

# Evidence for two genetic groups within the arctoalpine sedges *Carex bicolor* All. and *C. atrofusca* Schkuhr (Cyperaceae)

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**Abstract.** *C. bicolor* and *C. atrofusca* are sedge species characteristic for arctoalpine habitats. They have almost circumpolar distributions; they are found in Europe, Northern Asia, and North America, as well as in Middle Asia (*C. atrofusca*). We hypothesized that certain genetic and morphological differences between populations may be expected across this large area. We tested a set of geographically remote populations of *C. bicolor* and *C. atrofusca*. We sequenced a fragment of the plastid *matk* gene for 15 specimens of *C. bicolor* and 10 specimens for *C. atrofusca* from the Asian Russia, and also extracted sequences of this species from GenBank. We found that for both *C. bicolor* and *C. atrofusca*, plants from the Arctic and boreal zones of North America and Eurasia had identical *matk* sequences, while there was certain nucleotide diversity in the mountain ranges of the southern Siberia. Therefore, based on the obtained data we may hypothesize that the mountains of the East Siberia are the center of diversity for some arctoalpine sedge species, and might have served as the ancestral area of the populations colonizing the Arctic.

## 1 Introduction

The genus *Carex* L. (Cyperaceae Juss.) is one of the biggest genera of flowering plants. It includes over 2000 species. In Russia and adjacent states it is represented by 384 species and subspecies, 109 of them in the Arctic province [1, 2]. V.I. Kreczetovich [3] found that many of the arctoalpine sedges from the Polar and Northern Urals have disjunctive distributions, which may give valuable insights into the pathways and timing of the origin of northern flora. Kreczetovich believed that these species may be very old and that their ancient distributions may have been much larger than current ones. The glaciations, transgressions, and climate oscillations in the Pleistocene may have caused their dispersal to the south, into several mountain regions, which led to the colonization of their alpine belts [3]. The resulting vicariance may have led to the accumulation of genetic and morphological differences, as the result of both neutral evolution and natural selection.

The main goal of our study was to determine genetic differences among geographically distant populations of arctoalpine species. The study deals with two sedge species: *C.*

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*bicolor* All. and *C. atrofusca* Schkuhr with an almost circumpolar disjunctive distribution in Europe, Northern Asia, and Northern America, as well as in Middle Asia (*C. atrofusca*).

*C. bicolor* species is very rare. It is found on pebble and sand banks, damp meadows along the banks of rivers and lakes, sometimes near springs [4]. *C. atrofusca* is widespread on banks of rivers and streams, wet pebble slopes, wet moss and sedge tundras, larch woodlands, and on carbonate substrates. The distributions of *C. bicolor* and *C. atrofusca* have several disjunctions, including ones between the White Sea, the Urals, and Yenisei, as well as between the western and eastern coasts of Greenland.

## 2 Materials and methods

We investigated 15 specimens of *C. bicolor* and 10 specimens of *C. atrofusca*. Details on the specimens are given in Table 1. The universal primers for the amplification of the central portion of the plastid *matK* gene (matK-1, 5'-TTCAA-ATCCT-TCAAT-GCTGG-3'; matK-3, 5'-TGAGA-GGAAG-GACTG-GAACT-AA-3') were designed by us based on the sequences of the *matK* gene from GenBank.

PCR reactions were performed in BioRad PCR machines (USA); reaction mixtures contained 1.5 mM MgCl<sub>2</sub>, 65 mM Tris-HCl (pH 8.8), 16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.05% Tween-20, 0.2 mM of each dNTP, 0.3 mM of each primer, and 1 u. of Taq polymerase. Amplified products were sequenced with the following amplification profile: 5 min of denaturation at 95 °C; 37 cycles of 15 s at 95 °C, 15 s at 54 °C, 1 min at 72 °C; and the final elongation step of 5 min at 72 °C. The resulting amplification products were sequenced using the BigDye 3.1 Terminator kit (Applied Biosystems). The assembly and alignment of the resulting sequences was done manually.

**Table 1.** Sampling points *C. bicolor* and *C. atrofusca*. Reg., region; v., village

Specimen no.	Sampling locality	Collection date	Collectors
<i>Carex bicolor</i>			
C147	Sakha Rep. (Yakutia), Verkhnekolymy district, Momsy range, left bank of the river. Rassokhi	14.07.1983	N. Bolshakov, N. Vlasova
C148	Sakha Rep. (Yakutia), Tomponsky district, near v. Teplyu Kluch, left bank of the r. East Khandyga, no. 1190	17.07.1984	O. Nikiforova
C149	Sakha Rep. (Yakutia), Oleneksky district, r. Arga-Salaa	18.07.1979	N. Vodopyanova, E. Ammosov, R. Krogulevich
C150	Krasnoyarsk krai, near v. Taimba, pebble floodplain of the r. Taimba, 486	16.07.1980	N. Bolshakov
C151	Tuva Rep., Erzinsky district, Sangilen upland, below the mouth of the river. Solobelder	17.07.1973	I. Krasnoborov, L. Danilyuk
C152	Sakha Rep. (Yakutia), Taimyr Auth. district, v. Fomich	23.08.1979	N. Vodopyanova, R. Krogulevich, V. Nikolaeva
C153	Irkutsk oblast, Ziminsky district, on the banks of the river. Oka, no. 46	18.07.1971	A. Kiseleva, I. Sergienko
C154	Buryatia Rep., Eastern Sayan, mouth of the r. White Irkut, no. 1834	19.07.1986	L. Malyshev
C155	Buryatia Rep., Tunkinsky district, v. Mondy, valley of r. Irkut, no. 44	09.06.1966	G. Peshkova
C157	Buryatia Rep., Eastern Sayan, central part of the upper reaches of r. Sigach, no. 599	06.08.1962	L. Malyshev

C158	Buryatia Rep., Eastern Sayan, Tunka Alps, r. Shumak, Arshan	24.07.1957	L. Malyshev, N. Misyura
C172	Irkutsk oblast, Ziminsky district, village Saram, on the river bank, no. 242	26.06.1978	A. Kiseleva, I. Sergienko
C175	Buryatia Rep., Eastern Sayan, Udinsky ridge, r. Kara-Buren, Cross valley	07.07.1961	L. Malyshev, Z. Bespalova
C176	Buryatia Rep., Eastern Sayan, right bank of r. Urik (basin of the r. Angara), near v. Shanhar	02.07.1959	Krivogumenko
C177	Sakha Rep. (Yakutia), Mirinsky district, r. Mogdy	04.08.1978	N. Vodopyanova
<i>Carex atrofusca</i>			
C188	Buryatia Rep., Eastern Sayan, Kitoi Alps, middle reaches of r. Sagan-Saira, no. 88.	27.11. 1958	L. Malyshev
C190	Krasnoyarsk krai, Putorana plateau, lake Bokovoye, no. 2379	04.08.1972.	N. Vodopyanova, V. Parygin
C191	Sakha Rep. (Yakutia), Bulun district, near v. Checkurovka, no. 1537	12.08.1987	V. Zuev, Agaltsev
C192	Sakha Rep. (Yakutia), Taimyr Auth. district, v. Fomich	15.08.1979	N. Vodopyanova, R. Krogulevich, N. Friesen, V. Nikolaeva
C193	Tuva Rep., Erzinsky district, Sangilen uplands, upper reaches of r. Naryn, near the stream Brook Biche-Tel-Hen, altitude 2100 m	09.07.1973	I. Krasnoborov, M. Sakovich
C194	Altai, Kosh-Agach district, 40 km from v. Kosh-Agach, r. Tarhata	11.07.1982	N. Friesen, M. Doronkin
C195	Altai, Yuzhno-Chuisky ridge, upper reaches of r. Elangash, altitude 2450 m, no. 1196	14.08.1985	L. Malyshev
C196	Buryatia Rep., Eastern Sayan, Tunka Alps, Arshan, p. Kyngara, no. 527	23.11.1966	G. Peshkova, G. Petrochenko
C247	Altai, Chikhacheva ridge, r. Saylyugem, no. 1286	19.08.1985	L. Malyshev
C288	Krasnoyarsk krai, Putorana plateau, lake Hal Kyuel	04.08.1972	L. Malyshev

The construction of haplotype networks was performed using Network v. 10.2.0.0 [5]. In addition to the sequences obtained in this study we also used the following GenBank accessions of *C. bicolor*: LK021889 (Austria, Carinthia, Alps, Röser, 5875), KC474300 and KC474301 (Canada: Northwest Territories), KT021433 (Canada, Yukon), KC474302 (Canada, Yukon, Arctic Mainland), FJ548076 (Canada: Nunavut, Southampton Island). For *C. atrofusca*, we used the following sequences: FJ548071–FJ548075 Canada: Nunavut), JN966171–JN966172 (Canada: Manitoba), MF543490–MF543491 (Italy: Aosta Valley), MK925613 and MK925786 (United Kingdom).

### 3 Results and discussions

#### 3.1 Phylogenetic Analyses

In *C. bicolor*, the resulting alignment contained three polymorphic positions and no indels. A total of three haplotypes were found. All sequences from Canada (Yukon, Northwest

Territories, Nunawut) and Austria were identical to each other and to the majority of the specimens from Yakutia, as well as those from Taymyr and Stanovoy Uplands (northern Buryatia) (Fig. 1, A). A specimen from Krasnoyarsk krai (near village Taimba) differed from that group by one substitution. A group of southern specimens from the Eastern Sayan and the Sangilen uplands formed a separate group, differing by two nucleotide substitutions from the Yakutia group (table 2).

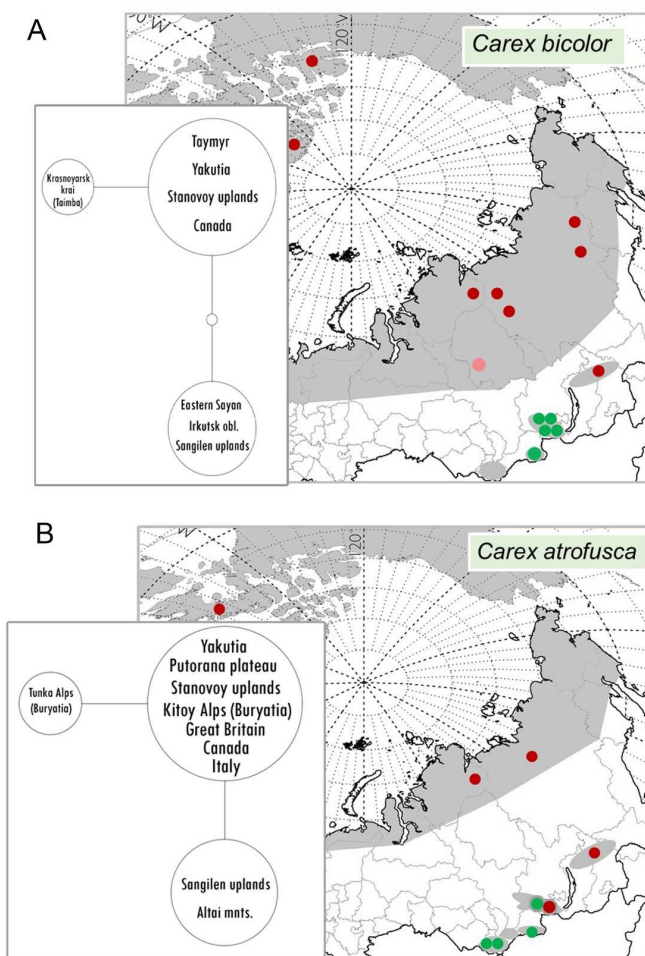
In *C. atrofusca*, the alignment also contained three polymorphic positions and three haplotypes. Again all North American and European specimens were identical to each other and to specimens from Yakutia (Fig. 1, B). However, the differences among the East Siberian populations were not as clear. The specimens from the Altai mountains and the Sangilen uplands (Tuva Republic) differed by one nucleotide substitution from the “Arctic” group. A specimen from the Tunka Alps (Buryatia Republic) differed by another substitution from the “Arctic” group. However, the *matk* gene of a plant from the adjacent Kitoi Alps was identical to those of the “Arctic” sequences.

In *C. bicolor* only minor variation was found in the main morphological character of inflorescences, utricles, and bracts (Fig. 2). Specimens from the East Sayan had smaller utricles, 2 mm long vs. 2.5–2.7 in the specimens from Yakutia, Taymyr, and Krasnoyarsk krai. The specimen from the Krasnoyarsk krai that formed a separate group on the haplotype network (Fig. 2), utricles were oblong ovoid vs. reverse ovoid in the rest of the specimens.

Morphological diversity within the *C. atrofusca* sample was also minor (Fig. 2). The specimen from Taymyr (C192) differed from the rest of the sample by having light-colored bracts with a transparent tip. In the plants from Tuva (C193) and Altai (C194, C195, C247), both utricles and bracts were dark brown.

**Table 2.** Nucleotide substitutions in the *matk* gene of *C. bicolor* and *C. atrofusca*

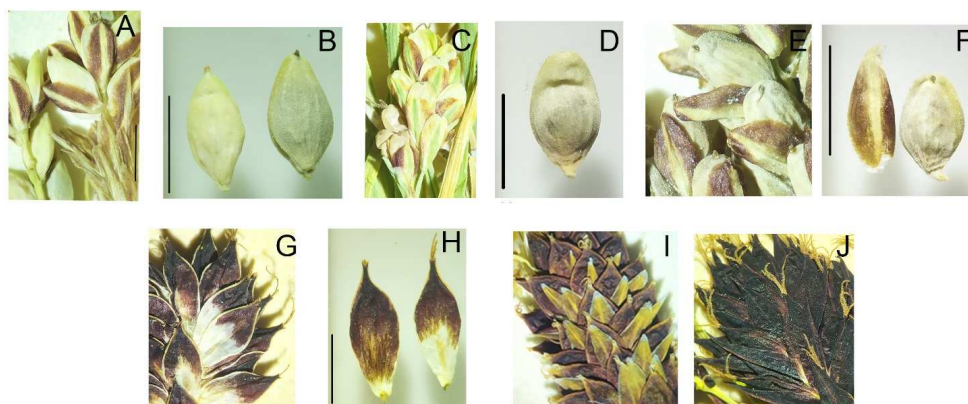
Locations	Position in the <i>matk</i> gene		
<i>C. bicolor</i>			
	137	165	375
Yakutia, Taymyr, Austria, Canada (C147, C148, C149, C152, C177, LK021889, KC474300, KC474301, KT021433, KC474302, FJ548076)	T	C	G
Krasnoyarsk krai (Taimba), C150	C	C	G
East Sayan, Sangilen Uplands, Irkutsk oblast (C151, C153, C154, C155, C157, C158, C172, C175, C176)	T	A	A
<i>C. atrofusca</i>			
	50	353	
Yakutia, Putorana plateau, Stanovoy uplands, Kitoi Alps, Great Britain, Canada, Italy (C188, C190, C191, C192, C288, FJ548071–FJ548075, JN966171–JN966172, MF543490, MF543491, MK925613, MK925786)	G	G	
Tunka Alps, Buryatia (C196)	A	G	
Sangilen Uplands, Altai mnts. (C193, C194, C155, C247)	G	T	



**Fig. 1.** Locations of the studied specimens and haplotype networks. Shaded area denotes species distributions; dots of different colors refer to different haplotypes. A – *C. bicolor*, B – *C. atrofusca*.

Therefore, we found that based on the substitution patterns in the *matk* gene two geographically distinct groups were detected in *C. bicolor*. One of them is the «Arctic» group; it includes plants from both Arctic Eurasia and North America, as well as a specimen from the Alps. The other group can be referred to as the «Alpine» group; it includes the populations from the south of East Siberia. Thus, we could hypothesize that there was an ancient disjunction event that split *C. bicolor* into two population group, one restricted to East Siberia and the other having a large circumpolar distribution.

For *C. atrofusca*, we found that the *matk* sequences of specimens from the Arctic and boreal zones, as well as European mountains were identical. However, there was certain nucleotide diversity in the mountain ranges of the southern Siberia. Therefore, based on the obtained data we may hypothesize that the mountains of the East Siberia is the center of diversity for some arctoalpine sedge species. Additional studies using advanced methods of molecular analysis are required to understand the history and evolution of current distributions of *C. bicolor* and *C. atrofusca*.



**Fig. 2.** Morphological characters of *C. bicolor* and *C. atrofusca*. Fragments of pistillate spikes and utricles: *C. bicolor* – A–F; *C. atrofusca* – G–J. Scale, 2mm. A, B – C150 specimen (Krasnoyarsk krai, near v. Taimba); C, D – C177 (Sakha Rep. (Yakutia), Mirinsky district, r. Mogdy); E, F – C154 (Buryatia Rep., Eastern Sayan); G, H – C196 (Buryatia Rep., Eastern Sayan, Tunka Alps, Arshan); I – C192 (Sakha Rep. (Yakutia), Taimyr Auth. district, v. Fomich); J – C193 (Tuva Rep., Erzinsky district, Sangilen uplands).

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