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Molecular signals for Late Tertiary/Early Quaternary range splits of an Eurasian steppe plant: *Clausia aprica* (Brassicaceae)

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Abstract

Several vegetation belts stretch continuously from Europe to Asia, taiga and steppe being most prominent. Numerous plant species within these belts share a conspicuous distribution area, which is longitudinally contracted or disrupted approximately along longitude 70° E. To date no hypothesis for this intriguing distribution pattern has been put forward. We detected molecular footprints in the contemporary genetic composition in nuclear DNA (ITS1, ITS2) and chloroplast DNA (*trn*L–*trn*F spacer region) of the steppe element *Clausia aprica* (Brassicaceae) providing evidence for a severe longitudinal range split and genetic differentiation east of the Ural Mountains about 1 million years ago caused by Quaternary climatic oscillations. *Clausia aprica* provides the first phylogeographical analysis on the intraspecific evolution of an Eurasian steppe plant.

Keywords: *Clausia aprica*, Eurasian steppe belt, ice ages, phylogeography

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Introduction

Recurrent climatic macrocycles have been typical of the earth's biosphere for the last 2 million years. Environmental responses in the form of repeated transformations and spatial shifts of vegetation belts account for the high biome dynamics, particularly observed in the northern hemisphere (e.g. Webb & Bartlein 1992; Lang 1994; Mai 1995; Williams *et al*. 1998; Velichko 1999). The severe palaeoclimatic oscillations also influenced genetic subdivision across species ranges. Testing the congruence of species phylogenies and landscape evolution has been greatly enhanced because molecular markers allow reconstruction of species histories and insights into population processes (Avise 2000; Hewitt 2001). In Europe and North America it is relatively easy to deduce Pleistocene range changes and their genetic consequences because of latitude and geographical structures, biogeographical knowledge, fossil records and molecular phylogeographical studies (Comes & Kadereit 1998; Taberlet *et al*. 1998; Hewitt 2000, 2004; Abbott & Brochmann 2003). In many other parts of the world the situation is not so straightforward. We have no molecular biogeographical studies on plant taxa of Asian–European vegetation zones along latitudinal belts in lowland plains, although this is exactly the type of distribution that is so characteristic for Eurasia (see distribution maps in Meusel *et al*. 1965, 1978; Hultén & Fries 1986; Meusel & Jäger 1992). Several vegetation belts, such as taiga and steppe, stretch over both continents. Within this zonal vegetation, numerous species are either confined to Europe or Asia, or they are distributed on both continents. Eurasian distribution areas are often contracted or disrupted between the Asian and European parts with a split in western Siberia/Kazakhstan, more or less along longitude 70° E (Meusel *et al*. 1965, 1978; Hultén & Fries 1986; Meusel & Jäger 1992). This specific distribution pattern is particularly obvious for the steppe belt, which extends from eastern Europe via Kazakhstan, Siberia, northwest China, Mongolia to the Amur region. The causes of this pattern are still poorly understood.

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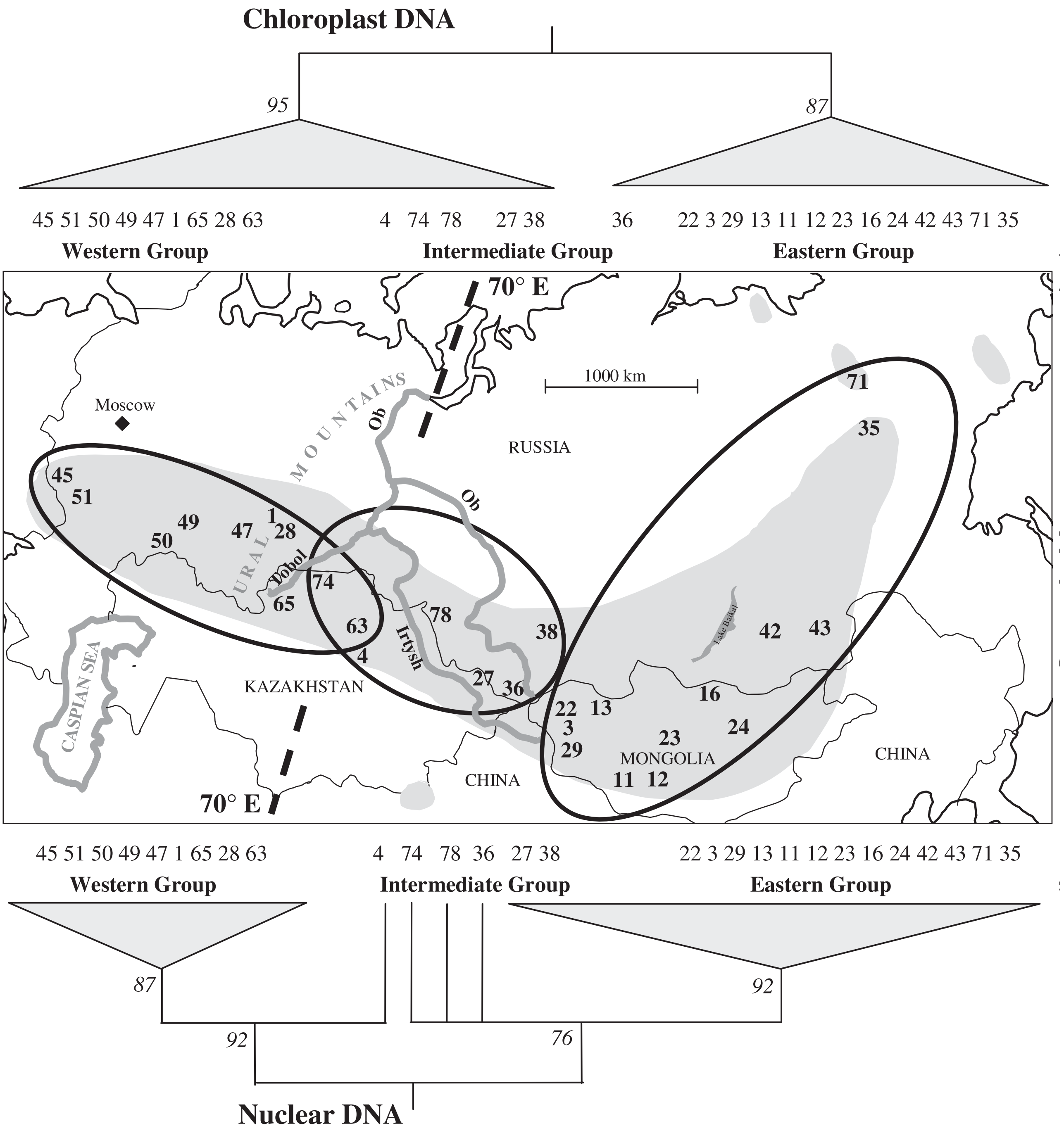

Fig. 1 Single most parsimonious chloroplast *trn*L–*trn*F tree (consistency index 1.0) and strict consensus ITS tree (consistency index 0.96) of *Clausia aprica* accessions from Eurasia. Numbers refer to the accessions studied (Table 1). *Clausia aprica* accessions were assigned to a western, an eastern and an intermediate group (ovals; see text). Triangles in the phylogenetic trees refer to unresolved clades containing the accessions mentioned. The position of the 70° E longitude line is indicated roughly marking the area where the steppe belt faced significant longitudinal splits during the ice ages (broken line). Bootstrap values > 75% are given. Trees were rooted by outgroup comparison. The overall distribution of *C. aprica* (according to Charkevicz 1988; Gajewski 1934; Malyschev & Peschkova 1994; Zhou *et al*. 2001) is indicated by the grey areas.

Litvinov (1890, 1902) was the first to interpret disjunct occurrences of steppe plants in European Russia as relicts from glacial periods, a view which was later supported by the investigations of Gajewski (1934) and others. Since then, biogeographical knowledge of eastern Europe and northern Asia has greatly increased, and considerable progress has been made in unravelling the climatic and environmental history of the steppe region during the last million years (Frenzel *et al*. 1992; Arkhipov & Volkova 1994; Velichko *et al*. 1999; Tarasov *et al*. 2000).

In the present study, we use molecular phylogenetics associated with a molecular clock approach to elucidate the causes of the distribution pattern of the steppe element *Clausia aprica* (Stephan) Korn.-Trotzky (Brassicaceae). *Clausia aprica* is a perennial herb, probably insect-pollinated but unfortunately nothing is known about its seed dispersal. The distribution of this plant is a typical example of the above mentioned general distribution pattern. It stretches from northern Ukraine, Middle Don, Volga, Kama region, southern Ural Mountains, Kazakhstan, west Siberia, Central Asia, east Siberia up to Yakutsia and the Russian Far East, with a latitudinally narrowed distribution and sparse population density in Kazakhstan (Fig. 1). This is the first phylogeographical analysis on the intraspecific evolution of an Eurasian steppe plant.

Materials and methods

Sequences of both nuclear ribosomal (internal transcribed spacers, ITS1, ITS2) and plastid DNA (*trn*L–*trn*F intergenic spacer) were produced for 28 accessions of *Clausia aprica* from almost the entire distribution area of this species (Fig. 1), and for one specimen of *Hesperis matronalis*, which was determined in initial analyses as a close relative (data not shown) and was therefore used as the outgroup. Methods for DNA extraction, polymerase chain reaction and direct sequencing of ITS and non-coding cpDNA are given elsewhere (Bowman *et al*. 1999; Mummenhoff *et al*. 2001). The resulting DNA sequence alignments are available from H.H. on request. Collection data and GenBank Accession numbers are given in Table 1. Parsimony analyses were performed in PAUP*, Version 4.0b.10 (Swofford 2001) using a heuristic search with 1000 random taxon additions,

Table 1 Numbers (Figs 1,2), origin and GenBank accession numbers of analysed accessions of *Clausia aprica* and *Hesperis matronalis*

					GenBank accession numbers		
No.	Accession* number	Provenance	Source†		ITS1	ITS2	*trn*L-*trn*F spacer
01	01-0071-50-00	Russia Urals‡	55°28′N/61°13′E	KAZ	AY–546109	AY–546138	AY–546173
03	00-0114-50-00	Mongolia	47°40′N/92°30′E	MW	AY–546121	AY–546150	AY–546182
04	00-0157-50-00	Kazakhstan, W. Siberia	51°10′N/71°30′E	MW	AY–546117	AY–546146	AY–546167
11	01-0256-50-00	Mongolia	45°30′N/97°05′E	UBA	AY–546124	AY–546153	AY–546185
12	01-0257-50-00	Mongolia	55°00′N/100°19′E	UBA	AY–546132	AY–546161	AY–546190
13	01-0258-50-00	Mongolia	49°30′N/96°00′E	UBA	AY–546131	AY–546160	AY–546189
16	01-0261-50-00	Mongolia	49°33′N/106°13′E	UBA	AY–546130	AY–546159	AY–546188
22	01-0267-50-00	Mongolia	48°53′N/95°45′E	UBA	AY–546129	AY–546158	AY–546187
23	01-0268-50-00	Mongolia	48°16′N/102°58′E	UBA	AY–546135	AY–546164	AY–546193
24	12362	Mongolia	48°16′N/106°33′E	OSBU	AY–546123	AY–546152	AY–546184
27	00-0160-50-00	Kazakhstan, W. Siberia	49°12′N/86°21′E	MW	AY–546126	AY–546155	AY–546168
28	01-0073-50-00	Russia, Urals	55°28′N/61°13′E	KAZ	AY–546111	AY–546140	AY–546177
29	00-0113-50-00	Mongolia	48°01′N/91°37′E	Chovd	AY–546133	AY–546162	AY–546191
35	00-0159-50-00	Russia, E. Siberia	62°25′N/131°48′E	MW	AY–546125	AY–546154	AY–546186
36	00-0164-50-00	Russia, W. Siberia	50°21′N/90°30′E	MW	AY–546118	AY–546147	AY–546169
38	00-0165-50-00	Russia, W. Siberia	53°49′N/91°24′E	MW	AY–546127	AY–546156	AY–546170
42	00-0162-50-00	Russia, E. Siberia	53°15′N/112°46′E	MW	AY–546122	AY–546151	AY–546183
43	00-0161-50-00	Russia, E. Siberia	52°22′N/115°34′E	MW	AY–546134	AY–546163	AY–546192
45	02-0085-50-00	Russia, E. Europe	41°42′N/44°55′E	MW	AY–546110	AY–546139	AY–546174
47	02-0089-50-00	Russia, E. Europe	52°53′N/56°36′E	MW	AY–546112	AY–546141	AY–546175
49	02-0091-50-00	Russia, E. Europe	53°25′N/49°30′E	MW	AY–546128	AY–546157	AY–546176
50	02-0086-50-00	Russia, E. Europe	51°40′N/49°23′E	MW	AY–546113	AY–546142	AY–546178
51	02-0087-50-00	Russia, E. Europe	51°40′N/39°28′E	MW	AY–546114	AY–546143	AY–546179
52	2744	*Hesperis matronalis*	48°56′N/10°58′E	OSBU	AY–546108	AY–546137	AY–546166
63	01-0078-50-00	NE Kazakhstan	53°22′N/76°18′E	KAZ	AY–546115	AY–546144	AY–546180
65	01-0076-50-00	N. Kazakhstan	52°28′N/61°52′E	KAZ	AY–546116	AY–546145	AY–546181
71	02-0184-50-00	Russia, E. Siberia	67°57′N/134°06′E	NSK	AY–546136	AY–546165	AY–546194
74	02-0187-50-00	N. Kazakhstan	54°23′N/64°47′E	NS	AY–546119	AY–546148	AY–546171
78	02-0191-50-00	Russia, W. Siberia	53°44′N/77°39′E	NS	AY–546120	AY–546149	AY–546172

*Accession numbers refer to the Brassicaceae collection of Systematic Botany, University of Osnabrück or the herbarium OSBU.

†Abbreviations refer to herbarium acronyms (*http://www.nybg.org/bsci/ih/ih.html*); Chovd refers to the Herbarium of the Mongolian National University Ulaanbaatar, Chovd branch, Mongolia.

‡Urals, Ural Mountains.

ACCTRAN, MULPARS, TBR, and STEEPEST DESCENT options with all characters unweighted. Bootstrap analyses were carried out with 100 replicates (heuristic search, simple taxon addition) to estimate tree stability.

Pairwise distance divergences were calculated in PAUP under Kimura's two-parameter model by using the deletion option for gaps and ambiguous data. Rate heterogeneity among lineages in the cpDNA and ITS trees was examined by using a tree-wide likelihood ratio test using MODELTEST 3.04 (Posada Crandall 1998). This test compares the log likelihoods (determined in PAUP* 4.0b10) of both constrained and unconstrained hypotheses under the most adequate substitution model that best fits the data (Huelsenbeck & Rannala 1997).

Results

After eliminating regions of ambiguous alignment, the ITS and *trn*L–*trn*F data matrix comprised 458 and 299 characters, of which 22 and five were parsimony informative, respectively. The single most parsimonious cpDNA tree (27 steps, consistency index 1.0), and the strict consensus tree of 46 709 maximally parsimonious ITS trees (107 steps, consistency index 0.96) is presented in Fig. 2. Well-supported clades correspond to the western and eastern parts of the distribution area of *Clausia aprica* (Fig. 1, western group, eastern group). However, there were conflicting topologies between both molecular trees concerning six accessions (intermediate group) representing the intermediate areas (approximately Kazakhstan) between the western and eastern parts of the distribution area (Fig. 1).

For the ITS regions (nuclear DNA), the Kimura's two-parameter pairwise sequence divergence among all the *Clausia aprica* accessions ranged between 0 and 2.19%. ITS divergence within the western group ranged from 0 to 0.05%, and within the eastern group from 0 to 1.9%. ITS sequence divergence between accessions of the western and eastern groups ranged from 1.45% to 2.19%, and rate constancy within the ITS phylogeny could not be rejected at $\alpha = 0.01$. Four accessions of the intermediate group (Fig. 1, Table 2) showed additivity at nucleotide positions that are variable between the western group and eastern group.

For the *trn*L–*trn*F data (cpDNA), the Kimura's two-parameter pairwise sequence divergence among all *C. aprica* accessions ranged between 0 and 2.0%. Sequence divergence between the western and eastern groups ranged from 1.7 to 2.0%. There was no trnL–trnF spacer divergence within the analysed accessions of the eastern group. Within the western group it ranged from 0 to 0.7%.

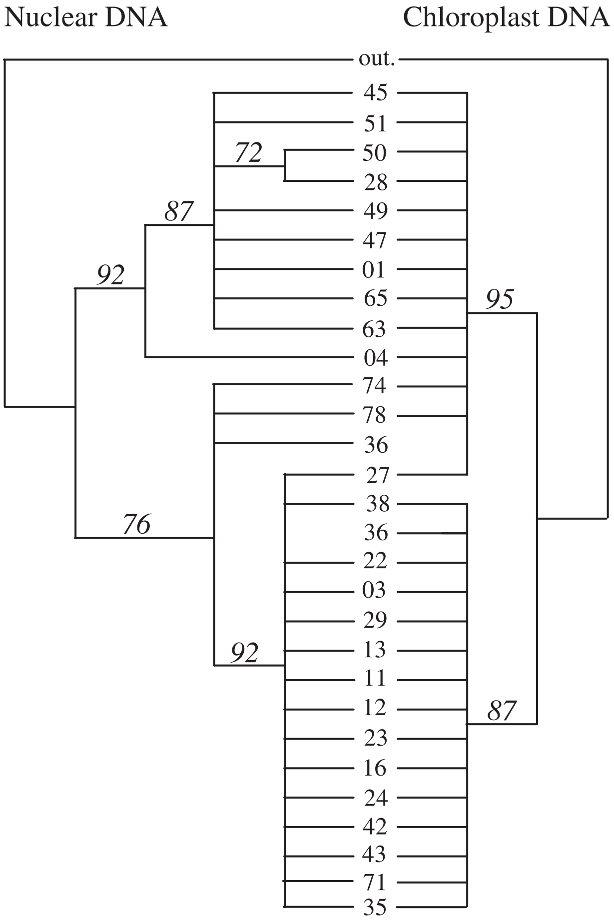

Fig. 2 Comparison of nuclear and chloroplast phylogeny of *Clausia aprica* accessions based on sequence analysis of nuclear ribosomal ITS and a noncoding cpDNA region (*trn*L–*trn*F spacer). Shown is the single most parsimonious cpDNA tree (27 steps, consistency index 1.0), and the strict consensus tree of 46 709 maximally parsimonious ITS trees (each 107 steps, consistency index 0.96). Bootstrap values are given above branches. Trees were rooted by outgroup (out.) comparison. Origin and code numbers of analysed accessions are given in Table 1.

Discussion

Chloroplast and nuclear DNA sequences clearly indicated intraspecific diversification of *Clausia aprica* into a western and an eastern group (Fig. 1). The split within *C. aprica* into two geographically defined lineages is estimated to have occurred 0.73–1.60 million years ago (Ma) taking the lowest ITS sequence divergence of 1.45% observed between accessions of the western and eastern groups and relying on estimates of approximately 1% ITS sequence divergence per 0.5–1.1 million years for Brassicaeae taxa (Koch *et al.* 1999; Mummenhoff *et al.* 2004). This agrees with ITS sequence substitution rates ranging from about 0.5 to 1% sequence divergence per million years for other taxa (Sang *et al.* 1995a; Wendel *et al.* 1995; von Hagen & Kadereit 2001; Zhang *et al.* 2001). The *trn*L–*trn*F spacer sequence divergence (1.7–2.0%) within the ingroup corresponded

Table 2 Positions of a complete ITS alignment that distinguishes accessions of *Clausia aprica* from the western and eastern groups (Fig. 1). Intermediate group accessions (Fig. 1) are characterized by additive nucleotide positions

	Nucleotide position in the ITS alignment													
Origin	ITS1							ITS2						
	0	0	1	1	2	2	2	2	2	3	3	3	3	3
	4	7	3	5	0	0	4	6	9	3	6	7	8	8
	9	4	8	7	2	6	2	4	4	7	5	7	8	9
Western group														
accessions nos 1, 28, 45, 47, 49, 50, 51, 63, 65	C	C	C	A	T	G	A	T	A	G	G	G	A	C
Intermediate group														
accession no. 4	**Y**	C	C	A	T	G	A	C	A	**R**	**R**	**R**	T	C
accession no. 78	T	T	T	C	C	A	C	C	A	G	G	**R**	**W**	**M**
accession no. 74	T	T	T	C	C	A	C	C	A	G	G	**R**	T	**M**
accession no. 36	T	T	T	**M**	C	A	C	**Y**	A	G	G	**R**	T	C
accession nos 27, 38	T	T	T	C	C	A	C	C	T	A	A	A	T	A
Eastern group														
accessions nos 3, 11, 12, 13, 16, 22, 24, 29, 35, 42, 43, 71	T	T	T	C	C	A	C	C	T	A	A	A	T	A

M = A & C; R = A & G; W = A & T; Y = C & T.

in magnitude with the ITS sequence divergence (1.45–2.19%). However, no reliable time estimates for rate changes for the *trn*L–*trn*F spacer are available. There was *trn*L–*trn*F sequence divergence within the western group (0–0.7%) but not in the eastern group, which might be a chance effect.

Given the above estimated divergence time, we conclude that Quaternary climatic oscillations led to intraspecific diversification through isolation by distance in *Clausia aprica*. At the end of the Pliocene (2.5–1.6 Ma) the climate cooled down significantly, and a continuous steppe belt formed from Central Asia to southeast Europe. The cool climate prevailed for much of the Pleistocene (1.6–0.4 Ma). Several climatic macrocycles with glacial and interglacial, stadial and interstadial phases have been proven in eastern Europe and Siberia associated with latitudinal range shifts of the steppe belt (Devyatkin 1993; Arkhipov & Volkova 1994; Akhmetyef *et al*. 1999; Arkhipov *et al*. 1999; Velichko *et al*. 1999; Tarasov *et al*. 2000). The steppe belt also faced significant longitudinal splits during the ice ages, approximately along 60–70° eastern longitude, which were probably involved in separating western and eastern *C. aprica* populations.

In eastern Europe, as the Pliocene became the Pleistocene, the Caspian Sea extended northwards several times, at its maximum up as far as the southern Ural Mountains and to the Volga–Kama basin (late Akčagyl transgressions *c*. 2.0–1.8 Ma, Apsheron transgressions *c*. 1.8–0.7 Ma). At the height of the Apsheron transgressions the Caspian Sea level was +80 m above sea level, compared to −28 m today. However, the area north of the Black Sea along the middle and lower Don (Ponto–Sea of Asov depression) was not affected by these transgressions, and consequently steppe vegetation was capable of surviving there.

In western Siberia, during the early Pleistocene (0.9–0.4 Ma, Mansi–Schaitan glacial), glaciers advanced south to 64–62° N. The river system of the Ob–Irtysh–Tobol was dammed by the glaciers, and backwaters reached the foothills of the Kazakhstan Highlands and of the Altai Mountains. The landscape was turned into a system of lakes and swampy areas interspersed with taiga vegetation that was not suitable for steppe plants (Arkhipov & Volkova 1994; Arkhipov *et al*. 1999). This may also account for the present day generally low floristic diversity in this area (Malyshev *et al*. 1999). At the end of the middle Pleistocene during the Samara–Tasa glacial (260 000–130 000 years ago), the Ob–Irtysh–Tobol river system was dammed again but the lake/swamp system described above was less extended and did not affect the Omsk and Kazakhstan steppe region (Arkhipov & Volkova 1994).

Incongruencies between phylogenetic tree topologies, based on maternally inherited cpDNA and biparentally inherited nuclear sequences, can provide evidence for reticulate evolution (Sang *et al*. 1995b; Lee *et al*. 2002; Mummenhoff *et al*. 2004). Thus, the conflicting topologies of the cpDNA and the ITS trees for the intermediate group of *C. aprica* (Fig. 1) may be explained by hybridization between representatives of the western and eastern groups as an outcome of a secondary contact which took place after deglaciation, during interglacials and/or in the Holocene. The hybridogenous constitution of the intermediate group is directly observed in the ITS sequences (Table 2). Accessions of the intermediate group were characterized by additive ITS nucleotide characters, indicating a hybrid origin of the intermediate group. Additive ITS nucleotide positions were documented in hybrids before, including Brassicaceae taxa of recent origin (O'Kane *et al*. 1996; Franzke & Mummenhoff 1999; Mummenhoff *et al*. 2004). The absence of monophyly in the intermediate group in the ITS tree (Figs 1 and 2) might indicate several cycles of range contraction, isolation and range expansions leading to repeated hybridization events. Four of the six intermediate group accessions (nos 4, 36, 74, 78; Table 2) showed additive ITS nucleotide positions. We hypothesize that concerted evolution has not yet completely homogenized ITS sequences, indicating more recent hybridizations. The other two accessions from the intermediate group (nos 27, 38; Table 2) did not show additivity at ITS nucleotide sites.

We argue that these accessions indicate older hybridizations and concerted evolution has led to complete homogenization of ITS. This hypothesis is supported by the repeated damming of the Ob–Irtysh–Tobol river system mentioned above (Arkhipov & Volkova 1994).

Bottle neck effects, in combination with demographic history, may account for the low ITS sequence divergence within the western group (0–0.05%). Fossil evidence provides proof of a steppe flora in the late Pliocene (1.8–1.6 Ma) north of the Sea of Asov near Rostov on the Don (Dorofeev 1966), whereas the contemporaneous palaeoflora from the northern end of the Caspian transgression showed no signs of steppe vegetation but proved to be boreal and nemoral vegetation (Velichkievich 1982). Rapid range expansion from the refugial area along the Don after the retreat of the Caspian transgression may explain reduced genetic diversity as has been reported in many species from Europe and North America (Hewitt 1999, 2004). The accumulation of genetic diversity among the eastern group (ITS sequence divergence ranged from 0 to 1.9%) suggests the survival of regional *C. aprica* genomes through several glacial cycles in different regions of Asia. This corresponds to the current view that the modern steppe is of Pre-Pleistocene origin (Kamelin 1998; Malyshev 2000; Peshkova 2001), and Yurtsev (1972) provided evidence based on floristic findings, that the steppe islands in Yakutsia and Chukotka, where *C. aprica* partly occurs, existed here throughout the Pleistocene.

Our study on *C. aprica* proves for the first time that genetic subdivisions within a European–Asian steppe plant exist east of the Ural Mountains, approximately along 70°E. We suggest that such a genetic boundary will also be found in other plants of zonal European–Asian distribution. The separation of the DNA clades of *C. aprica* seems to roughly coincide with genetic subdivisions within the dunlin (Wennerberg 2001), the collared lemming (Fedorov *et al*. 1999), and the root vole (Brunhoff *et al*. 2003). The coincidence of genome boundaries in such diverse taxa as mammals, birds and plants may reflect common biogeographical history over the ice ages. Biogeographical, fossil and molecular data suggest several glacial refugia in northern and Central Asia.

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References

Abbott RJ, Brochmann C (2003) History and evolution of the arctic flora: in the footsteps of Eric Hultén. *Molecular Ecology*, **12**, 299–313.

Akhmetyef MA, Dodonov AE, Sotnikova MV, Spasskaya II, Kremenetzky KV, Klimanov VA (1999) Kazakhstan and Central Asia — plains and foothills. In: *Climate and Environment Changes During the Last 65 Million Years; Cenozoic: from Paleocene to Holocene* (ed. Velichko AA), pp. 165–191. GEOS, Moscow. [In Russian].

Arkhipov SA, Volkova VS (1994) *Geological History of Pleistocene Landscapes and Climate in West Siberia*. Russian Academy of Sciences, Siberian Branch: United Institute of Geology, Geophysics and Mineralogy, Novosibirsk. [In Russian].

Arkhipov SA, Volkova VS, Zolnikov ID, Zyklina VS, Krukover AA, Kulkova IA (1999) West Siberia. In: *Climate and Environment Changes During the Last 65 Million Years; Cenozoic: from Paleocene to Holocene* (ed. Velichko AA), pp. 84–109. GEOS, Moscow. [In Russian].

Avise JC (2000) *Phylogeography: the History and Formation of Species*. Harvard University Press, Cambridge.

Bowman JL, Brüggemann H, Lee J-Y, Mummenhoff K (1999) Evolutionary changes in floral structure within *Lepidium* L. (Brassicaceae). *International Journal of Plant Sciences*, **160**, 917–929.

Brunhoff C, Galbreath KE, Fedorov VB, Cook JA, Jaarola M (2003) Holarctic phylogeography of the root vole (*Microtus oeconomus*): implications for late Quaternary biogeography of high latitudes. *Molecular Ecology*, **12**, 957–968.

Charkevicz SS (1988) *Plantae Vasculares Orientis Extremi Sovietici*, vol. 3. Nauka, Leningrad. [In Russian].

Comes H-P, Kadereit JW (1998) The effect of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Sciences*, **3**, 432–438.

Devyatkin EV (1993) Meridional distribution of Pleistocene ecosystems in Asia: basic problems. *Stratigraphy and Geological Correlation*, **1**, 77–83.

Dorofeev PI (1966) *Pliocene Flora of Matanova Sada on the Don*. Nauka, Moscow. [In Russian].

Fedorov VB, Fredga K, Jarrell GH (1999) Mitochondrial DNA variation and the evolutionary history of chromosome races of collared lemmings (*Dicrostomyx*) in the Eurasian Arctic. *Journal of Evolutionary Biology*, **12**, 134–145.

Franzke A, Mummenhoff K (1999) Recent hybrid speciation in *Cardamine* (Brassicaceae) — conversion of nuclear ribosomal ITS sequences in statu nascendi. *Theoretical and Applied Genetics*, **98**, 831–834.

Frenzel B, Pécsi M, Velichko AA (1992) *Atlas of Paleoclimates and Paleoenvironments of the Northern Hemisphere, Late Pleistocene — Holocene*. Fischer, Budapest.

Gajewski MW (1934) *Avenetum desertorum* a monographical study. *Bulletin International de l'Académie Polonaise des Sciences et des Lettres. Classe des Sciences Mathématiques et Naturelles, Série B: Sciences Naturelles*, **1**, 1–27.

Hewitt GM (1999) Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, **68**, 87–112.

Hewitt GM (2000) The genetic legacy of Quaternary ice ages. *Nature*, **405**, 907–913.

Hewitt GM (2001) Speciation, hybrid zones and phylogeography — or seeing genes in space and time. *Molecular Ecology*, **10**, 537–549.

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society London, B*, **359**, 183–195.

Huelsenbeck JP, Rannala B (1997) Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science*, **276**, 227–232.

Hultén E, Fries M (1986) *Atlas of North European Vascular Plants: North of the Tropic of Cancer*. Koeltz, Königstein.

Kamelin RV (1998) *Materials on the History of the Flora of Asia: the Altai Montane Country*. Altai University Press, Barnaul. [In Russian].

Koch M, Bishop J, Mitchell-Olds T (1999) Molecular systematics and evolution of *Arabidopsis* and *Arabis*. *Plant Biology*, **1**, 529–537.

Lang G (1994) *Quartäre Vegetationsgeschichte Europas*. Fischer, Jena.

Lee J-Y, Mummenhoff K, Bowman JL (2002) Alloploidization and evolution of species with reduced floral structures in *Lepidium* L. (Brassicaceae). *Proceedings of the National Academy of Sciences of the USA*, **99**, 16835–16840.

Litvinov DJ (1890) Geobotanical notes on the flora of European Russia. *Bulletin Societe Naturelles de Moscou*, **3**, 322–434. [In Russian].

Litvinov DJ (1902) About the relic character of stony slopes in European Russia. *Traveaux Musée Botanique Académie St. Petersburg*, **1**, 76–109. [In Russian].

Mai DH (1995) *Tertiäre Vegetationsgeschichte Europas*. Fischer, Stuttgart.

Malyschev LI, Peschkova GA, eds. (1994) *Flora Sibiriae* vol. 7. Nauka, Novosibirsk. [In Russian].

Malyshev LI (2000) Floristic division on the quantitative basis: Baikalian Siberia, Tuva and Outer Mongolia. *Flora*, **195**, 330–338.

Malyshev LI, Baikov KS, Doronkin VM (1999) Spatial diversity of the Siberian flora. *Flora*, **194**, 357–368.

Meusel H, Jäger E (1992) *Vergleichende Chorologie der zentraleuropäischen Flora*, vol. 3. Fischer, Jena.

Meusel H, Jäger E, Weinert E (1965) *Vergleichende Chorologie der Zentraleuropäischen Flora*. vol. 1. Fischer, Jena.

Meusel H, Jäger E, Rauschert S, Weinert E (1978) *Vergleichende Chorologie der Zentraleuropäischen Flora*. vol. 2. Fischer, Jena.

Mummenhoff K, Brüggemann H, Bowman JL (2001) Chloroplast DNA phylogeny and biogeography of *Lepidium* (Brassicaceae). *American Journal of Botany*, **88**, 2051–2063.

Mummenhoff K, Linder P, Friesen N, Bowman JL, Lee J-Y, Franzke A (2004) Molecular evidence for bicontinental hybridogenous genomic constitution in *Lepidium* sensu stricto (Brassicaceae) species from Australia and New Zealand. *American Journal of Botany*, **91**, 254–261.

O'Kane S, Schaal BA, Al-Shehbaz IA (1996) The origins of *Arabidopsis suecica* (Brassicaceae) as indicated by nuclear rDNA sequences. *Systematic Botany*, **21**, 559–566.

Peshkova GA (2001) *Florogenetic Analysis of the Steppe Flora of the South Siberian Mountains*. Nauka, Novosibirsk. [In Russian].

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Sang T, Crawford DJ, Stuessy TF, Silva OM (1995a) ITS sequences and the phylogeny of the genus *Robinsonia* (Asteraceae). *Systematic Botany*, **20**, 55–64.

Sang T, Crawford DJ, Stuessy TF (1995b) Documentation of reticulate evolution in paeonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. *Proceedings of the National Academy of Sciences of the USA*, **92**, 6813–6817.

Swofford DL (2001) *PAUP* Phylogenetic Analysis Using Parsimony (* and Other Methods)*, Version 4. 0b 10. Sinauer Associates, Sunderland.

Taberlet P, Fumagalli L, West-Saucy A-G, Cosson J-F (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.

Tarasov PE, Volkova VS, Webb T *et al.* (2000) Last glacial maximum biomes reconstructed from pollen and plant macrofossil data from northern Eurasia. *Journal of Biogeography*, **27**, 609–620.

Velichkievich FJ (1982) Main stages in the development of the Pliocene flora of the European part of the SSR. In: *Paleokarpological Studies in the Cenozoic Era*, pp. 104–118. Nauka and Technika, Minsk. [In Russian].

Velichko AA, ed. (1999) *Climate and Environment Changes during the last 65 Million Years; Cenozoic: from Paleocene to Holocene*. GEOS, Moscow. [In Russian].

Velichko AA, Akhlestina EF, Borisova OK *et al.* (1999) East European Plains. In: *Climate and Environment Changes During the Last 65 Million Years; Cenozoic: from Paleocene to Holocene* (ed. Velichko AA), pp. 43–83. GEOS, Moscow. [In Russian].

von Hagen KB, Kadereit JW (2001) The phylogeny of *Gentianella* (Gentianaceae) and its colonization of the southern hemisphere as revealed by nuclear and chloroplast DNA sequence variation. *Organisms Diversity and Evolution*, **1**, 61–79.

Webb T, Bartlein PJ (1992) Global changes during the last 3 million years: climatic controls and biotic responses. *Annual Review of Ecology and Systematics*, **23**, 141–173.

Wendel JF, Schnabel A, Seelanan T (1995) An unusual ribosomal DNA sequence from *Gossypium gossypioides* reveals ancient cryptic intergenomic introgression. *Molecular Phylogenetics and Evolution*, **4**, 298–313.

Wennerberg L (2001) Breeding origin and migration pattern of dunlin (*Calidris alpina*) revealed by mitochondrial DNA analysis. *Molecular Ecology*, **10**, 1111–1120.

Williams D, Dunkerley D, DeDecker P, Kershaw P, Chappell M (1998) *Quaternary Environments*. Arnold, London.

Yurtsev BA (1972) Phytogeography of north eastern Asia and the problem of Transberingian floristic interrelations. In: *Floristics and Paleofloristics of Asia and Eastern North America* (ed. Graham A), pp. 19–54. Elsevier, Amsterdam.

Zhang L-B, Comes H-P, Kadereit JW (2001) Phylogeny and quaternary history of European montane/alpine endemic *Soldanella* (Primulaceae) based on ITS and AFLP variation. *American Journal of Botany*, **8**, 2331–2345.

Zhou TY, Lu LL, Yang G, Al-Shehbaz IA (2001) Brassicaceae. In: *Flora of China* (eds Wu CY, Raven PH), vol. 8, pp. 1–193. Science Press, Beijing, Botanical Garden Press, St Louis MO.