Gene flow and mode of pollination in a dry-grassland species, *Filipendula vulgaris* (Rosaceae)

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Filipendula vulgaris is a characteristic species of dry nonacidic grasslands in Denmark. This habitat type occurs only on marginal areas not suitable for agriculture or urbanization and that are by their nature fragmented. The population genetic structure of *F. vulgaris* was investigated in 17 populations within two regions of Denmark, using isozyme electrophoresis. Small populations were found to have significantly fewer polymorphic loci than larger populations, but all populations maintained the same common allelic variants. The degree of isolation of individual populations did not affect the amount of genetic variation. Offspring arrays revealed a very high outcrossing rate (0.96). The field study demonstrated a very high level of gene flow between populations considering that small insects are thought to be the main pollinators of this species. An experiment to verify whether pollen transport by wind could explain the results from the field study demonstrated long-distance transport from isolated plants to bagged plants. *Filipendula vulgaris* pollen grains are very small and this explains why outcrossed progeny were found using pollination bags with small pore sizes. We conclude that wind pollination is indeed possible and together with insect pollination is causing the observed patterns of genetic variation. The substantial gene flow between populations may be reducing the effects of genetic drift in the small fragmented populations of *F. vulgaris*.

Keywords: fragmentation, gene flow, population size, wind pollination.

Introduction

Habitat disappearance is the prime cause for loss of species diversity in most parts of the world. Thus, indigenous and in many cases rare species that are occurring in habitats that decrease in abundance and size are threatened by extinction through stochastic demographic or environmental forces (e.g. Menges, 1991).

If populations are small and reproductively isolated, random genetic drift and inbreeding may diminish the amount of genetic and morphological variation (e.g. Ellstrand & Elam, 1993). This may be detrimental because maintenance of genetic variation is essential for the long-term survival of a species. The amounts of isozymic variation, as well as quantitative and morphological variation, have been demonstrated to be positively related to population size (van Treuren *et al.*, 1991; Raijman *et al.*, 1994; Weidema *et al.*,

1996; but see also Widén & Andersson, 1993 for a negative relationship).

The effects of population isolation are ambiguous. Fragmentation has been demonstrated to decrease levels of pollination and seed production for several species in forest fragments in a chaco dry forest regions in Argentina (Aizen & Feinsinger, 1994). In contrast, increases in interpopulation gene flow as a consequence of fragmentation have been hypothesized for the two wind-pollinated species *Acer saccharum* (Young *et al.*, 1993) and *Festuca ovina* (Berge *et al.*, 1998).

When habitat fragmentation affects plant species richness and density the insects that depend on various plants for food and shelter may be affected simultaneously (Olesen & Jain, 1994 and references therein). This could lead to changes in pollinator quantity and quality for the insect-pollinated species (Kwak *et al.*, 1998). The response of an individual species to habitat fragmentation and reduction in population size depends largely on the breeding system and the life history of the species but also on whether mating patterns are disrupted because of loss of pollen vectors.

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In Denmark, as well as in many other north European countries, intensification of agriculture has caused a general decrease of dry grassland areas during the last few centuries. Today dry, natural grassland covers approximately 0.6% of the land surface in Denmark (H. H. Bruun, pers. comm.). Dry grassland habitats thus occur as disconnected islands, in a sea of arable lands, forests and urban areas. Species extinction in these habitats is caused by fertilization, ceased grazing and shrub encroachment.

In this study we have investigated natural populations of *Filipendula vulgaris*, which is one of the habitat-specific herb species on the dry nonacidic grasslands in Denmark. We investigated two different areas of Denmark, Hornsherred on Zealand and Himmerland in Jutland. Both areas have experienced a reduction and fragmentation of dry grassland during the last two centuries. The development started earlier and more gradually on the densely populated island of Zealand, whereas the process has been more recent and abrupt on mainland Jutland (O. H. Caspersen, pers. comm.).

We have addressed the following questions. (i) Does population size affect the level of genetic variation, and are the processes of genetic drift and inbreeding prominent in the studied populations? (ii) Is the genetic diversity of the populations affected by the degree of geographical isolation? As a consequence of the information gained from the field study we performed an experiment to test (iii) if *F. vulgaris* can be successfully cross-pollinated as a result of wind dispersal of pollen or if insects are the only pollination vector.

Materials and methods

The species

Filipendula vulgaris Moench. (Rosaceae), dropwort, occurs in dry nonacidic grasslands over most of Europe (Clapham *et al.*, 1987). It is a perennial herb with short rhizomes and roots bearing tubers. The cream-white flowers are small and dish-shaped and occur in many-flowered inflorescences. The flowers lack nectar and are therefore supposed to be pollinated mainly by pollen-collecting insects such as various bee and syrphid species (Müller, 1883), or insects in general (Clapham *et al.*, 1987). In the absence of insects, self-pollination may take place (Müller, 1883; Clapham *et al.*, 1987). Each flower will produce six to 10 nutlets.

Fieldwork

Leaf samples from 17 populations of *F. vulgaris* were collected during the summers of 1996 and 1997. When possible, 60 individuals were collected from each population. Leaf material was sampled from mature individuals throughout the entire population, with a minimum spacing of at least 1 m to avoid multiple sampling of individual clones. The overall size of the populations was estimated, including nonflowering individuals. At one population, Hestbjerg on Zealand, nutlets were collected from five individual inflorescences.

Electrophoresis

Leaf material was homogenized directly upon return from the field. A leaf area of approximately 10×10 mm was homogenized in a Tris-HCl grinding buffer-PVP solution. The extract was absorbed onto filter paper wicks (Whatman no. 4). Gel electrophoresis was carried out in 12% starch gels (Connaught) using two different buffer systems: a histidine-citrate buffer system and a sodium-borate pH 8.5/Tris-citrate, pH 7.8 system (Weidema *et al.*, 1996). These gel systems ran for 4 h at 60 and 40 mA, respectively. The enzymes ACO (aconitase, EC 4.2.1.3), PGD (phosphogluconate dehydrogenase, EC 1.1.1.44), MDH (malate dehydrogenase, EC 1.1.1.37), and UGUT (UTP-glucose-1-phosphate uridylyltransferase, EC 2.7.7.9) were resolved on the histidine-citrate gel. The enzymes ADH (alcohol dehydrogenase, EC 1.1.1.1), PGI (phosphoglucoisomerase, EC 5.3.1.9) and TPI (triose-phosphate isomerase, EC 5.3.1.1) were resolved on the sodium-borate gel. For the enzymes ADH, ACO, PGD, PGI, TPI and MDH we followed standard staining procedures with only minor modifications (Weidema *et al.*, 1996). To resolve the enzyme UGUT, we used the recipe by van Someren *et al.* (1974). Eight loci were scored in all populations; where we observed more than one scorable locus on a gel the fastest was termed number one. The alleles have been numbered according to their mobility relative to the most common allele (100).

Wind dispersal experiment and outcrossing rates

In the summer of 1998 a wind dispersal experiment was performed in the Botanical Garden of Copenhagen. Forty-six plants, originating from the field-collected nutlets (Hestbjerg) were used for this experiment. To estimate the outcrossing rate, we used the following variable loci: *Aco*, *Adh*, *Pgi*, *Tpi* and *Ugut*. Plants were separated in four treatment groups according to their isozyme genotypes, in such a way that each group would have a unique marker gene combination.

The four main treatments were: (i) pollen-proof bags with invisible pores as control for self-pollination; (ii) 0.41 mm pores, no insect visitors available but wind pollination possible; (iii) 0.92 mm pores, very small insects and wind pollination possible; (iv) open-

pollinated (no bags). Furthermore, we positioned two open-pollinated plants with a special *Adh* marker allele each 20 m away in opposite directions.

Before the treatment was initiated the number of unopened flowers was counted for each plant, and any open flowers were cut away. The entire inflorescence was then bagged with the chosen bag-type for the first three treatments. After one month all flowers were withered and the pollination bags were removed. Entire inflorescences were harvested when ripe. All nutlets from these inflorescences were sown in sterilized potting soil and kept under uniform watering and light regimes. After 35 days the germination rate was recorded for each maternal individual as seedlings per number of initial flowers. Twelve offspring from each of 12 maternal plants were harvested and genotyped to obtain outcrossing rates for each treatment.

Statistical data analysis

Allelic frequencies, percentage of polymorphic loci (P_p), mean number of alleles per locus (A_p) and the gene diversity (H_e) were calculated for all populations using the program G-STAT ver. 3.1 (which is distributed by the author, Hans Siegismund, University of Copenhagen). The genotypic distribution at each locus was tested for a deviation from Hardy–Weinberg expectations by the $F\sqrt{N}$ statistic where F is Wright's fixation index and N is the sample size. Positive values of $F\sqrt{N}$ indicate an excess of homozygotes whereas negative values indicate an excess of heterozygotes.

Multilocus (t_m) and single-locus (t_s) outcrossing rates were estimated using the maximum likelihood procedures of Ritland & Jain (1981) and the MLTR program by Ritland (distributed by the author at http://forgen.forestry.ubc.ca/ritland/). The expectation-maximization procedure was used for the iterations. Variances of estimates were found by bootstrapping 1000 times over all progeny arrays. Parental genotypes were estimated from the original dataset (not bootstrap means). The same procedures were used to compute the population outcrossing rate from the field-collected offspring arrays. For these arrays the maternal genotypes were not known and the most likely maternal genotypes were deduced from the offspring arrays, using estimates from the original data set (not bootstrap means). We used the expectation-maximization method to find maximum likelihood estimates, and confidence limits were estimated by 100 bootstraps.

We tested whether allele counts at each locus were significantly different among all populations using a *G*-test. Rare alleles were pooled to obtain more than three as the expected number in a cell. A maximum likelihood tree was estimated under the assumption that the populations have diverged exclusively by genetic drift (Felsenstein, 1981) using the CONTML program from the PHYLIP package authored by Joseph Felsenstein (http://evolution.genetics.washington.edu/phylip.html). Distances, in km, between populations were correlated with their genetic distances and tested with a Mantel test with a permutation procedure using the G-STAT, ver. 3.1 program. We used Reynolds *et al.* (1983) genetic distance, which — like the method used for finding the maximum likelihood tree — assumes that populations diverge because of genetic drift.

To quantify population differentiation we conducted an *F*-statistics analysis (Wright, 1969) by using the algorithm of Weir (1990). We used the program POP-GENE, ver. 1.20 (distributed by Exeter Software, http://www.exetersoftware.com/cat/contents.html) to calculate the *F*-statistics with three hierarchies. The *F*-statistics were: the inbreeding coefficient, which is a measure of the deviation from random mating of the individuals within populations (F_{IP}), differentiation among populations within regions (F_{PR}), differentiation among regions within the total (F_{RT}), differentiation among populations within the total (F_{PT}) and differentiation of the individual relative to the entire data set (F_{IT}). The *F*-statistics are related by the equation:

$$(1 - F_{IT}) = (1 - F_{IP})(1 - F_{PR})(1 - F_{RT}),$$

where $(1 - F_{PT}) = (1 - F_{PR})(1 - F_{RT})$. To obtain standard deviations in order to test for significant deviations from zero we used the jackknife procedure over the eight involved loci (Weir, 1990; pp. 137–140).

Results

Genetic diversity at the population level

The average percentage of polymorphic loci, P_p (SD) was 55.88 (14.06) (rare allele only included if more common than 5%), the average number of alleles per locus, A_p (SD) was 1.96 (0.19), and the average gene diversity, H_e (SD) was 0.17 (0.04). There was, however, considerable variation among populations in these three genetic parameters. A positive correlation was found between log population size and fraction of polymorphic loci (Fig. 1) ($r = 0.568$, $P = 0.017$). Gene diversity (H_e) and mean number of alleles (A_p) were not significantly correlated with population size (data not shown).

Out of 107 possible tests for Hardy–Weinberg proportions only one locus in each of three populations showed significant deviation from the expected values ($F\sqrt{N}$-values in Table 1a,b), which is less than expected by chance alone. We can therefore conclude that Hardy–Weinberg distributions of alleles are observed in the
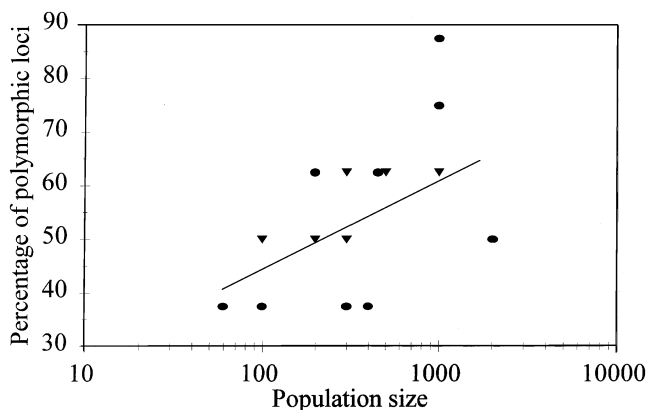
Fig. 1 Size of *Filipendula vulgaris* populations on a log scale related to percentage polymorphic loci ($r = 0.568$, $P = 0.017$). Dark circles denote populations on Zealand, and grey triangles denote populations from mainland Jutland. Two of the triangles represent double samples (300, 62.5; 500, 62.5).

studied populations. The result of Hardy–Weinberg proportions in all populations also underlines the fact that the sampling scheme was efficient in avoiding re-sampling.

Differentiation between populations and regions

From the allele frequencies presented in Table 1(a,b) it is seen that the populations of *F. vulgaris* are mostly dominated by the same common alleles, being differentiated only by a few rare alleles. The population samples were therefore compared with a homogeneity test to evaluate whether allele frequencies were different from each other. The loci *Pgd* and *Tpi-1* have been excluded from the homogeneity test, because even after pooling alleles they did not fulfil the statistical requirement of having an occurrence of more than three in the cell for the rarer alleles. The remaining loci all indicated highly significant differentiation among populations. The overall test for homogeneity summed across the loci accordingly showed that population samples were heterogeneous ($G_{96} = 929$, $P < 10^{-6}$).

Two alleles, *Pgi-75* and *Adh-80*, are found only in Hornsherred, Zealand, suggesting a difference in the two studied regions. This is clearly revealed in a maximum likelihood tree (Fig. 2). The relationships within each of the regions are less clear. Populations that are geographically close are also found adjoining, but the branch lengths between them reveal that they are just as related to other populations less closely positioned. The populations Åsbakke and Selsø, which are less than 2 km apart, exemplify this pattern (Fig. 2). These two populations are no more related to each other than they are to Skuldelev, which is 5 km away from both of them.

In both regions the Mantel test for relationship between geographical distance and genetic relatedness was found to be not significant at the 5% level. The *F*-statistics analysis was performed with three hierarchies (total, regions and populations) (Table 2). The overall F_{IT}, F_{PT}, F_{RT} and F_{PR} values are small but significantly different from zero, being larger than twice their standard deviation, whereas the overall within-population inbreeding coefficient, F_{IP}, is not significantly different from zero.

Wind dispersal experiment

Seedlings emerged from all treatments but in very unequal numbers. The control treatment (pollen-proof) had, as expected, a very low number of seedlings emerging. Seedlings resulted from the inflorescences that had been under both bag-types (small pores and large pores) as well as from the open-pollinated plants (Table 3). The proportions of seedlings per flower were, however, different in the three treatments (Kruskal–Wallis test, $K = 9.96$, $P = 0.01$). Within-treatment variation was high.

Analysis of the offspring genotypes revealed that in offspring arrays from three maternal plants some pollen grains were donated from the solitary far-away plants, whereas in the rest of the arrays the most likely pollen donors to all offspring were to be found among plants participating in the open-pollination treatment. Outcrossing rates were estimated for each offspring array and a large among-family variation was revealed (Table 3). Because individuals had been selected to have the same genotypes, the within-treatments cross-pollination is most likely underestimated. The difference between the single-locus outcrossing rate and the multilocus outcrossing rate ($t_m - t_s$) (SD) was found to be 0.08 (0.02), showing that the single-locus estimate describes breeding among plants with similar markers as well as selfing. In comparison, the $t_m - t_s$ difference was only found to be −0.003 (0.10) in offspring arrays originating from nutlets harvested in the field from the parental generation at Hestbjerg, indicating that little or no biparental inbreeding occurs in a natural situation. The average outcrossing rate (0.87) (0.07) that was observed in the experimental open-pollinated treatment is also somewhat lower than the population estimate for the field-collected offspring (0.96) (0.11).

Discussion

Distribution of genetic variation within populations

The evolutionary processes of genetic drift and inbreeding may lower the genetic diversity of fragmented

populations (Ellstrand & Elam, 1993), and furthermore insect-pollinated plants may suffer from a loss of the appropriate pollinators (e.g. Olesen & Jain, 1994; Kwak *et al.*, 1998).

However, in our study areas, the average percentage of polymorphic loci, mean number of alleles per locus and gene diversity were higher than normally found for perennial insect-pollinated herbs (Hamrick & Godt,

Table 1a Allele mobilities and frequencies, $F\sqrt{N}$ and sample sizes (N) for the *Filipendula vulgaris* populations in Hornsherred, Zealand. Deviations from Hardy–Weinberg expectations detected as $F\sqrt{N}$-values larger or smaller than ± 1.96 are shown in bold. Full population names can be seen in Fig. 2

Locus	Allele	Trol 1	Kynd 2	Ugle 3	Røsb 4	Åsba 5	Magl 6	Hest 7	Sels 8	Skul 9
						Locations on Zealand and population no.				
Aco	75	0	0	0	0	0	0	0	0.07	0
	95	0	0	0	0	0	0	0	0	0
	100	0.89	0.96	0.84	1.00	0.89	1.00	0.90	0.92	0.98
	105	0.11	0.04	0.16	0	0.11	0	0.10	0.02	0.02
	N	48	48	49	33	54	60	53	53	53
	F√N	−0.90	−0.30	−0.32	—	−0.46	—	−0.84	−0.68	−0.14
Adh	80	0.10	0.04	0.01	0.05	0.03	0.07	0.04	0.08	0.08
	85	0	0	0	0.04	0	0.03	0	0	0.01
	100	0.90	0.96	0.99	0.91	0.97	0.91	0.96	0.92	0.91
	N	50	57	52	50	60	60	60	60	60
	F√N	−0.79	−0.35	−0.07	−0.70	−0.27	−0.78	−0.34	−0.70	−0.78
Pgd	100	1.00	1.00	1.00	0.97	0.95	0.98	1.00	0.98	1.00
	115	0	0	0	0.03	0.05	0	0.02	0.02	0
	N	50	60	52	50	60	60	60	60	60
	F√N	—	—	—	−0.22	−0.41	−0.13	—	−0.13	—
Pgi	75	0	0	0	0.01	0.03	0	0.02	0	0
	100	0.94	0.75	0.97	0.96	0.89	0.70	0.93	0.89	0.90
	125	0.06	0.25	0.03	0.03	0.08	0.30	0.05	0.11	0.10
	N	48	59	52	50	60	60	59	58	57
	F√N	−0.46	1.71	−0.21	−0.29	−0.94	−0.25	1.50	0.36	**5.27**
Mdh	100	0.76	0.93	0.99	0.92	0.95	0.90	0.98	1.00	0.98
	115	0.24	0.08	0.01	0.08	0.05	0.10	0.02	0	0.03
	N	48	60	52	50	60	60	30	60	60
	F√N	0.99	−0.63	−0.07	1.31	**2.31**	0.57	−0.09	—	0.04
Tpi-1	85	0	0	0	0	0	0	0	0	0
	100	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	115	0.06	0	0	0	0	0	0	0	0
	N	50	60	52	50	60	60	60	60	60
	F√N	−0.45	—	—	—	—	—	—	—	—
Tpi-2	35	0.73	0.72	0.67	0.77	0.65	0.57	0.63	0.54	0.75
	100	0.27	0.28	0.33	0.23	0.35	0.43	0.37	0.46	0.25
	N	50	60	52	50	60	60	60	60	55
	F√N	0.25	0.12	−0.55	−0.52	0.37	−0.67	−0.04	0.73	−0.23
Ugut	75	0	0	0	0	0	0.01	0	0	0.01
	85	0.02	0.02	0	0.01	0.13	0.01	0	0.03	0.02
	100	0.75	0.89	0.89	0.96	0.81	0.85	0.97	0.90	0.96
	110	0.23	0.09	0.11	0.03	0.06	0.13	0.03	0.08	0.02
	N	48	57	50	49	60	46	57	60	57
	F√N	0.77	0.52	−0.87	−0.30	−1.00	−0.07	−0.20	0.57	−0.35

Table 1b Allele mobilities and frequencies, $F\sqrt{N}$ and sample sizes (N) for the *Filipendula vulgaris* populations in Himmerland, Jutland. Deviations from Hardy–Weinberg expectations detected as $F\sqrt{N}$-values larger or smaller than $\pm$ 1.96 are shown in bold. Full population names can be seen in Fig. 2

		Location in Jutland and population no.							
Locus	Allele	Nico 10	Hule 11	Tost 12	Ople 13	Stor 14	Røde 15	Lind 16	Kæll 17
Aco	75	0	0.12	0	0	0	0.03	0	0
	95	0.06	0	0	0	0	0	0	0
	100	0.77	0.81	0.88	0.92	0.82	0.86	0.84	0.93
	105	0.17	0.07	0.12	0.08	0.18	0.12	0.16	0.07
	N	32	47	39	38	52	39	37	34
	$F\sqrt{N}$	−0.75	0.26	−0.81	−0.53	−0.68	−1.03	−1.18	−0.46
Adh	80	0	0	0	0	0	0	0	0
	85	0.01	0.01	0	0	0.01	0.01	0.03	0.03
	100	0.99	0.99	1.00	1.00	0.99	0.99	0.98	0.97
	N	60	60	40	39	60	60	60	59
	$F\sqrt{N}$	−0.07	−0.07	—	—	−0.07	−0.07	−0.20	−0.27
Pgd	100	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00
	115	0	0	0	0	0	0.01	0	0
	N	60	60	40	39	60	60	60	60
	$F\sqrt{N}$	—	—	—	—	—	−0.07	—	—
Pgi	75	0	0	0	0	0	0	0	0
	100	0.69	0.75	0.88	0.74	0.77	0.61	0.88	0.74
	125	0.31	0.25	0.13	0.26	0.22	0.39	0.12	0.26
	N	59	60	40	38	60	59	60	59
	$F\sqrt{N}$	−1.53	−1.20	−0.90	1.14	−0.77	1.66	−1.02	0.62
Mdh	100	0.98	0.87	0.94	0.91	0.94	0.84	0.92	0.92
	115	0.02	0.13	0.06	0.09	0.06	0.16	0.08	0.08
	N	60	60	40	39	60	58	60	56
	$F\sqrt{N}$	−0.13	1.04	−0.42	−0.62	−0.48	−0.53	−0.70	−0.65
Tpi-1	85	0	0	0	0	0	0	0.01	0
	100	0.98	1.00	1.00	1.00	1.00	0.98	0.98	0.98
	115	0.03	0	0	0	0	0.02	0.01	0.02
	N	60	60	40	39	60	60	60	59
	$F\sqrt{N}$	−0.20	—	—	—	—	−0.13	−0.13	−0.13
Tpi-2	35	0.36	0.41	0.73	0.36	0.38	0.52	0.66	0.62
	100	0.64	0.59	0.27	0.64	0.62	0.48	0.34	0.38
	N	60	58	32	39	60	58	60	51
	$F\sqrt{N}$	−0.40	−0.28	−1.14	0.68	−1.54	−0.80	−1.72	**−2.05**
Ugut	75	0.01	0	0	0	0	0	0.02	0
	85	0.08	0.01	0	0	0	0	0	0
	100	0.84	0.96	0.96	0.92	0.85	0.73	0.92	0.89
	110	0.07	0.03	0.04	0.08	0.15	0.27	0.06	0.11
	N	60	57	37	38	47	58	59	53
	$F\sqrt{N}$	−1.46	−0.27	−0.26	1.70	−0.05	−0.10	1.21	1.81

1996). This observation could in part be explained by the choice of enzyme systems, because we investigated no loci that were exclusively monomorphic in our study. Polymorphism was always found in at least one population, although many loci such as *Adh* and *Tpi-2* were most often monomorphic (Table 1a,b). The genetic parameters observed for *F. vulgaris* are more comparable with data normally found for wind-pollinated species and species that are very long-lived, such as woody species.
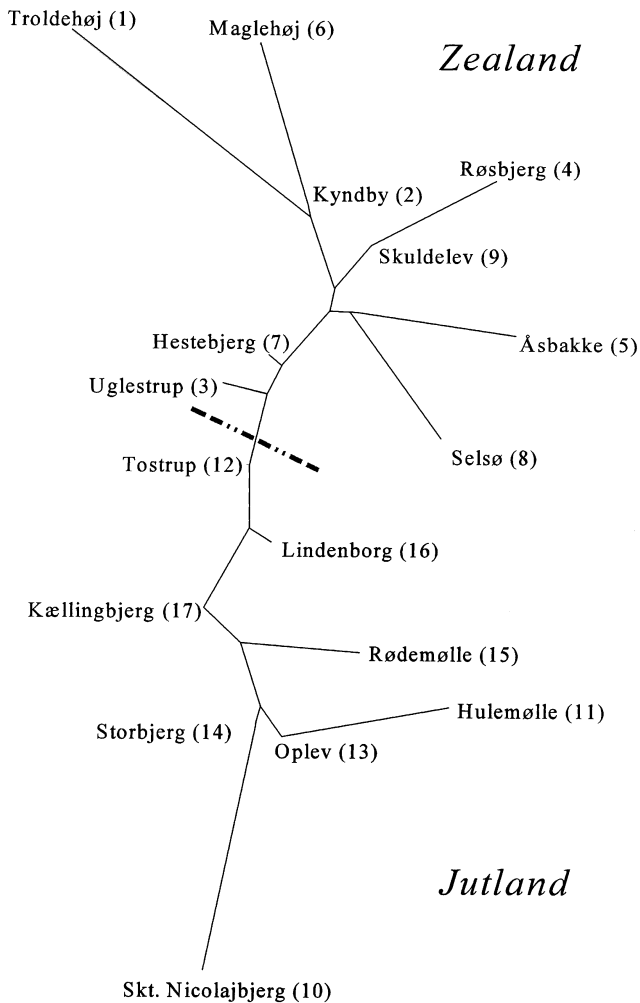
Fig. 2 Phylogenetic tree of the populations of *Filipendula vulgaris* based on a maximum likelihood tree. The tree is unrooted with branch lengths that indicate the expected accumulated variance resulting from drift. The angle between branches is arbitrary. Populations are marked with numbers that refer to Table 1; numbers 1–9 are from Zealand and numbers 10–17 are from Jutland.

Large populations of *F. vulgaris* hold more genetic variation than the small populations. This relationship has been demonstrated in several species, both rare and common (van Treuren *et al.*, 1991; Raijman *et al.*, 1994; Weidema *et al.*, 1996). *Filipendula vulgaris* has many loci where the distribution of alleles is strongly skewed, with one common allele and a few very rare alleles. It is at these loci that alleles are lost through random genetic drift, e.g. *Aco* at Røsbjerg and Maglehøj (Table 1a,b). In *F. vulgaris* the correlation between the measures of genetic diversity and population size is not so striking as has been demonstrated for some of the other species mentioned above. Only the percentage of polymorphic loci (P_P) was shown to relate to population size,

accounting for only 32% of the variation compared to, for example, the 50% found for *Scabiosa columbaria* (van Treuren *et al.*, 1991). Although small, the populations in our study are not very small, and random genetic drift is consequently less pronounced than in the populations studied by van Treuren *et al.* (1991). Another reason for the weak relationship between the genetic parameters and size could be that population size has only been estimated in one particular year. For a long-lived organism one should expect a difference between a point measure (N) and the effective population size (N_e) that participate in breeding over the years (e.g. Husband & Barrett, 1992). *Filipendula vulgaris* is a perennial with a life cycle of 14–16 years (Shmanova, 1991), and the species has root tubers that provide for storage and vegetative propagation. In four Ukrainian populations of *F. vulgaris*, regeneration from vegetative shoots was reported to be important together with regeneration from seeds (Shmanova, 1991). In the Danish populations investigated in this study, vegetative propagation was not found when sampling at distances of 1 m. The fine-scaled mosaic of genets observed in our study suggests that clonality does not cause the individual genets to move around. Clonality could, however, act to prolong the life of the genet, which survives beyond the life of the individual ramet. In this sense, clonality could act against genetic drift because the genetic diversity 'lingers' for a longer time.

Another feature maintaining a high genetic diversity in the populations could be the high outcrossing rate. The field-collected offspring arrays have an outcrossing rate of 0.96 which is relatively high for a self-compatible herb species, being comparable to data for wind-pollinated woody species (e.g. Merzeau *et al.*, 1994). Two factors contribute to this result. First, an experimental bias may be that pre- and postzygotic selection has eradicated some of the results of self-pollination, because screening of isozyme markers took place when the offspring had been growing one month. Secondly, each inflorescence is open over several days; thus different pollen sources may be available over the period of flowering. It can be concluded that *F. vulgaris* mainly outcrosses although self-fertilization is possible. The predominantly outcrossing mating system of the species contributes to the maintenance of variation within the populations and assures that inbreeding does not take place, not even in the small populations.

Distribution of genetic variation within and between regions

Within regions the genetic differentiation was unexpectedly low and the estimated migration rate between populations correspondingly high (Table 2). The

Table 2 *F*-statistics for Danish populations of *Filipendula vulgaris*, where the *F*-statistics are related by the equations $(1 - F_{IT}) = (1 - F_{IP})(1 - F_{PR})(1 - F_{RT})$ and $(1 - F_{PT}) = (1 - F_{PR})(1 - F_{RT})$. Standard deviations obtained by jackknifing over loci

Locus	F_{IT}	F_{PT}	F_{RT}	F_{IP}	F_{PR}
Aco	−0.0107	0.0490	0.0203	−0.0628	0.0292
Adh	−0.0148	0.0429	0.0356	−0.0603	0.0076
Pgd	−0.0028	0.0220	0.0062	−0.0253	0.0159
Pgi	0.1336	0.0950	0.0460	0.0427	0.0513
Mdh	0.0765	0.0407	−0.0033	0.0373	0.0438
Tpi-1	−0.0070	0.0199	−0.0027	−0.0274	0.0226
Tpi-2	0.0495	0.0960	0.0536	−0.0515	0.0448
Ugut	0.0774	0.0449	−0.0054	0.0340	0.0501
Overall	0.0611	0.0724	0.0316	−0.0123	0.0422
SD	0.0220	0.0143	0.0132	0.0256	0.0039

Table 3 Summary of wind dispersal experiment with *Filipendula vulgaris*

Treatment	Germination in pots/total number of pots	Seedlings per flower (±SE)‡	Outcrossing rate (t_m) (±SE) (no. of offspring arrays)	Hypothesized pollination mode
Pollen-proof bags (no pores)	4/11	0.01 (0.01)	0.09 (0) (1)	None
Bread-bags (pores: 0.41 mm)	9/10†	0.17 (0.07)	0.39 (0.22) (2)	Wind
Mosquito-bags (mesh: 0.92 mm)	11/11	0.33 (0.05)	0.73 (0.10) (5)	Wind, small insects
Open-pollinated (no bags)	9/11	0.60 (0.20)	0.87 (0.07) (4)	Wind, all insects

†One mother plant died in this treatment.
‡Mean number of seedlings per flower (including pots with no germination).
Pollen is less than 0.02 mm in diameter.

literature discussing pollination modes of *F. vulgaris* gives the impression that the species is pollinated by a coterie of small insects, with stronger flyers like bumblebees being under-represented because the flowers lack nectar (e.g. Müller, 1883; but Shmanova (1991) mentions 'Bees'). Therefore the substantial gene dispersal between the relatively isolated populations was a rather surprising result from our survey of natural populations.

A thorough analysis of species diversity has been conducted in the two study areas (Bruun, 1999). From this mapping of all populations of *F. vulgaris* it was seen that the studied populations were on average 1.68 (±0.84) km away from other habitats containing *F. vulgaris*. The longest distance between populations was 3 km. It was not possible to prove a relationship between degree of isolation from other populations containing *F. vulgaris* and the percentage of polymorphic loci (P_p), number of alleles per locus (A_P) or gene diversity (H_e). Furthermore no relationship between genetic and geographical distance of the populations was demonstrated, which is another indication of substantial migration of genes between populations.

The genetic differentiation between the two regions of *F. vulgaris* ($F_{RT} = 0.03$) and between populations ($F_{PR} = 0.04$) was surprisingly small compared to other studies at the same scale on herb species with insect pollination (e.g. van Treuren *et al.*, 1991; Weidema *et al.*, 1996). The overall F_{ST} value ($F_{PT} = 0.07$) obtained was within the range normally observed for woody, wind-pollinated species groups such as the Fagaceae or the Pinaceae (Hamrick & Godt, 1996). The absence of genetic differentiation both within and between regions prompts us to reconsider the possible pollination modes of *F. vulgaris*.

Evidence for wind pollination

Our data from the pollination exclusion experiment show that *F. vulgaris* can be wind-pollinated. The experimental data presented in this paper are based on relatively few offspring arrays, and the result should be interpreted with some caution. Still, there is strong evidence that pollen carried by wind can penetrate both the small-pored and the large-pored bags. Marker dispersal from the solitary far-away plants also supports

wind pollination. Evidence from several pollen-dispersal studies suggests high pollen productivity in north European members of the genus *Filipendula*, a typical adaptation to facultative wind pollination. Pollen from *Filipendula* spp. is invariably reported from Holocene (postglacial) pollen records from Danish lake sediments in mean frequencies of one to four out of 1000 terrestrial pollen grains (Odgaard, 1994; B. Odgaard, pers. comm.). This pollen could originate from either *F. vulgaris* or *F. ulmaria*, as pollen grains of the two species are indistinguishable under a light microscope. In a recent study by Hjelle (1997) *Filipendula* pollen was generally over-represented in moss samples relative to the occurrence of *F. ulmaria* in the vegetation. Its pollen representation pattern was similar to species like *Plantago major* and *Calluna vulgaris* that traditionally are reported as being exclusively or facultatively anemophilous (Faegri & Van der Pijl, 1971).

Filipendula pollen is very small (< 20 μm), about half the size of both *Calluna* and *Plantago* pollen (Moore *et al.*, 1991). The flowers of *F. vulgaris* are of the dish-shaped type *sensu* Faegri & Van der Pijl (1971) with copious amounts of pollen and well-exposed anthers, which is a prerequisite for wind pollination. Furthermore, the species occurs on open and very wind-exposed sites, where wind pollination is effective.

Further evidence for adaptation to a wind pollination strategy comes from the study of Dobson *et al.* (1996) investigating the flower and pollen odours of *F. vulgaris*. Dobson *et al.* found that the pollen odour of this species is dominated by 2-heptadecanone (63%), a substance that is suspected to deter or even to be toxic to insects. Dobson *et al.* (1996) hypothesized that *F. vulgaris* is evolving away from reliance on insects for pollination.

Our investigation of regional and local levels of genetic variation as well as the supplementary investigation of wind dispersal of pollen in *F. vulgaris* point in the same direction. In spite of the fact that most floras and textbooks mention *F. vulgaris* as being exclusively pollinated by insects, our study suggests that pollen transport takes place by wind with subsequent pollination.

Conclusions

We conclude that although there are regional differences in the allele frequencies, this study of *F. vulgaris* has shown that gene flow between populations takes place. The degree of fragmentation has apparently until now had no effect on the levels of diversity of isozyme markers. The size of the populations affected the level of gene diversity, in such a way that the larger populations were more variable. The small populations, however, contained the same common alleles as the larger populations, suggesting that genetic drift and local natural selection are not predominant in the species. The apparent absence of genetic drift in the small and fragmented populations of *F. vulgaris* may be caused by the opposing effects of a high gene flow.

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