

## Genetic Divergence of Eastern European and Tobol Populations of *Calluna vulgaris* (L.) Hull.

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**Abstract**—Analysis of geographic variation of chloroplast DNA in the eastern European and western Siberian (Tobol) groups of *Calluna vulgaris* populations isolated from each other for a long period of time has revealed distinct differentiation between them. It has been shown that three populations growing in the Russian Plain and Karelia share two haplotypes characteristic of central Europe, while three Tobol populations contain one haplotype *F* occurring in Sweden. These results confirm the hypothesis of genetic divergence between the Tobol and eastern European groups of *C. vulgaris* populations.

**Keywords:** *Calluna vulgaris*, population, chloroplast DNA, Eastern Europe, Western Siberia, isolation, genetic divergence

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Studies in population biology have revealed wide polymorphism in the ecogenetic population structure of many plant species, which is explained by specific differences in their centers of origin, routes and rates of migration, reproductive isolation, and habitat conditions. Using the example of conifers in the Northern Hemisphere, it has been shown that the level of polymorphism in their insular populations is lower, while the level of genetic differentiation between them is higher in the marginal than in the central parts of their ranges (Tigerstedt, 1973; Critchfield, 1984; Gullberg et al., 1985; Moran, Bell, and Eldridge, 1988; Semerikov and Lascoux, 2003; Sannikov et al., 2011). In general, however, specific ecogenetic features of marginal isolates and their role in speciation have not been studied sufficiently.

The group of endemic populations of heather (*Calluna vulgaris* [L.] Hull.) growing in the Tobol region, in the southwest of Western Siberia, is an interesting object for ecogeographic analysis of the role of long-term isolation in microevolutionary divergence of plants. The ridges and foothills of the Urals separate them from the main, European part of this monotypic species for more than 500 km (Beijerink, 1940; Hulten, 1958; Gorchakovskii, 1962). Scattered patches and colonies of heather in this region are closely associated with pine forests on sandy terraces above river floodplains and occur in a zone 150–170 km wide extending in the north–south direction from the

Konda River basin to the city of Kurgan (Gorchakovskii, 1962; Petrova et al., 2009).

Previous studies on *C. vulgaris* in Europe have provided comprehensive data on its geographic and ecological ranges (Beijerink, 1940; Hulten, 1958; Gimmingham, 1960, 1975), morphological and autecological features and seed reproduction (Nordhagen, 1937; Dommee, 1969; Gimmingham, 1975, 1988; Herrera, 1987; Legg, Maltby, and Proctor, 1992; Cumming and Legg, 1995), and differentiation into ecotypes depending on altitude (Grant and Hunter, 1962; Dommee, 1968). Methods of allozyme and chloroplast DNA (cpDNA) analysis have made it possible to reveal geographic variation and differentiation of populations in the direction from Pyrenean (probably refugial) to Scandinavian habitats (Mahy et al., 1997; Mahy, Ennos, and Jacquemart, 1999; Rendell and Ennos, 2002).

The results of comparative studies on *C. vulgaris* in geographically vicarious types of pine forests in the Tobol region and Russian Plain have shed light on cenocogeographic features of its ecological range, the structure of cenopopulations, and their heliophilia and competitive relationships with the edificator (*Pinus sylvestris*) tree stand (Petrova et al., 2009, 2010; Sannikova et al., 2012). It has been shown that the growth rate, abundance, and coverage of *C. vulgaris* under the canopy of similar pine forest types are higher in the Russian Plain than in Western Siberia. Moreover, this species does not grow at all on peat soils of

bogs in the Tobol region but commonly occurs on waterlogged sphagnum substrates in the eastern European part of its range, e.g., in the Carpathians and Baltic republics (Fedorchuk, Neshataev, and Kuznetsova, 2005; Petrova et al., 2009), as well as in western Europe (Gimingham, 1960, 1975). Taking into account these cenocological differences, strict and long-term isolation by distance, and specific climatic, soil, and biotic conditions in the arid continental Transural region, we have put forward the hypothesis concerning ecogenetic divergence of insular Tobol populations from European populations of *Calluna vulgaris* (Petrova et al., 2009).

This paper deals with a brief analysis of preliminary results of a study performed to test this hypothesis.

### OBJECTS AND METHODS

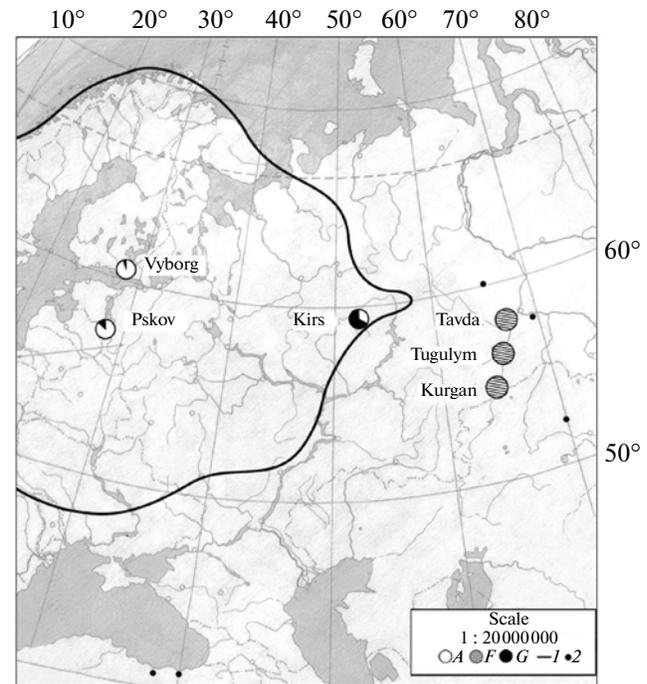
The comparative analysis of cpDNA variation was performed between two groups of geographically isolated *C. vulgaris* cenopopulations (below, referred to as populations), the Tobol group (populations from Tavda, Tugulym, and Kurgan) and the eastern European group (populations from Vyborg, Pskov, and Kirs), growing under the canopy of climatically vicarious, topologically similar pine forests of cowberry–heather–moss type (Petrova et al., 2009). Their geographic locations are shown in Fig. 1.

In October, 18–55 samples of leafy shoots were collected in each population from distinct, separately growing plants (ramets) located no less than 30–50 m from each other. Leaf tissues, fresh or frozen at  $-70^{\circ}\text{C}$ , were used in the study. Analysis of variation in cpDNA, which is maternally inherited in *C. vulgaris*, was performed by the PCR–RFLP method as described (Rendell and Ennos, 2002), except that 6% denaturing polyacrylamide gel was used instead of nondenaturing 8% gel. On the whole, 213 individual *C. vulgaris* plants were analyzed in the six samples.

The pattern of variation in marker cpDNA fragments was analyzed to determine chloroplast haplotypes, their number, composition, and occurrence frequency in the populations studied. Hierarchical analysis of cpDNA variation between the Tobol and eastern European groups, between populations within the groups, and between all samples included in the study was performed by the AMOVA method (Excoffier, Laval, and Schneider, 2006).

### RESULTS AND DISCUSSION

Analysis was performed for four markers variable in European populations of *C. vulgaris*: CS/*AluI*, FV/*HaeIII*, K1K2/*TaqI*, and TabCD/*TaqI* (Rendell and Ennos, 2002), but polymorphism was revealed only in TabCD/*TaqI* (Table 1). Its variation is due to two types of mutations, insertion and point replacement. Their combinations in all six populations allowed us to determine the occurrence and frequency



**Fig. 1.** Geographic range and locations of population samples and chloroplast DNA haplotypes (*A*, *F*, *G*) of *Calluna vulgaris* in eastern Europe and the Tobol region of Western Siberia. Tavda, Tugulym, and Kurgan are insular population groups in the Tobol region; (1) the boundary of the European part of the range (according to Gorchakovskii, 1962); (2) isolated locations.

of three chloroplast haplotypes corresponding to haplotypes *A*, *F*, and *G* (out of a total of 12) identified previously in 23 *C. vulgaris* population samples from western and central Europe (Rendell and Ennos, 2002). Thus, the total number of haplotypes in all *C. vulgaris* samples from the eastern part of its European range and the Tobol region of Western Siberia proved to be only one-fourth of that recorded in the main, western part of its range.

Analyzing the frequencies of chloroplast haplotypes, we revealed differences in their distribution between the two phylogeographic groups of *C. vulgaris* populations: haplotypes *A* and *G* characteristic of central Europe proved to occur in different combinations in populations of the eastern European group, while all three populations from the Tobol region contained only haplotype *F*, which was not identified in our samples from the Russian Plain or southern Karelia (Fig. 1) but was previously described in from Sweden (Rendell and Ennos, 2002). Such a contrasting (alternative) ratio of haplotypes is indicative of crucial differences and, probably of a certain boundary between these groups in the structure of the species gene pool. Haplotype *F*, which distinctly marks this boundary, can be termed “differential,” by analogy with differential plant species that make it possible to draw boundaries between phytocenoses (Scamoni, 1956).

**Table 1.** Haplotypes of *C. vulgaris* determined by restriction of four chloroplast DNA fragments

Haplotype	CS/ <i>AluI</i> band I, 307 bp	FV/ <i>HaeIII</i> band I, 1354 bp	K1K2/ <i>TaqI</i> band I, 330 bp	K1K2/ <i>TaqI</i> band II, 313 bp	K1K2/ <i>TaqI</i> band III, 275 bp	TabCD/ <i>TaqI</i> band I, 464 bp	TabCD/ <i>TaqI</i> band II, 221 bp
<i>A</i>	2	0	3	1	0	1	2
<i>F</i>	2	0	3	1	0	0	2
<i>G</i>	2	0	3	1	0	0	1

Note: Restriction fragments are numbered in order of decreasing molecular weight; zero sign indicates the absence of restriction fragment at the expected position in the gel.

**Table 2.** Hierarchical analysis (AMOVA) of variation and differentiation of the chloroplast genome in *C. vulgaris* populations

Source of variation	Parameters of variation					
	with regard to haplotype kinship			without regard to haplotype kinship		
	<i>N</i> -statistics	proportion of variance, %	<i>p</i>	<i>G</i> -statistics	proportion of variance, %	<i>p</i>
Between groups*	$N_{CT}$ : 0.443	44.29	0.10	$G_{CT}$ : 0.719	71.93	0.08
Between populations within group	$N_{ST}$ : 0.467	26.02	<0.001	$G_{SC}$ : 0.467	13.11	<0.001
Between all populations	$N_{ST}$ : 0.703	29.69	<0.001	$G_{ST}$ : 0.850	14.96	<0.001

\* Phylogeographic groups: Tobol group (Tavda, Tugulym, Kurgan) and eastern European group (Vyborg, Pskov, Kirs).

Parameters of intrapopulation cpDNA variation in the Tobol and eastern European population groups, respectively, are as follows: number of haplotypes ( $N_o$ ), 1 and 2; effective number of haplotypes ( $N_e$ ), 1.0 and 1.39; index of intrapopulation variation ( $H$ ), 0 and 0.27; i.e., these parameters obviously differ between the groups.

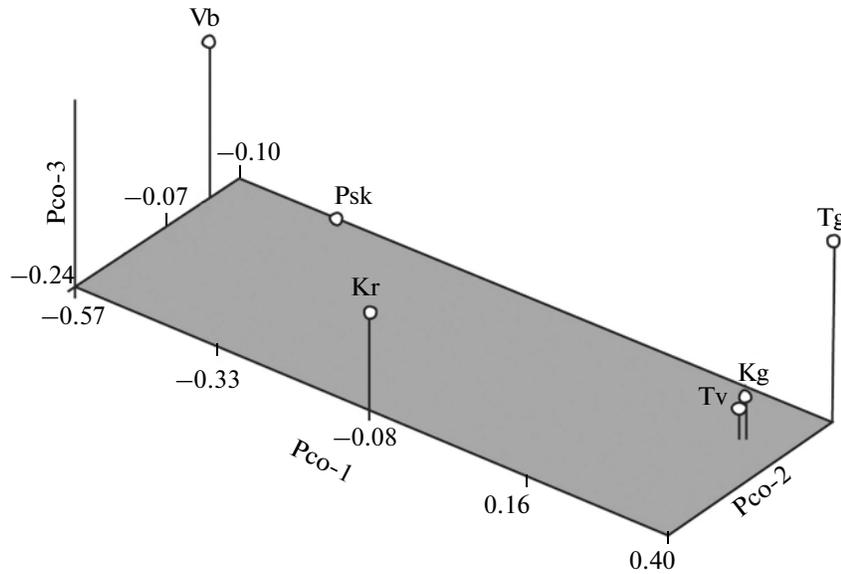
A hierarchical analysis of the distribution of cpDNA variation between the Tobol and eastern European groups of *C. vulgaris* populations, between populations within the groups, and between all the six populations was performed using the AMOVA test (Excoffier, Laval, and Schneider, 2006) for haplotype frequencies (Table 2). The results confirmed distinct differentiation between the two phylogeographic groups with respect to the distribution pattern of variation in chloroplast markers, especially in the variant where haplotype kinship was not taken into account:  $N_{CT} = 0.443$ ,  $G_{CT} = 0.719$ . The statistical significance of these values was insufficient ( $p = 0.08-0.10$ ) because of a small number of samples per group. Differences between populations within the groups in the two variants of analysis accounted for 26.0% and 13.1% of the total variance, and differences between all populations, for 29.7% and 15.0% ( $p \leq 0.001$ ); i.e., the proportion of explained variance was approximately three times smaller than in the above case. Thus, as follows from the difference revealed between the eastern European and Tobol groups, the geographic component obviously plays the dominant role

in differentiation of *C. vulgaris* populations with respect to cpDNA structure.

Ordination of the samples in a three-dimensional space of principal coordinates based on  $N_{ST}$  as a measure of genetic distance also provided fairly adequate evidence for genogeographic differentiation between European and Tobol populations of *C. vulgaris* (Fig. 2).

These results may be interpreted as follows. The *C. vulgaris* range in the Miocene probably covered not only Europe and East Atlantic islands but extended to continental regions of northern Eurasia, up to central Siberia. This follows from the occurrence of marginal, insular *C. vulgaris* populations scattered over the Azores, Faroe, and Shetland Islands, Iceland, and Western Siberia and single habitats of this species in Asia Minor, central Siberia, and central Kazakhstan (Gimingham, 1960; Gorchakovskii, 1962).

In the Eocene up to Oligocene, Siberian populations of *C. vulgaris* remained isolated from European populations for millions of years due to vast transgressions of the Kara and Tethys seas and, in the Pleistocene, by Ural glaciers and permafrost zones (up to 52° N). A long isolation of *C. vulgaris*, its exposure to disruptive selection in new directions under specific conditions of continental climate in the Transural region, and the effects of mutation, genetic drift, and other factors of microevolution provided for their divergence from European populations accompanied by segregation of the monomorphic "Transural" chloroplast haplotype *F*. It is noteworthy that this haplo-



**Fig. 2.** Ordination of *Calluna vulgaris* populations in a three-dimensional space of principal coordinates: (Vb) Vyborg, (Psk) Pskov, (Kr) Kirs, (Tv) Tavda, (Tg) Tugulyum, (Kg) Kurgan.

type was previously found in *C. vulgaris* from north-eastern Sweden (Rendell and Ennos, 2002), where it had probably been preserved since the Paleogene. The extreme impoverishment of *C. vulgaris* haplotype spectrum in insular pine forests of the Tobol region may be due to the effect of a series of population bottlenecks experienced by the species in this region during the Pleistocene.

It appears in first approximation that populations of the Tobol group have already diverged from eastern European populations to the subspecies level, which, in particular, is confirmed by their distinctive arealogical and cenoecological features (Petrova et al., 2009). A similar situation is observed, for example, with marginal Pleistocene isolates of *Larix kurilensis* ssp. *glabra* Dyl. in Kamchatka, in which monomorphic mitochondrial haplotypes are different from those in neighboring marginal populations of *L. cajanderi* Mayr (Polezhaeva, 2009).

## CONCLUSIONS

The analysis of geographic variation and differentiation of *C. vulgaris* chloroplast DNA haplotypes in the Tobol and eastern European groups of populations isolated from each other for a long time has revealed distinct genetic divergence between these groups.

Populations of the eastern European group from the Russian Plain and southern Karelia share two chloroplast haplotypes (*A* and *G*) characteristic of central Europe, whereas populations of the Tobol group have only one (monomorphic) haplotype *F*, which may be regarded as a differential haplotype. The proportion of total variance in the chloroplast haplotype

frequencies explained by genogeographic differences between the population groups is three times higher than that explained by differences between populations within the group and between all populations included in analysis.

Thus, preliminary results of comparative genogeographic analysis of marginal *C. vulgaris* populations from the Tobol region (Western Siberia), which have been long isolated by distance from the main part of the species range in Europe, confirm the hypothesis of their genetic divergence from European populations.

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