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ORIGINAL ARTICLE

Systemic fungal endophytes and ploidy level in *Festuca vivipara* populations in North European Islands

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Abstract Exploring the regional pattern of variation in traits driven by symbiotic interactions may provide insights to understand the evolutionary processes that operate over plant populations. Polyploidy, which is associated with fitness improvement, is expected to increase with latitude and altitude. However, it has never been explored in relation with the occurrence of epichloid fungal endophytes in plants. Both, variation in ploidy level and in the incidence of fungal endophytes, are known to occur in species of fine fescues. Here, we surveyed the occurrence of systemic fungal endophytes in natural *Festuca vivipara* populations in North European islands. In addition, we identified the fungal species associated with this grass and determined the predominant ploidy level for each population. Endophytes were found in four of six, two of three, and one of three populations for Faroe Islands, Iceland and Great Britain, respectively. With an average low incidence level of 15 % in infected populations, there was no relationship between infection level and either latitude or altitude. The phylogenetic analysis based on sequences ITS and the tub2 genes, supports that the endophytic species is *Epichloë festucae*, the same as in other fine fescues. We found no variation in ploidy level as all the plants were tetraploid (4X) with 28 chromosomes, a pattern which contrasts with the variation reported in previous antecedents. Our results suggest that apart from low and variable benefits of the endophyte to the plants, there would be a complex dynamics between epichloid endophytes and species of the fine fescue complex which merits further studies.

Keywords Pseudovivipary · Polyploidy · Symbiosis · Ecological patterns · *Epichloë festucae* · Fine fescues

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Introduction

Studies in plant traits and biological interactions across environmental gradients have aimed to understand the ecology and evolution of organisms (Saikkonen et al. 2004; Thrall et al. 2007; Schemske et al. 2009). Thus, occurrence of fitness correlated plant traits such as polyploidy (the increased number of chromosomes' copies) and pseudovivipary (vegetative propagation strategy consisting of growing leafy plantlets instead of sexually produced seeds) is observed to increase with latitude, while ecological interactions may be less diverse in higher latitudes compared to tropical environments (Brochmann et al. 2004; Leitch and Bennett 2004; Thrall et al. 2007; Schemske et al. 2009). However, general predictions for polyploidy, pseudovivipary and symbiotic interactions have not been explored simultaneously. Here, we focus on the grass-

endophyte symbiosis and ploidy level in the perennial grass *F. vivipara* (L.) Sm., a member of the fine fescue complex occurring in islands of Northern Europe.

The complex of fine fescues is widely distributed in Northern Europe comprising several species hard to differentiate by phenotypic or genetic comparisons (Flovik 1938; Gould and Shaw 1983; Jauhar 1993; Chiurugwi et al. 2011). Phenotypic traits of *F. vivipara* can easily be differentiated from its close relative *F. ovina* at reproductive stage, but it is difficult at vegetative stage (Flovik 1938; Watson 1958; Pils 1985). Sympatric populations of *F. vivipara* and *F. ovina* were found to be genetically more similar than distantly placed populations of the same 'phenotypic' species (Chiurugwi et al. 2011). Hypotheses on the origin and relationship between these two species propose that *F. vivipara* would have arisen from polyploidization processes of *F. ovina* plants and/or hybridization between the latter and *F. rubra* (Flovik 1938; Watson 1958; Beetle 1980; Pils 1985; Heide 1988). The former is particularly suggested to occur repeatedly in nature indicating that these two species form a complex (Chiurugwi et al. 2011). However, there exists great variation in the ploidy level in these species (Flovik 1938; Watson 1958; Pils 1985; Sarapul'tsev 2001; Šmarda and Kočí 2003; Šmarda et al. 2008). Ploidy levels of $2n = 14$ for *F. ovina* and $2n = 28$ for *F. vivipara* were recently observed (Pils 1985; Šmarda et al. 2008), while in particular for *F. vivipara*, earlier studies had reported 21, 28, 42, 35, 49 and 56 (Flovik 1938; Löve and Löve 1956, 1961; Watson 1958). A similar variability in the ploidy levels is reported for *F. vivipara*, *F. ovina* and *F. rubra* in the Missouri Botanical Garden's electronic database Tropicos® (Tropicos.org 2013). The variation in flowering behavior in *F. vivipara* has been suggested to be associated to the extant variation in ploidy level and/or other aspects such as the association with fungal endophytes (Beetle 1980; Clay 1986; Aiken et al. 1988; Wilkinson and Stace 1991; Elmqvist and Cox 1996).

Epichloid fungi (family Clavicipitaceae) of genera *Epichloë* and *Neotyphodium* (asexual form) establish perennial, asymptomatic symbiosis with grasses (subfamily Pooideae) including *Festuca* (Saikkonen et al. 1998; Clay and Schardl 2002; Schardl 2010). Whereas *Epichloë* species may be pathogenic by aborting host inflorescences (i.e., choke disease) during the sexual reproductive stage and horizontal transmission, *Neotyphodium* endophytes are only vertically transmitted from mother plant to offspring via seeds. Nonetheless, like *Neotyphodium* fungi, some *Epichloë* do not show the pathogenic symptom (stroma) relying mostly on the vertical transmission (Leuchtmann et al. 1994; Schardl 2001; Zabalgogeazcoa et al. 2006). Both groups of species can be parasitic under certain ecological conditions by depressing the fitness of host plants (Ahlholm et al. 2002; Zabalgogeazcoa et al. 2006). Therefore, the frequency of infected plants in a population depends on the relative fitness of infected and non-infected plants, as well as the success of vertical transmission of the fungus (Saikkonen et al. 2002; Gundel et al. 2008). The variability in infection frequency among populations may result from spatiotemporal variation in symbiosis outcome and/or the pollen-mediated gene flow that may be an opposite force to selection of partners' compatibility and, hence, disrupting the local genetic matching (Saikkonen et al. 1998, 2004, 2010).

Systemic fungal endophytes infecting species of the fine fescue complex have been described. In particular, *F. ovina* and *F. rubra* harbor the same systemic endophyte species (i.e., *Epichloë festucae*) (Tredway et al. 1999; Saikkonen et al. 2000; Craven et al. 2001; Schardl 2001; Zabalgogeazcoa et al. 2006). However, the endophyte species associated with *F. vivipara* is still unidentified. In a field survey carried out in continental Sweden, *F. vivipara* was only found at high altitudes, while *F. ovina* and *F. rubra* (incl. *F. richardsonii*, syn. *F. rubra* subsp. *arctica*) covered the whole altitudinal gradient. The frequency of endophyte infected *F. vivipara* plants with systemic fungal endophytes was low ranging from 12 to 37 %. However, the negative association between endophyte infection frequency and altitude was significant only for *F. rubra* (Granath et al. 2007). In Finland, the endophyte infection in populations of *F. rubra* and *F. ovina* was higher at higher latitudes, although variation among habitats was also found for *F. rubra* species at the northern extreme (Saikkonen et al. 2000; Wäli et al. 2007). If these species behave as a complex as suggested by genetic studies (see Chiurugwi et al. 2011), the distribution overlap may indicate that they may also behave as a host species complex sharing a common systemic endophyte species (see e.g., Tredway et al. 1999).

The aims in this article were: (1) to survey the occurrence of systemic fungal endophytes in natural *F. vivipara* populations, (2) identify the fungal species associated with this grass, and (3) determine the predominant ploidy level for each plant population. While symbiotic association with systemic fungal endophytes may be associated with the fitness benefits to the plants, their incidence in continental subarctic regions has been shown to be highly variable in fine fescues (Bazely et al. 2007; Granath et al. 2007; Wäli et al. 2007). In more oceanic environments, we predict the frequency of endophyte infected plants to increase with latitude and altitude. Similarly, given that polyploidy can be associated with higher fitness in harsh environments, we predict ploidy level to increase with latitude and altitude (Tyler et al. 1978; Leitch and Bennett 2004). Taken together, we might expect to find a positive association between endophyte infection level and polyploidy in populations of

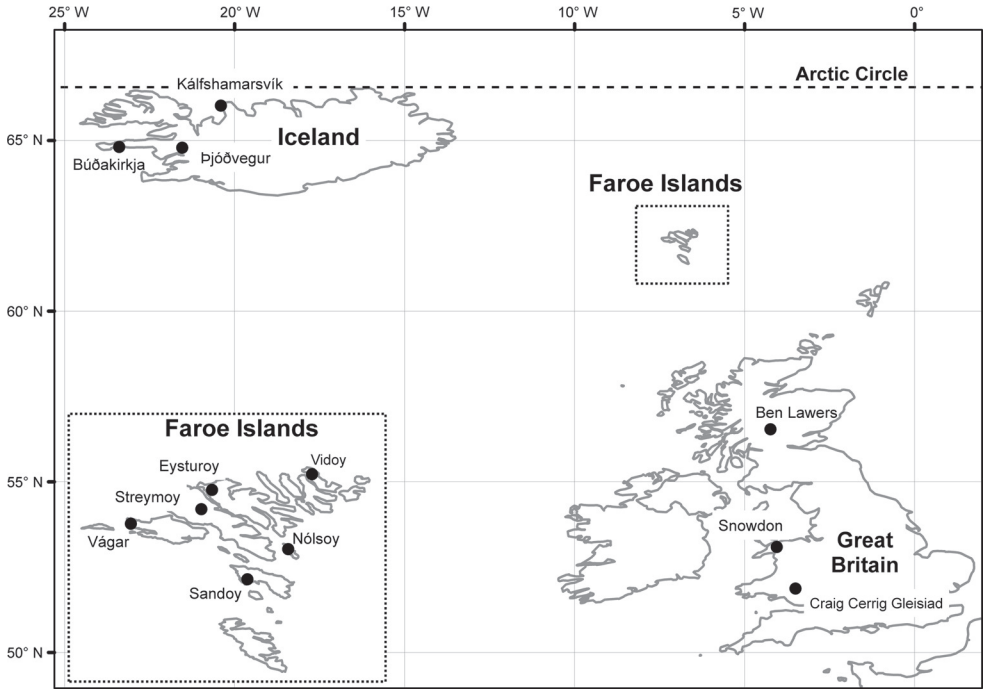

Fig. 1 Map showing the sites of collection of *Festuca vivipara* plant populations in Faroe Islands (framed in *dotted line*), Iceland and Great Britain

F. vivipara. We surveyed the occurrence of systemic fungal endophyte, identified the fungal species, and determined the predominant ploidy level in natural populations of *F. vivipara* in three North European islands or group of Islands: Faroe Islands, Iceland and Great Britain. Our work is among the first studies exploring the geographic diversity pattern of fescue ploidy levels in relation to the systemic fungal endophytes in wild plant populations.

Materials and methods

Collection of plant populations

In 2011, we collected about ten individual *F. vivipara* plants from six and three different populations from Faroe Islands and Iceland, respectively (Fig. 1; Table 1). All the collected plants presented the typical pseudoviviparous panicle with leafy plantlets. Populations were sampled from different islands in Faroe Islands and more than 100 km apart from each other in Iceland. The individual plants within each population were growing at least 2 m apart from each other. All the sites were grasslands overgrazed by sheep. The *F. vivipara* plants from Great Britain were collected from around three mountains: Ben Lawers (Mid Perthshire, Scotland), Snowdon (Caernarvonshire, North Wales) and Craig Cerrig Gleisiad (Breconshire, South Wales) (Fig. 1; Table 1). These plants were used in a previous paper in which the genetic relationship between populations of *F. ovina* and *F. vivipara* occurring in the same and in different locations was studied (Chiurugwi et al. 2011). All *F. vivipara* plants were grown in 300 cm^3 pots filled with peat moss, and kept in a greenhouse at MTT Agrifood Research, Jokioinen, Finland.

Fungal endophyte incidence

The endophyte colonization status of plants was determined by incubating three surface-sterilized green leaves from each plant (Wäli et al. 2007). Each leaf was cut into five pieces and placed in potato dextrose agar (5 % PDA) in a Petri dish and kept at room temperature for about a month until there were no more fungi growing out of the pieces. When typical systemic endophyte mycelium grew out from several leaf pieces, the plant was considered endophyte colonized (Wäli et al. 2007).

Species determination of fungal endophytes

The fungal strains used for species identification were: Färoe S1-4, Färoe S1-5 (From Vágar, Faroe Islands), Färoe S2-2, Färoe S2-4, Färoe S2-9 (from Vidoy, Faroe Islands); Iceland S2-6 (from Borgarnes, Iceland); and Great Britain 135P, Great Britain 153P (both from Ben Lawers, Great Britain) (Table 1). Molecular identification was based on the nucleotide sequence of the ITS1-5.8S rDNA-ITS2 (ITS) region, and of a 5′ region of the β-tubulin (tub2) gene containing part of its first intron, and the complete sequence of introns 2 and 3 (Byrd et al. 1990). A DNA extract from mycelium was obtained using a commercial kit for extraction and amplification of plant DNA (Sánchez Márquez et al. 2007). PCR amplification of the ITS region

Table 1 Sites of collection of *Festuca vivipara* plant populations in Faroe Islands, Iceland and Great Britain

Island	Population		Geographic coordinates	Altitude (m a.s.l.)[a]	*N*	Endophyte infection (%)
Faroe Islands	1	Vágar	N62°06′58″; W07°26′42″	245	11	18
	2	Vidoy	N62°22′03″; W06°32′31″	148	10	30
	3	Sandoy	N61°51′01″; W06°51′41″	167	9	0
	4	Nólsoy	N62°61′14″; W06°41′08″	55	10	0
	5	Eysturoy	N62°17′24″; W07°02′09″	316	11	9
	6	Streymoy	N62°11′43″; W07°05′20″	185	10	11
Iceland	1	Búðakirkja	N64°48′52″; W23°23′14″	10	10	10
	2	Þjóðvegur, Borgarnes	N64°47′34″; W21°32′0.4″	390	12	8
	3	Kálfshamarsvík	N66°01′20″; W20°23′39″	38	10	0
Great Britain	1	Ben Lawers	N56°32′24″; W04°13′52″	692	11	18
	2	Craig Cerrig Gleisiad	N51°52′34″; W03°29′48″	410	3	0
	3	Snowdon	N53°05′26″; W04°02′50″	725	13	0

Name and label of sites, geographic coordinates and altitude are reported in addition to endophyte infection level in each plant population

N is the number of sampled plants in each population

[a] *m a.s.l.* meters above sea level

was made using primers ITS4 and ITS5 (White et al. 1990), and the tub2 gene was amplified using the primers tubF: 5′ GTT TCG TCC GAG TTC TCG AC and tubR: 5′ ACC GAGA AAA ATG CGT GAG AT (Byrd et al. 1990). All amplicons obtained were sequenced. To detect differences among the ITS or the tub2 sequences obtained from the fungal strains, these were aligned using Clustal X software (Thompson et al. 1997). In addition, tubulin sequence chromatograms were carefully examined for the presence of ambiguous peaks, what could indicate heteroploidy, and the hybrid condition of a strain (Schardl et al. 1994). To ascribe the *F. vivipara* endophyte sequences to a taxonomic group, a phylogenetic trees based on ITS or tub2 nucleotide sequences were constructed using reference sequences of different *Epichloë* species obtained from the Genbank data base. The genetic distance between each pair of strains was estimated using the Kimura 2-parameter model, and the resulting matrix was used to construct a phylogenetic tree by the neighbor-joining method. The robustness of the relationships inferred from the trees was estimated by 1,000 bootstrap replicates. These phylogenetic analyses were conducted using MEGA version 5 software (Tamura et al. 2011).

Ploidy level determination

Ploidy level of plants was determined by the flow cytometry (FMC) standardized by comparing the DNA content with microscopically counted chromosome number in cells at mitotic metaphase of reference sample. For chromosome counting, fresh root-tips obtained from hydroponically grown *F. vivipara* plants were treated with 1 % alpha-monobromonaphtalene, then fixed in ethyl alcohol-acetic acid (3:1, v/v), hydrolyzed 1 N HCl, and stained in acetic orcein solution. The pieces were squashed in a drop of 45 % acetic acid on the slide, cover-slipped, and analyzed under microscope. Some preparations were mounted with enthalan after the metaphase photos were taken (Darlington and La Cour 1969).

For performing FCM, a small amount of leaf tissue ($\approx$0.5 cm^2) from each plant was placed in a glass Petri dish. One milliliter of ice-cold nuclei isolation buffer (LB01) was added to each Petri dish and the tissue was chopped immediately in the buffer with a razor blade, and filtered through a 42 µm nylon mesh into an Eppendorf tube. DNA fluorochrome stock solution propidium iodide (PI) was added simultaneously with the same amount of RNase (50 µl ml^{-1}), and incubated for 1 h (Dolezel et al. 2007). FCM analyses were run using FACSCalibur (Becton–Dickinson San Jose, USA) with external standard (using *F. vivipara* plants from which chromosome numbers had been observed by microscopy). A channel was determined as a G1 peak for each sample. *Pisum sativum* L. cv. Ctirad (2C DNA value = 9.09 pg) was used for determining DNA quantity as pg (Provided by Laboratory of Molecular Cytogenetics and Cytometry, Czech Republic) (Dolezel et al. 2007). At least 5,000 nuclei were analyzed per sample considering CV value <5 for each G1 peak. The statistical analysis for FCM data was performed by the Flowing Software version 2.4.1 (Perttu Terho, Turku Centre for Biotechnology, Finland, www.flowingsoftware.com). The highest peak (G1 population) of the channel of measuring Propidium Iodide was found for each data file.

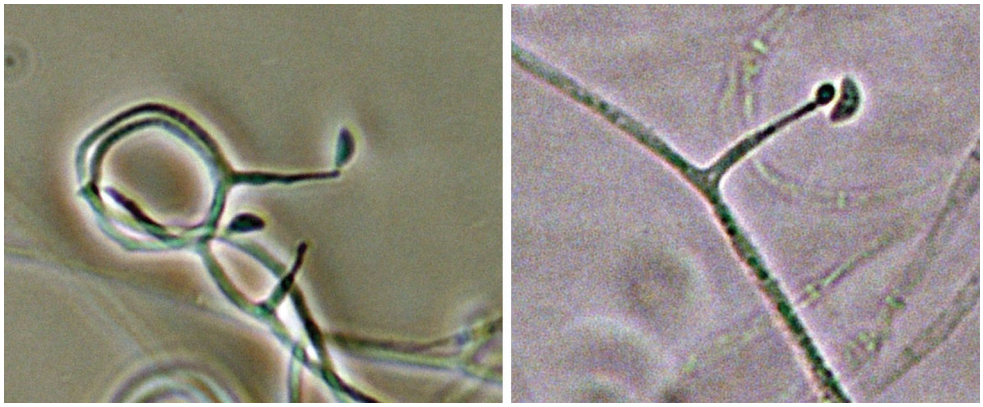

Fig. 2 Conidiophores and conidia of endophytic fungal strain isolated from *Festuca vivipara* from Great Britain 135P (400X) showing typical *Epichloë* morphology

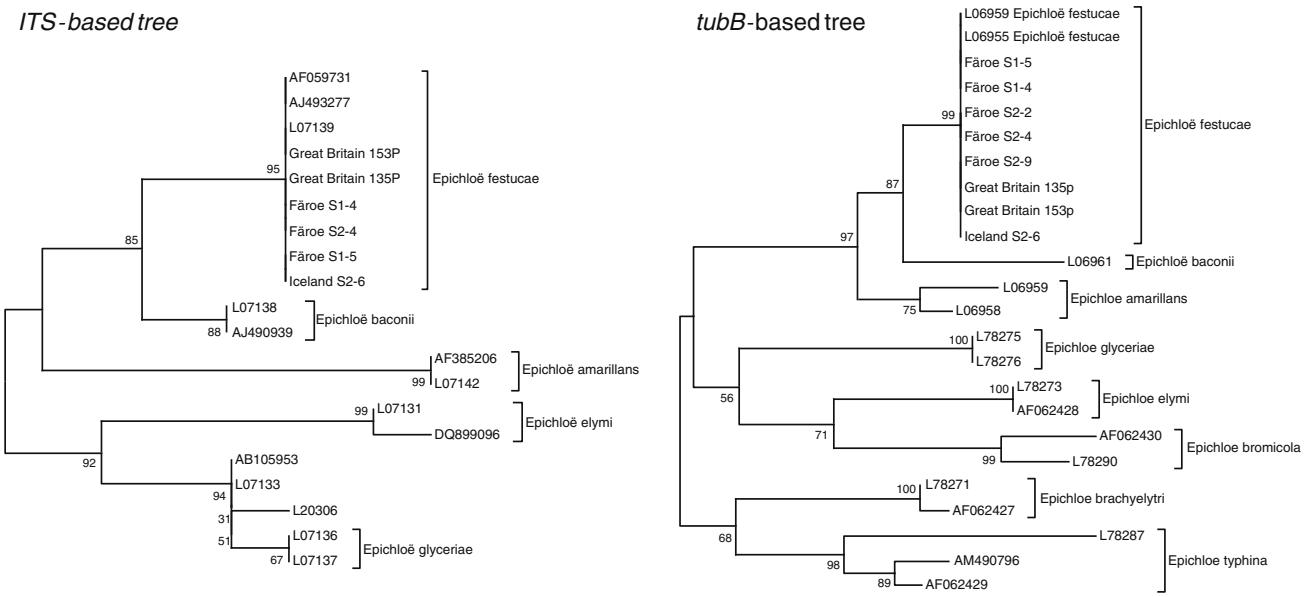

Fig. 3 Neighbor-joining phylogenetic trees based on ITS sequences (*on the left*) and on β-tubulin sequences (*on the right*) obtained from the endophyte isolated from *F. vivipara* and other *Epichloë* species. Nomenclature: Färoe S1-5, Färoe S1-4, Färoe S2-4, Färoe S2-2, and Färoe S2-9 are isolates from Vágar (S1) and Vidoy (S2), Faroe Islands; Iceland S2-6, is an isolate from Þjóðvegur, Borgarnes, Iceland; and Great Britain 135P and Great Britain 153P are isolates from Ben Lawers, Great Britain

Results

Systemic endophytic fungi were found in all geographic locations, but not in all the sampled populations (Table 1). Endophytes were found in four out of six, two out of three, and one out of three populations for Faroe Islands, Iceland and Great Britain, respectively. The average infection level among infected populations was 14 %, with a minimum in 10 % recorded for three populations and maximum of 30 % for Vidoy of Faroe Islands (Table 1). The linear regression analyses testing for relationship between endophyte infection level and either latitude or altitude were not significant ($P = 0.59$, and $P = 0.98$, respectively) (Table 1).

The ITS and β-tubulin sequences of the eight isolates analyzed (five from Faroe Islands, one from Iceland, and two from Great Britain) were identical, and no ambiguous peaks were observed in any sequence chromatogram suggesting that the strains were not hybrid and belonged to the same taxon. The microscopic observation of the strains revealed the presence of conidiophores bearing single reniform conidia typical of *Epichloë* species (Leuchtmann et al. 1994; Schardl 2001) (Fig. 2). In the sequence-based phylogenetic analysis, the sequences of both the ITS and the tub2 genes were included in a cluster with reference *Epichloë festucae* sequences (Fig. 3).

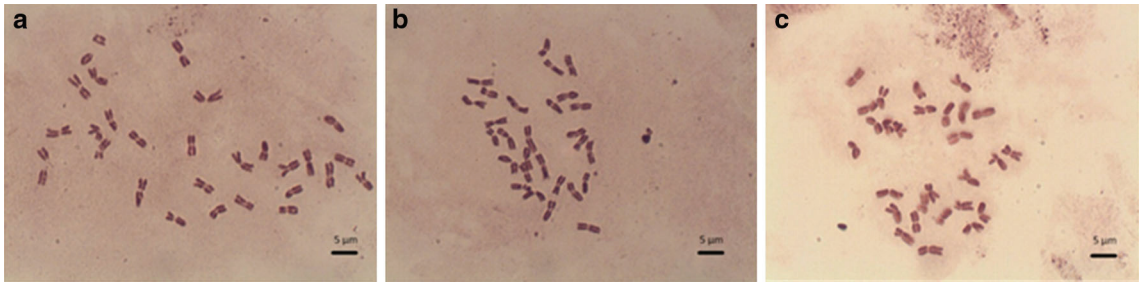

Fig. 4 Photos of number of chromosomes in root-tip cells at mitotic metaphases (**a**, **b**, **c**) in three randomly selected *Festuca vivipara* plant which were used as reference in the correlation between number of chromosomes and quantity of DNA. Number of chromosomes: $2n = 4X = 28$

Table 2 DNA content (pg) per nucleus (2C), estimated ploidy level and chromosome number in *Festuca vivipara* plant populations collected from different sites in Faroe Islands, Iceland and Great Britain

Island	Population	DNA (pg)/ Nucleus Mean ± SE	Ploidy level	2*n*
Faroe Islands	Vágar	9.59 ± 0.08	4x	28
	Vidoy	9.38 ± 0.05	4x	28
	Sandoy	9.52 ± 0.11	4x	28
	Nólsoy	9.22 ± 0.09	4x	28
	Eysturoy	9.29 ± 0.10	4x	28
	Streymoy	9.73 ± 0.12	4x	28
Iceland	Búðakirkja	9.27 ± 0.11	4x	28
	Þjóðvegur, Borgarnes	9.49 ± 0.05	4x	28
	Kálfshamarsvík	9.45 ± 0.09	4x	28
Great Britain	Ben Lawers	9.18 ± 0.14	4x	28
	Craig Cerrig Gleisiad	9.32 ± 0.07	4x	28
	Snowdon	9.45 ± 0.25	4x	28

The number of plants examined within each population is reported in Table 1

There was no difference in DNA content/nucleus among plants, and the overall total mean was 9.40 with 9.34 and 9.46 as lower and upper limit of the confidence interval. All the analyzed plants ($N = 117$) were tetraploid (4X) with 28 chromosomes (Fig. 4). Thus, there was no variation in ploidy levels of the *F. vivipara* plants among populations and islands (Table 2). Therefore, no statistical analysis was performed to test for any relationship between endophyte infection frequency and ploidy level.

Discussion

We surveyed infection frequencies of systemic fungal endophytes and plant ploidy level, two features supposedly associated with plant fitness, in the alpine-arctic perennial grass *F. vivipara* at various altitudes and latitudes in north European islands. Despite the low number of sampled plants per population, the very low endophyte infection frequencies observed in *F. vivipara* populations are consistent with earlier reports from alpine Sweden (Granath et al. 2007). In fact, the overall low infection frequencies found by our sampling (characterized by low number of plants per population) are likely to be overestimations of the actual incidence of endophytes in this species. In addition, the phylogenetic trees support that the endophytic species symbiotic with *F. vivipara* is the fungus *Epichloë festucae*, the endophyte species detected in fine fescues, including red fescue (Leuchtmann et al. 1994). Many alpine-arctic species descend close to sea level in northern oceanic climate and *F. vivipara* follows this distribution pattern (Elven 2005; Rønning 1996). In less oceanic climate, in Kiölen Mountains of Sweden, *F. vivipara* occurs only above 750 m from the sea level (Granath et al. 2007). In Finland, the species is not found lower than 475 m a.s.l., and is most commonly detected between 800 and 1,100 m a.s.l. (Väre and Partanen 2012). Near the sea level, growing season is longer than at higher altitudes, and climate is more favorable. However, beyond the climate largely dictating the home range of *F. vivipara*, the association with systemic fungal endophytes seems to be unrelated to the climatic conditions since many populations were endophyte-free or the frequency of endophyte infected grasses was very low and uncorrelated with the latitude or altitude.

Pseudovivipary has been proposed to be an ecological advantageous reproduction strategy for grasses enabling them to cope with the cold climate and very short growing season of the subarctic regions (Harmer and Lee 1978; Beetle 1980; Lee and Harmer 1980; Heide 1988; Elmqvist and Cox 1996; Sarapul'tsev 2001). Systemic fungal endophytes can be expected to promote pseudovivipary which should provide more opportunities and higher fitness for vertical transmission of the fungus compared to seminiferous panicles (Saikkonen et al. 2004). However, although the endophytic fungus *Balansia cyperi* inducing pseudovivipary in the sedge *Cyperus virens* supports this idea

(Clay 1986), evidence linking the symbiosis with vertically transmitted fungal endophytes and pseudovivipary is scarce (Beetle 1980; Saikkonen et al. 2004). As for *Festuca* species, there are pairs of taxa that potentially exhibit similar patterns, *Deschampsia alpina* and *D. cespitosa*, *Poa alpina* ssp. *alpina* and *P. alpina* ssp. *vivipara* (Lee and Harmer 1980; Elmqvist and Cox 1996). Although symbioses with systemic fungal endophytes have been reported in these genera (White and Cole 1986; Saikkonen et al. 2000; Iannone et al. 2012), there are no reports on endophytes associated to their pseudoviviparous variants.

Epichloë fungal endophytes infect new hosts by germinating ascospores in open flowers and growing into developing seeds (Bultman and White 1988; Schardl 2001; Clay and Schardl 2002). Although we cannot rule out other avenues of contagious spreading of the fungus such as the transmission of asexual spores from epiphytic mycelium infecting new hosts (see Tadych et al. 2007), there would be few chances for *Epichloë* endophytes to infect uncolonized plants of *F. vivipara* producing vegetative plantlets instead of flowers. When *F. ovina* and *F. vivipara* that were transplanted from different altitudes at NW Finland, Enontekiö into common garden in Oulu (Botanical Garden, Oulu University), *F. ovina* remained seminiferous and *F. vivipara* pseudoviviparous for the three years the plants were monitored (Väre, unpublished). Therefore, even though changing the photoperiodic conditions experimentally can promote the occurrence of seminiferous panicles in *F. vivipara* and induce pseudoviviparity in *F. ovina* (Heide 1988), these species seem to keep their typical reproduction behavior in their original distribution range. Thus, pseudoviviparity appears as a constant feature of *F. vivipara* and, consequently, flowers cannot be colonized by endophytic fungi in such cases. Considering that *F. vivipara* would have repeatedly arisen from *F. ovina* (Flovik 1938; Watson 1958; Beetle 1980; Pils 1985; Heide 1988; Chiurugwi et al. 2011), and the latter is a common host to the fungal endophyte *E. festucae* (Leuchtmann 1992; Schardl 2001; Granath et al. 2007), it would be possible that *F. vivipara* inherits its endophyte from its ancestors. Thus, the accumulated failures in the vertical transmission of the fungus to the new generations and difficulties for horizontal transmission may explain the observed zero or very low infection levels in both continental and oceanic populations of *F. vivipara*.

To understand the importance of pseudoviviparous reproduction for endophyte symbiosis requires research determining success of endophyte transmission from plant to pseudoviviparous plantlets. We may assume that the endophyte is transmitted to plantlets equally successfully as it is transmitted to new tillers. However, the establishment of plantlets is highly dependent on prevailing environmental conditions, particularly moisture in soil (Harmer and Lee 1978). In contrast, seeds can survive over years in seed bank although it is well documented that the seed and endophyte viability decreases over time and is negatively affected by relative humidity (see e.g., Gundel et al. 2010). Thus, the subarctic and oceanic Northern Europe is likely to promote the vertical transmission of endophyte via pseudoviviparous plantlets contrary to environments with hot and dry summers (Gundel et al. 2011).

In contrast to past literature, we found no variation in ploidy level (Flovik 1938; Löve and Löve 1956, 1961; Watson 1958). All the examined plants had 28 chromosomes ($2n$). The differences in ploidy level between populations collected in north European islands and populations collected in the continent can be explained by polytopic origin and/or distribution history of the species after glacial period. Furthermore, our results suggest relatively low, but variable benefits from the endophyte to the host plants which, in addition, may depend on the environmental conditions and gene flow between partners' populations (Saikkonen et al. 1998, 2004; Gundel et al. 2011). The challenge for the future is to understand to what extent the detected endophyte infection frequencies mirror the prevailing or past selection pressures and whether they are promoted by vertical transmission of the fungus via pseudoviviparous plantlets. Future experiments will help to disentangle the role of endophyte on the fitness of *F. vivipara* plant in these particular oceanic environments as well as to estimate the success of vertical transmission from plant to plantlets, two processes that determine the abundance and distribution of endophyte in nature (Saikkonen et al. 2002, 2004; Gundel et al. 2008, 2011).

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