Each bar represents the probability of an individual for assignment to each of the six genealogical classes. The green and red bars below the plot are as described in Fig. 1 and represent individuals sampled in the heathland and invaded area, respectively.

Table 1 Posterior distribution of parameters from DIYABC based on 36 nuclear single nucleotide polymorphic markers. For the estimation of absolute time, we used a generation time of three years (Parker 1997)

Model	Parameter	Median (95% credibility interval)
	Population size of native population	3620 (876, 8680)
	Population size of invasive population	5550 (1750, 9400)
	Population size of hybrid population	4430 (1130, 9210)
	t1 generations	3.27 (0.21, 10.7)
	t1 absolute time	9.81 (0.62, 32.1)
	t1/mean population size	2.33e^{-15} (2.64e^{-22}, 6.69e^{-8})
	t2 generations	655 (116, 4130)
	t2 absolute time	1965 (348, 12390)
	t2/mean population size	1.08e^{-2} (3.31e^{-3}, 1.04e^{-1})
	ra (ratio of admixture)	0.525 (0.342, 0.698)

Discussion

Empirical and experimental results support that hybridization in many cases plays a role in increasing fitness of invasive plant taxa (Hovick & Whitney 2014). Much research has focused on the role of interspecific hybridization in invasion biology (Todesco *et al.* 2016). However, failure to recognize invasions at lower taxonomic levels may lead us to overlook those invasions, as exemplified in Saltonstall's (2002) landmark paper on cryptic invasion in *Phragmites australis*. Furthermore, most studies of invading species have focused on intercontinental invasion only (but see Bleeker 2003; Valtuena *et al.* 2011) because they are easy to identify. In the present case of the intracontinental invader *Cytisus scoparius*, we study hybridization between invasive and native gene pools at a subspecies taxonomic level. We show that native *C. scoparius* in the vulnerable heathlands are strongly influenced by hybridization with invasive genotypes from surrounding areas. The genetic invasion of the native *C. scoparius* genome causes a change in the morphology of invaded plants towards the more erect phenotype of the invasive type. We also document that taller plants produce more flowers and seed pods; hence, our study supports the hypothesis that hybridization can lead to increased

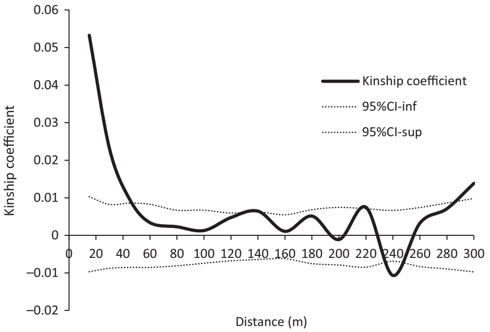
Table 2 Genetic characteristics (microsatellites and SNPs) of *Cytisus scoparius* from heathland and invaded areas

		N	N_A	N_e	H_o	H_e	F_{IS}
Microsatellites							
Heathland	Mean	180.8	16.20	5.50	0.67	0.74	0.09
	SE	0.58	4.02	1.96	0.05	0.06	0.03
Invasive	Mean	65.4	14.20	5.41	0.59	0.75	0.21
	SE	0.40	2.40	1.83	0.04	0.06	0.03
SNPs							
Heathland	Mean	181.1	1.97	1.40	0.24	0.26	0.06
	SE	0.45	0.03	0.04	0.02	0.02	0.02
Invasive	Mean	65.1	2.00	1.45	0.26	0.29	0.10
	SE	0.37	0	0.04	0.02	0.02	0.03

N = sample size, N_A = number of alleles per locus, N_e = effective number of alleles per locus, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{IS} = fixation index.

fitness even at this low taxonomic level. The potential increase in invasiveness in the heathland will be in disguise: our combination of nuclear and chloroplast makers reveals that while the tall invasive *C. scoparius* phenotypes spreading along roadsides and disturbed areas most likely originate from historical seed introductions, the tall, advanced-generation hybrids in the heathland in most cases must originate from native heathland plants initially hybridized with invasive plants via pollen.

(a) SNP and microsatellite markers



(b) plastid markers

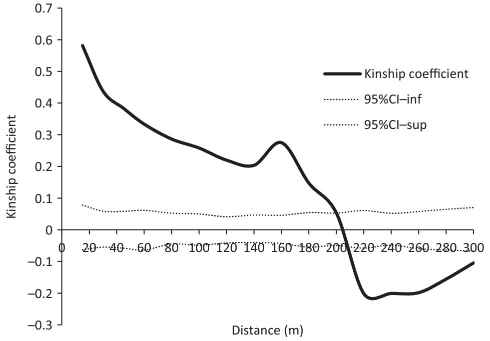


Fig. 6 Spatial genetic structure among individuals of *Cytisus scoparius* in the heathland measured as kinship coefficients at distance classes up to 300 m. The solid line indicates the observed kinship values for all heathland plants, and the upper and lower 95% confidence intervals are displayed as broken lines. (a) Estimates based on nuclear markers (SNPs and microsatellites). (b) Estimates based on plastid markers.

Gene flow between native and invasive gene pools of Cytisus scoparius

Gene flow from invasive to native species is now recognized as widespread (see, e.g., Burgess *et al.* 2005; Prentis *et al.* 2007; Kellner *et al.* 2012; Balao *et al.* 2015 and recently reviewed in Todesco *et al.* 2016). However, few studies demonstrate gene flow between conspecific invasive alien and native gene pools (but see, e.g., Saltonstall 2002). This is most likely due to the difficulty in identifying good study cases as the nature of the invasion is cryptic, and the prevalence of this process remains unknown. Our results outline a scenario where native *C. scoparius* in a vulnerable heathland is surrounded by invasive, heavy flowering *C. scoparius* leading to massive gene flow from the invasive to the native gene pool. The invasive *C. scoparius* most likely originates from large-scale introductions during the last 50–60 years. Besides being used for soil improvement owing to the ability of *C. scoparius* to fix nitrogen (Fogarty & Facelli 1999), the introduced tall types have been promoted widely in western Denmark to provide food and shelter for animals (Schlätzer 1965). Böcher & Larsen (1958) suggested that native and invasive types already hybridized in the 1950s at some localities in western Denmark, but clear documentation has been lacking until now. In the present study, we find that hybridization between the two gene pools is frequent and that the invasive genome has already been introgressed into individuals in the heathland to a large extent (Fig. 3b). We observe a hybrid swarm with complex genomic compositions where hybrids were not limited to the first generation, indicating a large potential for gene transfer between the two gene pools. We estimated the onset of hybridization to be 3.27 generations ago, which corresponds to 10 years using a generation time of three years (Parker 1997; Table 1). This is somewhat later than the assumed time of introduction. However, the generation time may be longer on the

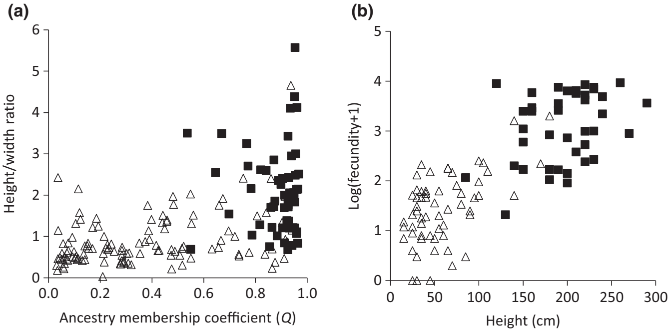


Fig. 7 Phenotypic variation in *Cytisus scoparius*. (a) Height/width ratio in relation to genetic classification (admixture coefficient; *Q*) (b) fecundity (log-transformed number of flowers/pods) in relation to height. Open triangles = plants in the heathland population, closed squares = plants from the invading population.

poor, sandy soils and the age of the invasive population at the study site is unknown. Furthermore, a lag phase for the establishment and spreading of the introduced type can be expected (Aikio *et al.* 2010). In New Zealand, *C. scoparius* had a lag phase of 97 years (Aikio *et al.* 2010). Our results are in line with Böcher & Larsen (1958) and suggest a history of hybridization and introgression dating back several generations. A similar scenario has been observed in *Eucalyptus* with a long history of gene flow between the species *E. aggregata* and *E. rubida* (Field *et al.* 2011).

Gene flow between native and invasive gene pools is asymmetric and pollen-mediated

Our results suggest more gene flow from the invasive gene pool into the heathland than the other way around (Figs 3 and 4). Forty-one (22%) individuals in the heathland had an ancestry membership coefficient (*Q*) above 0.5 (invasive genome), whereas only one individual (1.5%) in the invaded area had a *Q*-value below 0.5 (native genome). Asymmetric gene flow from invasive to native species has long been recognized as a potential threat to native species (Rhymer & Simberloff 1996) and demonstrated in several studies (e.g. Burgess *et al.* 2005; Prentis *et al.* 2007; also reviewed in Todesco *et al.* 2016). Nevertheless, only few studies have detailed how asymmetric gene flow creates extinction threat to native species (Ayres *et al.* 2004; Zika 2006; Balao *et al.* 2015). Differences in fecundity or female fitness between local and invading populations can play an important role in determining the direction and magnitude of introgression (Anttila *et al.* 1998). In *C. scoparius*, the plants of the invading type developed more flowers and seed pods compared to plants of the native type (Fig. 7b), which increases the potential for gene flow into the heathland from adjacent dense stands of invasive individuals. Other factors may have contributed to asymmetric gene flow, including different pre- and postzygotic mechanisms. Postzygotic mechanisms could include selection (Lexer *et al.* 2005), demographic processes (Currat *et al.* 2008) or the relative abundance of parent species (Cianchi *et al.* 2003). Prezygotic mechanisms may include asymmetric genetic incompatibility as has been studied in *Quercus* (Bacilieri *et al.* 1996; Olrik & Kjaer 2007), but such factors are more likely to play a role at the species level than at the lower taxonomic level investigated here. As invading *C. scoparius* is predominantly associated with fertile soils in farms surrounding heathlands, it is possible that the poor sandy soil in heathlands prevents seedling establishment of invading types. More research is needed to clarify whether there is ongoing selection against seeds from the invasive type driven by differences in soil composition.

The fact that the majority of individuals in the heathland have the native plastid haplotype suggests that gene flow from the invaded area into the heathland occurs almost entirely through pollen. Excellent examples of interspecific invasion through pollen-mediated gene flow are given by Potts & Reid (1988) for two *Eucalyptus* species and Petit *et al.* (2004) for two species of *Quercus*. Our estimate of the sevenfold increased range of pollen dispersal compared to seed dispersal can explain the high proportion of plants in the heathland scored to be pure introduced genotypes ($Q > 0.9$) that nevertheless had the native plastid haplotype. This suggests that the genomes of these individuals originate from generations of continuous introgression from invasive to native heathland populations through pollen flow. Malo (2004) found that seed dispersal by gravity, ballistics and ants in *C. scoparius* is only 5–10 m per generation. Therefore, the sevenfold increase in pollen dispersal compared to seed dispersal may reach pollen flow distances of 35–70 m in the heathland, which is in line with an average pollination distance of 64 m (with occasional pollen movement of several hundred metres) found in a Danish study of the insect pollinated *Malus* *sylvestris* (Larsen & Kjaer 2009). A few individuals in the heathland containing plastid haplotypes representing invasive *C. scoparius* were found at two sites close to the border to the invaded area. These might be the result of a few seed introductions followed by mating with plants of the native type reducing their *Q*-values. This suggests that demographic and fecundity differences, rather than incompatibility mechanisms per se, create the observed asymmetric introgression in *C. scoparius*.

The large number of individuals in the heathland with a *Q*-value above 0.5 indicates that there are mechanisms reinforcing gene flow within the heathland between individuals with high level of ancestry in the invasive gene pool. This can be a result of more efficient pollination from individuals with high level of ancestry in the invasive gene pool, possibly also in combination with transgressive segregation of invasive traits, giving hybrids an advantage in establishment and survival. Both have been invoked as explanations for asymmetric gene flow (Anttila *et al.* 1998; Rieseberg *et al.* 1999, 2003; Latta *et al.* 2007; Field *et al.* 2011). Eventually, these individuals will serve as stepping stones for introgression from the invasive genome, and over time the native heathland *C. scoparius* runs a high risk of being swamped.

In a previous study, Rosenmeier *et al.* (2013) identified a population in Villingerød that consisted of invasive types based on one plastid and five nuclear SSR markers. We re-analysed two individuals from this population and both contained the native plastid haplotype (Brandes *et al.*, unpublished). It is known from the literature that *C. scoparius* has been growing there for at least 150 years (Buchwald 2008), and it is tempting to speculate that a former native population in this area has been eradicated through swamping by the invasive *C. scoparius*, leaving only traces of the native type in form of the native plastid haplotype. This is particularly worrisome, as it may be an omen of what is also expected to happen with the native heathland population in our study and possibly other native heathland populations.

Genome invasion alters the phenotype

We found that the invading genome appears to change the phenotype of its carriers, leading to a tall, slender growth form compared to pure native individuals in the heathland that phenotypically are short and wide. Because of the higher fecundity of such tall plants (Fig. 7b), we expect the presence of a positive feedback process, whereby hybridized plants in the heathland obtain increased fitness and therefore contribute disproportionally more to the next generation. This would explain the reinforcement of gene flow from the invasive gene pool because individuals with high invasive genome content in the heathland act as stepping stones, further increasing gene flow and thus speeding up the introgression process. A similar scenario has been described in *Spartina*, where the invader *S. alterniflora* threatens to swamp the native *S. foliosa* through superior male fitness, which contributes to increased seed set in native (introgressed) individuals (Anttila *et al.* 1998).

As an example of a major invasive species, *C. scoparius* is of interest because of the potential adverse effect on the native flora both at the species and subspecies level. The spread of a species outside its native range and its potential consequences on the native flora is easy to detect, but the effects of dispersal and introgression of non-native genome into native gene pools are in most cases difficult to study. However, introgression of introduced *C. scoparius* into native populations can be detected based on the distinct genetic clusters congruent with phenotypic differences, where the change in phenotypes can have important ecological implications for local vegetation and associated fauna. Our data indicate that pure native individuals in the heathland had a height/width ratio of 0.5 compared to 1.3 among pure invasive individuals in the heathland (see Fig. 7a). The potential for changes in the heathland is therefore substantial if the short and wide native *C. scoparius* individuals are replaced by tall and slender individuals as a result of genomic invasion. In another study, we found that dense stands of invasive *C. scoparius* reduced the amount of dwarf shrubs in a heathland in Norway (Brandes *et al.*, unpublished). Specific studies quantifying the ecological consequences of introgression in invaded areas are needed, and future studies that compare sites with variable degrees of introgression are therefore important. Of further importance is to study the fitness of introgressed plants compared to plants of the pure native type in more detail. Comparison of selective advantages at different zygotic and sexual stages can be based on a combination of in situ studies supported by genetic markers and transplanting experiments with controlled crosses. In this sense, *C. scoparius* may prove to be an important model species that can contribute to the general understanding of the cryptic parts of invasive ecology.

The future for native Cytisus scoparius

We show that native and invasive *C. scoparius* most likely have a long history of hybridization and introgression in the vulnerable heathland. Indications of seed flow into the heathland, which possibly could have been facilitated by humans, are limited in this area. However, efficient gene flow via pollen from sources outside the heathland in combination with backcrossing to individuals of the invasive phenotype and high invasive genome content in the heathland makes management challenging. Removal of plants with the tall, slender phenotype in the heathland is likely to reduce the frequency of highly introgressed individuals, but caution should be taken that such operations and disturbance do *not* lead to unintended seed dispersion into the heathland by machines or humans. We expect to find similar unidirectional introgression at other localities where the two types meet. Removal of invaders from surrounding areas is important, but will require large amounts of resources and repeated efforts because seeds from the seed bank can germinate in disturbed areas. Again, special caution must be taken to ensure that such activities do not lead to seed dispersal of the invasive type into vulnerable areas, as limited natural seed dispersal capacity may be the major obstacle for seeding plants to spread rapidly into neighbouring heathlands. At present, we predict that we will see an increased degree of swamping of the native gene pool in the future. For in situ conservation, it will be important to identify those native populations that are still comfortably isolated by distance from invasive types. Any invasive types in the vicinity of such genomically conserved populations must be removed. Genomic invasion of the native *C. scoparius* may have more than genetic consequences. Negative ecological consequences may also occur in the form of a changing landscape, species composition and ecosystem functioning of heathlands.

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L.R.N., E.D.K. and S.F. designed the study. L.R.N., U.B., E.D.K. and S.F. did the field work. L.R.N. and U.B. performed genetic analyses. L.R.N., U.B., E.D.K. and S.F. analysed the data. L.R.N., U.B., E.D.K. and S.F. wrote the manuscript.

Data accessibility

Data available from the Dryad Digital Repository: doi:10.5061/dryad.p49q0.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Likelihood and Evanno Δ*K* (Evanno *et al.* 2005) plots for evaluation of most likely number of genetic groups summarized over 10 independent STRUCTURE runs for *K* = 1–10.

Fig. S2 Prior and posterior distribution of parameters estimated with DIYABC based on 36 nuclear SNP markers. Median and 95% credibility intervals are given for each parameter. *N* is the population size, *t* is the time in generations and *ra* is the admixture rate. The model used in simulations is shown in Table 1.

Table S1 Prior distribution of historical and demographic parameters used for DIYABC

Paper III

Impact of *Cytisus scoparius* on coastal heathland vegetation

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Abstract

The impact caused by biological invasions differs for each alien species, the invaded habitat and other conditions. In order to justify and prioritise the management of alien species their impact needs to be assessed for each specific case of invasion. We studied the impact caused by the *Cytisus scoparius* on a threatened coastal heathland in Norway. This shrubs ability to fix nitrogen is of particular concern for such low nutrient ecosystems. We surveyed the plant species composition in quadrats with different density of canopy cover by *C. scoparius*. We further estimated environmental conditions for each plot based on the average Ellenberg and CSR values of each species present graded by its abundance.

Our results show a slight reduction in biodiversity and pronounced reduction of most typical heathland species, including a significant reduction in cover of all dwarf shrub species. *C. scoparius* appears to increase the availability of nitrogen, reduces soil moisture and increases pH. Unexpectedly, we found more light demanding plants under the canopy of *C. scoparius.* Grime's CSR strategies changed only slightly towards less stress and more competitive strategy in the presence of *C. scoparius*.

We found no evidence of species extirpation from the invasion of *C. scoparius* into coastal heathland, but the ecosystem itself is threatened by the decline in abundance of its typical species, particularly dwarf shrubs. While some environmental impact of *C. scoparius* is expected to revert with its removal, in particular the increased soil nitrogen may require

additional management to preserve the heathland ecosystem. In general, the impact of *C. scoparius* increased with its abundance.

Introduction

Invasive species play a major part in the reduction of biodiversity globally (Murphy & Romanuk, 2014; Pereira, Navarro, & Martins, 2012). As the number of alien and invasive species is continuously increasing (Seebens et al., 2017) and measures to remove them are resource intensive, it becomes evident that eradication measures need to be focussed on the most harmful alien species (e.g. Byers et al., 2002). In order to justify and prioritise alien species management efforts, more knowledge and understanding of their impact is needed (Blackburn et al., 2014; McGeoch et al., 2016; Parker et al., 1999). Yet, type and severity of impact depends of the species that is invading as well as the invaded habitat and might change over time (Blackburn et al., 2014; Foxcroft, Pyšek, Richardson, Genovesi, & MacFadyen, 2017; Hejda, Chytrý, Pergl, & Pyšek, 2015; Mollot, Pantel, & Romanuk, 2017).

In this study, we evaluated the impact of the invasive shrub *Cytisus scoparius* (Scotch broom (L.) Link) on threatened coastal heathland in southern Norway. The species is native to continental Europe and the British Isles, but has been introduced across the globe (North- and South America, Africa, Australasia, Eurasia outside of native range) and is widely classified as invasive (Cordero, Torchelsen, Overbeck, & Anand, 2016; Downey & Brown, 2000; Mkhize, Mhlambi, & Nanni, 2013; Neubert & Parker, 2004; Srinivasan, Shenoy, & Gleeson, 2007). Following numerous introductions of *C. scoparius* to Norway (Brandes et al., unpublished), it is currently expanding its northern-most distribution range, mostly along roadsides in the coastal areas (Elven, 2018; Rosef & Heegaard, unpublished). It has currently invaded at least one coastal heathland site, and may pose an increasing threat to this vulnerable nature type. The species it is likely to continue to spread, first into man-made and then also into natural and protected habitats. It is also likely to further increase its dominance in the invaded habitats, and intensify its impact.

Coastal heathlands are semi natural ecosystems, as they require regular burning and grazing, a tradition going back millennia in Norway and Western Europe. It can be found along the coast of Norway from Lofoten to Kristiansand (Lindgaard & Henriksen, 2011), but in 1997 only 10 % of its original extent remained (Hjeltnes 1997 in Lindgaard and Henriksen 2011). Coastal heathland is important for the biological diversity at the landscape scale, because of its specific plant species and a rich invertebrate fauna including threatened species (Bargmann, Heegaard, Hatteland, Chipperfield, & Grytnes, 2016; Fremstad & Moen, 2001). In Europe, coastal heathlands extend along the Atlantic coast, southward into Spain, while Norway represents its northern most expansion and maintains a distinctive species composition (Fremstad & Moen, 2001). Coastal heathland is dominated by species that can persist burning and grazing. The soil is usually poor in nutrients and more acidic, and it can range from dry to moist water conditions (Fremstad, 1997). Typical vegetation is dominated by *Calluna vulgaris* and other dwarf shrubs but also contains some species of grasses and herbs (Fremstad, 1997). The largest threat on coastal heathland in Norway is the abandonment of its use, allowing encroachment of shrubs and trees, but also habitat fragmentation and increased nutrient input (Fremstad, 1997).

C. scoparius may interact with the heathland vegetation change the environmental conditions. We expect that this shrub would replace light demanding lower growing heathland. A major concern is an increase in soil nitrogen, as *C. scoparius* can fix nitrogen in symbiosis with rhizobia bacteria (Sajnaga, Malek, Lotocka, Stepkowski, & Legocki, 2001; Wheeler, Perry, Helgerson, & Gordon, 1979). Nitrogen fixing invasive plant species have been shown to have a dramatic impact on invaded ecosystems, and therefore may be classified as ecosystem engineers (Jones, Lawton, & Shachak, 1996, 1997; Simmering, Waldhardt, & Otte, 2001; Wright & Jones, 2006) or ecosystem transformers (Richardson et al., 2000). An increase in nitrogen availability weakens the competitive advantages of the typical heathland species that are adapted to low nitrogen environments (Borchard et al., 2017). Such changes in the soil chemistry can remain for a long time after the removal of *C. scoparius*, and may require additional soil treatments to restore coastal heathland (Slesak, Harrington, & D'Amato, 2016). Other studies on *C. scoparius* effect on soil nitrogen have found an increase in some cases (Fogarty & Facelli, 1999; Haubensak & Parker, 2004) but not always (Srinivasan, 2012). These soil measurements might underlie spatial and temporal variations and may not capture the transfer of released nitrogen that is quickly taken up by co-occurring plants with high affinity (Shaben & Myers, 2010). However, another study found that the increased nitrogen in the soil from *C. scoparius* was not taken up by *Pinus radiata* because the legume also suppressed pine growth by drying out the soil (Watt et al. 2003). This indicates how other soil characteristics affect the nitrogen availability; for example, soil texture is affecting nitrogen leaching (Slesak et al., 2016).

Environmental conditions can change across short distances and fluctuate rapidly, thus it can be challenging to measure them directly. If such changes continue over an extended period, the plant community will respond accordingly. As plants are commonly adapted to specific environmental conditions, in which they have an optimal growth and fitness, we can use the change in abundance of plant species, in combination with each species' environmental preferences, to interpret changes in environmental conditions (Diekmann, 2003; Ellenberg, Düll, Wirth, Werner, & Paulissen, 1992). Similarly, we expect that these changes correlate with the change in the levels of environmental stress, disturbance and plant-plant competition, and thus cause a shift in species abundances according to their specific ecological strategies, according to Grime (J. Grime, Hodgson, & Hunt, 1988; J Philip Grime, 1974).

This study tests the impact of *C. scoparius* invasion on the vegetation composition in a Norwegian coastal heathland. We hypothesize that *C. scoparius* : a) reduces species diversity, b) reduces the abundance of the typical heathland species (*Calluna vulgaris, Vaccinium uliginosum*, *Deschampsia flexuosa*,*Empetrum nigrum, Salix repens, Vaccinium vitis-ideae*, *Anthoxantum odoratum, Carex nigra and Carex pilulifera*), c) changes local environmental conditions, such as increased shade, soil nitrogen, and pH but decreased soil moisture. We measure the environmental impact indirectly from the ecological requirements of the vegetation observed, following the concepts of Ellenberg and Grime.

Methods

Study area

The study area is situated coastal heathland ecosystem of Einarsneset (58° 3'46.89"N, 6°47'16.01"E), a small peninsula of approximately 1 km^2, located at the southern coastline of Norway, in the Farsund municipality, Vest-Agder county (Figure 1). Coastal heathland dominates the interior of Einarsneset, with a transition to sand dune vegetation towards the shoreline. Einarsneset is legally protected due to its high diversity of plants and bird species. The particular site was one of the 23 coastal heathland reference areas for Norway (Kaland & Kvamme, 2013). It has been considered unique to Norway because it contains *C. scoparius* at its northern most distribution. However, *C. scoparius* has colonised vigorously since the 1970s and is now recognised as an alien introduction (Brandes et al., unpublished; Elven, 2018; Kaland & Kvamme, 2013). After the main field season of this project in 2013, the county governor of Agder started a management project trying to remove *C. scoparius* from Einarsneset.

Einarsneset is situated in the nemoral vegetation zone and the highly oceanic vegetation section with mild climate for Norway (Moen, 1999). The heathland of Einarsneset groups with the Calcium-poor coastal heathland (T34-C-2) based on the Norwegian classification of nature types (Bratli et al., 2017). The closest meteorological station is Lista Fyr, which recorded yearly average temperatures of 7.4 °C, with February as the coldest month with average of 0.5 °C and August as the warmest month with average of 14.6 °C (Aune, 1993). The annual average precipitation is 1147 mm, with April as the driest month with an average precipitation of 58 mm, and October as the average wettest month with 151 mm (Førland, 1993). The plant growing season (temperatures over 5 °C) extends to over 210 days (Moen, 1999). The bedrock consists of amphibolite and gneiss and the soils consist of exposed bedrock and sand low in nutrients (Geological Survey of Norway, www.ngu.no).

Field survey

We surveyed the vegetation of the costal Einarsneset heathland invaded by *C. scoparius* to compare the species composition with and without *C. scoparius* shrubs in 2012 and 2013.

We focussed on the impact of mature shrubs of *C. scoparius*, which have developed a canopy of 0.5 to 2 m height. Some of the mature shrubs of *C. scoparius* grew as solitary shrubs surrounded by the heathland, while other grew as thickets of many shrubs densely clustered together. We distinguished between single shrubs or thickets, as they may differ in their impact.

Quadrats to survey were placed on transects that cover equal lengths with and without mature shrubs of *C. scoparius.* The placement of the quadrats along the transects was adjusted for individual shrubs or thickets of shrubs as described below (Figure 2). For each single shrub, a total of four quadrats were placed along one transect, which crosses the trunk of the bush (Figure 2). Two quadrats were placed on the transect directly at both sides of the trunk of the shrub, thus below the canopy of *C. scoparius* (partial canopy). The other two quadrats were placed at 1 m distance from the central quadrats and to the periphery of the transect, thus outside the shrub canopy (no canopy). For thickets (groups of shrubs) of *C. scoparius*, each transect was placed perpendicular across the edge of the thicket, and each transect had five quadrats with 1 m distance between them (Figure 2). The central quadrat was placed at the edge of the *C. scoparius* thicket (partial canopy), two quadrats where placed further into the thicket (full canopy), while two quadrats were placed outside of the thicket (no canopy). On larger thickets, several transects where placed at least 5 m apart. In one case, only one quadrat was measured within the thicket, because a second quadrat would have been too close to the other transects. The number of quadrats sampled in each class of *C. scoparius* cover differed and full canopy quadrats were only present in thickets. (Table 1, Figure 2). We surveyed 20 transects on solitary shrubs and 25 transects on ten different thickets, resulting in 204 quadrats. The size of the quadrats was 25 cm x 25 cm, divided into 16 sub-quadrats. Only vascular species where recorded and their abundance was estimated by counting the presence of each species in the 16 sub-quadrats. Vascular plants were identified to the species level, when possible (Lid & Lid, 2007).

Data analysis and plant species indicator values (Ellenberg, Grime)

We explored for three types of impact of *C. scoparius* shrubs on the heathland vascular vegetation: a) overall species richness and diversity, b) abundance of typical heathland species, c) local environmental conditions as estimated based on Ellenberg species indicator values and Grime CSR strategies.

For the impact on species diversity (a) we evaluate species richness by the number of species in each quadrat and species diversity as the Shannon-Weaver diversity index (M. O. Hill, 1973). The latter was calculated in the VEGAN package (Oksanen et al., 2018).

In the assessment of (b) the typical heathland species of our dataset we selected *Calluna vulgaris, Vaccinium uliginosum*, *Deschampsia flexuosa*,*Empetrum nigrum, Salix repens, Vaccinium vitis-ideae*, *Anthoxantum odoratum, Carex nigra and Carex pilulifera* (based on Bratli et al. (2017). Additionally, the effect of Cytisus cover on the number of *C. scoparius* seedlings was observed, to see if the presence of mature *C. scoparius* fosters further establishment.

In addition, we assessed (c) the abiotic environmental conditions as indicated by the vegetation composition. Each plant species has an ecological optimum along the environmental gradients. These optima are given by the Ellenberg indicator values (EIV) for light, moisture, pH (reaction) and nitrogen (Ellenberg et al., 1992; Mark O Hill, Mountford, Roy, & Bunce, 1999), and the environmental condition of each plot is calculated as the weighted average, using the abundance of each species as weight. EIVs range as integers from 1 to 9 or for moisture from 1 to 12, where low values represent low requirements of this resource or for the pH value the ability to grow in more acidic soils (Mark O Hill et al., 1999). Using the plant species as indicators for environmental conditions we can assess the effect of *C. scoparius* viewed by the environmental traits of the different species combined. Hence, we gain insight into which factors are structural important regarding the species composition.

To infer how *C. scoparius* may influence the dynamics of a species composition is to assess the structural differences in species strategies under the various conditions of *C. scoparius* canopy cover. Here we applied the competitive - stress tolerant – ruderal strategies (CSR) developed for vascular plants (J Philip Grime, 1974; J. P. Grime, 1977). Grime classified plant species by their traits and how they can cope with different levels of the three main conditions of: competition (C), stress (S) and disturbance (R). Environments vary with respect to the level of fertility and the degree of disturbance. The combination of low fertility and high disturbance environments eliminates plant growth, whereas high fertility and low disturbance environments allow highly competitive plants to thrive (C-strategy), high fertility and high disturbance favours ruderal plants (R-strategy) and low fertility with low disturbance favours stress tolerant species (S-strategy) (J. Grime et al., 1988). For this study we sourced the CSR values for each species from Hunt et al. (2004); Pierce et al. (2017) and Hanslin & Kollmann (2016); Riibak et al. (2015). The weighted average CSR values were calculated for each quadrat based on the species CSR values in the quadrat. These CSR values per plot were visualised in ternary plots using the packages ggplot2 v.2.2.1 (Hamilton, 2018) and ggturn v.2.2.1 (Wickham, 2016).

Statistical modelling

We further tested if the differences in vegetation composition in the presence of mature *C. scoparius* are statistically significant. The statistical tools follow a Bayesian inference, and we applied an Integrated Nested Laplace Approximation to establish the posterior distributions of the effects specified in the models (Blangiardo & Cameletti, 2015). These models tested differences of either two (cover vs. no cover) or three (full cover, partial cover or no cover) classes of density and of the two types of transects (transect on a single shrub or transects on a thicket of shrubs; Figure 2, Table 1). Transect identity was used as a random contribution, to control for effects associated with grouped observations. The model for species abundances uses a binomial distribution for each of the 16 subplots per quadrat, with an added quadrant-specific random contribution working as a dispersion parameter. The model was applied for the more abundant of the typical heathland species (presence in more than 30 quadrats). The best fitting models based on the lowest

Watanabe's information criterion (WAIC) were chosen for further estimation of the level of significance and the direction. The models for the Ellenberg indicator values (EIV) assumes a Gaussian distribution. All data visualisations and statistical analysis was conducted in R v.3.5.0 (R Core Team, 2018).

Results

In the 204 quadrats, we identified 54 species of vascular plants.

a) Species richness (i.e. number of species) and species diversity (Shannon-Weaver) were both significantly negatively affected by the presence of *C. scoparius* shrubs, and this effect was stronger for thickets than for single shrubs (Figure 3, Table 2 and 3). Three quadrats were bare of vascular plant species at ground level and instead where either completely covered in litter or partly in litter and partly in bryophytes. Two of those quadrats were under dense canopy of *C. scoparius*, the third was under dense *Calluna vulgaris* cover but without *C. scoparius*. The highest number of 15 taxa was observed in two quadrats with no *C. scoparius* shrubs. However, the differences in species richness and diversity are not very large (Figure 3). Those species that were absent from dense cover of *C. scoparius* but relatively common (found in more than 5% of the survey plots, i.e. more than 10) were mostly species more typical for semi-natural meadows not heathland (*Luzula multiflora, Plantago maritima, Hieracium pilosella*).

b) In this survey, we found lower abundances of the typical heathland species, under *C. scoparius* (Figure 4). All dwarf shrubs (*C. vulgaris, E. nigrum, S. repens, V. uliginosum and V. vitis-ideae*) and half of the graminoids (*A. odoratum, C. nigra*) declined in mean abundance (Figure 4). This decline correlated with the three classes of increasingly dense canopy cover of *C. scoparius* shrubs (Figure 4). Only two graminoid species (*D. flexuosa, C. pilulifera*) followed no clear trend. Four species (*C. vulgaris, E. nigrum, S. repens, A. odoratum*) were sufficiently abundant for modelling and their reduced abundance with increasing canopy of *C. scoparius* is significant at least between two of the three classes (Table 2 and 3, Figure 4). The best fitting model for each species is described in the following with significant levels at $p < 0.05$. *Calluna vulgaris* abundance was significantly lower under partial canopy of *C. scoparius* compared to no canopy and again under full canopy compared to partial canopy (Table 3, Figure 4). *Empetrum nigrum* showed significant differences between no canopy to partial canopy cover but no difference between partial and full canopy, and there was a significant lower abundance of *E. nigrum* in quadrats on thickets compared to single shrubs (Table 3, Figure 4). *Salix repens* abundance was significantly lower under *C. scoparius* canopy of any density compared to no canopy, and was also lower in quadrats on transect on thickets compared to transects on single shrubs (Table 3, Figure 4).

The opposite trend was apparent for the abundance of *C. scoparius* seedlings, which significantly increased in quadrats under *C. scoparius* canopy of any density compared to quadrats without canopy, and also for transects on thickets compared to those on single shrubs of *C. scoparius* (Table 3, Figure 4).

c) The analysis of the Ellenberg indicator values (EIV) indicated that *C. scoparius* does affect the environmental conditions (Table 2 and 3, Figure 5). Unexpectedly, the presence of mature *C. scoparius* increases the frequency of species with higher EIV for light significantly ($p<0.05$), i.e. light demanding species appeared to grow better under *C. scoparius* canopy (Table 2 and 3, Light indicator in Figure 5). There was no difference between transects on single shrubs and on thickets or between partial and full canopy of *C. scoparius*. The mean values of EIV for nitrogen resulted in significant changes across the three classes (no, partial or full) of *C. scoparius* canopy cover (Table 2 and 3, Nitrogen indicator in Figure 5). The denser the canopy, the more abundant were species with higher affinity to nitrogen. Beyond this, we found no significant difference in the average EIV values for nitrogen between the transects on single shrubs and on thickets. The mean values of EIV for moisture were significantly lower for transect on thickets than on single shrubs and within thicket transects they were significantly lower under *C. scoparius* than outside (Table 2 and 3, Moisture indicator in Figure 5). The EIV for pH is significantly higher under the canopy of *C. scoparius* on transects across thickets (meaning plants prefer less acidic soils), but not significantly different for transects across single shrubs outside (Table 2 and 3, pH indicator in Figure 5).

The three CSR plant strategies by Grime (1974), calculated as weighted averages for each plot, show slight changes in correlation with the three classes of *C. scoparius* canopy cover (Figure 6). In the absence of *C. scoparius,* most quadrats show a higher frequency of stress tolerating plants with plot average values in the upper range between 40 and 70 % (Figure 6a). With increasing cover of *C. scoparius,* the number of plots with high averages for stress tolerance (S > 50 %) is decreasing and more plots resulted in lower averages of stress tolerance (S < 40 %; Figure 6b and 6c). This indicates an overall decrease of stress tolerating strategies in plants with increasing *C. scoparius* cover. The frequency of competitive strategists averaged by plot (C) only slightly increased with increasing *C. scoparius* canopy, with some plots averaging C-values over 50 % in dense canopy only (Figure 6). Finally, plots with extreme low frequencies of ruderal tolerating plants (R < 10 %) are much more common for plots without *C. scoparius* cover than with partial or full cover (Figure 6).

Discussion

The aim of this study was to explore if the presence of *C. scoparius* in a coastal heathland in southern Norway has an impact on this ecosystem (vegetation and environmental conditions) and to characterise this impact further. This species has been of concern for heathland conservation as it has expanded its range and become locally dominant in recent decades. Our results indicate that *C. scoparius* causes a shift in the plant community composition and in environmental conditions, such as nitrogen availability, soil moisture and pH, and light (however it appears to promote light demanding species). In particular, the abundant and typical heathland species (mostly dwarf shrubs) were significantly reduced with increasing *C. scoparius*. Species richness and diversity was also reduced to some degree, which matches the general view that invasive species reduce biodiversity (Hejda, Pyšek, & Jarošík, 2009; Jucker, Carboni, & Acosta, 2013; Mollot et al., 2017; Pyšek et al., 2012).

The decline in heathland species can be the result of direct or indirect impact of *C. scoparius*. Direct impact might be by competition for resources or by excreting secondary compounds that inhibit growth in other plant species (allelopathy). Our analysis of the plants C-S-R strategies (J. Grime et al., 1988) showed some increase in the average competition strategy in the presence of *C. scoparius*, indicating that its invasion increases competitive interactions between the remaining plants. *C. scoparius* has high competitive abilities considering its physiological characters, such as its taller growth compared to other heathland species and its deep reaching roots (Simmering et al., 2001). Whenever mineral nitrogen is available, it can reduce the energetically costly fixation of atmospheric nitrogen by rhizobia (Pérez-Fernández, Calvo-Magro, Rodríguex-Sánchez, & Valentine, 2017). Yet, if necessary, it can fixate atmospheric nitrogen in other limited environmental circumstances than many other plants, such as limited phosphorus (Pérez-Fernández et al., 2017), or when it sheds leaves during drought or winter (Wheeler, Helgerson, Perry, & Gordon, 1987; Wheeler et al., 1979). The species' ability to photosynthesise efficiency in its stem tissue gives more flexibility to grow under adverse environmental conditions, such as cold temperatures, drought or high herbivory (Bossard & Rejmanek, 1992). Apart from its high competitive ability, *C. scoparius* has been shown to produce allelopathic compounds (Gresser, Witte, Dedkov, & Czygan, 1996; Wink et al., 1983) and this has been used to explain its negative effect on the growth of other plant species, grown in the soil (Haubensak & Parker, 2004) or litter (Sara Grove, Haubensak, & Parker, 2012) from *C. scoparius*. Grove et al. (2012) found indications that *C. scoparius* might affect the mycorrhizal community structure in the soil and their interaction with other plant species. The application of *C. scoparius* leaf litter reduced ectomycorrhizal fungi in the soil and on roots of Douglas-fir trees (S. Grove, Parker, & Haubensak, 2017). While *C. scoparius* itself associates mostly with arbuscular mycorrhiza, most heathland species grow in symbiosis with ericoid mycorrhiza (Finlay, 2008). Unfortunately, we did not analyse the soil microbial community.

This study however, indicates that the change in species composition may be affected indirectly by the environmental changes caused by *C. scoparius*. Heathlands usually develops in conditions of poor nutrients, low pH and high light availability (Böcker, Bornkamm, & Kowarik, 1983; Borchard et al., 2017; Saure, Vandvik, Hassel, Vetaas, &

Scheiner, 2013). The encroachments of shrubs and trees into the heathland threaten to increase shading on the lower growing heathland species, but we observed the opposite for *C. scoparius*. The Ellenberg values indicated on average more light demanding species under *C. scoparius* than outside. While *C. scoparius* grows taller than any native heathland species, those heathland species can also develop dense canopies that shade other plants, especially at juvenile stages. In particular, *Calluna vulgaris* forms a very thick and light impermeable canopy. *C. vulgaris* is much more abundant where *C. scoparius* is absent, so the shading effect of the former might outweigh the latter. In comparison to *C. vulgaris,* the canopy structure of *C. scoparius* is much more open. Simmering et al. (2001) observed a high variability of the light conditions under *C. scoparius* canopy and found some species with high light requirements persisting even under dense canopy. The shading intensity of *C. scoparius* also varies over time and dense stands of this shrub usually thin out within a few years as the individuals shrubs become senescent and die back (Simmering et al., 2001). In addition, *C. scoparius* may shed its leaves in response to drought, cold temperatures or herbivory (Bossard & Rejmanek, 1992). This may allow for sufficient light reaching the ground level vegetation for time periods long enough to enable light demanding plants to persist, though they might not be able to reproduce and under long term cover from *C. scoparius*. It is also possible that the shift of EIV for light we observed, is not directly affected by shading from *C. scoparius*, but is a trade-off for other changes in environmental conditions, such as nutrients. However, the species' EIV for light and nitrogen do not correlate (data not shown).

The invasion of *C. scoparius* into the heathland is of particular concern for heathland conservation, because it can undergo symbiosis with nitrogen fixing bacteria. Our results show a shift in vegetation composition towards more nitrogen demanding species in the presence of *C. scoparius*, indicating an increase in nitrogen availability for co-occurring species. This was also observed for *C. scoparius* expansion in its native range, based on the same methodology (estimating Ellenberg Nitrogen values per plot based on the vegetation composition) by Simmering et al. (2001). Comparing our results with other studies that resulted in either nitrogen increase or no changes, might help to entangle the additional aspect, which affect if and how *C. scoparius* influences nitrogen input and flow in invaded ecosystems (see introduction). Broadbent et al. (2017) further showed that soil nitrogen increase correlates gradually with increasing density of *C. scoparius*, matching with our results. The typical heathland species are adapted to low nitrogen availability (Ellenberg nitrogen values of 1-3) (Mark O Hill et al., 1999) and will be outcompeted by species that can grow quicker and thus use up any available nitrogen source (Fogarty & Facelli, 1999).

Low soil moisture might play a minor role for heathland species, but in this study *C. scoparius* caused the plant composition to shift towards less soil moisture demanding species abundances. Previous studies in forestry have shown that *C. scoparius* dries out the soil (Michael S Watt, Whitehead, Mason, Richardson, & Kimberley, 2003; M. S. Watt, Whitehead, Richardson, Mason, & Leckie, 2003). Water is taken up by the shrubs' deep rooting system (Simmering et al., 2001). We also found that thickets had a larger effect on decreasing soil moisture than the single shrubs, indicating that a high density of *C. scoparius* may affect soil water beyond its above ground cover.

Simmering et a. (2001) has shown that *C. scoparius* can transfer cations from deeper to higher soil layers with its deep reaching roots (Simmering et al., 2001). This may explain our results, that dense thickets of *C. scoparius* slightly increased the EIV for soil pH. We did not take direct measurement of pH values. The changes in plant composition leading to our observation of increased EIV for soil pH may be caused indirectly as plants with higher nitrogen requirements are more likely indicators for less acidic soils. Increased pH could pose a minor threat to the heathland ecosystem, as all but one of the characteristic species (based on T34-C-2 in Bratli et al. (2017)) are adapted to more acidic soils (EIV for pH: 2–4, where 0 is extremely acidic and 9 is basic; J. Grime et al., 1988; Mark O Hill et al., 1999). Böcker et al. (1983) estimated a EIV for pH of 2.4 for a Calluno-Vaccinietum plant community. While *C. scoparius* prefers a lightly acidic pH of four, several grasses and herbs indicating more neutral of alkaline soil increase their abundance in dense thickets of the shrub in this study. Other studies on soil pH changes by *C. scoparius* invasion have either resulted in increase in pH (Mohr, Simon, & Topp, 2005) or in the opposite case, a reduction of cations (Slesak et al., 2016).

The invasion of *C. scoparius* causes some shifts in the occurring plant strategies according to competition (C), stress (S) and disturbance (R), as defined by Grime (1974). These changes match our findings based on the Ellenberg indicator values. Heathland has been described as a high stress habitat in the literature, because it is poor in nutrients and high in acidity (Böcker et al., 1983; Borchard et al., 2017). Eight of the nine typical heathland species in Einarsneset are primarily adapted to the stress strategy (S=0.75 for *C. vulgaris, D. flexuosa, V. uliginosum, V. vitis-ideae* and *C. nigra*; S=0.66 for *C. pilolifera*; S=0.5 for *E. nigrum* and *S. repens*; where 0 is lowest and 1 is highest adaptation; Hunt et al., 2004; Pierce et al., 2017). Quadrats without *C. scoparius* had relatively high stress values which were decreasing with increasing cover of *C. scoparius*. This could be explained by the increased nitrogen availability and increase in pH, both of which we observed with the Ellenberg indicator values and discussed above. The reduction of stress tolerant plants also matches with the positive effect of *C. scoparius* on light, observed in this study. J. P. Grime (1977) has included shading as an aspect of stress in an environment. The observed reduction in soil moisture by *C. scoparius* appear to play none or a minor role as a stress factor. Most of the same shifts in Ellenberg values, which can explain the reduced stress tolerance with *C. scoparius* invasion, can also explain the increased competitive strategy in the plant composition. Reduced stress usually allows for higher productivity and that again increases the requirement for competitive strategies. Thus, increased nutrient availability and the more benign soil pH can explain the observed reduction of plants with low competitive abilities. Heathland species are less competitive, as they grow slowly and instead invest into durable and persisting organs (e.g. foliage). Further, *C. scoparius* itself is defined as a SC strategist (Hunt et al., 2004). In the vegetation survey, we did not record the mature shrubs of *C. scoparius* but included the seedlings, and they are more abundant under the shrubs. Disturbances in the heathland stem primarily from human management such as burning and cutting. With increasing abundance *C. scoparius* the decrease of quadrats resulting in less than 10% of average disturbance strategy might be due to the increase in fire intensity with *C. scoparius* shrubs. Most typical heathland species are not adapted to strong disturbance which are typical for ruderal habitats (Hunt et al., 2004) and are stronger S-strategists than R-strategists (see Appendix S1 in Supporting Information).

There are shortcomings of our study design, as it allows only for correlative relationships between the abundance of *C. scoparius* and that of the native species, as well as the environmental conditions. We have no knowledge about the past changes in the distribution of *C. scoparius* and if it really has the possibility to expand further and to grow on our control quadrats, which were free of mature shrubs at the time of the survey. Thus, we cannot rule out the possibility that the difference in species composition and Ellenberg indicator values under *C. scoparius* shrubs may have been caused by other factors. Small-scale environmental variations might have been present before the spread of *C. scoparius* and may affect its establishment as well as other species' abundance. During the survey we tried to place the transects across locations that were similar in soil characteristics, slope and aspect, but we could not consider temporary differences, such as periodical flooding. We also avoided sampling in plant communities different from heathland, to keep our focus on this ecosystem. We assumed that *C. scoparius* is able to grow in the entire area that was subject to our survey. We cannot completely exclude the possibility that environmental conditions and also the abundance of heathland species might suppress the establishment and growth of *C. scoparius*. For example, heathland species are known to produce allelopathic compounds in their leaves as well (González et al., 2015). However, seedlings and small plants of *C. scoparius* have been commonly observed under mature plants of *C. vulgaris*, thus they appear to survive in competition with dense *C. vulgaris* abundance.

Conclusions

We could show that the invasion of *C. scoparius* causes impact on the coastal heathland ecosystem in southern Norway. While we have no indication of species' extirpation, the heathland habitat is degraded by *C. scoparius* because its dominant and typical species are reduced. However, plants can often persist for long times under unfavourable conditions, thus extirpations may occur in the future.

The degradation of the protected coastal heathland by *C. scoparius* invasion should justify its removal efforts. Timely removal may minimise the impact caused. After the removal of *C. scoparius* shrubs and seedlings, the legacy of its impact needs to be considered. Especially the increased nutrient availability to the co-occurring vegetation and increased pH might require additional management to restore the coastal heathland ecosystem. *C. scoparius*' symbiotic rhizobia may also affect the soil microbial community and their interaction with other plant species, and monitoring this would also be beneficial.

Studying the impact of invasive species must be based on case by case studies, considering the invasive species as well as the invaded ecosystem and additional conditions. Impact can be the result of several direct and indirect effects of invasion and they may interact. Impact can also differ based on density and time of the invasion and may prevail after the removal of the invasive species. More case studies and more focus on the complex system of interactions may help to understand the overall question on how invasive species affect native ecosystems.

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Tables

Table 1 Number of quadrats classified in groups of different density (no canopy, with canopy and the latter split into partial canopy or full canopy) and different type (single shrub or thicket) of C. scoparius *canopy. Not that due to the smaller size of shrubs they do not provide full canopy cover.*

Number of quadrat/ Type of *C. scoparius* cover	**No canopy cover**	**With canopy cover**		**Total**
		Partial canopy	**Full canopy**	
Single shrub	40	40	0	80
Thicket of shrubs	50	25	49	124
Total	90	65	49	204

Table 2 Watanabe's information criterion (WAIC) for modelling the effects from Cytisus scoparius *canopy. The lowest value (marked in bold) represents the best fitting model. The following models were tested: Intercept (effect of each transect), Thicket (transect on thickets versus transects single shrubs), Canopy (quadrats with versus without C. scoparius canopy), T + C (additive effect of Thicket and Canopy), T * C (interactive effect of Thicket and Canopy), Can3 (three classes of canopy of C. scoparius: no, partial or full), T + Can3 (additive effect of Thicket and Can3), T* Can3 (interactive effect of Thicket and Can3). See the methods section for more information on the modelling.*

	Calluna vulgaris	*Empetru m nigrum*	*Salix repens*	*Descham p-sia flexuosa*	*Anthoxan -tum odoratum*	*Cytisus scoparius* seedlings	Species Richness	Shannon-Weaver div idx	Light	Nitrogen	Moisture	Reaction/ pH
Intercept	777.973	667.374	228.986	504.183	291.970	318.568	857.626	239.278	230.500	475.300	391.900	591.000
Thicket	805.849	657.143	228.577	343.942	292.343	310.461	857.626	239.660	232.400	474.000	391.000	590.400
Canopy	610.897	548.728	227.554	508.138	**291.492**	315.229	828.307	208.019	**221.700**	419.100	371.100	553.700
T + C	605.572	537.055	226.925	344.090	291.989	307.411	828.598	208.367	224.000	420.200	369.000	553.300
T * C	583.991	529.978	**225.331**	485.290	293.720	307.495	**825.672**	**206.441**	223.100	420.500	**364.300**	**544.300**
Can3	**575.399**	525.092	228.565	356.841	292.790	306.061	829.487	210.375	225.250	**418.370**	367.960	548.780
T + Can3	575.920	522.439	227.582	510.934	293.144	304.290	829.855	210.843	226.050	418.770	366.150	548.830
T * Can3	579.980	**521.066**	226.119	**340.705**	292.577	**300.993**	827.911	208.643	225.770	421.660	365.850	545.480

Table 3 Results of the best fitting model (based on table 2) under different cover of Cytisus scoparius *canopy cover. Values in bold are significant results at p≤0.5. The following models were tested: Intercept (effect of each transect), Thicket (transect on thickets versus transects on single shrubs), Canopy (quadrats with versus without* C. scoparius *canopy), T * C (interactive effect of Thicket and Canopy), Can3 (effect of the three classes of canopy of* C. scoparius*: no, partial or full), T * Can3 (interactive effect of Thicket and Can3).Expe: expected values; P (E<0): the probability that the effects are less than zero.*

	Calluna vulgaris		*Empetrum nigrum*		*Salix repens*		*Deschampsia flexuosa*		*Anthoxantum odoratum*		*Cytisus scoparius* seedlings	
	Expe	P(E<0)	Expe	P(E<0)	Expe	P(E<0)	Expe	P(E<0)	Expe	P(E<0)	Expe	P(E<0)
Intercept	-0.961	**0.947**	-1.361	0.942	-4.690	**1.000**	-7.675	**1.000**	-6.147	**1.000**	-7.212	**1.000**
Thicket			-0.815	0.754	-5.619	**1.000**	1.934	0.091			2.614	**<0.001**
Canopy					-2.429	**0.023**			-1.406	**0.994**		
T * C					2.422	**0.023**						
Can3.1	-3.536	**1.000**	-1.954	**0.996**			-1.068	0.857			2.362	**0.001**
Can3.2	-10.190	**1.000**	-4.784	0.585			-0.321	0.506			0.591	0.490
T*Can3.1			-2.498	**0.978**			-0.285	0.579			-3.525	**1.000**
T*Can3.2			-4.784	0.585			-0.321	0.506			0.591	0.490

	Species richness		Shannon-Weaver div idx		Light		Nitrogen		Moisture		Reaction (pH)	
	Expe	P(E<0)	Expe	P(E<0)	Expe	P(E<0)	Expe	P(E<0)	Expe	P(E<0)	Expe	P(E<0)
Intercept	4.900	**<0.001**	1.275	**<0.001**	7.272	**<0.001**	2.410	**<0.001**	5.525	**<0.001**	3.540	**<0.001**
Thicket	0.660	0.156	0.095	0.240					-0.359	**0.989**	0.130	0.310
Canopy	-0.650	**0.962**	-0.167	**0.981**	0.170	**0.001**			-0.127	0.849	0.308	0.054
T * C	-0.949	**0.978**	-0.186	**0.963**					-0.387	**0.992**	0.755	**0.001**
Can3.1							0.486	**<0.001**				
Can3.2							0.791	**<0.001**				

Figures

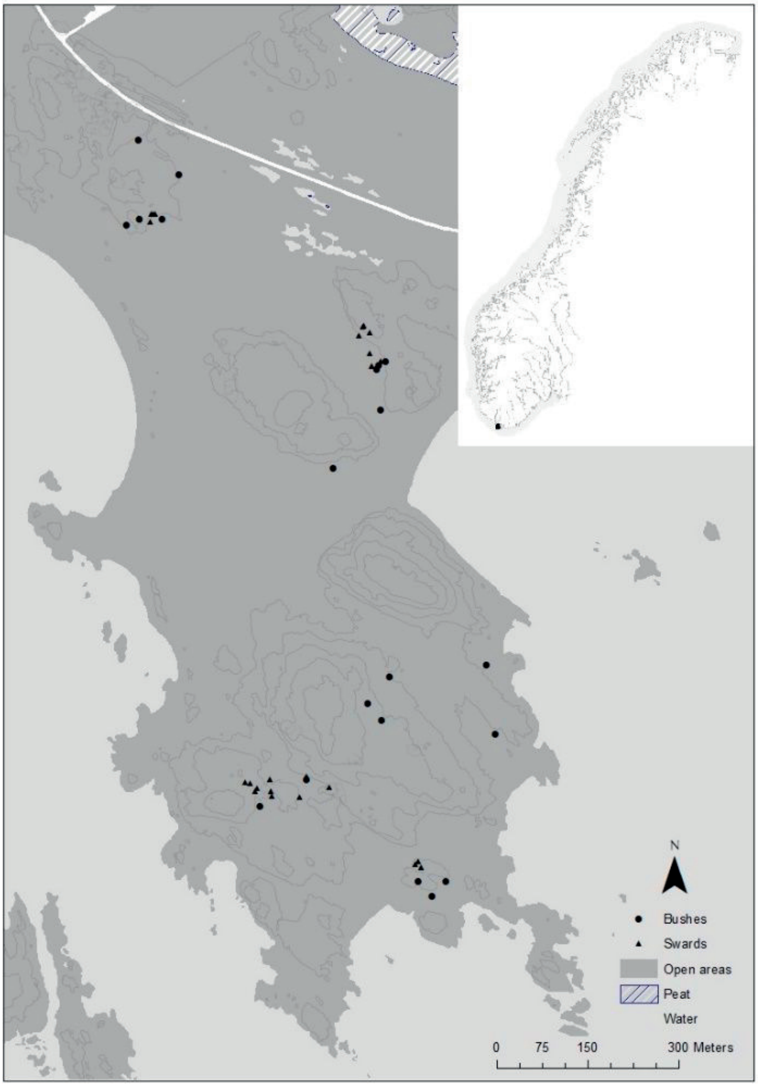


Figure 1 Maps of Einarsneset with location of transects across individual shrubs (circles) and across thickets (triangles).

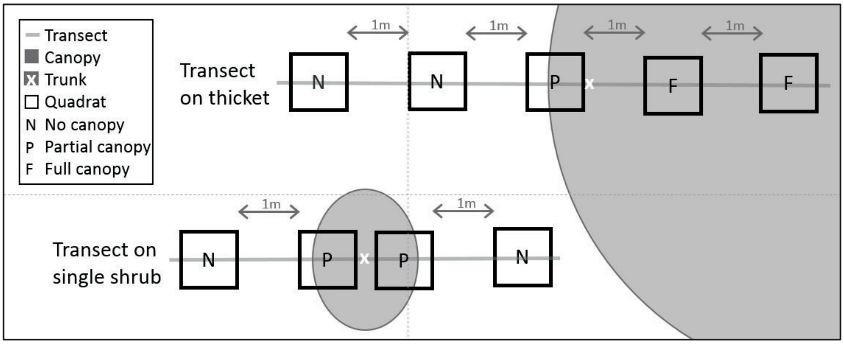


Figure 2 Experimental setup of vegetation survey. Grey areas represent areas that are covered in canopy of Cytisus scoparius *of either a single mature shrub or a thicket of several mature shrubs. Transects were aligned either with the trunk of a single shrub of* C. scoparius *at the centre (for single shrubs) or with the trunk of a shrub at the edge of a larger thicket of* C. scoparius *and running perpendicular along the edge of the thicket. The surveyed quadrats can be grouped into three classes (N: No canopy, P: Partial canopy, F: Full canopy) or in two classes (N: No canopy, P and F: With canopy).*

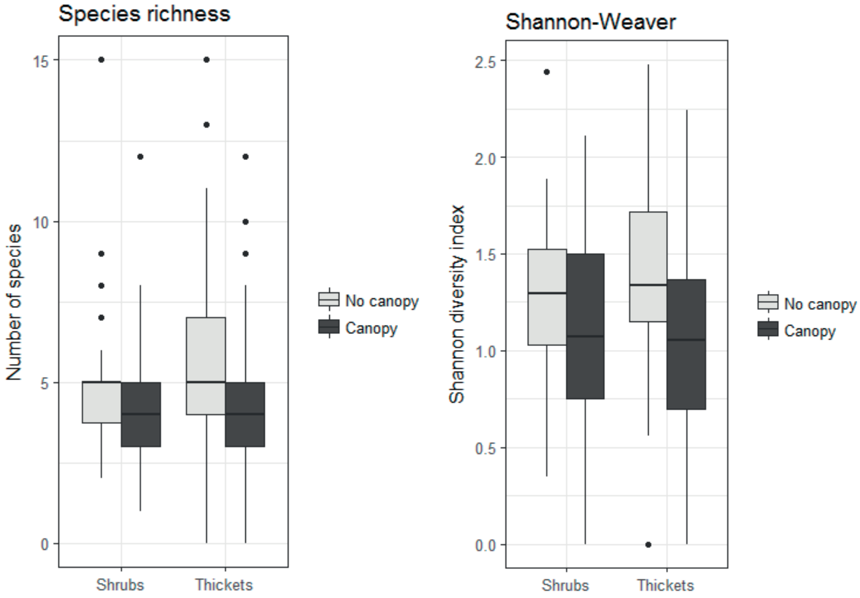


Figure 3 Species richness and Shannon-Weaver diversity index of the quadrats grouped by transects across single shrubs or thickets, and divided into quadrats with or without canopy of Cytisus scoparius*.*

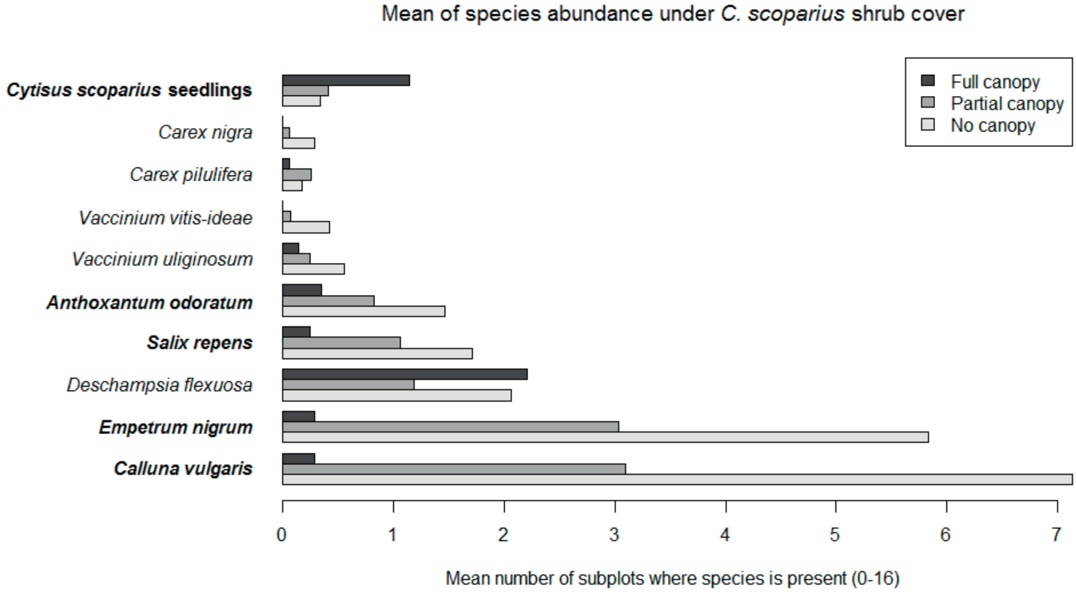


Figure 4 Mean abundance (number of subplots present) of typical heathland species in quadrats under no, partial or full canopy cover of Cytisus scoparius*. Species in bold showed significant differences (p<0.05) between at least two of the groups.*

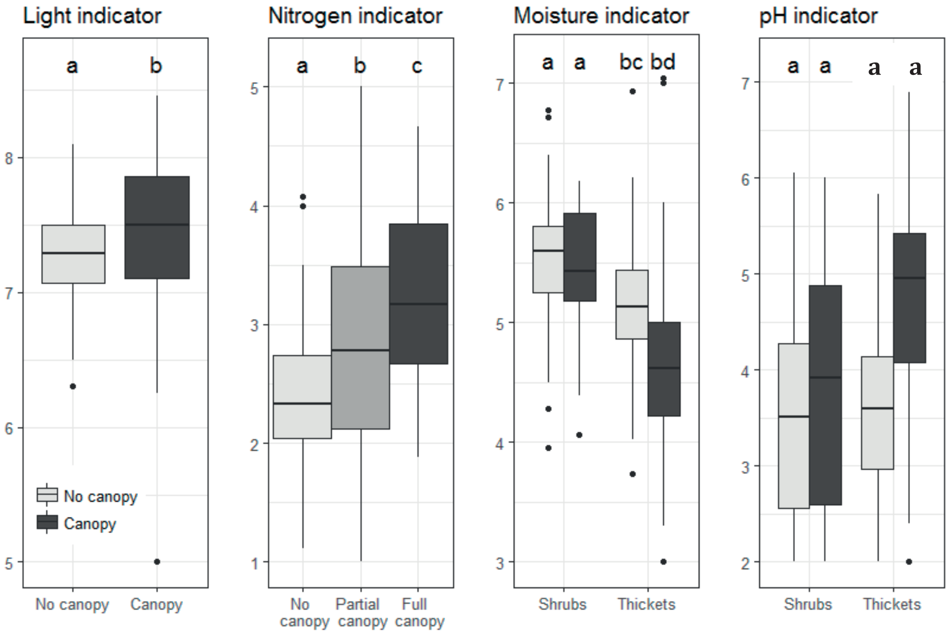
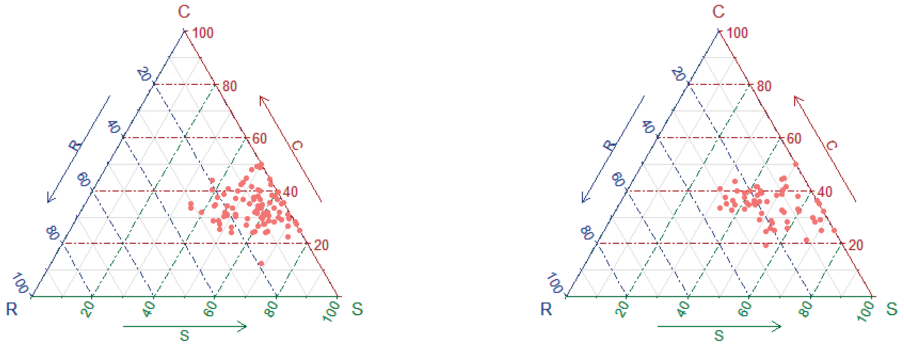
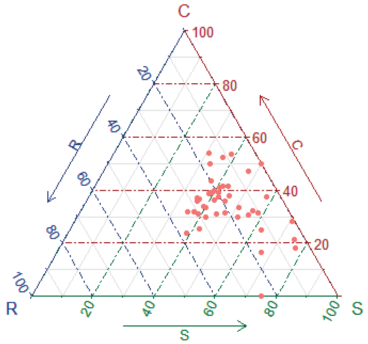


Figure 5 The graded averaged plot values of four Ellenberg indicator values (EIV) as boxplots: Light, nitrogen, moisture and pH. EIV range from 1 (lowest) to 9 (highest), except for moisture which ranges from 1 to 12 (see Mark O Hill et al. (1999) for details). The quadrats are grouped by Cytisus scoparius *cover type, based on the best fitting model. The best fitting models were: for light: presence or absence of* C. scoparius *canopy; for nitrogen: no partial or full canopy cover of* C. scoparius*; for moisture and pH: presence or absences of* C. scoparius *canopy, further distinguished between single shrubs or thickets (see also Table 2).*



a) No canopy of *C. scoparius*

b) Partial canopy of *C. scoparius*



c) Full canopy of *C. scoparius*

Figure 6 Graded averaged CSR values per plots with (a) no, (b) partial or (c) full canopy of Cytisus scoparius*.*

Supplementary material

S1 List of species with Ellenberg values and Grime's CSR values

Species	**Sum of abundancies**	**Sum of quadrats present**	**Light**	**Moist**	**pH**	**Nitrogen**	**C**	**S**	**R**	**Strategy type**	**Reference**
Carex arenaria	888	122	8	3	5	2	0.417	0.417	0.167	SC/CSR	Hunt et al. (2004)
Calluna vulgaris	857	93	7	6	2	2	0.250	0.750	0.000	S/SC	Hunt et al. (2004)
Festuca rubra	815	100	8	5	6	5	0.333	0.333	0.333	CSR	Hunt et al. (2004)
Empetrum nigrum	736	84	7	6	2	1	0.500	0.500	0.000	SC	Hunt et al. (2004)
Deschampsia flexuosa	371	52	6	5	2	3	0.250	0.750	0.000	S/SC	Hunt et al. (2004)
Salix repens	235	39	8	7	6	3	0.500	0.500	0.000	SC	Hunt et al. (2004)
Anthoxanthum odoratum	203	41	7	6	4	3	0.167	0.417	0.417	SR/CSR	Hunt et al. (2004)
Pimpinella saxifraga	182	34	7	4	7	3	0.167	0.417	0.417	SR/CSR	Hunt et al. (2004)
Cytisus scoparius	114	50	8	5	4	4	0.500	0.500	0.000	SC	Hunt et al. (2004)
Lotus corniculatus	79	22	7	4	6	2	0.167	0.667	0.167	S/CSR	Hunt et al. (2004)
Hieracium pilosella	76	11	8	4	7	2	0.167	0.417	0.417	SR/CSR	Pierce et al. (2017)
Vaccinium uliginosum	73	12	7	6	2	2	0.000	0.750	0.250	S/SR	Pierce et al. (2017)
Leymus arenarius	65	12	9	5	7	6	0.500	0.500	0.000	SC	Hunt et al. (2004)
Poa pratensis	64	13	7	5	6	5	0.333	0.333	0.333	CSR	Hunt et al. (2004)
Calamagrostis stricta	61	10	*NA*	*NA*	*NA*	*NA*	0.750	0.250	0.000	C/SC	Hunt et al. (2004)
Rumex acetosa	53	8	7	5	5	4	0.333	0.333	0.333	CSR	Hunt et al. (2004)
Galium verum	50	8	7	4	6	2	0.417	0.417	0.167	SC/CSR	Hunt et al. (2004)
Luzula multiflora	45	14	7	6	3	3	0.167	0.667	0.167	S/CSR	Hunt et al. (2004)
Cerastium fontanum	45	13	7	5	5	4	0.250	0.000	0.750	R/CSR	Hunt et al. (2004)
Vaccinium vitis-idaea	43	8	6	5	2	2	0.250	0.750	0.000	S/SC	Hunt et al. (2004)
Plantago maritima	40	14	8	7	6	4	0.000	1.000	0.000	S	Hanslin & Kollmann (2016)

Carex pilulifera	36	8	7	5	3	2	0.167	0.667	0.167	S/CSR	Hunt et al. (2004)
Carex nigra	30	4	7	8	4	2	0.250	0.750	0.000	S/SC	Hunt et al. (2004)
Polypodium vulgare	26	7	5	5	4	3	0.167	0.667	0.167	S/CSR	Hunt et al. (2004)
Anthyllis vulneraria	26	7	8	4	7	2	0.167	0.417	0.417	SR/CSR	Hunt et al. (2004)
Rumex acetosella	26	5	7	5	4	3	0.167	0.417	0.417	SR/CSR	Hunt et al. (2004)
Geranium sanguineum	24	4	7	4	7	3	0.167	0.667	0.167	S/CSR	Hunt et al. (2004)
Rhinanthus serotinus	24	7	7	6	7	2	0.333	0.333	0.333	CSR	Riibak et al. (2014)
Campanula rotundifolia	22	6	7	4	5	2	0.167	0.667	0.167	S/CSR	Hunt et al. (2004)
Melampyrum sylvaticum	21	7	4	5	2	2	0.250	0.000	0.750	R/CR	Pierce et al. (2017)
Festuca ovina	20	2	7	5	4	2	0.000	1.000	0.000	S	Hunt et al. (2004)
Aira praecox	18	4	8	2	4	2	0.000	0.500	0.500	SR	Hunt et al. (2004)
Luzula pilosa	16	8	5	5	5	3	0.167	0.667	0.167	S/CSR	Hunt et al. (2004)
Vicia cracca	16	6	7	6	7	5	0.667	0.167	0.167	C/CSR	Hunt et al. (2004)
Festuca rubra ssp. *arenaria*	15	6	8	4	5	3	0.333	0.333	0.333	CSR	Hunt et al. (2004)
Lathyrus japonicus	14	5	9	5	7	6	0.500	0.000	0.500	CS	Hanslin & Kollmann (2016)
Achillea millefolium	13	2	7	5	6	4	0.333	0.333	0.333	CSR	Hunt et al. (2004)
Polygala vulgaris	12	3	8	5	6	3	0.167	0.667	0.167	S/CSR	Hunt et al. (2004)
Corynephorus canescens	11	4	9	1	3	1	0.000	0.750	0.250	S/SR	Pierce et al. (2017)
Botrychium lunaria	11	1	8	4	6	2	0.167	0.417	0.417	SR/CSR	Hunt et al. (2004)
Trientalis europaea	10	5	5	6	3	3	0.167	0.667	0.167	S/CSR	Hunt et al. (2004)
Silene vulgaris	9	1	7	4	8	5	0.333	0.333	0.333	CSR	Hunt et al. (2004)
Elytriga repens	7	2	7	5	7	7	0.750	0.000	0.250	C/CR	Hunt et al. (2004)
Quercus robur	6	2	7	5	5	4	0.500	0.500	0.000	SC	Hunt et al. (2004)
Ammophila arenaria	4	1	9	4	6	3	0.500	0.500	0.000	SC	Hunt et al. (2004)
Silene viscaria	4	1	8	3	4	2	0.333	0.333	0.333	CSR	Riibak et al. (2014)
Angelica sylvestris	4	2	7	8	6	5	0.750	0.000	0.250	C/CR	Hunt et al. (2004)
Artemisia vulgaris	4	1	7	4	8	7	0.750	0.000	0.250	C/CR	Hunt et al. (2004)
Succisa pratensis	3	1	7	7	5	2	0.167	0.667	0.167	S/CSR	Hunt et al. (2004)

Jasione montana	2	1	7	4	4	2	0.167	0.417	0.417	SR/CSR	Hunt et al. (2004)
Viola tricolor	2	2	8	4	6	4	0.000	0.000	1.000	R	Pierce et al. (2017)
Solidago virgaurea	1	1	5	5	4	3	0.167	0.667	0.167	S/CSR	Hunt et al. (2004)
Galium album ssp. *mollugo*	1	1	7	4	7	4	0.667	0.167	0.167	C/CSR	Hunt et al. (2004)
Scorzoneroides autumnalis	1	1	8	6	6	4	0.250	0.000	0.750	R/CSR	Hunt et al. (2004)
Senecio vulgaris	1	1	7	5	7	7	0.000	0.000	1.000	R	Hunt et al. (2004)

Errata list

Page	Line	Original text	Corrected text
Title page	3	innfødt	stedegen
v	12	naturlige	stedegene
v	24	utvanning	innblanding
3	8f	… any naturalise species that do not become invasive or if they are just undergo a very long…	…any naturalised species that do not become invasive or if they are just undergoing a very long…
4	6	… focussed on how alien species become invasive…	… focussed on the traits that allow alien species to become invasive…
10	26	invasion depth	invasion debt
13	8f	… and to the vegetation composition of the heathland and we investigate if…	… and the vegetation composition of the heathland and we investigated if…
13 - 14	27ff	Samples were collected from 38 populations in Norway, 15 populations in Europe, 7 populations in Denmark and 27 herbarium specimens…	Samples were collected from 36 populations in Norway, 17 populations in Europe, 8 populations in Denmark and 108 herbarium specimens…
14	18	Error! Reference source not found.	Figure 2
15	21 (figure caption)	Figure 2 Expected spatial distribution patterns of natural of human facilitated range expansion.	Figure 2 Conceptional map of the spatial distribution patterns expected from either natural or human facilitated range expansion. The different patterns of the dots represent different haplotypes.
18	8	Neither does it shows no 'invasive' behaviour…	Neither does it show 'invasive' behaviour…
19	29	Based on our results that populations of *C. scoparius*	*- delete -*
21	9	Error! Reference source not found.	Figure 3
24	30	…would be selected for trades to grow…	…would be selected for traits to grow…
Paper I, page 1	13	…support a natural spread human introductions.	…support a natural spread or human introductions.
Paper I, page 5	14	…separable from plants of low plants and are…	…separable from plants with dwarf morphology and are…
Paper I, page 6	5ff	…individuals and 13 populations with single samples. The native European range was represented by 15 populations with five to 20 individuals, sourced…	…individuals and 11 populations with single samples. The native European range was represented by 12 populations with five to 20 individuals and seven populations with one individual, sourced…
Paper I, page 6	12	…and the Netherlands where combined…	…and the Netherlands were combined…
Paper I, page 6	14	…from the garden centre where excluded.	…from the garden centre were excluded.
Paper I, page 10	4f	…where calculated in ARLEQUIN 3.5…	…were calculated in ARLEQUIN 3.5…
Paper I, page 10	16	…Hd values, where estimated for Norway,…	…Hd values, were estimated for Norway,…
Paper I, page 12	9	…for which several individuals where analysed,…	…for which several individuals were analysed,…

Paper I, Page 1	3	*- list of authors missing -*	Ursula Brandes[1], Einar Heegaard[2], Line Rosef[3] 1) Department of Plant Sciences, Norwegian University of Life Sciences, Ås NO-1432, Norway; 2) Forestry and Forest Resources, Norwegian Institute of Bioeconomy Research, Fanaflaten 4, N-5244 Fana, Norway 3) Faculty of Landscape and Society, Norwegian University of Life Sciences, Ås NO-1432, Norway
Paper III, Page 3	11f	We expect that this shrub will replace light demanding lower growing heathland.	We expected that this shrub would replace light demanding lower growing heathland species.
Paper III, Page 8	14	…(full cover, half cover…	…(full cover, partial cover…
Paper III, Page 9	15	…abundancies…	…abundances…

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