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.
**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'-TTCAAA-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$_2$, 65 mM Tris-HCl (pH 8.8), 16 mM (NH$_4$)$_2$SO$_4$, 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 ºC; 37 cycles of 15 s at 95 ºC, 15 s at 54 ºC, 1 min at 72 ºC; and the final elongation step of 5 min at 72 ºC. 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 |
|-------------|-------------------|----------------|------------|
| C147 | Sakha Rep. (Yakutia), Verkhneolymsky district, Momsky 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. Krasnоборов, 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 |
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, Nunavut) 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 Kitoy 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 *matk* gene |
|-----------|-----------------------------|
| **C. bicolor** | |
| Yakutia, Taymyr, Austria, Canada (C147, C148, C149, C152, C177, LK021889, KC474300, KC474301, KT021433, KC474302, FJ548076) | 137 T | 165 C | 375 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 |
| **C. atrofusca** | |
| Yakutia, Putorana plateau, Stanovoy uplands, Kitoy Alps, Great Britain, Canada, Italy (C188, C190, C191, C192, C288, FJ548071–FJ548075, JN966171–JN966172, MF543490, MF543491, MK925613, MK925786) | 50 G | 353 G |
| Tunka Alps, Buryatia (C196) | A | G |
| Sangilen Uplands, Altai mnts. (C193, C194, C155, C247) | G | T |
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. atrofusc*a. Fragments of pistillate spikes and utricles: *C. bicolor* – A–F; *C. atrofusc*a – 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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