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Allozyme differentiation among populations of *Pinus sylvestris* (L.) from Sweden and China

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Allozyme differentiation at 14 loci was studied among 7 populations of *Pinus sylvestris* representing one geographic variety *lapponica* from Sweden and two varieties, *mongolica* and *sylvestriformis*, from China. The expected and observed heterozygosities were high and ranged between 0.165 and 0.282, and between 0.164 and 0.278 respectively. With the exception of the var. *sylvestriformis*, the patterns of genetic variation among populations were in accordance with the patterns of their geographic distribution. Nei's genetic diversity statistics showed that 7.5 % of the total diversity was due to differences among varieties. Pronounced differences were found between the var. *sylvestriformis* population and the populations representing the other two varieties. On the other hand, less differentiation was found between the geographically very distant var. *lapponica* and var. *mongolica*. It appears that as a result of past events the patterns of differentiation among populations of *P. sylvestris* are more complex than previously thought. It is possible that the individual geographic varieties of this species have a different evolutionary history.

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Pinus sylvestris is among the most important timber trees in many countries. Thus, since long it has been subject to detailed genetic scrutiny with respect to various characters (WRIGHT and BULL 1963; SZMIDT 1984). However, most of the earlier studies of allozyme variation in this species were concentrated on seed orchards (MÜLLER-STARCK 1982; SZMIDT 1986; and others), or on cultivated populations (SZMIDT and MUONA 1985; YAZDANI et al. 1985; RUDIN et al. 1986; MUONA et al. 1988; and others). In addition, the earlier studies of allozyme variation in natural populations were limited to the European part of *P. sylvestris* distribution (GULLBERG et al. 1985; MEJNARTOWICZ and BERGMANN 1985; MUONA and SZMIDT 1985; KINLOCH et al. 1986). To our knowledge, there are no reports describing allozyme variation in the natural populations of *P. sylvestris* from Asia.

P. sylvestris is the most widely distributed of pines. It grows throughout northern Eurasia from Scotland to northern China and is characterized by immense morphological and physiological variability (CRITCHFIELD and LITTLE 1966; MIROV 1967; OUDEN and BOOM 1978). So far, systematic division of this species is still unclear and various authors describe different numbers of taxonomic units (BIALOBOK 1970). The Swedish populations of *P. sylvestris* occurring north of 62° latitude belong to the variety *lapponica* (OUDEN and BOOM 1978; RUBY and WRIGHT 1976). In China, at least two geographic varieties of *P. sylvestris* are recognized: var. *mongolica* and var. *sylvestriformis* (CHENG and FU 1978). The var. *mongolica* occurs in the sandy and mountainous areas of the extreme north-eastern China where it forms two distinct ecotypes (CHENG 1983). The var. *sylvestriformis* occurs in a limited region on the northern slope of Changbai mountains in the Jilin province. The systematic position of this variety is not settled. However, there is general agreement among authors that it differs from the var. *mongolica* with respect to various morphological and ecological characters (CHENG and FU 1978; CHOU et al. 1986). The patterns of genetic variation in these two varieties have neither been studied previously nor compared to the var. *lapponica* from Sweden. Analysis of allozyme variation is useful in discerning patterns of genetic differentiation among closely related taxa (WHEELER et al. 1983; MILLAR et al. 1988; KARALAMANGALA and NICKRENT 1989; WANG et al. 1990). Information gained from these studies has been helpful in discerning evolutionary mechanisms of forest tree

Table 1. Geographic origin of *P. sylvestris* seed samples used in allozyme analyses

Variety	Locality	Symbol	Sample size	Latitude (N)	Longitude (E)
lapponica	Jokkmokk	Pl-1	135	66°40'	20°00'
lapponica	Norrsjö	Pl-2	134	65°30'	16°59'
lapponica	Tallsjö	Pl-3	133	64°30'	17°00'
mongolica	Honghuarji[`]	Pm-1	120	48°20'	120°20'
mongolica	Jagdaqi[m]	Pm-2	120	51°00'	125°00'
mongolica	Mohe[m]	Pm-3	120	53°00'	122°00'
sylvestriformis	Fusong	Ps-1	120	42°30'	127°30'

[`] Denotes sandy ecotype
[m] Denotes mountainous ecotype (see text for explanation)

Table 2. Enzyme systems, their Enzyme Commission (EC) numbers, and the number of loci scored

Enzyme	Abbreviation	E.C. No.	No. of loci scored
Aconitase	*Aco*	4.2.1.3	1
Acid phosphatase	*Aph*	3.1.3.2	1
Fluorescent esterase	*Fes*	3.1.1.1	1
Glutamate dehydrogenase	*Gdh*	1.4.1.2	1
Glutamate oxaloacetate transaminase	*Got*	2.6.1.1	2
Leucineaminopeptidase	*Lap*	3.4.11.1	2
Malate dehydrogenase	*Mdh*	1.1.1.37	2
Phosphoglucose isomerase	*Pgi*	5.3.1.9	2
Shikimate dehydrogenase	*Sdh*	1.1.1.25	2

speciation (e.g., WHEELER and GURIES 1987; WANG et al. 1990).

In the present study we examined the patterns of allozyme differentiation within and among all these three varieties of *P. sylvestris*. The purpose of our study is to provide new information about the patterns of genetic differentiation among adjacent as well as very distant populations of this species. Such information can help explaining the mechanisms of *P. sylvestris* evolution in Eurasia.

Material and methods

Seed material

Bulked seed samples were obtained from three Chinese populations of *P. sylvestris* var. *mongolica*, including one population representing the sandy ecotype and two populations representing the mountainous ecotype, and from one population of *P. sylvestris* var. *sylvestriformis*. In order to obtain information about allozyme differentiation among European and Asian populations we have also included three Swedish natural populations of *P. sylvestris* var. *lapponica* that were described previously in other contexts (MUONA and SZMIDT 1985; SZMIDT and MUONA 1985). The geographic origins of the sampled populations are presented in Table 1. Collections from the Swedish populations were made by the Institute of Forest Improvement at Sävar. Collections from the Chinese populations were made by the Department of Forestry of the Nanjing Forestry University and by the Forest Research Institute of the Chinese Academy of Forestry in Beijing. The exact number of trees included in these collections is unknown, but was apparently greater than 50. Seeds were kept at 0°C until analysis. Random seed samples were taken from each of these collections and regarded as random samples of the zygote population.

Electrophoresis

Before analysis, seeds were germinated for approximately 10 days. Isoenzyme extraction and separation was made as described previously (SZMIDT 1984). Nine enzyme systems coded by 14 loci were analyzed. Staining procedures and the inheritance of individual isoenzymes have been described elsewhere (SZMIDT 1984). Information concerning enzyme systems analyzed and the number of loci scored is given in Table 2. All 14 loci were assessed simultaneously in each macrogametophyte and the corresponding embryo.

Table 3. Allele frequencies in the investigated populations

Locus	Population						
	P1-1	P1-2	P1-3	Pm-1	Pm-2	Pm-3	Ps-1
Aco							
(N)	134	134	132	118	120	120	116
1	0.004	0.015	0.027	0.000	0.025	0.004	0.194
2	0.944	0.925	0.939	0.860	0.750	0.871	0.440
3	0.052	0.060	0.034	0.140	0.225	0.125	0.366
Aph							
(N)	134	132	131	120	119	113	118
1	0.000	0.008	0.008	0.000	0.000	0.000	0.004
2	0.011	0.004	0.000	0.000	0.000	0.000	0.064
3	0.858	0.860	0.901	0.933	0.836	0.867	0.585
4	0.123	0.110	0.080	0.054	0.067	0.053	0.347
5	0.007	0.015	0.011	0.013	0.097	0.080	0.000
6	0.000	0.004	0.000	0.000	0.000	0.000	0.000
Fes							
(N)	133	134	133	118	119	119	120
1	0.019	0.000	0.011	0.000	0.004	0.000	0.063
2	0.726	0.765	0.767	0.720	0.727	0.790	0.646
3	0.154	0.119	0.094	0.076	0.139	0.084	0.271
4	0.102	0.116	0.128	0.203	0.130	0.126	0.021
Gdh							
(N)	131	132	131	118	120	120	120
1	0.405	0.413	0.363	0.195	0.262	0.229	0.008
2	0.595	0.587	0.637	0.805	0.738	0.771	0.992
Got-1							
(N)	135	134	132	120	119	120	120
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Got-2							
(N)	134	134	133	120	120	117	120
1	0.228	0.097	0.086	0.013	0.021	0.017	0.004
2	0.291	0.399	0.380	0.404	0.242	0.286	0.792
3	0.481	0.504	0.534	0.583	0.738	0.697	0.204
Lap-1							
(N)	135	134	132	120	120	120	120
1	0.044	0.022	0.000	0.000	0.000	0.000	0.000
2	0.900	0.948	0.981	1.000	1.000	1.000	0.958
3	0.041	0.019	0.011	0.000	0.000	0.000	0.042
4	0.015	0.011	0.008	0.000	0.000	0.000	0.000
Lap-2							
(N)	135	134	131	120	120	120	114
1	0.015	0.015	0.004	0.008	0.004	0.000	0.000
2	0.922	0.940	0.958	0.979	0.971	0.962	0.654
3	0.059	0.037	0.031	0.013	0.025	0.038	0.346
4	0.004	0.007	0.008	0.000	0.000	0.000	0.000
Mdh-1							
(N)	135	134	133	120	120	120	120
1	0.067	0.063	0.075	0.025	0.033	0.033	0.000
2	0.933	0.937	0.925	0.975	0.967	0.967	1.000
Mdh-2							
(N)	133	128	131	113	120	120	117
1	0.658	0.680	0.710	0.925	0.887	0.942	0.231
2	0.342	0.320	0.290	0.075	0.092	0.025	0.765
3	0.000	0.000	0.000	0.000	0.000	0.008	0.000
4	0.000	0.000	0.000	0.000	0.021	0.017	0.000
5	0.000	0.000	0.000	0.000	0.000	0.008	0.004

Table 3. Continued

Locus	Population						
	P1-1	P1-2	P1-3	Pm-1	Pm-2	Pm-3	Ps-1
Pgi-1							
(N)	135	134	133	120	120	120	120
1	0.993	1.000	1.000	1.000	1.000	1.000	1.000
2	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Pgi-2							
(N)	135	134	133	120	120	115	120
1	0.022	0.011	0.000	0.000	0.000	0.000	0.000
2	0.000	0.011	0.008	0.050	0.042	0.052	0.096
3	0.930	0.959	0.977	0.938	0.950	0.939	0.900
4	0.048	0.019	0.015	0.013	0.008	0.009	0.004
Sdh-1							
(N)	135	134	133	116	120	120	57
1	0.000	0.026	0.023	0.060	0.067	0.067	0.421
2	0.848	0.813	0.846	0.845	0.817	0.788	0.307
3	0.100	0.101	0.083	0.082	0.108	0.121	0.061
4	0.052	0.060	0.049	0.013	0.008	0.025	0.035
5	0.000	0.000	0.000	0.000	0.000	0.000	0.123
6	0.000	0.000	0.000	0.000	0.000	0.000	0.053
Sdh-2							
(N)	131	134	133	120	120	118	118
1	0.057	0.030	0.015	0.000	0.000	0.000	0.000
2	0.004	0.011	0.000	0.033	0.075	0.072	0.030
3	0.931	0.959	0.985	0.967	0.925	0.928	0.936
4	0.008	0.000	0.000	0.000	0.000	0.000	0.034

Statistical methods

Allozyme frequencies, expected (NEI 1978) and observed heterozygosities, unbiased genetic distance measures (NEI 1978), and cluster analysis using the unweighted pair-group method algorithm (UPGMA; SNEATH and SOKAL 1973) were made using release 1.7 of the BIOSYS-1 program (SWOFFORD and SELANDER 1981). A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. Hierarchical analysis of gene diversity in the investigated varieties and populations of *P. sylvestris* was made following NEI (1973) and CHAKRABORTY (1980). The analysis included twelve loci which were polymorphic (0.95 criterion) in at least one population. The total gene diversity (H_T) was calculated as the expected heterozygosity when all populations were treated as a panmictic unit. The average gene diversities for varieties (H_V) and populations (H_P) were calculated in similar way. The fractions of allelic variation that reside between varieties (G_{VT}) and between populations within varieties (G_{PT}) were calculated using formulas 1 and 2 respectively:

$$G_{VT} = (H_T - H_V) / H_T \quad [1]$$
$$G_{PT} = (H_V - H_P) / H_T \quad [2]$$

The proportion of diversity that resides within populations was calculated as H_P/H_T.

Results

Of the fourteen analyzed loci, twelve loci (86 %) were polymorphic in at least one population (0.95 criterion) and seven loci (50 %) were polymorphic in all populations. Among the most polymorphic loci were *Got*-2, *Fes*, *Gdh*, *Mdh*-2 and *Sdh*-1. Little or no polymorphism was found at *Got*-1, *Lap*-1, *Mdh*-1 and *Pgi*-1 loci. Allozyme frequencies found in the investigated populations are given in Table 3. Considerable differences were found with respect to allele frequencies at some loci, usually indicating distinct split among the investigated varieties. Several alleles were unique to individual varieties. For instance, allele 4 at the *Lap*-1 locus and allele 1 at the *Sdh*-2 locus were unique to var. *lapponica* populations; alleles 3 and 4 at the *Mdh*-2 locus were unique to the var. *mongolica* populations, and the alleles 5 and 6 at the *Sdh*-1 locus were unique to the var. *sylvestriformis* population (Table 3).

Summary of estimates of genetic variability at 14 loci in the investigated populations is given in Table

Table 4. Genetic variability at 14 loci in the investigated populations; standard errors are given in parentheses

Population	Mean sample size per locus	Mean no. of alleles per locus	Percentage of polymorphic loci[a]	Mean heterozygosity	
				Observed	Expected[b]
Pl-1	133.9 (0.4)	2.9 (0.3)	85.7	0.203 (0.042)	0.241 (0.051)
Pl-2	133.3 (0.5)	3.0 (0.4)	71.4	0.197 (0.044)	0.222 (0.051)
Pl-3	132.2 (0.2)	2.7 (0.3)	57.1	0.179 (0.047)	0.196 (0.051)
Pm-1	118.8 (0.6)	2.3 (0.2)	57.1	0.164 (0.044)	0.165 (0.044)
Pm-2	119.8 (0.1)	2.5 (0.3)	64.3	0.205 (0.046)	0.199 (0.045)
Pm-3	118.7 (0.6)	2.5 (0.3)	64.3	0.171 (0.040)	0.177 (0.040)
Ps-1	114.3 (4.4)	2.7 (0.4)	64.3	0.278 (0.067)	0.282 (0.068)

[a] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95
[b] Unbiased estimate (Nei 1978)

Table 5. Gene diversity within and among varieties and populations of *P. sylvestris*

Locus	H_T[a]	H_V	H_P	G_{VT}	G_{PV}	H_P/H_T
Aco	0.297	0.267	0.264	0.101	0.010	0.889
Aph	0.286	0.268	0.266	0.063	0.007	0.930
Fes	0.428	0.422	0.420	0.014	0.005	0.981
Gdh	0.397	0.358	0.357	0.098	0.003	0.899
Got-2	0.554	0.497	0.490	0.103	0.013	0.884
Lap-1	0.063	0.058	0.058	0.079	0.000	0.921
Lap-2	0.157	0.138	0.138	0.121	0.000	0.879
Mdh-1	0.084	0.080	0.080	0.048	0.000	0.952
Mdh-2	0.409	0.303	0.302	0.259	0.002	0.738
Pgi-2	0.111	0.110	0.110	0.009	0.000	0.991
Sdh-1	0.361	0.361	0.361	0.000	0.000	1.000
Sdh-2	0.100	0.100	0.100	0.000	0.000	1.000
Mean	0.271	0.247	0.246	0.075	0.003	0.922

[a] Abbreviations are explained in Materials and methods

4. The mean number of alleles per polymorphic locus was high and ranged from 2.3 and 3.0. The proportion of polymorphic loci ranged from 57.1 % to 85.7 %. Observed heterozygosity and unbiased estimate (Nei 1978) of expected heterozygosity in embryos ranged from 0.164 to 0.278 and from 0.165 to 0.282 respectively, and were highest in the var. *sylvestriformis* population.

Results of hierarchical partitioning of the gene diversity statistics are presented in Table 5. The total diversity H_T was 0.271. On the average, 92.2 % of gene diversity resided within individual populations, 0.3 % between populations within varieties, and 7.5 % between varieties. The relatively high diversity between varieties was mainly due to the contribution from the var. *sylvestriformis*. When this variety was excluded the genetic diversity between the other two varieties was only 2.8 %.

The values of unbiased estimates of genetic distance (Nei 1978) among the investigated populations are given in Table 6. A particularly large genetic distance was observed in comparisons between the var. *sylvestriformis* population and the populations of the other two varieties and ranged from 0.106 to 0.133. Genetic distance between var. *lapponica* populations and the var. *mongolica* populations was smaller and ranged from 0.008 to 0.017. Still lower values were obtained in comparisons among populations representing the same variety. With the exception of the var. *sylvestriformis*, genetic distance increased with geographic distance among the investigated populations. When populations were considered as operational taxonomic units regardless of their variety assignment and subjected to cluster analysis based on genetic distance, the var. *sylvestriformis* population again appeared as the most divergent population (Fig. 1). The remaining six populations formed two clusters corresponding to their geographic and taxonomic origin, i.e., one comprising var. *lapponica* populations from Sweden and another comprising var. *mongolica* populations from China.

Table 6. Matrix of unbiased genetic distance (NEI 1978) among individual populations

Population	Pl-1	Pl-2	Pl-3	Pm-1	Pm-2	Pm-3
Pl-1	****					
Pl-2	0.001	****				
Pl-3	0.002	0.000	****			
Pm-1	0.015	0.011	0.008	****		
Pm-2	0.016	0.013	0.011	0.004	****	
Pm-3	0.017	0.013	0.010	0.002	0.001	****
Ps-1	0.114	0.106	0.111	0.120	0.125	0.133

Discussion

To our knowledge, allozyme variation in the Asian varieties of *P. sylvestris* has not been analyzed prior to this study. We compared differentiation of populations representing three different geograhic varieties of *P. sylvestris* originating from Sweden and China.

The main finding of our study is substantial genetic distinctiveness of the var. *sylvestriformis* population. The observed discrete character of this population supports the other evidence indicating the separate taxonomic status of this variety (CHOU et al. 1986). Some authors have suggested that the var. *sylvestriformis* is more related to *P. densiflora* than to *P. sylvestris* or that it represents a new species (TAKENOUCHI 1942; CHOU et al. 1986). The current distribution of the var. *sylvestriformis* populations is limited to the Changbai mountains in northeastern China. There it grows in small stands at elevations between 800 and 1600 m, partly overlapping with *P. densiflora* populations, which occur below 900 m (CHENG and FU 1978). It does not overlap, however, with the distribution of the var. *mongolica* populations, which are confined to the northeastern extremes of China (CHOU et al. 1986). Despite a limited distribution of the var. *sylvestriformis*, the investigated population exhibited more diversity than the var. *lapponica* and var. *mongolica* populations. This suggests that drift and restricted gene flow have had no distinct effect on its allelic composition.

At least three different suggestions can be put forward to explain the observed distinctiveness of the var. *sylvestriformis* population. First, it may represent an old endemic population resulting from the previous fragmentation of the var. *mongolica* range. Second, it may represent an inter-specific hybrid. Third, as suggested by some botanists, it may represent a variety of its sympatric neighbor *P. densiflora* (TAKENOUCHI 1942; CHOU et al. 1986). Taking into account the highly dissimilar character of the var. *sylvestriformis* population, its derivation from a splinter of the var. *mongolica* range must be doubted. In addition, the observed considerable genetic diversity of the var. *sylvestriformis* population is not compatible with the decrease of diversity that is expected for small isolated populations due to founder effect and random drift. Our present data do not explicitly favor any of the latter two hypotheses. However, morphological affinity of the var. *sylvestriformis* to the var. *mongolica* (CHENG and FU 1978), the occurrence in a sympatric region with *P. densiflora* and the discrete vertical distribution lend some support to the second hypothesis suggesting a hybrid origin of this taxon. As revealed by our previous studies, interspecific hybridization has indeed played a significant role in the evolution of the genus *Pinus* in Asia (WANG et al. 1990; WANG and SZMIDT 1990). We must emphasize, however, that only a single population of the var. *sylvestriformis* was analyzed in this study. Thus, our results cannot be generalized. More studies, including additional populations of the var. *sylvestriformis* as well as sympatric populations of *P. densiflora* are required to elucidate the evolutionary origin of the var. *sylvestriformis*.

From the earlier assessments of allozyme variation, it appeared that while the *P. sylvestris* in Sweden harbors a considerable amount of genetic diversity, most of it resides within individual populations whereas very little differentiation occurs among populations (GULLBERG et al. 1985; SZMIDT and MUONA 1985). With the exception of the var. *sylvestriformis* population, our results show similar patterns of allozyme variation in the investigated populations from Sweden and China. GULLBERG et al. (1985) suggested that the post-glacial history of *P. sylvestris* in Sweden was too short to permit substantial differentiation among individual popula-

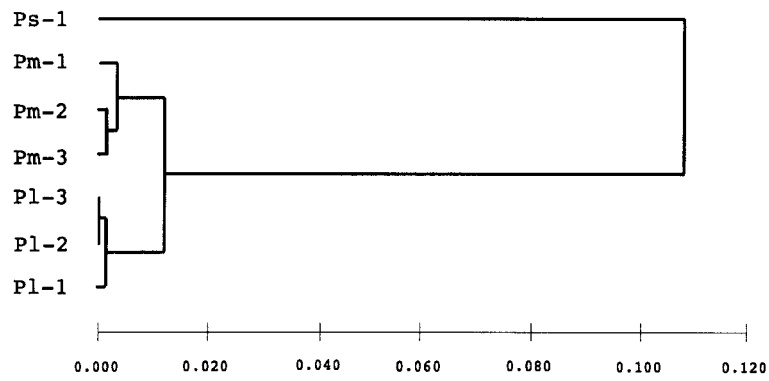

Fig. 1. Cluster analysis using UPGMA method (SNEATH and SOKAL 1973) of *P. sylvestris* populations representing three geographic varieties, *lapponica* (Pl), *mongolica* (Pm), and *sylvestriformis* (Ps), based on the unbiased genetic distance (NEI 1978).

tions. A similar suggestion can be advanced to explain the low level of genetic differentiation among the var. *lapponica* populations found in this study. The var. *mongolica* populations occur in areas that were also invaded by glacier (WRIGHT and BULL 1963; FRENZEL 1968). Therefore, they probably experienced a similar post-glacial life history as the Swedish populations. Here also, this history may have been too short to permit substantial differentiation among these populations.

Despite the huge geographic distance separating the var. *lapponica* and var. *mongolica* populations their genetic divergence was similar to that observed among populations from Poland and Scotland (MEJNARTOWICZ and BERGMANN 1985; KINLOCH et al. 1986). When only var. *lapponica* and var. *mongolica* were included in the analysis, only 2.8 % of the total diversity was due to differences between these two varieties. The relatively low levels of genetic differentiation found among the var. *lapponica* and var. *mongolica* may suggest that they originated from a common ancestral population after the retreat of the glacier, and that their present-day divergence developed during postglacial isolation.

Nevertheless, it was possible to discriminate between varieties based on the calculated genetic distance and clustering patterns following UPGMA analysis. In addition, slight but distinct increase of genetic distance was observed between the two ecotypes of the var. *mongolica* included in the present study. The present-day distribution of the sand and mountain ecotypes is discontinuous (CHINESE VEGETATION 1980). This geographic isolation is likely to limit the gene exchange between these ecotypes and to promote their divergence due to drift and/or selection. Taking into account the limited size of our material, the aforementioned suggestion must be corroborated by additional studies including more populations representing these two ecotypes.

Results of our study indicate that the patterns of genetic differentiation in *P. sylvestris* are more complex than previously thought. While some geographically distant populations appear little differentiated as in the case of var. *lapponica* and var. *mongolica,* unexpectedly large differences may occur among adjacent populations. The evolutionary history of *P. sylvestris* appears to have affected individual populations and localities in a heterogeneous way. Further studies, including more populations and different regions are imperative for a better understanding of the patterns of genetic variation and evolution in this species.

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