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Variation in the *Festuca brachyphylla* (Poaceae) complex in Svalbard, elucidated by chromosome numbers and isozymes

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Contrasting with former taxonomic treatments, chromosome numbers and isozyme data support the delimitation of the seminiferous representatives of the *Festuca brachyphylla* complex in Svalbard into four species: *F. baffinensis*, *F. brachyphylla*, *F. hyperborea* and *F. edlundiae*. Unique enzyme markers were found for all species. *Festuca brachyphylla* proved hexaploid, and the others, tetraploid. The chromosome numbers of *F. hyperborea* and *F. brachyphylla* (as circumscribed at present) are new to Svalbard. *Festuca baffinensis* is the most distinct species within the complex, probably representing a separate evolutionary lineage. The three other species seem closely related, showing mutually equidistant relationships. Some deviating plants found on disturbed ground might represent hybrid derivatives or an introduced foreign strain of the elsewhere variable *F. brachyphylla*. Materials of diploid *F. ovina* from northern Fennoscandia was enzymatically closely related to the *F. brachyphylla* complex in Svalbard. *Festuca brachyphylla*, *F. edlundiae*, and *F. hyperborea* all had a stronger affinity to *F. ovina* than to *F. baffinensis*, indicating that the *F. brachyphylla* complex is an artificial taxonomic group. There are reasons to believe that the origin of the polyploid taxa of the *F. brachyphylla* complex can be traced to diploid species of the *F. brachyphylla* and *F. ovina* complexes.

ADDITIONAL KEY WORDS: genetic variation – polyploidy – species delimitation.

INTRODUCTION

The *Festuca brachyphylla* complex is an arctic-alpine group vicariant to the montane-temperate *F. ovina* complex. Both belong to section *Festuca*. Studies of cross sections of the leaf blades have emphasized sclerenchyma differences to be important for species delimitation within the section, and sclerenchyma characters are also frequently used to separate the two complexes (Skvorcov in Tolmatchev, 1964; Tzvelev, 1976; Markgraf-Dannenberg, 1980; Frederiksen, 1982). Whereas plants of the *F. brachyphylla* complex display weakly developed sclerenchyma in 3–7 strands on the midline and margins of the leaf blade, plants belonging to the *F. ovina* complex have strongly developed sclerenchyma in a continuous ring beneath the epidermis. Length of anthers is also often used to discriminate between the two complexes: short anthers (<2 mm) in the *F. brachyphylla* complex, in contrast to longer anthers (2–3 mm) in the *F. ovina* complex.

Up to 1934, all tussock-forming fescues in Svalbard were referred to *F. ovina* L. *s.l.* (e.g. Resvoll-Holmsen, 1927) or *F. brevifolia* R. Br. (e.g. Asplund, 1918). Viviparous plants were most often treated as a separate species, *F. vivipara* (L.) Sm., or as a viviparous race of *F. ovina*. The viviparous fescue was recognized as *F. vivipara* ssp. *glabra* by Frederiksen (1977), and raised to species rank by Pavlick (1984), i.e. *F. viviparoidea* Krajina ex Pavlick. According to Pavlick, this taxon belongs to the *F. brachyphylla* complex as the plants have the sclerenchyma characters of that group. Only the seminiferous species will be treated in this study.

The seminiferous material in Svalbard was accepted as *F. brachyphylla* Schultes & Schultes fil. by Scholander (1934). Although he pointed at a wide variation in size, colour, floret density in spikelets, hairiness of the culm and leaf structure within this taxon, he did not report any discontinuities within the material. He described, however, a variety from Greenland, *F.*

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brachyphylla var. *groenlandica* (Scholander, 1934), including *F. ovina* L. var. *supina* (Schur) Hackel pro parte.

Festuca supina was reported 6 years later from one site in Adventdalen, central Spitsbergen (Hadač, 1944), which indicated that the tussock-forming, seminiferous Svalbard materials contained more than one entity. Furthermore, Hadač accepted Scholander's concept of *F. brachyphylla*.

In his revision of *Festuca* in Svalbard, Rønning (1961) concluded that all material previously reported as *F. brachyphylla* Schultes & Schultes should be divided into three entities: *Festuca baffinensis* Polunin, *F. hyperborea* Holmen ex Frederiksen and *F. brachyphylla s.s.*, the latter including Hadač's *F. supina*. The three species were mapped for Svalbard by Rønning (1972). Since then, *F. baffinensis* has been accepted and recognized as a species in Svalbard. Rønning (1961) stated that *F. brachyphylla* and *F. hyperborea* are closely related, but distinguished by the glossy appearance of the former and the glaucousness of the latter. Frederiksen (1977), who studied the Svalbard fescues in connection with her work on the same species in Greenland, mainly supported Rønning's conclusions.

Most field botanists during the 1980s were uncomfortable with the treatments of Rønning (1961, 1972, 1996) and Frederiksen (1977), as they did not find any consistent differences between *F. brachyphylla* and *F. hyperborea*, but found that parts of the variation pattern did not fit either species. Furthermore, the key characters given by Rønning and Frederiksen did not separate the Svalbard material into two distinct groups. In this period, material was deposited in Herbarium Osloensis (O) under *F. hyperborea*, mainly because two chromosome number reports from Svalbard (Flovik, 1938; Engelskjøn, 1979) corresponded with the chromosome numbers ($2n=28$) reported for *F. hyperborea* as circumscribed by Holmen (1952) and Frederiksen (1977), not with *F. brachyphylla*. An additional complication was the finding of some plants corresponding to Hadač's *F. supina* and to *F. brachyphylla* as circumscribed from other regions.

The research group of Dr Susan Aiken has worked with Canadian material of the *F. brachyphylla* complex, reporting chromosome numbers, morphological variation, and variation in allozyme and isozyme profiles (Aiken & Fedak, 1992; Aiken *et al.*, 1993, 1994; Aiken, Consaul & Lefkovitch, 1995; and Ramesar-Fortner, Aiken & Dengler, 1995). In one study, comparing *F. hyperborea* with *F. brachyphylla* and *F. baffinensis*, 12 individuals sampled as *F. hyperborea* showed a distinct isozyme profile deviating from *F. hyperborea* in all the five enzyme systems investigated (Aiken *et al.*, 1994). Further morphological and isozyme investigations revealed that these plants belonged to a distinct taxon, described as *F. edlundiae* S. Aiken, Consaul & Lefkovich. Additional herbarium studies reported *F. edlundiae* from 30 other localities, including sites in northern Greenland, east Siberia and Svalbard.

The publication of a new species within the *F. brachyphylla* complex stimulated further investigations of the Svalbard taxa. With the description of *Festuca edlundiae*, there seemed to be enough taxa to cover the observed variation in Svalbard, as pointed out by Elven & Elvebakk (1996). Characters separating *F. hyperborea* and *F. brachyphylla* were also clarified by Aiken *et al.* (1995), facilitating the species delimitation within the complex.

In a circumpolar context, several other species are also considered as belonging to the *F. brachyphylla* complex. The tetraploid *F. minutiflora* Rydb. (Rydberg, 1905) and the diploid *F. brevissima* Yurtz. (Yurtzev & Tzvelev, 1972) in the Beringian-Pacific area were included in the *F. brachyphylla* complex by Tzvelev (1984). In an isozyme study of Canadian material, Aiken *et al.* (1993) found strong affinities between *F. minutiflora* and *F. baffinensis*.

From Greenland, *F. groenlandica* (Schol.) Frederiksen (Frederiksen, 1982) and *F. jensenii* Gjærevoll & Ryvarden (Gjærevoll & Ryvarden, 1977) have been reported as belonging to the complex. *Festuca groenlandica* was first described as a variety of *F. brachyphylla* (Scholander, 1934) but later raised to the species level (Frederiksen, 1982). *Festuca jensenii*, on the other hand, was identified by Frederiksen (1982) as a pale form of *F. brachyphylla* (f. *flavida*), in accordance with Holmen (1952).

The main aim of this study has been to investigate species delimitation within the *F. brachyphylla* complex in Svalbard by means of chromosome numbers and molecular markers (enzymes) and to compare the results from Svalbard with the results from Canada (Aiken *et al.*, 1995). A further aim has been to study infraspecific variation in Svalbard. As the evolutionary history of the *F. brachyphylla* complex is uncertain and a possible relationship with northern mainland *F. ovina* has been suggested (Elven in Lid & Lid, 1994), material of *F. ovina* from northernmost Norway was included in the analyses to elucidate this hypothetical relationship.

THE SPECIES

FESTUCA BRACHYPHYLLA

The species was first described from Melville Island in the Canadian Arctic archipelago (Brown, 1823) as *F. brevifolia* R. Br., a homonym of *F. brevifolia* Muhl. (Muhlenberg, 1817), and later changed to *F. brachyphylla* (Schultes & Schultes fil., 1827), with reference to the type and the original description by Brown.

The hexaploid chromosome number ($2n=42$) has been reported by several authors (e.g. Holmen, 1952, 1964; Jörgensen, Sörensen & Westergaard, 1958; Bowden, 1960; Zhukova, 1965a, b; Mosquin & Hayley, 1966; Johnson & Packer, 1968; Packer & McPherson, 1974; Zhukova & Petrovsky, 1980; Frederiksen, 1981; Wade, 1986; Alexeev, Sokolovskaya & Probatova, 1987; Aiken & Fedak, 1992), but the tetraploid number ($2n=28$) is also reported (Flovik, 1938; Sokolovskaya & Probatova in Tzvelev, 1976; Engelskjøn, 1979). However, tetraploid numbers were recorded before the recognition of *F. edlundiae* and probably refer to this species.

Festuca brachyphylla has a circumpolar distribution. It is reported as widespread in Greenland but rare in the northern and southeastern parts (Frederiksen, 1977). In North America, it extends as far south as Arizona (Aiken, pers. comm.). It is found throughout northern Russia, from the Bering Strait west to Novaya Zemlya and the northeasternmost European Russian mainland (Tzvelev, 1976). It is also reported to occur in Svalbard (Markgraf-Dannenberg, 1980).

FESTUCA BAFFINENSIS

The species was described by Polunin (1940), based on plants collected on Baffin Island, Arctic Canada. It was considered to be well defined by Holmen (1957, 1964), Böcher, Holmen & Jacobsen (1957), Tzvelev (1976), Rønning (1961 and later), Markgraf-Dannenberg (1980), Elven in Lid & Lid (1994), and Elven & Elvebakk (1996), but as more doubtful by Skvortsov (in Tolmatchev, 1964).

The numerous chromosome counts are all tetraploid, $2n=28$ (e.g. Holmen, 1952, 1964; Jörgensen *et al.*, 1958; Bowden, 1960; Zhukova, 1965b; Mosquin & Hayley, 1966; Johnson & Packer, 1968; Zhukova & Petrovsky, 1971; Löve & Löve, 1975; Yurtsev & Zhukova, 1978; Engelskjøn, 1979).

Festuca baffinensis occurs in arctic and alpine areas (Markgraf-Dannenberg, 1980; Frederiksen, 1977, 1982; Aiken *et al.*, 1995). In Greenland, it has been reported from northern areas on relatively basic rocks (Frederiksen, 1977). In North America, it is found as far south as Colorado (Weber, 1961). In Russia, it is reported to occur in Novaya Zemlya, Wrangel Island and the Chukotka peninsula (Tzvelev, 1976). From Europe, it is reported only from Svalbard (Markgraf-Dannenberg, 1980).

FESTUCA HYPERBOREA

In his cytological studies of the flora of northern Greenland, Holmen (1952) found two groups within his materials sampled as *F. brachyphylla*, corresponding to one hexaploid and one tetraploid. He suggested that the hexaploids represented *F. brachyphylla*, whereas the tetraploids should be regarded as a distinct species: *F. hyperborea*. The name was not validly published, but the species was nevertheless accepted and is the name used by most botanists working with the Arctic flora. Frederiksen (1977) made the first formal description, but Holmen's short description of *F. hyperborea* was the basis of Rønning's (1961) treatment of the species in Svalbard.

Festuca hyperborea has been reported as tetraploid (i.e. $2n=28$) elsewhere (Zhukova, Petrovsky & Plieva, 1973; Petrovsky & Zhukova, 1981; Alexeev *et al.*, 1987). The hexaploid number ($2n=42$) reported in Petrovsky & Zhukova (1981) and Tzvelev (1976) may refer to *F. brachyphylla* as presently circumscribed.

Festuca hyperborea has been reported from the Canadian Arctic Archipelago, Greenland, Svalbard, and eastern Siberia including Chukotka and Wrangel Island (Aiken *et al.*, 1995). However, the Russian distribution reported by Aiken and co-workers has not been confirmed by more recent studies (Elven, pers. comm.). This taxon seems to be restricted to the Polar Desert and the North Arctic Tundra Zone, and is typically found in snowbeds, on damp soil polygons, and in open habitats in mountain plateaus. It is also found in more or less disturbed mountainsides (Guldahl & Elven, 1999).

FESTUCA EDLUNDIAE

This species was described from Bathurst Island in the Canadian Arctic Archipelago as late as 1995 (Aiken *et al.*, 1995). It has been reported to be tetraploid, with $2n=28$ (Aiken *et al.*, 1995). Some of the previous reports of $2n=28$ for *F. hyperborea* may refer to this taxon.

Festuca edlundiae is reported from Arctic Canada, Greenland, Svalbard and north-eastern Siberia. It is found in a variety of habitats; often on fine-grained calcareous substrates, dry gravelly ridges, and shore terraces (Aiken *et al.*, 1995).

FESTUCA OVINA

On the coast of northern Norway, *F. ovina* has been found to differ morphologically from *F. ovina* elsewhere in the country. Moreover, this northern *F. ovina* resembles materials of the *F. brachyphylla* complex in Svalbard in general appearance (Elven in Lid & Lid, 1994). It has been suggested that these northern specimens might be connected to the *F. brachyphylla* complex in Svalbard (Elven in Lid & Lid, 1994).

Festuca ovina is a perennial tufted grass. The culms are thin and erect. The leaves are grey green, slender, curved, finely papillated, with 5–7 veins and sclerenchyma forming a complete ring. The panicles are slender with short and finely papillated branches. The spikelets have 3–8 florets and are green, glaucous or

violet-tinged. The anthers are 2–3 mm long. Following the concept of Wilkinson & Stace (1991), both diploid ($2n = 14$) and tetraploid ($2n = 28$) races of *F. ovina* are known. However, only diploids have been reported in Norway (Engelskjøn, 1979).

Festuca ovina is found from the nemoral to the high-alpine regions, and it is widely distributed throughout northern and central Europe. It occurs in a variety of habitats, particularly in unproductive grasslands and in drought-exposed habitats (Grime, Hodgson & Hunt, 1988; Prentice *et al.*, 1995).

MATERIAL AND METHODS

SITES

Most plants were sampled from sites in the Isfjorden area, Central Spitsbergen, Svalbard in 1997 by B. Andersen, R. Elven, S. Fjellheim, A. S. Guldahl, J. Haugen, A. C. Scheen, and N. W. Steen (Fig. 1, Table 1).

Festuca ovina plants were sampled from Finnmark, northern Norway and Inarin Lappi, northern Finland by I. Nordal and R. Elven (Fig. 1, Table 1).

PLANT MATERIALS

A total of 50 populations was sampled in Svalbard. From most populations, plants were collected within an area of about 150 × 150 m, if possible with at least 3 m between each plant. An initial test was made to check enzymatic variation within populations. From each of the putative taxa, electrophoresis was applied to one large population, consisting of 20 individuals (populations BA1, BR1, ED1 and HY1, see Table 1). As the variation at the population level was negligible, samples of five individuals per population were regarded as sufficient for further sampling.

Live plants were collected for isoenzyme and cytological studies. Ecological data were recorded in the field for 33 populations (Guldahl & Elven, 1999).

The plants were grown in pots outdoor in Longyearbyen. The plants were transplanted to phytotrone chambers at the University of Oslo in late September, with an alternating summer and winter regime, 12 weeks of each.

Six populations of *F. ovina* were sampled in Finnmark. Four populations (OV1, OV4–OV6) were sampled on the coast of Finnmark to represent the northern *F. ovina* type, which deviates morphologically from the *F. ovina* found elsewhere in Norway, and is represented in this study by populations OV2 and OV3. Live plants were brought to Oslo and cultivated in phytotrone chambers at the University of Oslo, under the same conditions as the Svalbard plants.

METHODS

Chromosome counts

Root-tips were pretreated in 8-hydroxyquinoline for 2 h at room temperature, and for 2 h at 4°C. The root tips were then fixed for 24 h in absolute ethanol:acetic acid (3:1), washed three times in 70% ethanol, stored in 70% ethanol at −20°C, stained in Snow's carmine (Snow, 1963) for 48–62 h at room temperature, hydrolysed for 10 min in 45% acetic acid at 60°C, and squashed in 45% acetic acid.

Enzyme electrophoresis

Enzyme electrophoresis was performed at the University Courses in Svalbard (UNIS), in general following the methods outlined by Morden, Doebley & Schertz (1987) and Wendel & Weeden (1989). Leaves were crushed in a grinding buffer and stored at −80°C. The homogenates were absorbed onto paper wicks and applicated on 12.4% starch gels (3% sucrose). Horizontal eletrophoresis was run at 4°C.

Two different gel- and electrode buffer systems were used; the AB-system (with boric acid, Tris and lithium hydroxide, pH 8.3) and the D-system (with L-histidine and citric acid, pH 6.5). AB-system gels were run for at least 8 h at 60 mA each, and D-systems gels were run for at least 4 h at 50 mA each.

Five enzyme systems were used: DIA (Diaphorase), 6-PGD (6-Phosphogluconate dehydrogenase), PGM (Phosphoglucomutase), GPI (Glucose-6-phosphate isomerase) and TPI (Triose-phosphate isomerase). D-systems gels were stained for DIA, 6-PGD, and PGM. AB-system gels were stained for GPI and TPI.

Genetic interpretation of banding patterns in polyploids is difficult due to the duplications of entire genomes (Kephart, 1990). Furthermore, the distinction between allozymes/isozymes and alleles/loci is difficult without crossing experiments and analyses of progeny segregation (Werth, 1989; Brochmann, Soltis & Soltis, 1992). The term 'enzyme band' is therefore used in the polyploid materials instead of allozymes/isozymes. Enzyme bands grouped together on the gel were interpreted as one 'locus', meaning that they correspond to one single locus in the closely related diploid species, even though these bands most likely represent several duplicated loci (Brochmann *et al.*, 1992). Exact dosage differences of otherwise identical enzyme bands were not interpreted. The electrophoretic variants found in polyploids were therefore designated 'phenotypes' rather than 'genotypes', and variants referring to all 'loci' were designated 'multilocus phenotypes'. In the diploid material, the terms 'allele', 'locus', 'genotype' and 'multilocus genotypes' were used.

The most anodally migrating 'locus' was designated '1'. Supposed homomeric and monomeric enzyme bands were given allelic designations (a, b, c and so on). Mean

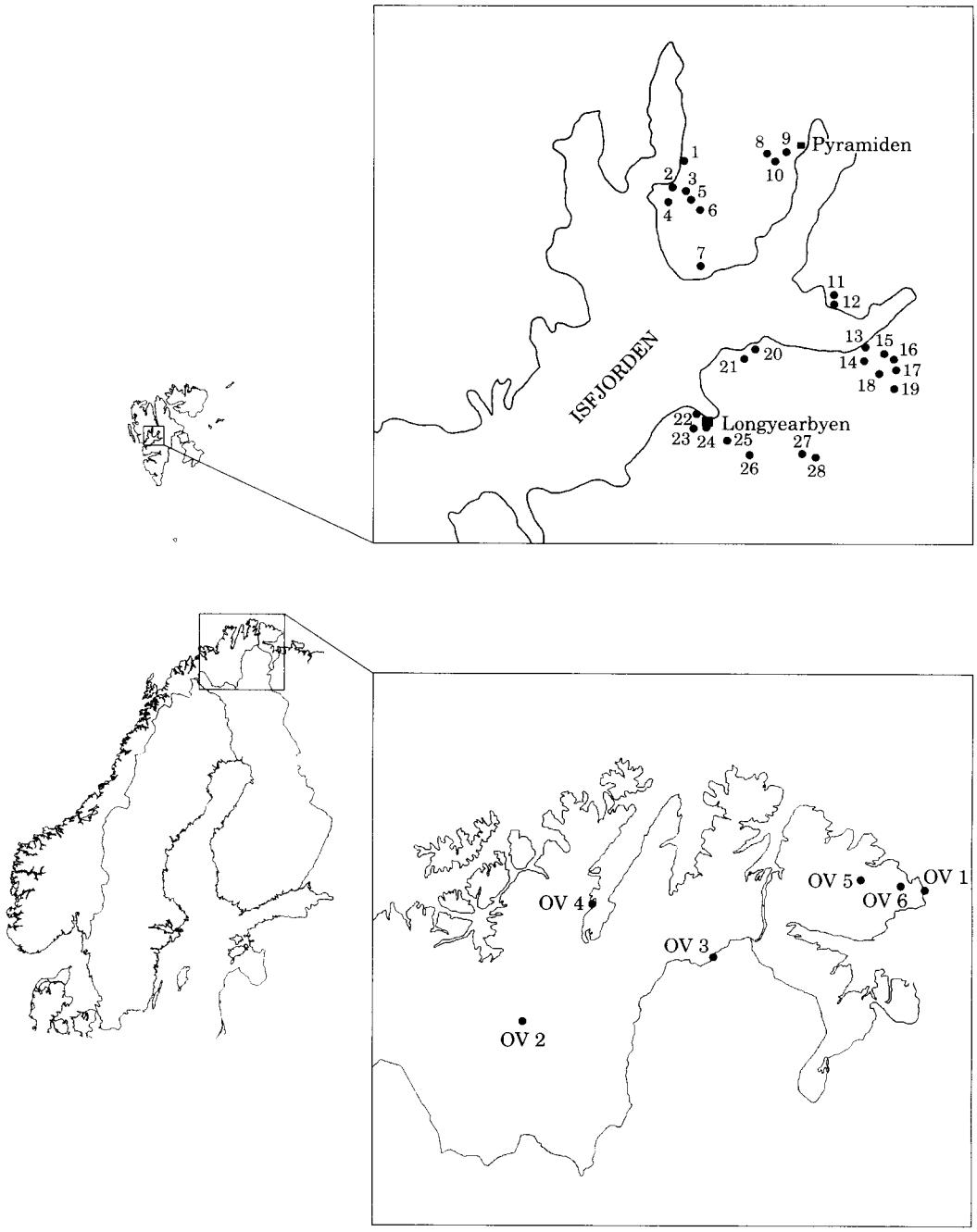

Figure 1. Sampling sites from Isfjorden on Central Spitsbergen, Finnmark in northern Norway, and Inarin Lappi in northern Finland.

migration values were calculated for all enzyme bands, relative to the origin-front distances of the gel.

The data set resulting from the isozyme electrophoresis was analysed by two methods: unweighted Pair-Group Method using Arithmetic averages (UPGMA) clustering of 309 individuals, using NTSYS-pc, version 2.02h (Rohlf, 1998); and PCO ordination of 309 individuals, in NTSYS-pc, version 2.0 (Rohlf, 1998).

Different similarity or distance coefficients were applied (Rohlf, 1998): Dice, SM (simple matching), and J (Jaccard). The Dice coefficient for two-state data are given by $\mathbf{2a/(2a+b+c)}$, where **a** is the number of shared bands, **b** and **c** are the number of bands present

Table 1. Analysed material with population code, number of analysed plants and reference code to chromosome-counted individuals, and number of individuals analysed by means of isozyme electrophoresis.

Species	Population	Chromosome analyses		Isozyme electrophoresis
		N	Individual no.	*N*
F. baffinensis	BA1	—	—	18
	BA2	1	BA2-2	5
	BA3	1	BA3-3	5
	BA4	—	—	5
	BA5	—	—	5
	BA6	1	BA6-3	3
	BA7	—	—	5
	BA8	1	BA8-7	5
	BA9	—	—	5
	BA10	—	—	5
	BA11	—	—	5
	BA12	—	—	5
F. brachyphylla	BR1	—	—	20
	BR2	1	BR2-1	5
	BR3	1	BR3-4	5
	BR4	1	BR4-2	5
F. edlundiae	ED1	—	—	20
	ED2	1	ED2-3	5
	ED3	—	—	5
	ED4	1	ED4-5	5
	ED5	—	—	2
	ED6	—	—	2
	ED7	—	—	5
	ED8	—	—	5
	ED9	—	—	5
	ED10	—	—	1
	ED11	—	—	5
	ED12	—	—	5
	ED13	—	—	5
	ED14	—	—	5
	ED15	—	—	1
	ED16	—	—	5
	ED17	—	—	1
	ED18	—	—	5
	ED19	1	ED19-3	5
	ED20	—	—	5
	ED21	—	—	5
	ED22	—	—	5
	ED23	—	—	5
	ED24	1	ED24-2	5
	ED25	—	—	5
	ED26	—	—	5
	ED27	—	—	5
F. hyperborea	HY1	—	—	20
	HY2	1	HY2-3	4
	HY3	1	HY3-2	5
	HY4	1	HY4-2	5
	HY5	—	—	1
F. species	SP1	—	—	3
	SP2	1	SP2-5	4
F. ovina	OV1	—	—	4
	OV2	—	—	5
	OV3	—	—	4
	OV4	2	OV4-3, OV4-4	8
	OV5	—	—	5
	OV6	2	OV6-4, OV6-5	5

Table 2. Chromosome numbers and geographic origin of the investigated populations

Taxon	Ploidy level	Specimen	Locality S = Svalbard F = Fennoscandia	Chromosome number	
				2*n*	No. of metaphasic counts
F. baffinensis	4x	BA2-2	S: Templet	28	2
		BA3-3	S: Odinfjellet	28	2
		BA6-3	S: Kapp Wijk	28	3
		BA8-7	S: Gjelrabbene	28	3
F. brachyphylla	6x	BR14-1	S: Endalen	42	3
		BR16-4	S: Adventdalen	42	3
		BR17-2	S: Adventdalen	42	3
F. edlundiae	4x	ED20-3	S: Gipsvika	28	3
		ED22-5	S: Planteryggen	28	3
		ED37-3	S: Kapp Wijk	28	3
		ED42-2	S: Skjørlokstupet	28	3
F. hyperborea	4x	HY47-3	S: Todalen	28	3
		ED48-2	S: Gjelrabbene	28	3
		ED49-2	S: Coloradofjellet	28	3
F. species	6x	SP2-5	S: Flyplassen	42	3
F. ovina	2x	OV4-3	F: Trollholmsund	14	3
		OV4-4	F: Trollholmsund	14	3
		OV6-4	F: Persfjord	14	3
		OV6-5	F: Persfjord	14	3

in one sample but absent in the other. Shared absence of bands is thus not taken into account. The SM coefficient is given by $(\mathbf{a}+\mathbf{d})/(\mathbf{a}+\mathbf{b}+\mathbf{c}+\mathbf{d})$, where **a**, **b** and **c** are defined as above, whereas **d** is the number of bands absent in both samples. The Jaccard coefficient is given by $\mathbf{a}/(\mathbf{n}-\mathbf{d})$, where **a** and **d** are as defined above, and **n** is the total sample size. The different coefficients gave more or less identical results, and only the analyses based on SM similarity are shown.

RESULTS

CHROMOSOME NUMBERS

Chromosome numbers were determined for 19 plants, and a total of 55 mitotic counts was obtained. All chromosome numbers were consistent with those previously reported in the species as currently circumscribed: *Festuca baffinensis*, *F. edlundiae* and *F. hyperborea* proved to be tetraploid, with the chromosome number $2n = 28$. *Festuca brachyphylla* proved to be hexaploid, with the chromosome number $2n = 42$, whereas *F. ovina* proved to be diploid, with $2n = 14$ (Table 2). The chromosomes were large, and most of the chromosomes were metacentric.

ENZYME VARIATION

A total of 313 plants from Svalbard and Finnmark was analysed using enzyme electrophoresis. All populations were easily referred to one or other species, except populations SP1 and SP2 ('*Festuca species*', sampled at Longyearbyen Airport). The multilocus phenotypes representing these plants, except individual SP1-3, were electrophoretically identical and were given the designation U1. The unique multilocus phenotype of individual SP1-3 were given the designation U2.

Variation at individual 'loci'

All five enzyme systems (DIA, 6-PGD, PGM, GPI, TPI) displayed activity and more or less legible bands. NADH Diaphorase (DIA) gave unique enzyme phenotypes for all taxa but was nevertheless omitted from the further analyses, due to uncertainties concerning the enzyme structure (mono/di/tetrameric, see Kephart, 1990), and difficulties with the interpretation in the polyploids. Five more enzyme systems were tested, AMP, AAT, MDH, IDH, and SKD, but were not successful. AMP and IDH gave no bands at all, AAT and MDH gave no variation for the materials tested, and SKD was difficult to interpret.

6-phosphogluconate dehydrogenase (6-PGD). This is a dimeric enzyme (Kephart, 1990). Two areas of activity appeared on the gels, probably corresponding to a plastid and a cytosol isozyme. Only the most anodal area, labelled '*6-Pgd-1*', was interpretable. (Figs 2, 3). Five homomeric bands gave a total of five different phenotypes. One homozygous phenotype, **b**, was observed in all *F. brachyphylla* and *F. hyperborea* plants,

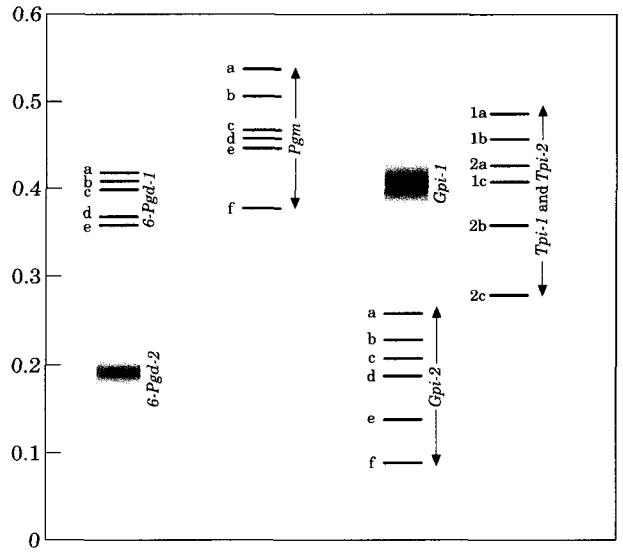

Figure 2. Migration values of the 23 interpreted allelic bands of *Festuca* relative to the anodal migration front (R_m values) for the four enzyme systems 6-PGD, PGM, GPI and TPI. Uninterpreted regions of activity (i.e. *'6-Pgd-2'* and *'Gpi-1'*) are shaded.

and in the *F. species* plants, except of one specimen, SP 1-3, which showed phenotype **be**, with an unbalanced dosage. All *F. edlundiae* plants were fixed heterozygous, with phenotype **be**. All the *F. baffinensis* plants were also fixed heterozygous and most populations had the **ad** phenotype, whereas population BA10 and three individuals from BA1 had the **cd** phenotype. The diploid *F. ovina* populations from Finnmark displayed two alleles, **b** being most frequent. Most plants were homozygous **bb**, whereas **a** was found in a heterozygous combination only.

Phosphoglucomutase (PGM). This monomeric enzyme is most often coded by two loci in plants (Kephart, 1990). However, only one area of activity appeared on the gel. A possible interpretation is that two loci overlap in this area. The separation of these two putative loci was difficult, and the area of activity was therefore interpreted as one complex locus, labelled *'Pgm'* (Figs 2, 3). Six monomeric bands gave a total of seven different phenotypes. All *F. edlundiae* plants were homozygous, with phenotype **e**. All *F. brachyphylla* plants were fixed heterozygous with phenotype **ef**. Also the unidentified *F. species* plants had the **ef** phenotype. The *F. hyperborea* plants were fixed heterozygous with the **de** phenotype. The **be** phenotype dominated in the *F. baffinensis* plants, but one plant had some rare 'alleles', with phenotype **abce** (i.e. BA8-2), and another plant was homozygous, with phenotype **e** (BA7-4). Most *F. ovina* plants were homozygous, with genotype **ee**. The rare **c** allele was found in one homozygote and two heterozygotes, not violating Hardy–Weinberg equilibrium.

Glucose 6 phosphate isomerase (GPI). This enzyme is dimeric (Kephart, 1990). Two areas of activity on the gels probably corresponded to a plastid and a cytosolic

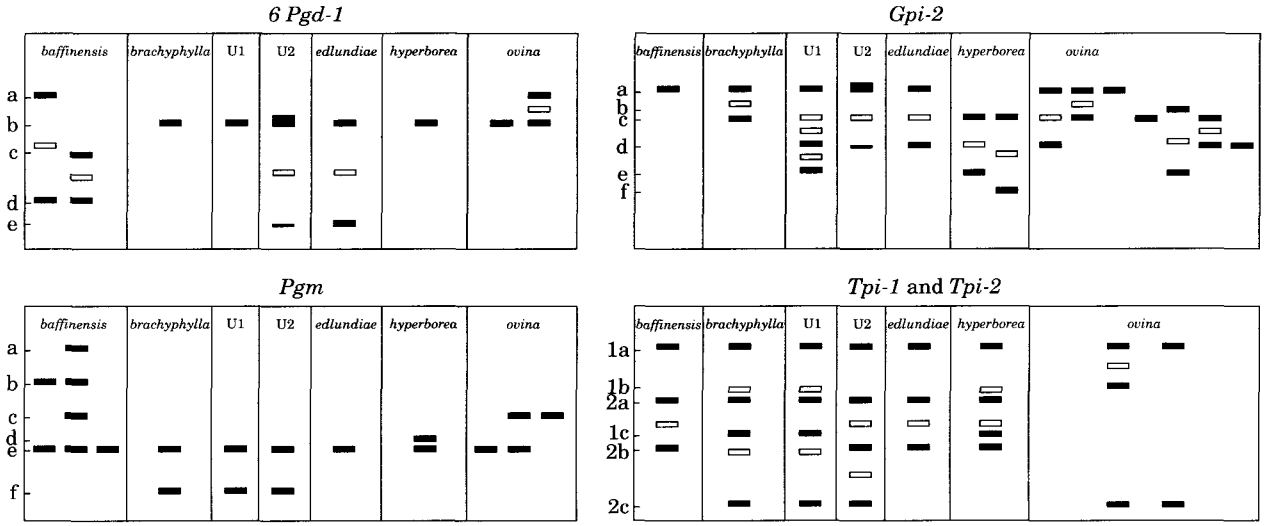

Figure 3. Interpreted enzyme phenotypes (polyploids) and genotypes (diploids) of the five *Festuca* species and the possible hybrids (SP1 and SP2) at five different 'loci'. Supposed mono- or homomeric bands are given allelic designations (a, b and so on) and are illustrated by filled boxes, whereas open boxes illustrate the supposed heterodimeric bands.

isozyme. However, only the most cathodal area, *'Gpi-2'*, was interpretable (Figs 2, 3). Six enzyme bands were observed, giving a total of six different phenotypes in the polyploids. The three-banded patterns (e.g. phenotype **ac** in *F. brachyphylla*) were interpreted as two homodimers and an intermediate heterodimer. The six-banded pattern in the **ade** phenotype found in the unidentified *F. species* plants was interpreted as three homodimers and three heterodimers. Specimen SP1-3 had the **ad** phenotype, with an unbalanced dosage. All *F. baffinensis* plants were homozygous, having phenotype **a**. The *F. brachyphylla* plants had the **ac** phenotype. The *F. edlundiae* plants were fixed heterozygous, with phenotype **ad**. The *F. hyperborea* plants were also heterozygous, with either phenotype **ce** or **cf**. *Festuca ovina* showed much variation in GPI. Six alleles were present, most often in heterozygous condition. The **a** and **c** alleles were most frequent (Figs 2, 3; Table 3).

Triose phosphate isomerase (TPI). This dimeric enzyme is most often coded by two loci in plants (Kephart, 1990). One area of activity appeared on the gels. Due to the lack of heterodimeric bands intermediate between supposed homomeric bands, the area of activity was interpreted as two overlapping 'loci', probably corresponding to a plastid and a cytosolic isozyme. They were labelled *'Tpi-1'* and *'Tpi-2'* (Figs 2, 3). A total of six enzyme bands were observed, giving four different enzyme phenotypes in the polyploids. The most common enzyme phenotype was **1a/2ab**; and this four banded pattern was found in all *F. edlundiae* and all *F. baffinensis* plants, interpreted as homozygous for *'Tpi-1'* and fixed heterozygous for *'Tpi-2'*. The *F. brachyphylla* plants were fixed heterozygous for both 'loci', with a six-banded pattern; **1ac/2ac**. Also the unidentified *F. species* displayed the phenotype **1ac/2ac**. Specimen SP 1-3 also displayed a six-banded pattern, but this pattern was interpreted as **1a/2abc**, i.e. homozygous for *'Tpi-1'*, and with three bands present in *'Tpi-2'*. Only one enzyme phenotype (**1ac/2ab**) was found within the *F. hyperborea* plants which all showed a six-banded pattern resulting from fixed heterozygosity at both 'loci'. Within the *F. ovina* material, two genotypes were found; the frequent **1aa/2cc**-genotype and the rare **1ab/2cc** genotype (Figs 2, 3; Table 3).

Multilocus phenotypes

A total of 26 multilocus phenotypes was observed. In the 50 populations of polyploid plants from Svalbard, 10 multilocus phenotypes were found, whereas 16 multilocus genotypes were found in the four diploid populations from Finnmark (Table 3). All multilocus phenotypes were species-specific. Considering the Svalbard materials, the multilocus phenotypes generally had a skewed distribution within each species, with one common type and a few rare ones. Moreover, each population usually showed one multilocus phenotype only. In Svalbard, a total of 21 bands was observed, of which 11 were unique to single species. Seven unique bands were fixed and diagnostic to different species. Diagnostic bands were observed for all four Svalbard species (see below).

Festuca baffinensis. Two 'loci' were polymorphic; *'6-Pgd-1'* and *'Pgm'*, with two phenotypes at *'6-Pgd-1'*, and three at *'Pgm'*. One phenotype was found at each of the remaining 'loci'. Four multilocus phenotypes were observed. 'baffinensis 1' dominated and was found in 77% of the plants and from most of the localities. 'baffinensis 4' was found in 20% of the materials, but from one locality only (Fig. 1, site no. 13). The remaining two phenotypes were found in one single plant each. In Svalbard, all three bands observed for *6-Pgd-1* (**a**, **c** and **d**) were unique to *F. baffinensis*. The bands **a** and **d** were found in all *F. baffinensis* plants, whereas **c** was observed in the plants from site no. 13 only. Band **a**, however, was also observed in some of the *F. ovina* plants from Finnmark. At *'Pgm'*, band **b** was observed in all plants except one, and not found in any of the other species.

Festuca brachyphylla. All 35 plants analysed had the same banding pattern. At *'Pgm'*, band **f** was fixed and diagnostic, i.e. not found in any of the other species. In Svalbard, band **c** was also fixed and diagnostic for this species at *'Tpi-2'*. However, band **c** was also observed in all *F. ovina* plants from the mainland.

Festuca edlundiae. No variation was detected among the 128 plants analysed. At *'6-Pgd-1'*, band **e** was unique, and thus diagnostic to this species. At *'Gpi-2'*, all plants were heterozygous, having the phenotype **ad**, observed in *F. edlundiae* only.

Festuca hyperborea. Most 'loci' were monomorphic, except *'Gpi-2'*, where two phenotypes were observed. In contrast to the other Svalbard species, the distribution of the two multilocus phenotypes in *F. hyperborea* was about equal; 'hyperborea 1' constituted 46% of the materials, 'hyperborea 2' 54%. Band **d** at *'Pgm'* was observed in this species only and occurred in all plants. At *'Gpi-2'*, band **f** in 'hyperborea 2' was not found in any of the other species.

Festuca species 1. Two individuals originally sampled as *F. edlundiae* (SP1-1, SP1-2) and four individuals originally sampled as *F. brachyphylla* (SP2) displayed

Table 3. Multilocus phenotypes/genotypes related to species and population. Each multilocus phenotype/genotype is defined by presence/absence of mono- or homomeric enzyme bands. For simplicity, the supposed homo- or monomeric bands are given allelic designations (a, b, c and so on) with the most anodally migrating band labelled 'a'. Supposed heteromeric bands are left out. Populations labelled 'OV' are sampled in northern Fennoscandia, the others in Svalbard. Populations including more than one multilocus phenotype are denoted by asterisks. Specimens are indicated in parentheses

Multilocus/ genotypes	Populations	*N*	Freq. %	*6-pgd-1*					*Pgm*						*Gpi-2*						*Tpi-2*			*Tpi-2*		
				a	b	c	d	e	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	a	b	c
baffinensis 1	BA1*, BA2–BA6, BA7*, BA8*, BA9–BA12	54	77	a			d			b			e		a						a			a	b	
baffinensis 2	BA7* (4)	1	1	a			d						e		a						a			a	b	
baffinensis 3	BA8 (2)	1	1	a			d		a	b	c		e		a						a			a	b	
baffinensis 4	BA1* (1–15)	14	20			c	d			b			e		a						a			a	b	
brachyphylla	all	35	100		b								e	f	a		c				a		c	a		c
edlundiae	all	130	100		b			e					e		a			d			a			a	b	
hyperborea 1	HY1* (2, 11, 16–17, 19), HY2–HY3, HY4* (2), HY5	16	46		b							d	e				c		e		a		c	a	b	
hyperborea 2	HY1* (1, 3–10, 12–15, 18, 20), HY4* (1, 3–5)	19	54		b							d	e				c			f	a		c	a	b	
U1	SP1* (1–2), SP2	6	100		b								e	f	a			d	e		a		c	a		c
U2	SP1* (5)	1	100		b			e					e	f	a			d			a			a	b	c
ovina 1	OV1–OV5	9	29		b								e		a						a					c
ovina 2	OV1	2	7		b								e		a			d			a	b				c
ovina 3	OV1	1	3	a	b								e				c				a					c
ovina 4	OV1	1	3		b						c		e				c	d			a					c
ovina 5	OV2, OV4	2	7	a	b								e		a						a					c
ovina 6	OV2	2	7	a	b								e		a		c				a					c
ovina 7	OV2	2	7		b								e			b			e		a					c
ovina 8	OV3	1	3		b								e					d			a					c
ovina 9	OV3	4	13		b								e		a		c				a					c
ovina 10	OV4	1	3		b								e				c	d			a					c
ovina 11	OV4	1	3		b								e				c				a					c
ovina 12	OV4	1	3		b								e		a			d			a					c
ovina 13	OV4	1	3	a	b								e		a			d			a					c
ovina 14	OV5	1	3	a	b						c						c				a					c
ovina 15	OV6	1	3		b						c		e		a		c				a					c
ovina 16	OV6	1	3		b								e		a						a					c

banding patterns similar to those of *F. brachyphylla* in all 'loci', except *'Gpi-2'*, where the plants displayed the bands **a**, **b** and **e**. The **a** band was common in Svalbard plants, whereas the **d** band was otherwise found in *F. edlundiae* and *F. species 2* only, and the **e** band in *F. hyperborea* only. This particular multilocus phenotype was labeled 'U1'.

Festuca species 2. One specimen originally sampled as *F. edlundiae* (SP1-3) displayed banding patterns similar to those of *F. edlundiae* in three enzyme systems (*'6-Pgd-1', 'Gpi-2', 'Tpi-1'*) and to that of *F. brachyphylla* in *'Pgm'*. *'Tpi-2'*, showed an additive banding pattern, combining the bands of *F. edlundiae* (bands **a** and **b**) with those of *F. brachyphylla* (bands **a** and **c**). This multilocus phenotype was labeled 'U2'.

Festuca ovina. In contrast to the polyploid materials from Svalbard, the six populations of *F. ovina* in northern Norway (31 plants) were very variable. Altogether, 16 multilocus genotypes were observed. All loci were heteromorphic, except *Tpi-2*, where all plants were homozygous **cc**. The observed banding patterns were in agreement with those of a regular outcrossing plant with more or less random mating. Numbers of homozygous and heterozygous individuals were in Hardy–Weinberg equilibrium, in spite of small population samples. The two populations OV2 and OV3 (representing southern and inland materials of *F. ovina*), were not separable from the northern and coastal populations (OV1, OV4–OV6) on the basis of enzymes.

Variation among taxa

In the UPGMA dendrogram including *F. ovina* (Fig. 4), *F. baffinensis* is the most distinct species, separated from the others at a similarity coefficient of 0.62. *Festuca hyperborea* is the second most distinct species, separated at 0.65. The *F. ovina* cluster is separated from the rest at a coefficient of 0.74. *Festuca edlundiae* and *F. brachyphylla* cluster together with the two multilocus phenotypes (U1 and U2) representing the deviating plants from populations SP1 and SP2. *Festuca brachyphylla* and U1 are separated from *F. edlundiae* and U2 at 0.76. Further separation within *F. brachyphylla* and *F. edlundiae* is found at similarity coefficients of 0.87 and 0.92, which corresponds to levels of infraspecific variation in the other species, i.e. *F. baffinensis* and *F. ovina*.

Exclusion of the mainland *F. ovina* materials from the analysis did not change the mutual relationships among the Svalbard taxa. *Festuca baffinensis* still came out as the most distinct species. Considering the three other species, *F. hyperborea* was most distinct, separated from the rest at 0.67. *Festuca brachyphylla* and U1 were separated from *F. edlundiae* and U2 at a coefficient of 0.76.

Excluding both *F. ovina* and multilocus phenotypes U1 and U2 from the analysis gave clearer mutual relationships among the four Svalbard species (Fig. 5). Compared with the cluster analysis excluding *F. ovina* only, the close affinity between *F. brachyphylla* and *F. edlundiae* disappeared. Two main clusters were found at a similarity coefficient of 0.63; one of *F. baffinensis* and *F. edlundiae*, and another of *F. brachyphylla* and *F. hyperborea*. At a similarity coefficient of 0,75, all four species appeared as distinct clusters. U1 and U2 seemed accordingly to bridge *F. edlundiae* and *F. brachyphylla*.

The PCO analysis (Fig. 6) gave much the same results as the UPGMA analysis (Fig. 4). The structure in the plot was consistent with the current view of species delimitations within the *F. brachyphylla* complex. *Festuca ovina* and *F. baffinensis* appeared as most distinct and defined the span of axis one. *Festuca hyperborea* and *F. edlundiae* were positioned intermediately along this axis, whereas *F. brachyphylla* was placed close to the *F. ovina* cluster. PCO axis two separated *F. hyperborea* from the other species, whereas the third axis (not shown) separated *F. edlundiae* from the others. In the plot of the two first axes (Fig. 6), the deviating U1 plants were placed close to *F. brachyphylla,* whereas the U2 plant was placed closest to *F. edlundiae*. Plotting the first axis against the third (not shown), the U1 plants were equally close to *F. brachyphylla* and *F. edlundiae*, whereas the U2 individual was equally close to *F. edlundiae* and U1. Axis one, two and three explained 32%, 16% and 15% of the variance, respectively (altogether 64%).

As in the UPGMA analysis, exclusion of *F. ovina* from the ordination did not change the relationships among the Svalbard species. The four putative species still came out as distinct entities. *Festuca baffinensis* and *F. brachyphylla* spanned the first axis. *Festuca hyperborea* was close to *F. brachyphylla,* whereas *F. edlundiae* was intermediate between *F. brachyphylla* and *F. baffinensis*. Along the second axis, *F. hyperborea* was separated from the others. The third axis (not shown) separated *F. edlundiae* further from *F. brachyphylla* and *F. baffinensis*. PCO axes one, two and three explained 43%, 24% and 12% of the variance, respectively (altogether 79%).

Band sharing

A band sharing matrix (Table 4) shows that *F. brachyphylla* shared a large proportion of bands with both *F. hyperborea* and *F. edlundiae*. Among the four taxa, *F. brachyphylla* had the highest number of enzyme bands, corresponding to the highest ploidy level. However, the proportion of bands shared between *F. brachyphylla* and *F. baffinensis* was relatively small. *Festuca baffinensis* also had a relatively small proportion of shared

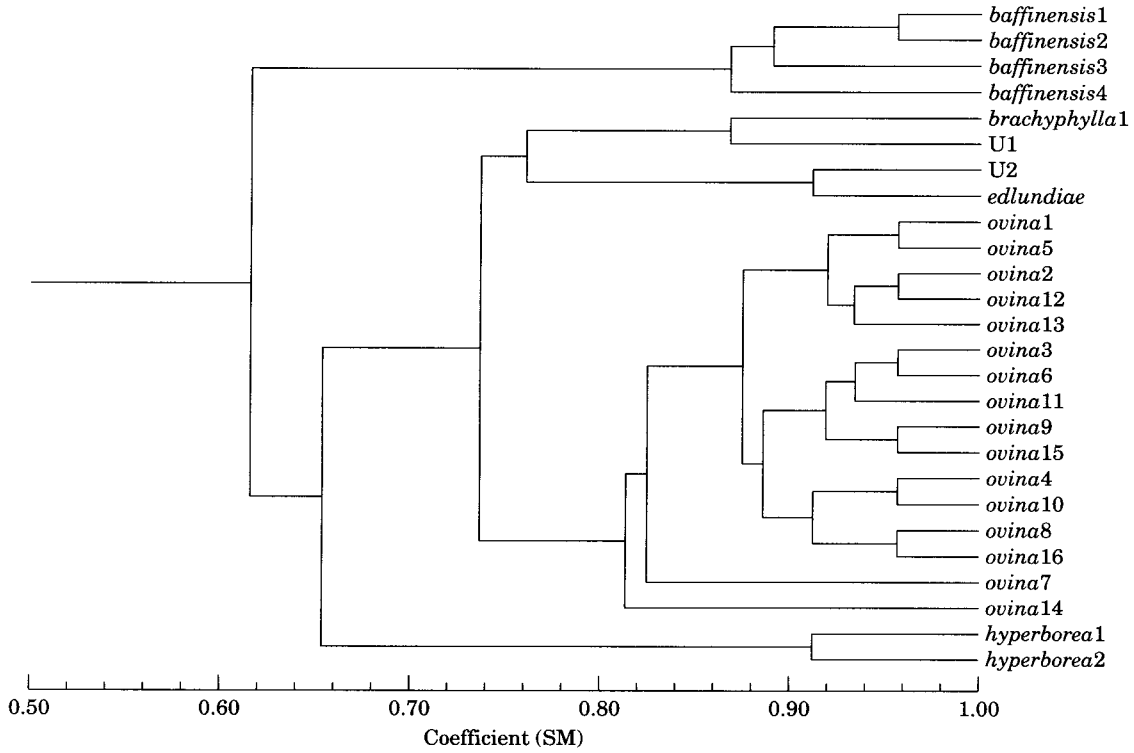

Figure 4. An UPGMA dendrogram based on presence-absence data of the 23 enzyme markers, constructed for the 26 genotypes/multilocus phenotypes of *Festuca* listed in Table 3. Simple Matching (SM) is applied as a similarity coefficient. U1 and U2 designate the multilocus phenotypes of the deviating plants from the airport populations (SP1 and Sp2).

bands with *F. hyperborea* and the deviating plants with multilocus phenotype U1.

A band sharing table (Table 5) shows that *F. ovina* had most enzyme bands in common with *F. brachyphylla* and the deviating plants with multilocus phenotypes U1 and U2, whereas the number of shared bands with *F. baffinensis* was low.

DISCUSSION

PLOIDY LEVELS

The basic chromosome number for the genus *Festuca* is x = 7 (Löve & Löve, 1975). Chromosome numbers reported in this study show that *F. brachyphylla* is hexaploid, with the chromosome number $2n = 42$, whereas *F. baffinensis*, *F. edlundiae* and *F. hyperborea* all proved tetraploid, $2n = 28$, in accordance with chromosome numbers reported from elsewhere, e.g. Holmen (1952), Frederiksen (1977), Aiken *et al.* (1994, 1995). According to the present species circumscriptions, the tetraploid report in *F. hyperborea* is new to Svalbard, and so is the hexaploid report in *F. brachyphylla*. For all four species, counts were made from geographically separated populations, strongly suggesting that the species have constant chromosome numbers in Svalbard.

ISOZYME VARIATION

With few exceptions, the investigated Svalbard materials represent four distinct enzymatic entities, corresponding to the four putative species of the area. Unique enzyme markers were found for all entities. The four enzyme systems used in this study were also used by Aiken *et al.* (1994, 1995) on Canadian material. The number of interpreted enzyme bands is 21 in the present study, and in the Canadian study it was 18. Most likely, several of the enzyme bands reported from Canada generally correspond to the enzyme bands found in Svalbard. However, because Aiken *et al.* (1994, 1995) did not provide relative migration values for their bands, it is not possible to make precise and detailed comparisons between the Canadian and Svalbard materials.

In the enzyme system 6-PGD, Aiken *et al.* (1994) found the presence of one band and the absence of another to be diagnostic for *F. baffinensis*. In Svalbard materials, these bands probably correspond to the enzyme band 1a, which is unique, although not fixed,

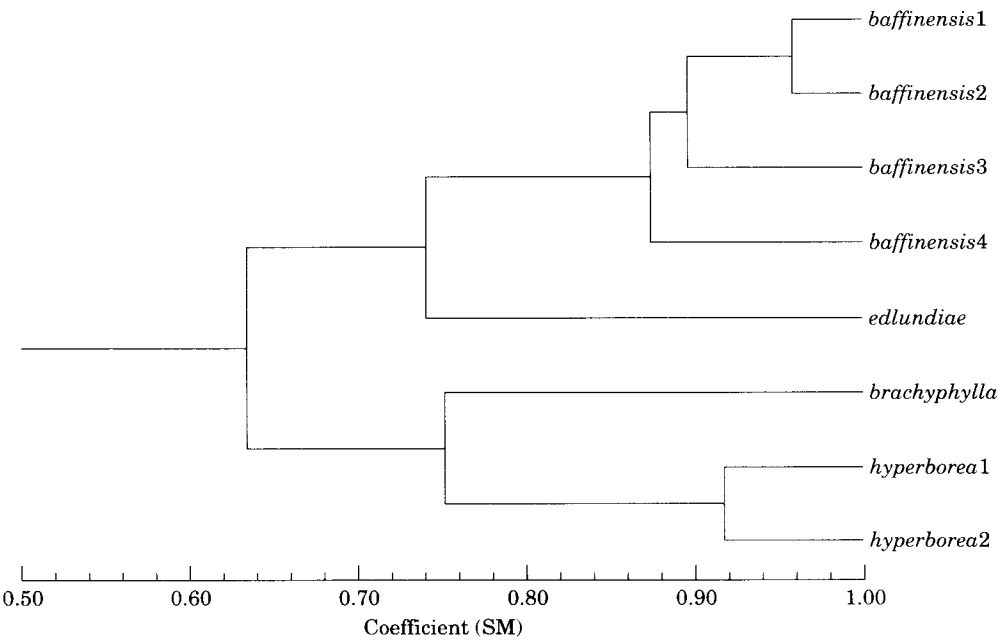

Figure 5. An UPGMA dendrogram based on presence-absence data of the 23 enzyme markers, constructed for the eight multilocus phenotypes of Svalbard *Festuca*, excluding multilocus phenotypes U1 and U2 and the genotypes of *F. ovina*. Simple Matching (SM) is applied as a similarity coefficient.

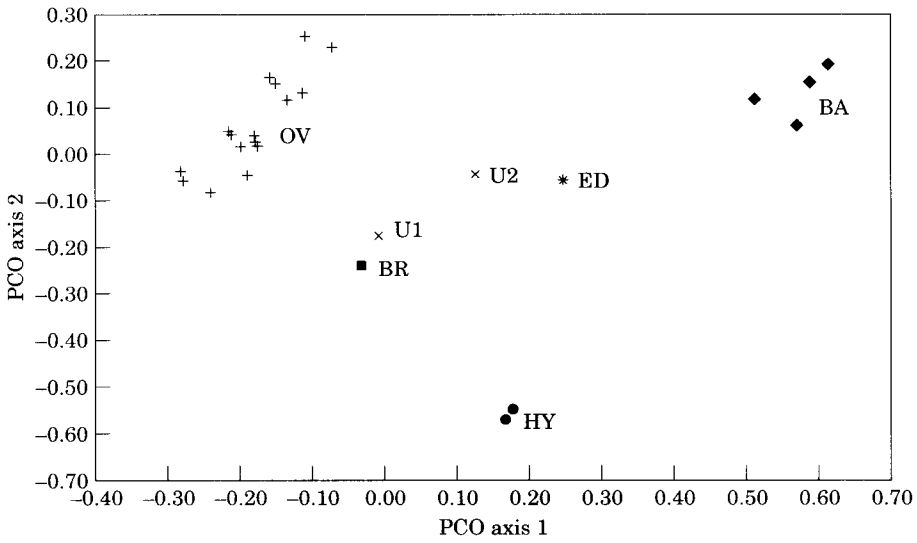

Figure 6. PCO ordination based on presence-absence data of the 23 enzyme markers, constructed for the 26 genotypes/multilocus phenotypes of *Festuca* (Table 3). Simple Matching (SM) is applied as a similarity coefficient. Abbreviations: BA = *F. baffinensis*, BR = *F. brachyphylla*, U1 and U2 = multilocus phenotypes of the deviating plants (populations SP1 and SP2) from Longyearbyen Airport, ED = *F. edlundiae*, HY = *F. hyperborea*, and OV = *F. ovina*.

in *F. baffinensis*, and 1b, which is absent from *F. baffinensis* only. In the present study, two more unique bands (c and d) were found in *F. baffinensis*, and one (d) was fixed and diagnostic. According to the results of Aiken *et al.* (1994), none of the other species can be differentiated on the basis of 6-PGD bands. In the Svalbard material, *F. edlundiae* was also distinct from the others by having one unique

Table 4. Percentage of enzyme bands shared between the different Svalbard entities. Bands shared by all entities are not accounted for

	F. baffinensis	*F. brachyphylla*	*F. edlundiae*	*F. hyperborea*	U1
F. brachyphylla	19				
F. edlundiae	24	24			
F. hyperborea	19	29	24		
U1	19	38	29	29	
U2	24	33	33	24	38

Table 5. Percentage of enzyme bands shared between the different Svalbard entities and *Festuca ovina*. Bands shared by all entities/taxa are not accounted for

Svalbard entities	*F. ovina*
F. baffinensis	17
F. brachyphylla	26
F. edlundiae	22
F. hyperborea	22
U1	30
U2	26

band (e), which was fixed and thus diagnostic for this species.

The enzyme system PGM gave several enzyme markers; the band f was diagnostic for *F. brachyphylla*, d for *F. hyperborea* and b considered as diagnostic for *F. baffinensis*, although lacking in one individual. Aiken and coworkers (1994, 1995) also reported enzyme markers in *F. baffinensis* and *F. hyperborea*, but no diagnostic band was found in *F. brachyphylla*. For GPI, no enzyme markers have been found, either by Aiken *et al.* (1994) or by us.

Due to differences in the interpretations, a comparison of the TPI-results is difficult. Aiken and coworkers reported one locus, whereas here the TPI banding pattern has been interpreted as two 'loci' (Table 3; Figs 3, 4). However, one band was reported as diagnostic for *F. brachyphylla* by Aiken *et al.*, as found by us. These bands are probably identical. Another band reported as diagnostic for *F. hyperborea* in Canada was not observed in Svalbard.

The isozyme results of this study are more or less in accordance with the results by Aiken *et al.* (1994, 1995). Four distinct entities are recognized in both studies, and it is likely that most enzyme markers found in the Canadian material correspond to those found in the Svalbard material. However, some additional markers were detected in Svalbard (6-PGD, PGM) and one was observed only in Canada (TPI).

In the present study, unique combinations of bands were found at most 'loci' for each species (possible hybrids not taken into account). This means that different combinations of enzyme bands are fixed in different species. Distinct and unique banding patterns occur at *'Pgm'* and *'Gpi-2'* for all four species. At *'6-Pgd-1'* and *'Tpi-1'*, *F. brachyphylla* and *F. hyperborea* have the same banding patterns. *Festuca baffinensis* and *F. edlundiae* have the same banding pattern at *'Tpi-1'*, and *F. baffinensis*, *F. edlundiae* and *F. hyperborea* share the same banding pattern at *'Tpi-2'*. Unique combinations of bands were also reported for all four species by Aiken and co-workers (1994, 1995).

The present enzyme data correspond fairly well with the other data sets for the Svalbard materials. Numerical analyses of RAPD data show clusters more or less distinct and consistent with current species circumscriptions (Fjellheim *et al.*, 1999, 2001). Also the morphometric analyses divide the material into the same four entities, confirming the species circumscriptions made by Aiken and co-workers (1994, 1995), and separating characters have been found for all four species (Fjellheim *et al.*, 1999, 2001).

RELATIONSHIPS AMONG THE SPECIES

Enzymatically, *F. baffinensis* seems to be the most distinct species, with a low percentage of shared bands (Table 4) and a separate position in both the UPGMA analysis (Fig. 4) and the PCO plot (Fig. 6). However, when the multilocus phenotypes U1 and U2 (representing possible hybrids and later offspring) are excluded, *F. baffinensis* clusters with *F. edlundiae* at a high similarity level in the UPGMA analysis, whereas *F. brachyphylla* clusters with *F. hyperborea* (Fig. 5). In spite of this fact, *F. baffinensis* must be regarded as the most distinct entity within the complex, as also confirmed by the analyses of RAPDs and morphometry (Fjellheim *et al.*, 1999, 2001).

The relationships among the three other species are more obscure. Considering proportions of band sharing, *F. brachyphylla* and *F. hyperborea* have most bands in common (29%). The *F. species* plants share a relative large proportion of bands with *F. brachyphylla* (38%); except of specimen SP1-3, which shares equal proportions of enzyme bands with both *F. brachyphylla*

and *F. edlundiae* (33%). The two UPGMA dendrograms based on enzyme data are also contradicting. According to the one including all Svalbard multilocus phenotypes (not shown), *F. edlundiae* and *F. brachyphylla* are most similar, whereas in the ordination plot, *F. brachyphylla* and *F. hyperborea* appear to be the two most similar species (Fig. 6). The *F. species* plants, which represent possible hybrids or introgressants (see below), appear to bridge *F. edlundiae* and *F. brachyphylla* in this plot.

Whereas the PCO analysis of the enzyme data supports the first cluster analysis with *F. hyperborea* as the most distinct species within the *brachyphylla/edlundiae/hyperborea* group, the RAPD data support *F. edlundiae* as the most distinct species within this group (Fjellheim *et al.*, 1999, in press). *Festuca brachyphylla* and *F. hyperborea* are not fully separated in the RAPD cluster analysis; three individuals, corresponding to those having enzyme multilocus phenotypes U1 and U2, seem to link the two clusters. On the other hand, *F. brachyphylla* is slightly more distinct than the two others in morphology (Fjellheim *et al.*, 1999, 2001).

The incongruent results obtained by the different data sets and different numerical analyses probably reflect an equidistant similarity and, possibly, an equidistant relationship among the three species *F. brachyphylla, F. edlundiae* and *F. hyperborea*. Thus, on basis of the present data, it seems difficult to judge the mutual relationships between these three species.

INFRASPECIFIC VARIATION

In Svalbard, infraspecific enzyme variation, although small, is present in *F. baffinensis* and *F. hyperborea*, whereas *F. edlundiae* and *F. brachyphylla* are enzymatically invariable.

In Canada, the widely distributed *F. brachyphylla* is reported to be the enzymatically most variable species within the *F. brachyphylla* complex (Aiken *et al.*, 1994, 1995). The low variation seen in *F. brachyphylla* in Svalbard thus suggests that the species may have been exposed to genetic bottlenecks during its postglacial migration to Svalbard. Furthermore, distance from glacial refugia is another factor likely to affect the genetic variation within a taxon. During species dispersion, most of the variation is retained in the source area, because the new colonies are recruited mainly from the marginal parts of the main distribution area. This may lead to consecutive founder events along the migration front, and to genetic depauperation in the new colonies (Barrett & Kohn, 1991), as also postulated for other Svalbard species (Odasz & Savolainen, 1996). Autogamy and ecological cleistogamy has been proved in Russian *F. baffinensis* and *F. brachyphylla* (Levkovsky, Tikhmenev & Levkovsky, 1981). Selfing would enhance the effect of the above-mentioned factors, as it lowers the genetic variability within populations (e.g. Stace, 1989). However, given that the species originally was variable, one should expect to find variation among the populations, but this is not the case in Svalbard.

In contrast to Canadian *F. brachyphylla*, the enzymatic variability was low in Canadian materials of *F. edlundiae* and *F. hyperborea* and intermediate in Canadian *F. baffinensis* (Aiken *et al.*, 1994, 1995), confirming the pattern of variability seen in the Svalbard material of these species. Original low enzymatic variability in combination with autogamy (and even cleistogamy) may explain these patterns.

Enzyme electrophoresis and RAPDs gave, however, different results regarding the variation patterns within taxa (Fjellheim *et al.*, 1999, 2001; Guldahl, 1999). The isozyme data gave a total of eight multilocus phenotypes; four in *F. baffinensis*, two in *F. hyperborea* and one in each of *F. brachyphylla* and *F. edlundiae*. The RAPD data revealed no variation in *F. baffinensis* nor in *F. hyperborea*, but some variation in *F. brachyphylla* and much variation within *F. edlundiae* (Fjellheim *et al.* 1999, 2001).

Based on the results discussed above, it seems likely that *F. brachyphylla, F. edlundiae* and *F. hyperborea* have one genome in common. This possibility is reflected in band-sharing and numerical analyses of isozyme data, as well as numerical analyses of RAPD data and morphometric analyses (Fjellheim *et al.*, 1999, 2001). *Festuca brachyphylla* and *F. hyperborea* may also have another genome in common, in addition to the one they share with *F. edlundiae*. This is supported by the rare enzyme bands found in these two species only. Allozyme markers give no clear indications that any of the species of the *F. brachyphylla* complex are directly involved (parentally) in the evolution of each other, even if shared genomes are probable. On the other hand, the relationships among *F. baffinensis* and the other taxa are apparently more distant. *Festuca baffinensis* may thus represent a separate evolutionary lineage within the complex.

POSSIBLE HYBRIDS

Judged from enzymes, DNA, and morphological variation together, there are a few deviating plants which more or less obscure the relationships within the *F. brachyphylla* complex. These plants, with population codes SP1 and SP2 (i.e. multilocus phenotypes U1 and U2), are most likely of hybrid origin. Sampled near Longyearbyen Airport, population SP1 was originally classified in the field as *F. edlundiae*, and found intermingled with population ED5. Population SP2, classified in the field as *F. brachyphylla*, was found on disturbed ground next to the parking area at the airport, on the opposite side of the runway to SP1. SP 2 was found intermingled with population ED11.

Additive enzyme banding patterns in the seven deviating plants of SP1 and SP2 suggest that these are hybrids between *F. brachyphylla* and *F. edlundiae*. In addition, six of the plants had one enzyme band (*Gpi-2e*) uniquely shared with *F. hyperborea*, indicating that *F. hyperborea* may also have been involved in the ancestry of these plants. Furthermore, RAPD data place the deviating plants as intermediates between *F. brachyphylla* and *F. hyperborea* (Fjellheim *et al.* 1999, 2001). Additive RAPD banding patterns indicating hybridity were not found.

Since first generation hybrids (F_1) are expected to display additive enzyme banding patterns of the putative parental species in all loci (Crawford, 1990; Rieseberg & Ellstrand, 1993), these deviant plants probably represent later-generation hybrids or introgressants involving all three species to a various degree. Furthermore, the chromosome number of individual SP2-4 is hexaploid ($2n=42$) like *F. brachyphylla*, not pentaploid ($2n=35$) as expected in a primary hybrid between *F. edlundiae* and *F. brachyphylla* (cf. Stace, 1989; Rieseberg & Ellstrand, 1993). Morphologically, these plants are intermediates between *F. brachyphylla* and *F. edlundiae*, or clusters with *F. brachyphylla* (Fjellheim *et al.*, 1999, 2001). Some reduction in fertility is expected in hybrids, but the putative hybrids appear to have fully developed pollen grains, indicating normal fertility (Fjellheim *et al.*, 1999, in press).

Thus, it is most likely that these individuals represent fully stabilized hybrid products involving both *F. brachyphylla*, *F. edlundiae* and *F. hyperborea* but with the strongest affinity to *F. brachyphylla*. Furthermore, reproductive independency from the parents is indicated by the apparently, retained fertility and the occurrence in two, albeit adjacent, populations.

Disturbed habitats are known to promote interspecific hybridization and to facilitate the maintainance of hybrid populations (e.g. Anderson, 1949; Rieseberg & Ellstrand, 1993). As there are many indications that hybridization and introgression have contributed to the formation of the deviating fescues in the airport area, introgression still might be going on in this disturbed area. However, a relatively high age of these putative hybrid products may be indicated by the apparent swamping of some genes from *F. hyperborea*, which is not known from the adjacent area today. Long-distance dispersal of pollen of *F. hyperborea* is improbable due to the reproductive system of these fescues. Autogamy, with a transition to cleistogamy, has been reported as an important mode of reproduction in the *F. brachyphylla* complex (Levkovsky *et al.*, 1981). Of course, autogamy and cleistogamous selfing greatly reduces the possibility of hybridization, but this mode of reproduction may allow hybrids to stabilize sympatrically with the parental taxa. In other angiosperms, cleistogamy has been shown to have strong potentials of hybrid stabilization (e. g. *Viola*; Marcussen & Borgen, 2000). It is therefore likely that the evolutionary history of the *F. brachyphylla* complex has elements of reticulation. Not only have species apparently originated through polyploidy, but hybridization and introgression appears to occur, even across ploidy levels, as previously shown in Arctic *Draba* (cf. Brochmann *et al.*, 1992) and also across genera (*Festuca* and *Vulpia*, cf. Stace & Ainscough, 1984).

However, an alternative theory to hybridization might be that the deviating plants represent an introduction of a foreign strand of *F. brachyphylla*, elsewhere known as a variable species.

RELATIONSHIPS BETWEEN THE *F. BRACHYPHYLLA* COMPLEX AND *F. OVINA*

As comparison of *F. ovina* from northern Fennoscandia and the *F. brachyphylla* complex in Svalbard involves enzymatic variation only, and the samples of *F. ovina* were small, conclusions regarding possible relationships cannot be drawn. In northern Fennoscandia, the morphologically deviating *F. ovina* plants are mainly found near the coast. Two of the *F. ovina* populations included in this study (OV2, OV3) were sampled in the interior of Finnmark, to represent the southern *ovina* morphotype. No enzymatic differences between the interior and the coastal plants were found. Thus, the observed morphological differences between northern and southern *F. ovina* may reflect phenotypic plasticity or different ecotypes only.

Based on the limited materials included in this study, it seems that the genomic compatibility between the hexaploid *F. brachyphylla* in Svalbard and the diploid *F. ovina* from northern Fennoscandia is high. The genomic similarity between *F. ovina* and *F. edlundiae*/*F. hyperborea* also seems relatively high. *Festuca brachyphylla*, *F. edlundiae* and *F. hyperborea* probably share a larger proportion of their genomes with *F. ovina* than with *F. baffinensis*. The nesting of *F. ovina* within the *F. brachyphylla* complex, as seen in the UPGMA dendrogram, strengthens the impression that the *F. brachyphylla* complex is an artificial taxonomic group. As already pointed out, *F. baffinensis* may represent an isolated lineage compared with the rest of the complex. There is reason to believe that the origin of polyploid taxa of the *F. brachyphylla* complex can be traced to (possibly ancestral) diploid species from the *F. brachyphylla* and *F. ovina* complexes. From the known circumpolar distribution of the *F. brachyphylla* complex, possible parental species might be found in the Beringian area, where both the *F. ovina* and the *F. brachyphylla* complexes probably have a longer history of contact, and where several diploids of the *F.*

ovina complex are reported, i.e. the mutually closely related and possibly conspecific *F. auriculata* Drob., *F. kolymensis* Drob., and *F. lenensis* Drob., in addition to the diploid *F. brevissima* from the *F. brachyphylla* complex.

A striking feature of *F. ovina* as opposed to the Svalbard taxa is its considerable amount of enzymatic variation. In a sample of 31 individuals, 16 multilocus genotypes were found. In contrast, a total of 10 multilocus phenotypes was found in the material from Svalbard, consisting of 277 individuals from four taxa. There is reason to believe that the explanation for this is the different breeding strategies. Diploid *F. ovina* has been found to be self-incompatible (Watson, 1958), and the great enzymatic diversity observed coincides well with what one would expect in a regular outcrosser. The polyploid Svalbard fescues, on the other hand, appear to be self-fertile and possibly inbreeders, resulting in low genetic diversity at the species level. Cleistogamy has been studied in several grasses in the Wrangel Island, and proved to be characteristic of *F. brachyphylla, F. baffinensis* and *F. brevissima* (Levkovsky *et al.*, 1981). As already pointed out, founder events and bottleneck effects have probably also contributed much to the observed genetic depauperation in the Svalbard polyploids.

SPECIES CIRCUMSCRIPTIONS

The isozyme results clearly support a new taxonomic delimitation within the *F. brachyphylla* complex in Svalbard. The species circumscriptions accepted in this study imply a major change compared with the treatments of Rønning (1961, 1972, 1996), with respect to both the number of species and their distributions within the archipelago. Therefore, new and precise descriptions are given below. The figures representing variation in quantitative morphological characters are based on Aiken (1995) and Fjellheim *et al.* (2001).

Most diagnostic characters used in the literature to discriminate between *F. brachyphylla* and *F. hyperborea* are probably not completely correct, since they both are likely to include *F. edlundiae* (see below).

Festuca brachyphylla

Plants 10–20 cm tall, growing in caespitose erect tufts. Culms glabrous and somewhat shiny; inflorescence culms often much longer than the basal leaves. Flag leaf blade long (1.3–15.0 mm) and linear. Leaves inrolled or tightly folded, green without bloom. Panicle slender and pale violet, bilateral. Spikelets 5.5–7.6 mm long, glumes lanceolate, unequal, lower glume 2.2–3.7 mm × 0.5–1.8 mm, upper glume 2.8–5.0 mm × 0.8–3.0 mm; lemma 3.8–4.9 mm; awn straight, 0.6–2.5 mm; ovary apex glabrous. Chromosome number $2n = 42$.

Habitat. In Svalbard, *F. brachyphylla* appears to have a narrow range of habitats. Plants grow in relatively acidophilic, dry and open habitats; often on disturbed ground; on level ground or in south-facing slopes. The substrate consists of Tertiary sandstones.

Festuca baffinensis

Plants 5–20 (–27) cm tall, growing in caespitose tufts. Culms erect with dense, curved hairs near the inflorescence. Flag leaf blade usually 1 cm or longer. Leaves loosely folded; green without bloom; usually erect; shorter than or of the same length as the culms. Panicle dark purple; short and broad; more or less unilateral. Spikelets 4.3–5.7 mm long; glumes lanceolate, lower glume 2.2–3.7 mm × 0.5–1.8 mm, upper glume 2.8–5.0 mm × 0.8–3.0 mm; lemma 2.8–4.0 mm; awn straight, 0.9–3.1 mm; ovary apex with a few, sparse hairs. Chromosome number $2n = 28$.

Habitat. The species is distinctly basophilous. In Svalbard, plants grow in relatively species-rich habitats with closed vegetation; in south-facing slopes with dense vegetation cover, often beneath bird-cliffs; or on river outwash areas and riverbanks, considered to be secondary habitats.

Festuca hyperborea

Plants 2.5–10 cm tall, growing in caespitose tufts. Culms erect; glabrous or sparsely hairy. Flag leaf characteristically short (0.8–3.8 mm) and spoon-shaped. Leaves often usually folded; recurved; green without bloom, often with some violet pigmentation. Panicle violet and bilateral. Spikelets 3.4–4.9 mm long; lower glume 1.3–2.0 mm × 0.4–0.7 mm, upper glume 2.0–2.8 mm × 0.6–1.2 mm; lemma 2.6–3.1 mm; awns twisted, 0.4–1.5 mm; ovary apex glabrous. Chromosome number $2n = 28$.

Habitat. In Svalbard, plants grow in relatively closed vegetation with much mosses; in damp sites in cold areas; on polygon soil; in snowbeds; in open habitats in mountain plateaus; in more or less disturbed mountainsides. The habitats are species-poor, and thermophilous species are lacking.

Festuca edlundiae

Plants 2.5–14 cm high; semi-prostrate; growing in caespitose tufts. Culms glabrous; semi-prostrate. Flag leaf blades often more than 1 cm long; linear. Leaves almost flat; somewhat prostrate; bluish-green with bloom. Panicle slender; violet and bilateral. Spikelets 2.9–8.1 mm long; glumes lanceolate, lower glume 1.6–3.2 mm × 0.4–1.2 mm, upper glume 2.7–4.4 mm ×

KEY TO SPECIES

1.	Culms densely hairy; ovary apex with a few, sparse hairs; panicle unilateral, dense and dark purple coloured	*F. baffinensis*
	Culms glabrous or sparsely haired; ovary apex glabrous; panicle violet and bilateral	2
2.	Plants prostrate to semi-prostrate; base of spikelet lacking trichomes; leaves bluish-green with glaucous bloom, may have some violet pigmentation	*F. edlundiae*
	Plants erect; trichomes at base of spikelet; leaves green without glaucous bloom, may have some violet pigmentation	3
3.	Blade of flag leaf short (0.8–3.8 mm) and spoon-shaped; awn short (0.3–1.5 mm) and twisted; leaves relatively short and loosely folded	*F. hyperborea*
	Blade of flag leaf long (1.3–15.0 mm) and linear; awn long (0.9–3.1 mm) and straight; leaves relatively long and tightly inrolled	*F. brachyphylla*

0.8–1.6 mm; lemma 3.4–4.6 mm; awn straight, 0.6–2.5 mm; ovary apex glabrous. Chromosome number $2n = 28$.

Habitat. In Svalbard, *F. edlundiae* has a wide range, including most of the habitats of the other species within the *F. brachyphylla* complex. Plants grow on exposed, gravelly ridges; raised gravel beaches; soil polygons; snowbeds on mountain plateaus; warm and dry habitats. Some degree of disturbance is often present.

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