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Population structure of a riparian willow species, *Salix viminalis* L.

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Summary

Material sampled along 11 rivers of the western part of *Salix viminalis* L. natural range (in Poland, Germany and Austria), as well as in stands in Sweden and Belgium, was assayed for 15 isozyme loci and cuttings were installed in two field experiments located in a nursery south of Uppsala, where growth traits were measured. These data were used to test hypotheses on the origin of Swedish populations, on the part played by rivers in the genetic differentiation and on the relative differentiation at isozyme and quantitative trait loci. Although significant, the overall population differentiation was low, the F_{ST} value being around 4%. Much higher F_{ST} values were observed between subpopulations from southern (Skåne) and central Sweden. This strong population differentiation, accompanied by significant linkage disequilibria, suggests the recent and diverse origin of Swedish populations. Degrees of differentiation between and within Polish river systems were of the same magnitude, indicating the presence of gene flow between river systems. Flow-regulated waterways, associated with higher human disturbance, may well explain why populations along rivers of the western part of the study area exhibited significant differentiation patterns while no differentiation could be detected along the less disturbed riparian habitats of eastern Poland. Finally, higher F_{ST} values were obtained for quantitative trait loci than for isozyme loci but, with two notable exceptions, their 95% confidence intervals overlapped.

1. Introduction

Salix viminalis L., the basket willow or common osier, is a dioecious riparian shrub with a large distribution area, ranging from the Atlantic Ocean eastward to Siberia and from Sweden southward to the Mediterranean Sea. This vast range may be a consequence of the long use of *S. viminalis* L. for all kinds of wickerwork. The Ancient Greeks and the Romans developed willow husbandry to provide the flourishing basket industry with raw material (Pohjonen, 1984). However, extensive use of willows as a crop did not occur until the eighteenth century, when the cultivated area in France reached a peak of 70000 ha (Frankowski *et al.* 1961). Cultivation then apparently declined, although Poland could still claim as much as 43000 ha of cultivated willows in 1925. *S. viminalis* L. made up a significant part of these plantations but other species were also used, including American ones such as *S. cordata* (Frankowski *et al.* 1961). It is usually claimed that *S. viminalis* L. was indigenous in southern Russia and was spread in the rest of Europe by man, although evidence for this is still limited (Skvortsov, 1968; Meikle, 1984). Only in some instances are the date as well as the vector of introduction of the species to a novel area known with good accuracy. For example, willows along the Fyris river, south of Uppsala, were first introduced by man in the middle of the eighteenth century. Populations in southern Sweden are said to date at least from the seventeenth century, if not earlier, but again only in a few cases are the date and the vector of introduction documented. Because Poland was a net exporter of osier sticks (that can be used to create new stands through vegetative propagation) in the 1920s (Frankowski *et al.* 1961) one can speculate that many of the present-day populations in neighbouring countries may comprise a significant proportion of Polish material.

Since the end of the last century cultivation of *S. viminalis* L. has declined in most countries, following the decline in the wickerwork industry, and most populations today consist of small stands disseminated along rivers, some of which may well be the remnants

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of cultivated willow fields. Hence, the genetic structure of contemporary *S. viminalis* L. populations is the direct consequence of intermittent species domestication, and a pattern unambiguously linked to major historical events or ecological variables is unfortunately not to be expected. For instance, the part played by rivers in connecting different riparian populations may have been blurred by the introduction of foreign material. Because of past local selection for adaptive value and growth-related traits such as numbers of shoots, we may also expect to observe a more pronounced differentiation patterns for quantitative traits than for isozyme loci. Differentiation for quantitative traits and isozymes can be compared by assuming that both are neutral and computing Wright's measure, F_{ST}, of population differentiation for each of them (Wright, 1969; Lewontin, 1984, 1986; Rogers, 1986; Felsenstein, 1986; Prout & Barker, 1993; Spitze, 1993; Podolsky & Holtsford, 1995). A significant difference between F_{ST} values would then suggest that population differentiation for the two types of characters was due to different evolutionary processes.

Variation at isozyme loci and quantitative traits was assessed in material sampled in the western part of the natural range of *S. viminalis* L. These data are used to test hypotheses on the part played by river systems in shaping the genetic structure of *S. viminalis* L. populations, on the origin of the Swedish material, and on the amount of differentiation detected with isozyme and quantitative traits. More precisely we shall test the following hypotheses: (i) Population differentiation is related to the river system: stands along a river and its tributaries are genetically closer than stands along unconnected rivers. (ii) Along rivers likely to have been little disturbed by humans no differentiation or a clear pattern of isolation by distance is expected, whereas a strong differentiation pattern is expected along rivers in densely population areas. If due to human intervention, this pattern is not expected to show a clear pattern of isolation by distance. (iii) Swedish material originates from present-day Polish populations and has diverged from its putative ancestors. (iv) The pattern of differentiation for quantitative traits is more pronounced than that observed at protein loci.

2. Materials and methods

(i) *Sampling sites*

Samples of *Salix viminalis* L. were collected in Central Europe in 1986 (Fig. 1). Different river systems were followed in a North to South or East to North direction and samples were taken in populations 30–50 km apart. With the exception of a couple of large stands, most sampled stands contained from 1 to 10 individuals. The Swedish collection includes most of the Swedish *S. viminalis* L. stands.

(ii) Quantitative trait experiments

The material collected comprised cuttings from young branches which we planted in pots and held in a separate greenhouse for quarantine. This material was then propagated by first taking green cuttings from the original plant and then dormant cuttings from the green cutting plants. Ten cuttings per individual were thus obtained. The material was planted in two field experiments, called thereafter experiment 1 and experiment 2, located at Pustnäs, south of Uppsala (58° N, 17° E). All populations except one (Schelde, Belgium) were represented in experiment 1, while only populations where at least 12 or more individuals could be collected were represented in experiment 2. Both experiments were established in 1988 according to an incomplete block design with 10 replicates. The spacing between plants was 0·75 m × 0·75 m. The plants were harvested on two occasions (1988 and 1991), the final harvest taking place in the winter of 1991/2. The number of shoots in 1988, 1990 and 1991 and the basal diameter of the longest shoot in 1991 were measured. In experiment 1 the height of the longest shoot was also assessed.

(iii) Electrophoresis

A total of 743 individuals were analysed by electrophoresis. Cuttings were taken from dormant branches, planted in a greenhouse and sampled after 2 weeks. The first two or three fully grown leaves from the top of each shoot were collected and homogenized in approximately 250 μl cold extraction buffer (Coulhart & Denford, 1982) using a power-driven pestle. Microglass pearls were added during homogenization to facilitate tissue breakdown. The homogenate was centrifuged for 10 min in a refrigerated centrifuge at 7000 rpm and then stored at −70 °C until electrophoretic analysis. Electrophoretic separation techniques followed by Cheliak & Pitel (1984) and Lagercrantz *et al.* (1988), using 12·5% starch gels. Separation buffer systems are described in Ashton & Braden (1961) for AAT (aspartate aminotransferase, EC 2.6.1.1), ACP (acid phosphatase, EC. 3.1.3.2) and ACON (aconitase, EC 4.2.1.3), in Cheliak & Pitel (1984) for MDH (malate dehydrogenase, EC 1.1.1.37), PGD (phosphogluconate dehydrogenase, EC 1.1.1.44) and SKD (shikimate dehydrogenase, EC 1.1.1.25), and in Clayton & Tretiak (1972) for GPI (glucose-6-phosphate isomerase, EC 5.3.1.9). Enzyme staining recipes were from Cheliak & Pitel (1984) with some modifications, mainly in relation to the adoption of the 'agar-overlay' technique (Harris & Hopkinson, 1976). Tissue extractions and enzyme electrophoresis were repeated at least twice for each clone. Nine polymorphic loci were scored: *Aat-1, Acon, Acp, Mdh-1, Mdh-3, Pgdh-1, Pgdh-2, Pgi-1, Shdh.* Mendelian determinism of the enzyme loci was verified in Thorsén

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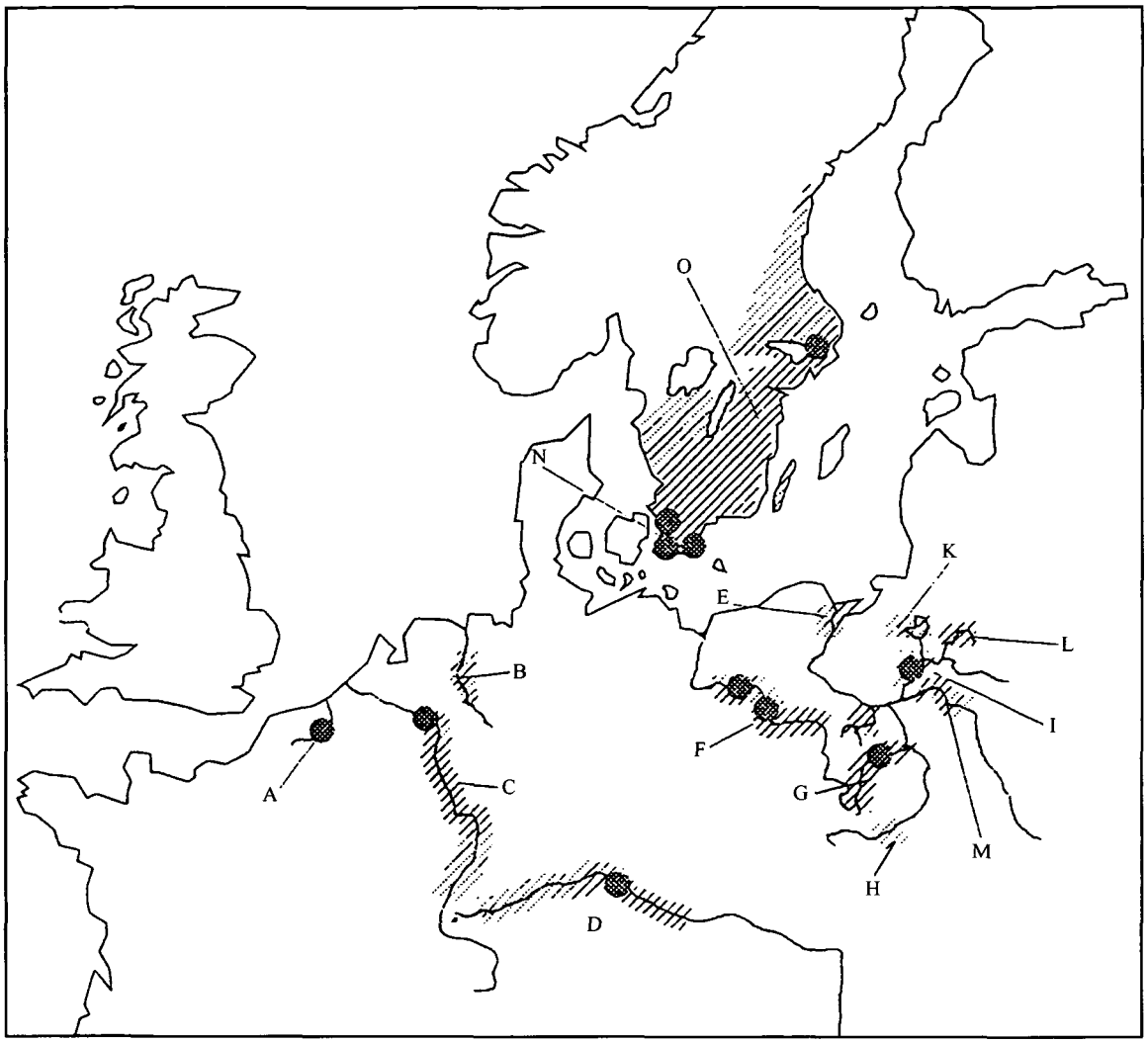

Fig. 1. Map of the main populations included in the study. Each population is in turn subdivided into subpopulations within the same area or along the same river. Dots gives the locations of the largest subpopulations. A, Schelde; B, Ems; C, Rhine; D, Danube; E, North Vistula; F, Warta; G, Piliça; H, Vistula; I, Narew; K, Mazury; L, Bierbza; M, Bug; N, Skåne; O, Central Sweden.

et al. (1995). Designation of loci and alleles followed the description in Thorsén *et al.* (1996).

(iv) *Methods*

Isozyme data were analysed with Genepop (version 2) (Raymond & Rousset, 1995*b*) and Fstat (version 1·2) (Goudet, 1995); quantitative trait data were analysed with SAS software (SAS Institute, 1982).

(a) *Isozyme data*

Hardy–Weinberg expectations. The fit of genotypic distributions to Hardy–Weinberg expectations was tested by the exact test proposed by Haldane (1954), using the algorithm of Louis & Dempster (1987). The overall significance for each locus was estimated by Fisher's combined probability test (Fisher, 1954). According to this test, if P values are obtained for each locus separately under the null hypothesis, then $-2\Sigma_{i=1}^{n}\log(P_i)$ is distributed according to a chi-squared distribution with n degree of freedom where n is the number of loci (Sokal & Rohlf, 1994). F_{IS} values, where F_{IS} is the correlation between two uniting gametes within a subpopulation, were estimated according to Weir & Cockerham (1984). Heterozygote deficits or excesses were tested using an exact test (Rousset & Raymond, 1995).

Linkage disequilibrium. For each population, the non-random association between pairs of loci, or linkage disequilibrium, was tested using Fisher's exact test on contingency tables. Contingency tables are created for all pairs of loci in each population and an unbiased estimate of the exact probability is obtained by using a Markov chain Monte Carlo method (Raymond & Rousset, 1995*a*). Tests are not affected by a potential departure from Hardy–Weinberg expectations because each contingency table considers the genotypic composition, not the allelic composition. For each pair of loci, a global measure was obtained by averaging across populations and a global test was obtained using Fisher's combined test.

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Ohta's indices were used to discriminate between genetic drift and selection (Ohta, 1982). The variance in observed and expected frequencies of allele combinations is described using five 'D-statistics'. These indices were computed with the program LINKDOS (Garnier-Gere & Dillmann, 1982).

Population differentiation. Genetic differentiation between populations or groups of populations was tested for each locus separately using Fisher's exact test on contingency tables. As for linkage disequilibrium, a Markov chain Monte Carlo method permits the attainment of an unbiased estimate of the exact probability (Raymond & Rousset, 1995*a*). Wright's F statistics (F_{IS}, F_{IT} and F_{ST}) were estimated according to Weir & Cockerham (1984) and a 95% confidence interval was estimated by bootstrapping over loci. F_{IS} and F_{IT} are the correlations between two uniting gametes relative to the subpopulation and relative to the total population, respectively, and F_{ST} is the correlation between two gametes drawn at random from each subpopulation and measures the degree of genetic differentiation of subpopulations (Nei, 1987). Only statistically independent loci which did not depart from Hardy–Weinberg proportions were retained.

Migration and isolation by distance. The number of effective migrants (Nm) between populations was estimated using the relationship $Nm = (1/F_{ST}-1)/4$ that holds for an island model (Wright, 1969) and by the private allele method (Slatkin, 1985). Finally, isolation by distance along the Rhine, Danube, Warta and Narew rivers was analysed according to Slatkin (1993). The distance between populations was estimated using a subroutine of ARC/INFO (Anonymous, 1991) that translates latitude and longitude into Cartesian coordinates according to a given projection. Two projections (adapted Lambert 1 and equidistant points) were used that gave very similar and satisfying results.

(b) *Quantitative trait data*

Variance components were estimated using the model:

$$Y_{ijklm} = \mu + R_i + B_j + P_k + C_l + \epsilon_{ijklm}, \quad (1)$$

where μ is the overall mean, R_i is the replicate effect, B_j is the block effect, P_k is the population effect, C_l is the clone effect and ϵ_{ijklm} is the error.

For a neutral trait, with purely additive variance, and assuming random mating within populations, Wright showed that:

$$F_{ST} = \frac{\sigma^2_{ST}}{\sigma^2_{ST} + 2\sigma^2_{IS}},$$

where σ^2_{ST} is the variance between populations due solely to genetic causes and σ^2_{IS} is the genetic variance within population (Wright, 1969, pp. 446–448). Our dataset does not permit us to have estimates of these parameters but only approximate values of them if we use the variance between population, σ^2_{pop}, in place of σ^2_{ST} and the clonal variance, σ^2_{clone}, in place of σ^2_{IS}. Because plants were grown in the same environment, assuming that σ^2_{pop} reflects only genetic differences is plausible. However, both variances contain extra terms (dominance variance, maternal effects) and therefore are likely to be overestimates of σ^2_{ST} and σ^2_{IS}, respectively. Consequently the resulting estimator of F_{ST},

$$t' = \frac{\sigma^2_{pop}}{\sigma^2_{pop} + 2\sigma^2_{clone}},$$

will most likely be biased downward. The 95% confidence intervals were obtained using the method proposed by Podolsky & Holtsford (1995).

3. Results

(i) Polymorphism

Nine of the 15 loci assayed were polymorphic. The proportion of polymorphic loci varied from 46% to 60% across populations and the heterozygosity varied between 0·10 and 0·19. The number of alleles at polymorphic loci ranged from 2 to 4. The average number of alleles per population varied between 1·9 and 2·5 (Table 1).

(ii) *Statistical independence among loci*

Highly significant linkage disequilibrium was observed only in Skåne and central Sweden (3 and 10 pairs of loci, respectively; results not shown). Otherwise, the Fisher exact test indicated the independence between loci in all other populations. The correlation structure between loci led us to remove *PGDH1* from further analyses.

Ohta's statistics were computed only in populations where significant linkage disequilibrium was detected at the overall level, i.e. Skåne and central Sweden. The variance in the observed frequency of allele combinations within subpopulations was always much greater than the same variance in the overall population. The variance in expected frequency of allele combinations within subpopulations was always much smaller than the variance in observed frequency of allele combinations in the overall population. This suggests that allelic associations were not due to epistatic selection but instead resulted from random drift (data not shown).

(iii) *Hardy–Weinberg equilibrium*

The hypothesis of Hardy–Weinberg equilibrium was rejected in 9 cases out of 117 and most of these cases involved the *ACP* and *SHDH* loci. Only for these two loci did the combined Fisher test lead to the rejection

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Table 1. *Average heterozygosity (H), proportion of polymorphic loci (P) and average number of alleles (A) for all 14 main populations*

Populations	Sample size	No. of subpopulations		H	P(%)	A
Ems	10	5	*(1–3)**	0·108	46·6	2·0
Rhine	42	15	*(1–17)*	0·138	46·6	2·1
Danube	99	23	*(1–32)*	0·158	46·6	2·2
Narew	58	5	*(3–40)*	0·170	60·0	2·3
Bug	28	5	*(4–7)*	0·145	53·3	2·1
Vistula	18	5	*(2–5)*	0·166	46·6	2·0
Piliça	75	10	*(1–44)*	0·168	60·0	2·4
Warta	96	11	*(4–28)*	0·184	60·0	2·5
North Vistula	13	7	*(1–5)*	0·149	53·3	2·1
Mazury	5	3	*(1–2)*	0·194	46·6	2·0
Biebrza	7	6	*(1–2)*	0·145	46·6	2·0
Skåne	111	25	*(1–12)*	0·188	60·0	2·3
Central Sweden	158	40	*(1–102)*	0·145	53·3	2·2
Schelde	20	1	—	0·137	46·6	1·9

H and P are based on the 15 loci assayed whereas A is based only on polymorphic loci.
* Figures within brackets are the smallest and largest subpopulation sizes.

Table 2. *Analysis of departure from Hardy–Weinberg equilibrium for loci* ACP *and* SHDH

	ACP				*SHDH*			
Populations	F_{IS}	General	Deficit	Excess	F_{IS}	General	Deficit	Excess
Rhine	+0·2903	0·0094	ns	ns	+0·3723	0·0006	0·0033	ns
Bug	+0·5398	0·0017	0·0071	ns	+0·0074	ns	ns	ns
Vistula	+0·3462	ns	0·0205	ns	−0·2143	0·0072	0·0303	ns
Warta	+0·3579	0·0000	0·0002	ns	−0·0425	ns	ns	ns
North Vistula	−0·0588	ns	ns	ns	+0·2593	ns	0·0283	ns
Central Sweden	−0·2474	0·0040	ns	0·0009	+0·2686	0·0000	0·0001	ns
All*	—	0·0000	0·0032	ns	—	0·0000	0·0000	ns

F_{IS} values and type I error probabilities of rejecting Hardy–Weinberg proportions for all possible reasons (General) or for only heterozygote deficit (Deficit) or excess (Excess) are given. Only populations for which either significant deficit or excess was found ($P < 0·05$) are shown.
ns, probabilities larger than 0·05.
* Fisher's combined probability test across samples.

of the hypothesis of Hardy–Weinberg equilibrium, whether or not there was multiple testing. With the exception of *ACP* in the central Sweden population, departure from Hardy–Weinberg at these loci was due to a heterozygote deficit (Table 2). Consequently, these two loci were removed from subsequent analyses. Because there was no global departure from Hardy–Weinberg but only departure from Hardy–Weinberg in specific populations, loci *ACON* and *MDH1* were removed from the Danube and Narew populations, respectively. Otherwise, all further analyses were carried out with loci *AAT1, ACON, MDH1, MDH3, PGDH2* and *PGI1*.

(iv) *Population differentiation*
(a) *Isozymes*

Despite a low F_{ST} value (0·041), the overall differentiation was highly significant (Table 3). When loci were considered separately, all F_{ST} values were of similar magnitude but there were marked differences between the corresponding F_{IT} and F_{IS} values. Overall F_{IS} was slightly negative while F_{IT} was slightly positive.

The differentiation was also studied hierarchically by considering differentiation between populations at different levels. First, at the main populations level, F_{ST} values showed that four large groups could be delineated: central Sweden, Skåne, Germany (Ems + Rhine), Poland and Danube. The last can in turn be subdivided into eastern Poland (Narew + Bug + Bierbza) and Danube, central Poland (Pilica + Vistula), western Poland (Warta), although the differentiation between these groups is generally weak. Subpopulations in central Sweden and Skåne were extremely differentiated. Both central Sweden and Skåne are also significantly differentiated from all putative ancestor populations in this study, even though differentiation between Skåne and western

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Table 3. *Wright's F statistics when all main populations are considered*

Locus	F_{IT}	F_{ST}	F_{IS}	P
AAT1	+0·082	+0·038	+0·045	0·00000
ACON	+0·037	+0·029	+0·008	0·00000
MDH1	+0·055	+0·061	−0·006	0·00000
MDH3	−0·029	+0·011	−0·040	0·00914
PGDH2	−0·018	+0·036	−0·056	0·00000
PGI1	−0·049	+0·055	−0·111	0·00000
Overall	+0·020	+0·041	−0·023	$< 10^{-5}$
95% CI	−0·025–0·058	0·024–0·055	−0·069–0·017	

P gives the corresponding probability of Fisher's exact test on contingency tables. The overall P value refers to the Fisher's combined probability test.

Table 4. *Allelic differentiation among subpopulations of the main geographical groups and between geographical groups*

Comparison	F_{ST}	P
Among subpopulations within		
Central Sweden	0·1556	$< 10^{-5}$
Skåne	0·1625	$< 10^{-5}$
Germany	0·0177	0·5321
Poland	0·0092	0·0014
Danube	0·0636	$< 10^{-5}$
Between		
Western Poland–eastern Poland–central Poland	0·0068	0·0039
Skåne–central Sweden	0·0480	$< 10^{-5}$
Skåne–Germany	0·0332	$< 10^{-5}$
Skåne–eastern Poland	0·0168	0·0005
Skåne–western Poland	0·0051	0·0014
Skåne–central Poland	0·0216	$< 10^{-5}$
Central Sweden–Germany	0·0210	$< 10^{-5}$
Central Sweden–eastern Poland	0·0863	$< 10^{-5}$
Central Sweden–western Poland	0·0748	$< 10^{-5}$
Central Sweden–central Poland	0·0802	$< 10^{-5}$
Germany–Belgium	0·0558	0·0037
Germany–Danube	0·0524	$< 10^{-5}$
Germany–western Poland	0·0581	$< 10^{-5}$
Germany–central Poland	0·0701	$< 10^{-5}$
Germany–eastern Poland	0·0796	$< 10^{-5}$
Danube–eastern Poland	0·0009	0·0203
Danube–western Poland	0·0043	0·0077
Danube–central Poland	0·0138	0·0014

F_{ST} is calculated according to Weir & Cockerham (1984). P is the P value of the Fisher combined test. Probabilities for loci considered separately were obtained using Fisher's exact test on contingency tables. Eastern Poland is the river system Narew–Bug–Bierbza, central Poland is the river system Piliza–Vistula–North Vistula, western Poland corresponds to the Warta river, and Germany consists of the Rhine and Ems.

Table 5. *Allelic differentiation along rivers*

Rivers	P
Western rivers	
Rhine	0·0009
Danube	$< 10^{-5}$
Warta	0·0000
Eastern rivers	
Vistula + tributaries	0·5289
Bug	0·0671
Narew	0·6288

P is Fisher combined probability test; probabilities for loci considered separately were obtained using Fisher's exact test on contingency table.

and eastern Poland was less marked than with Germany and central Poland (Table 4).

Secondly, comparisons between Polish river systems indicate that, contrary to our expectations, there was no more differentiation between than within Polish river systems. Indeed, the largest F_{ST} value was observed within the Warta river ($F_{ST} = 0·075$). Thirdly, marked differentiation was generally detected between subpopulations located along western rivers (Rhine, Danube and Warta), whereas no significant differentiation could be detected along the eastern rivers (Vistula + Pilica, Bug and Narew) (Table 5).

(b) *Quantitative traits*

Analysis of variance for the growth traits indicated that all effects were highly significant (data not shown). Computed t' values were generally higher than F_{ST} values obtained from isozyme data, but confidence intervals overlapped in most cases. The same pattern was observed in the two experiments: population differentiation was most pronounced for diameter and t' values for number of shoots decreased with ageing of the stumps (Table 6). The latter was due to a proportionally steeper rise in clonal variance than in population variance.

(v) *Migration*

The absolute number of migrants (Nm) varied greatly according to the populations considered. Wright's F_{ST} and Slatkin's private alleles methods led to similar

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Table 6. *Population differentiation for quantitative traits: variance estimates and t′ values with their 95% confidence intervals (95% CI) for quantitative traits using model (1) in experiment 1 and experiment 2 (see text)*

Trait	Replicate	Block	Population	Clone	Error	t'	95% CI of t'
Experiment 1							
Shoot 88	0·0003	0·0325	0·0146	0·0607	0·7362	0·107	−0·040 to 0·256
Shoot 90	0·0271	0·1067	0·0645	0·4749	2·6354	0·063	−0·001 to 0·128
Shoot 91	0·1038	0·6988	0·1612	1·8307	8·1974	0·042	−0·007 to 0·091
Diam. 91	2·7028	1·0561	0·5546	2·4127	5·8258	0·103	0·032 to 0·173
Height 90	752·96	194·78	38·968	517·60	1509·11	0·036	−0·009 to 0·082
Experiment 2							
Shoot 88	0·0013	0·0237	0·0812	0·1366	0·7938	0·229*	0·077 to 0·380
Shoot 90	0·2515	0·1391	0·2884	1·7279	4·5754	0·077	−0·004 to 0·158
Shoot 91	0·4546	0·2609	0·2872	2·0471	4·9461	0·065	−0·009 to 0·140
Diam. 91	912·22	1·0589	4·9894	3·4700	9·4835	0·418*	0·213 to 0·623

Shoot 88 is the number of shoots in 1988, Diam. 91 is the diameter of the tallest shoot in 1991 and Height 90 is the height of the tallest shoot in 1990, etc.
* $P < 0·05$ from estimates of F_{ST} obtained with isozyme data (see Table 3).

Table 7. *Estimates of the number of migrants between different populations, estimated from both Wright's F_{ST} and Slatkin's private allele method*

	Estimation of *Nm* from	
Comparison	F_{ST}	Private allele
Overall	5·81	55·3
Skåne–central Sweden	4·96	7·03
Skåne–Germany	7·26	0·50
Skåne–eastern Poland	14·6	27·5
Skåne–western Poland	48·7	14·0
Skåne–central Poland	11·3	0·71
Central Sweden–Germany	11·6	No private allele
Central Sweden–eastern Poland	2·64	0·38
Central Sweden–western Poland	3·09	0·94
Central Sweden–central Poland	2·86	0·83
Germany–Belgium	4·22	No private allele
Germany–Danube	4·51	No private allele
Germany–western Poland	4·04	1·63
Germany–central Poland	3·31	1·40
Germany–Eastern Poland	2·88	0·72

values (Table 7). Isolation by distance was tested for four rivers (Rhine, Danube, Warta and Narew). No isolation by distance was detected when the number of migrants was plotted against the distance between population on a logarithmic scale.

4. Discussion

The amount of population differentiation in *Salix viminalis* L. is fairly low and does not differ very much from that of species with similar life history characteristics, most estimates of F_{ST} reported for Salicaceae being between 1% and 7% (Weber & Stettler, 1981; Huyn *et al.* 1987; Jelinski & Cheliak, 1992; Farmer *et al.* 1988, Légionnet and Lefèvre, 1996). Both differentiation and diversity are also close to those observed for forest tree species with large and continuous stands, such as spruces, pines or oaks (Kremer, 1994). In the case of *S. viminalis* L. this may be explained partly by the numerous population transfers in the past, and possibly by the occurrence of long-range dispersal via either pollen (*S. viminalis* L. can apparently be wind pollinated; C. Alström, unpublished results) or seeds carried by the flood of rivers with unregulated water regimes. There could also be extensive vegetative propagation by means of twigs broken from existing plants floating down the river and rooting when they find appropriate conditions. Vegetative propagation does occur (Lascoux, personal observations) but it is unclear whether it is frequent or not.

(i) *Differentiation within and between river systems*

The lack of differentiation observed along rivers of the eastern part of the range of *S. viminalis* L. may indicate that gene flow does indeed take place along waterways, while the significant, but unstructured, differentiation pattern observed among large and disturbed rivers of the western part of the range may be explained by the joint effects of water regulation that limits successful gene flow through seeds, and introduced willow stands. While transfer of material between populations of central Europe probably occurred, it has been difficult to document and quantify. On the other hand, there is good experimental evidence in other willow species that the water regime plays a significant part in the regeneration of riparian stands: in most riparian willow species, regeneration relies on the existence of sand banks or gravel bars and seedling survival critically depends on the soil structure and moisture (Krasny *et*

al. 1988; McBride & Strahan, 1984; Marty, 1984; Thébaud & Debussche, 1991; Sacchi & Price, 1992). Finally, it should be noted that the lack of differentiation along the Bug and Narew rivers cannot be attributed to the small number of populations, because taking tributaries into consideration, and thus increasing the number of subpopulations, does not increase the F_{ST} value. Also, although numerous (n = 16), the populations along the Vistula and its tributaries were undifferentiated.

(ii) *Origin of Swedish populations and divergence from putative ancestors*

Skåne and central Sweden differ from all other populations in at least three respects: (i) their subpopulations are highly differentiated (F_{ST} values being 0·15 an 0·16, respectively *v.* a global value of 0·04); (ii) there are significant linkage disequilibria (at the level of the main population as well as within the largest subpopulation in central Sweden) and, (iii) one of the loci (*ACP*) that departs from Hardy–Weinberg expectations showed a highly significant excess of heterozygotes.

The significant amount of linkage disequilibrium observed in Skåne and central Sweden suggests that these are admixed populations, resulting from the pooling of populations that differ in allele frequencies but can themselves be in linkage equilibrium (Nei & Li, 1973). An alternative hypothesis is that the observed linkage disequilibrium is the result of natural selection. However, the analysis of Ohta's parameters suggests that it is not the case. Note, however, that Ohta's analysis assumes that the populations studied are in equilibrium, but this is unlikely in the present study. In any case, considering the small number of generations that have elapsed since the founding of the populations and the selection intensities commonly observed in nature (Endler, 1986), selection can be ruled out as the sole explanation of the observed linkage disequilibrium. In the absence of selection, the presence of linkage disequilibrium indicates the fairly recent origin of these populations.

This interpretation is consistent with the high degree of differentiation among subpopulations, which can occur from the pooling of material from different origins. Had most stands been founded by individuals from the same population, we would observe an F_{ST} value among them of the same magnitude as the one observed among central European populations. Some linkage disequilibrium was also found within some of the subpopulations (data not shown) such as the one along the Fyris river, south of Uppsala, where growth in population size probably resulted from the introduction of new material rather than natural regeneration during the last 10 years. This lack, or at least infrequency of natural regeneration, combined with the different origins of the individuals, may also explain the departure from Hardy–Weinberg proportions observed in this population.

Among the putative ancestor populations analysed in the present study, sources from Germany (Rhine + Ems) and western Poland seemed to have been the main contributors to the populations in central Sweden and Skåne, respectively. However, estimates of the number of migrants indicates also that populations from Skåne, which historically preceded the ones of central Sweden, also contributed to their foundation. Finally, much higher migration rates between Swedish populations and central European ones than among the latter may be the consequence of the important human intervention in the founding of the Swedish populations. Conversely, because these transfers have certainly occurred, it implies that similar transfers were less common between continental populations, at least in the westernmost part and over the last three centuries. Had they been as common a migration rate as high as the ones observed between Sweden and its putative ancestors would be observed.

Despite their recent origin, the Swedish populations have apparently begun to diverge from their ancestors. No clear trend emerges from the analysis of growth traits, but some of the populations used in these studies have also been used in experiments on resistance to leaf rust (*Melamspora* spp.) and to gall midge (*Dasineura margenimtorquens* Brem.). In both cases the Swedish populations differ from other populations, although, surprisingly, in opposite directions. The rust develops faster on Swedish willows than on introduced ones, whereas Swedish material is less prone than foreign willows to attacks by the midge (Gullberg & Ryttman, 1993; Fritz *et al.*, unpublished data). In field tests, Polish material was among the most susceptible of the introduced willows to Swedish *Melampsora* strains (Gullberg & Ryttman, 1993; Lascoux, unpublished observations). These results, together with the isozyme differentiation patterns reported above, suggest that Polish populations, especially the north-western ones may have been extensively used in establishing the present Swedish willow populations, especially the most southern ones. However, it is not possible at this point to identify the cause of divergence, even though the comparison between quantitative traits and isozyme differentiation patterns suggests that neutrality of both types of trait cannot be rejected.

(iii) *Comparison of differentiation at isozyme and quantitative trait loci*

Higher F_{ST} values were obtained for quantitative trait loci than for isozyme loci. Therefore, the differentiation pattern in *S. viminalis* L. does not depart in this respect from the one observed in other species (Prout & Barker, 1993; Spitze, 1993; Podolsky & Holtsford, 1995). However, with the exception of two traits,

Shoot 88 and Diam 91 in experiment 2, the 95% confidence intervals overlapped. A first explanation would be that our estimator, t', severely underestimated F_{ST}. That could be the case if there were a large dominance variance or strong 'maternal' effects. In a separate experiment the dominance variance was estimated to be 3% of the total genetic variance for number of shoots and up to 21% for weight (Rönnberg-Wästljung *et al.* 1994). The first possibility cannot be ruled out in the absence of further estimates but the low inbreeding depression observed after three generations of full-sib mating suggests that this is apparently not the case (unpublished results). Because vegetative copies were used in both experiments, the second possibility is also not very plausible. The presence of a strong maternal effect is inconsistent with the increase in clonal variance that accompanied the ageing of the stumps. The cause of this increase that was in turn responsible for the decrease in F_{ST} with the ageing remains unclear. Because it was observed in both experiments, it is not likely to be an artefact. It can be due to a change in genetic control accompanying ageing. Such changes have been documented in other tree species (Barnes & Schweppenhauser, 1978) but have not yet been extensively studied in willows. In any case, the observed lack of differentiation may not be an artefact and, assuming that the isozyme loci are neutral, our results suggest that the characters studied here may not depart significantly from neutrality. While this may be expected for traits such as shoot length, whose relation to fitness is not obvious, it is more surprising for traits such as number of shoots that can be more readily related to fecundity.

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