Microevolutionary relationships between biotypes of *Elymus confusus*, *E. peschkovae*, and *E. sibiricus* (Poaceae) according to hybridization and sequencing of the nuclear gene **GBSS1** (*waxy*)

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Abstract. According to descriptions, the Siberian species *E. peschkovae* and *E. confusus* differ in the presence or absence of trichomes on lemma and rachilla surfaces only. Two methods were used for study microevolutionary relationships between these species: hybridization of biotypes from different locations and analysis of nucleotide sequences of the low-copy nuclear gene **GBSS1**. Created and grown hybrids in combinations AMU-8804 × BER-0807 and AMU-8804 × BUK-1109 showed complete seed sterility in two field vegetations. Taking into account wide species areas, 3 hybrids were created between Magadan biotypes from a common habitat in the combination *E. confusus* MOL-1887 × *E. peschkovae* MOL-1882. The plants showed seed fertility (SF) of 0–3 %. This result does not obscure the possibility to assess SF in the F2 generation and the character of inheritance of diagnostic traits. A comparative study of the **GBSS1** gene sequences in accessions was carried out in comparison with clones of reference species. Differentiation of clones by the St2 subgenome in *E. confusus*, *E. peschkovae*, *E. sibiricus* and *E. caninus* did not reveal a clear relationships between the species. Meanwhile, a certain species specificity for the H1 subgenome was noted, confirming the existence of microevolutionary isolation of these species.

1 Introduction

*Elymus sibiricus* L. and *E. confusus* (Roshev.) Tzvel. are spread everywhere in southern regions of Siberia [1]. In the Russian Far East *E. confusus* area is shifted to the North, where it totally replaces *E. sibiricus* [2]. Both species are characterized by glabrous lemmas, but *E. sibiricus* rarely have shortly pilous lemmas. It was shown in the previous experiment that interspecific introgression is possible under the joint growth of these two species in Siberia, as a result of sexual hybridization [3].

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Created and analyzed hybrids of East-Sajan biotypes in combination *E. sibiricus* BUR-0569 × *E. confusus* BUM-0505 in generation *F*₁ showed mostly opened anthers, values of seed fertility (SF) accounted for 2–7 seeds per spike.

Among 20 plants of the *F₃* generation, grown on the opened plot, 17 corresponded to *E. sibiricus* on the trait of doubled spikelets on spike ledge, 3 plants carried single spikelets on all spikes. These results gave reason to refer *E. sibiricus* and *E. confusus* to a unified recombination gene pool with reproductive compatibility at the *α₂* level at least within the Southern Siberia area.

*Elymus peschkovae* Tzvelev (— *Agropyron confusum* var. *pubiflorum* Roshev. — *Elymus confusus* var. *pubiflorus* (Roschev.) Tzvelev — *E. pubiflorus* (Roshev.) Peschkova) differs from *E. confusus* by pilose lemmas (Fig. 1). In Siberia the area of *E. peschkovae* is distinctly shifted to the North-East part in comparison with *E. confusus*.

![Image of seeds](image-url)

**Fig. 1.** Seeds (lemmas) of accessions *E. confusus* MOL-1887 (1) and *E. peschkovae* MOL-1882 (2)

The purpose of this research was to study microevolutionary relationships between *E. peschkovae* and *E. confusus* biotypes. Two experimental methods were used: hybridization of biotypes from different points of species area, and analysis of nucleotide sequences of low-copy nuclear gene *waxy* (granule-bound starch synthase 1, *GBSS1*).

## 2 Materials and Methods

Parental biotypes for hybridization were grown from seeds of wild-growing accessions collected at different points of areas, or from seeds of their offspring grown under conditions of self-pollination. Plants were hybridized according to the previously developed technique [4]. Techniques of probe preparation and sequences study of the *GBSS1* gene in three species were performed as described earlier [5]. The origin of accessions and clone numbers submitted to the GenBank NCBI (http://www.ncbi.nlm.nih.gov/nuccore) are shown in Table 1. In addition to accessions of three species and monogenic markers, clones of *E. caninus* (L.) L. from GenBank NCBI, as a morphologically related species, were included in the study of *GBSS1* sequences (Table 2). The construction of dendrograms by the Neighbor-Joining method was carried out in the TREECON ver. 1.3b soft [6].
Table 1. Accessions and clones of *GBSS1* gene sequences in *E. confusus*, *E. peschkovae*, *E. sibiricus* which have been submitted to the GenBank NCBI. Accessions taken for hybridization are marked by bold.

| Accession | Clones (sequences) in NCBI | Location of collecting |
|-----------|-----------------------------|------------------------|
| TAR-0730  | conTAR30_1-St MN164827       | The Tyva Republic, Tes-Khemsky District, alt. 1545 м N 50° 36.870' E 95° 10.729' |
|           | conTAR30_4-H MN164828       |                        |
| BER-0807  | conBER07_2-St MN164825      | The Republic of Buryatia, Eravensky District, alt. 958 м N 53° 00.776' E 112° 03.673' |
|           | conBER07_1-H MN164826       |                        |
| BUR-0557  | conBUR57_7-St MN605026      | The Republic of Buryatia, Okinsky District, alt. 1585 м N 51° 57.383' E 100° 33.564' |
|           | conBUR576a-H MN605025       |                        |
| BUM-0505  | conBUM05_1-St MN605028      | The Republic of Buryatia, Eravensky District, alt. 1738 м N 51° 42.610' E 100° 59.967' |
|           | conBUM05_7-H MN605027       |                        |
| BUK-1109  | conBUK09_8-St MN605030      | The Republic of Buryatia, Tunkinsky District, alt. 750 м N 50° 32.762' E 107° 47.435' |
|           | conBUK09_6-H MN605029       |                        |
| MOL-1887  | conMOL87_2-H MN605031       | Magadan Region, Olsky District, alt. 6 м N 59° 34.913' E 151° 16.585' |
|           | pesMOL82_1-St MN6344450     |                        |
|           | pesMOL82_7-H MN634449       |                        |
| MOK-1895  | conMOK95_3-St MN605033      | Magadan Region, Olsky District, alt. 24 м N 59° 36.706' E 150° 27.629' |
|           | conMOK95_4-H MN605032       |                        |

**Table 2.** Species accessions and their clones drawn from the GenBank NCBI.

| Species     | Accession (origin) | Sequence in NCBI | Designation on dendrograms |
|-------------|--------------------|------------------|----------------------------|
| *Elymus sibiricus* | PI 628699, Russia | St HM035280 H HM035281 | sib_5280_S sib_5281_H |
| *E. sibiricus*   | PI 499461, China   | St HM035282 H HM035283 | sib_5282_S sib_5283_H |
| *E. caninus*    | ABZ-1654, Russia   | St MN164839 H MN164840 | canABZ54_2 canABZ54_1 |
3 Results and Discussions

Hybrids between *E. peschkovae* (Amur region) and *E. confusus* (The Republic of Buryatia), created and grown by us in combinations **AMU-8804 × BER-0807** (2 plants) and **AMU-8804 × BUK-1109** (2 plants), have shown total seed sterility. This phenomenon forced us to return to the problem of phylogenetical relationships between three species, especially considering their wide distribution areas within the Asian part of Russia.

In vegetation period 2020 we have created 3 hybrids between Magadan biotypes from the common ecotope in the valley of river Ola in combination **E. confusus** MOL-1887 × **E. peschkovae** MOL-1882. In the greenbox all three plants had a little part of opened anthers and showed low fertility (SF) 0-3%, which was confirmed on the open experimental plot. This result does not obscure the possibility to estimate inheritance of the distinctive (diagnostic) traits and dynamics of SF in the F2 generation.

At the same time we carried out a comparative research of the GBSS1 gene sequences in collection accessions **E. peschkovae** and **E. confusus** from the different points of area compared to clones of reference species. Dendrogram, built on the data of GBSS1 gene sequences (exons from 9 to 14 only), is shown on the Fig. 2.

Based on these results, the following conclusions were made:

1. The StH-genomic constitution was confirmed for **E. confusus**, **E. peschkovae** and **E. sibiricus**.

2. All gene variants of St subgenome in studied species are phylogenetically close to the North American ancestral line **St2** of Pseudoroegneria genus, unlike the St subgenome of reference Asian StY-genomic species **E. pendulinus** and **E. gmelinii**. The group of **St2** clones of Siberian **E. confusus** accessions separated from the larger group of **E. confusus**, **E. peschkovae** and **E. sibiricus** species with the bootstrap support value 87.
3. As for the H subgenome, all studied species showed relation only within the Asian group \( H_1 \), which is phylogenetically close to \( H. jubatum \). Wherein all \( E. caninus \) clones separated from other species with the bootstrap support value 58.

4. Three of the four \( E. peschkovae \) clones belonging to the H subgenome were separated from the \( E. confusus \) clones into a particular clade, which is probably not an accidental event and supports the microevolutionary isolation of the two species. We can suppose an independent origination of the trait «pilose lemmas» in the mountane biotype \( E. peschkovae \) JTO-1708, which is located in the other separated branch \( E. confusus \) – \( E. sibiricus \).

5. The largest group of \( H_1 \) subgenome comprised all \( E. sibiricus \) clones except the one from Yakutia–Sakha, and four \( E. confusus \) clones. This fact can hypothetically explain the high reproductive compatibility of the \( E. confusus \) BUM-0505 accession when hybridized with the accession \( E. sibiricus \) BUR-0569 from Buryatia (Agafonov, Gerus, 2009).

Fig. 2. NJ dendrogram, based on the results of analysis of \( GBSS1 \) gene sequences (only exons 9-14) in accessions \( E. confusus \), \( E. peschkovae \), \( E. sibiricus \) compared to reference species from the GenBank NCBI. Monogenomic species that carry subgenomes St and H are marked by asterisks.

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