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Polymorphism of alien *Erigeron canadensis* L. (Asteraceae) along the Trans-Siberian Railway

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Abstract. Transport corridors serve as one of the main vectors of plant invasion over long distances. The Trans-Siberian Railway, connecting two parts of the world with a different set of native species, is a unique research object for analyzing the distribution of alien plants on a global scale. The invasive species of North American origin, *Erigeron canadensis* L., found throughout the Trans-Siberian Railway has been set as a model object. This species grows directly on the railway track and on the adjacent slopes, therefore, its spreading is likely along the transport corridor, but not repeatedly from settlements located nearby the railway. All plants have been divided into three haplotypes in accordance to the structure of chloroplast DNA sites (rpl32–trnL and trnL–trnF). The first two haplotypes are represented in the samples from European Russia, the third one includes all samples from the Urals, Western Siberia, the Far East, and all the rest of material collected in European part of Russia. These data confirm our hypothesis about the leading role of the Trans-Siberian Railway in the distribution of *E. canadensis* in Russia from west to east. However, the isolated haplotypes indicate a low degree of polymorphism of the studied genome regions of *E. canadensis*. Therefore, its successful invasion is mainly associated with modification variability.

Keywords: Canadian horseweed, railway flora, invasive species, populations, ITS1–2, rpl32–trnL, trnL–trnF, modification variability

Introduction

Erigeron species, or horseweed (section *Conyza*), have naturalized in Europe: *E. bilbaoanus* (J. Rémy) Cabrera, *E. blakei* Cabrera, *E. bonariensis* L., *E. canadensis* L., and *E. sumatrensis* Retz.; *E. trilobus* (Decne) Boiss. is also reported as ephemerophyte (Vinogradova, 2012). *E. bonariensis*, *E. canadensis*, and *E. sumatrensis* are three most common species, they are currently recorded in Russia as well (Galkina and Vinogradova, 2011; Vinogradova, 2012).

Canadian horseweed, *E. canadensis*, is an annual or winter biennial plant of North American origin. It is characterized by seed reproduction, and even small individuals of *E. canadensis* can produce about 2000 seeds per growing season. This explains active invasion of the species to the new territories, so it has been included in the top-100 most dangerous invasive species in Russia in 2018 (Dgebuadze et al., 2018; Vinogradova et al., 2018). According to DAISIE (Handbook..., 2009) and GT IBMA databases, the species is included in the top ten most aggressive invasive species in Europe. The identification of such plant taxa and the study of their adaptive capabilities is an urgent task of modern botany. Invasive species are the second largest threat to natural biodiversity after habitat destruction (Bellard et al., 2016; Olmstead, 2006). In phytocenoses, where first invasive species have penetrated, the other invaders are easily introduced (Hess et al., 2019).

All species of plants on the Earth, both in their native area and the secondary distribution range, are subject to microevolutionary processes. The polymorphism of the populations of the species in any part of the range indicates a high degree of genome variability, which may be associated with the influence of both abiotic and biotic environmental factors. In turn, high degree of genome variability indicates high plasticity and a wide adaptive potential, which may be associated with a wide range of responses and modification variability, or with a high degree of polymorphism. The intensity of these processes, as well as the initiating reasons, may be completely different. The influence of phytophagous animals is reported as one of such reasons for other representatives of the Asteraceae family, such as *Hieracium* × *robustum* Fr. and phytophagous gall-forming *Aulacidea hieracii* (L., 1758) (Kritskaya et al., 2019). The other reason is the transition to apomixis, as in some representatives of the genera *Chondrilla* and *Taraxacum* (Kashin et al., 2019; Van Dijk et al., 2020).

Therefore, considering the populations of the same species that are sufficiently distant from each other makes it possible to assess the ecological flexibility of the species and to trace the degree of genome variability. Railroads and ground roads

play a dual role: on the one hand, they allow plants to spread freely over long distances, on the other, they serve as foci and donor sites, from which unintentionally introduced alien species spread to nearby phytocenoses (Christen and Matlack, 2006; Galkina et al., 2021; Vinogradova et al., 2020; Wagner et al., 2021). The Trans-Siberian Railway connects Europe and Asia, which have a different set of native species, but the invasive species *E. canadensis* is noted nowadays along its entire length. Since railway conditions are characterized by a high degree of stability, the studied populations of this species are affected by exclusively abiotic factors associated with climate. *E. canadensis* most often grows on the railway track and adjacent slopes, but sometimes it is also found in the right-of-way. In our opinion, the growth of this species directly on the railway track indicates that it spreads directly along the Trans-Siberian Railway, secondary dispersal to nearby settlements takes place from the railroad. This hypothesis has been tested in the present study by molecular genetic analysis.

The study aims to identify genetic differences between individuals of *Erigeron canadensis* growing in different parts of the Trans-Siberian Railway.

Materials and methods

DNA was isolated from the leaves of herbarium specimens *E. canadensis* collected along the railroad tracks in the European part of Russia (Yaroslavl, Kostroma, and Vladimir oblasts), in the Urals (Sverdlovsk Oblast), in Western Siberia (Tyumen Oblast) and in the Far East (Khabarovsk Krai and Primorsky Krai) (Table 1; Fig. 1). DNA was extracted using the DNA-Extran-3 kit (CJSC Sintol, Russia). Polymerase chain reaction (PCR) was carried out in an amplifier BioRad T-100 (USA). Primers nnc18s10 (forward) and c26A (reverse) were used for the nuclear ribosomal internal transcribed spacer 1–2 (ITS1–2) at an annealing temperature of 58 °C. Primers rpl32F (forward) and trnL UAG (reverse) were used for chloroplast highly variable non-coding intergenic rpl32–trnL spacer at an annealing temperature of 57 °C; for the second chloroplast intergenic spacer trnL–trnF, these were primers C and F at temperatures from 0.3 to 65 °C according to the J. Shaw method (Shaw et al., 2007). The PCR product for sequencing was purified in a mixture of ammonium acetate and ethanol. DNA nucleotide sequences were read an automatic sequencer (Sintol, Russia). Further processing of nucleotide sequences was carried out in BioEdit programs. v. 7.0.5.3. (Hall, 1999) and TCS 1.21 (Clement et al., 2000). The obtained data were downloaded to the GenBank (NCBI) database.

Results and discussion

In all the studied individuals, the nuclear region of ITS1–2 was similar, but slight differences were noted in the chloroplast regions. Nucleotide substitutions and deletions were found in intergenic spacers *rpl32–trnL* and *trnL–trnF* (Fig. 2A). The Siberian populations differ only in deletion in the *rpl32–trnL* region. In the Far East, only *E. canadensis* population from Dalnerechensk was characterized by polymorphism. Individuals collected in European Russia has polymorphism as well: for example, the ET1 specimen from the Vladimir Oblast was distinguished by the structure of the *trnL–trnF* region (Fig. 2B). However, these differences in the structure of the chloroplast regions in some samples were not associated with their belonging to a certain region, and sometimes to a specific population. Thus, there are differences in samples ET6a and ET6b belonging to the same population, collected in the right-of-way in the city of Galich, Kostroma region, as well as in samples ET2a and ET2b from a population growing near the Rostov-Yaroslavy station in Rostov town between the tracks (Fig. 2). This means that *E. canadensis* has undergone microevolutionary changes in the secondary range, at least in the Russian part of the range.

After processing the data on the structure of both chloroplast DNA regions in the TCS 1.21 program, all individuals were divided into three haplotypes

(Fig. 3). The first haplotype included an ET1 sample from the Vladimir Oblast (Bogolyubovo railway station), the second one, a sample collected in the Yaroslavl Oblast (ET2a, Rostov-Yaroslavy station), the third one, the remaining 23 samples from the European part of Russia, the Urals, Western Siberia, and the Far East. This haplotype network testifies that, on the one hand, the population polymorphism of *E. canadensis* in European Russia is higher than in Siberia and the Far East, on the other hand, most of the identified intrapopulation and interpopulation differences are not significant. Higher polymorphism of the European population is presumably explained by the fact that this species has appeared in the European part of Russia much earlier than in the Siberian and Far Eastern parts of the secondary range. Therefore, microevolutionary changes in this population are logically more pronounced.

E. canadensis dissipated along the Trans-Siberian Railway eastwards. The Asian populations of this invasive species have also been influenced by microevolutionary processes, but these changes are currently insignificant. Since all Far Eastern and Siberian collections belong to the same haplotype together with most European samples, our hypothesis about the railway as the main vector of *E. canadensis* dispersal to remote areas of Siberia and the Far East is confirmed.



Fig. 1. Sampling sites of *Erigeron canadensis* at the Trans-Siberian Railway.

Table 1. Samples of *Erigeron canadensis* used for molecular genetic analysis.

Region	Sample no.	No. in GenBank		No. of herbarium sample	Sampling site	Year of sampling
		ITS1–2	rpl32–trnL trnL–trnF			
European part of Russia	ET1	OL853467	OL913131	OM731595	MHA0166597	Vladimir Oblast, Bogolyubovo railway station
	ET2a	OL853468	OL913132	OM731596	MHA0166599	Yaroslavl Oblast, Rostov town, near the Rostov-Yaroslavsky railway station
	ET2b	OL853469	OL913133	OM731597	–	
	ET 3	OL853470	OL913134	OM731598	MHA0166598	Yaroslavl Oblast, Rostov town
	ET4	OL853471	OL913135	OM731599	MHA0166603	Kostroma Oblast, Kostroma city, Malyshkovo railway station
	ET5a	OL853472	OL913136	OM731600	MHA0166602	Sendega railway station
	ET5b	OL853473	OL913137	OM731601	MHA0166601	
	ET6a	OL853474	OL913138	OM731602	MHA0166600	Kostroma Oblast, Galich town
	ET6b	OL853475	OL913139	OM731603	MHA0166604	
Ural	ET16a	OL853487	OL913151	OM731617	MHA0412853	Sverdlovsk Oblast, Pervouralsk town
	ET16b	OL853488	OL913152	OM731618	MHA0412855	
	ET17	OL853489	OL913153	OM731619	MHA0412854	Sverdlovsk Oblast, Ekaterinburg
Western Siberia	ET13	–	OM731592	OM731614	–	Tyumen Oblast, Tyumen city, Voynovka railway station
	ET14a	–	OM731593	OM731615	–	
	ET14b	–	OM731594	OM731616	–	Tyumen Oblast, Tyumen city, Utyashevo railway station
Far East	ET7a	OL853476	OL913140	OM731604	MHA0333516	Khabarovsk Krai, Khabarovsk city, Khabarovsk-1 railway station
	ET7b	OL853478	OL913142	OM731605	–	
	ET8	OL853478	OL913142	OM731606	MHA0333514	Khabarovsk Krai, Khabarovsk city, Locomotive Depot railway station
	ET9a	OL853479	OL913143	OM731607	MHA0333510	
	ET9b	OL853480	OL913144	OM731608	MHA0333509	Primorsky Krai, Dalnerechensk town
	ET9c	OL853481	OL913145	OM731609	MHA0333508	
	ET10	OL853482	OL913146	OM731610	–	Primorsky Krai, Vladivostok city, main railway station
	ET11a	OL853483	OL913147	OM731611	MHA0333512	
	ET11b	OL853484	OL913148	OM731612	MHA0333511	Primorsky Krai, Ussuriysk city, Sakhzavod railway station
	ET12	OL853485	OL913149	OM731613	MHA0333513	Primorsky Krai, Ussuriysk city

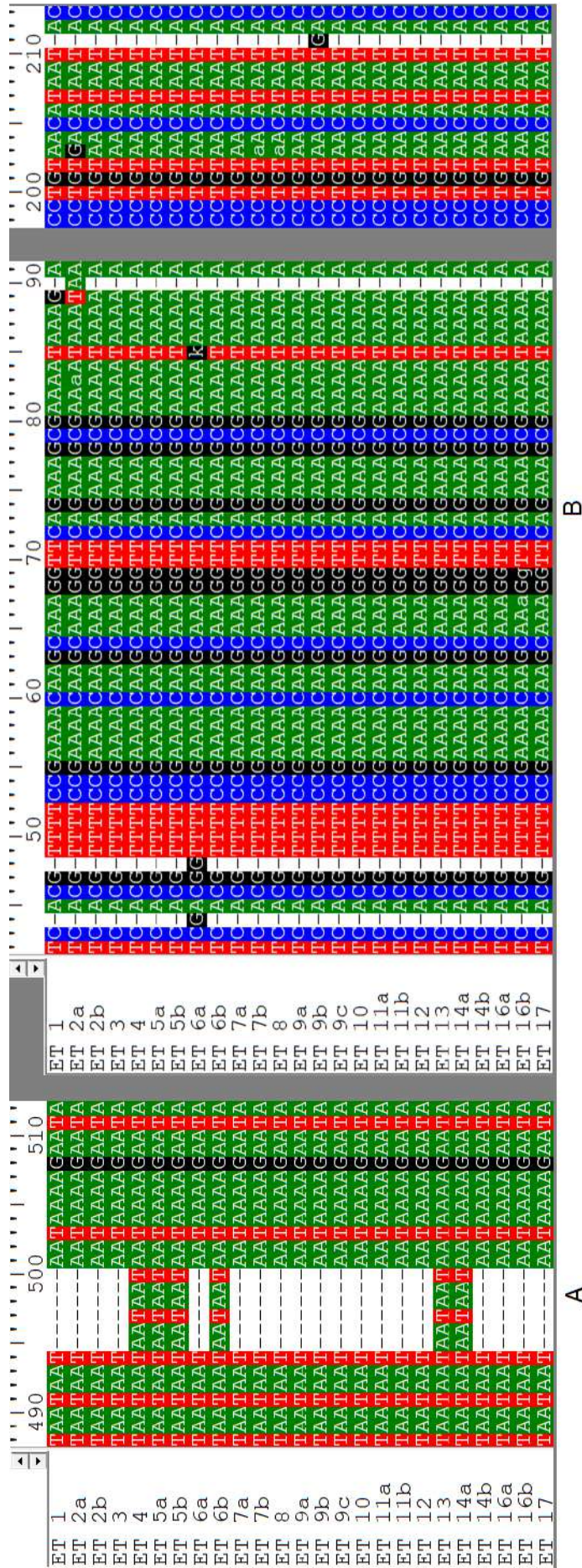


Fig. 2. Alignment fragment of chloroplast DNA sites of *Erigeron canadensis* collected along the Trans-Siberian Railway: **A** – rpl 32-trnL, **B** – trnL-trnF.

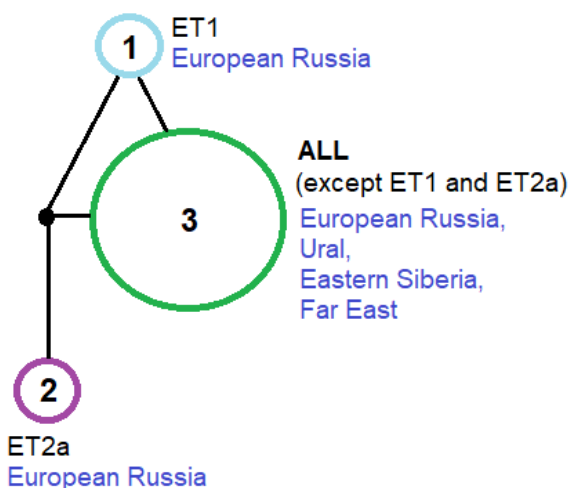


Fig. 3. Haplotype network of *Erigeron canadensis* from different parts of the Trans-Siberian Railway. ET1 – sample from the Vladimir Oblast, ET2a – sample from the Yaroslavl Oblast.

Conclusions

The isolated haplotypes indicate a low degree of polymorphism of the studied genome regions of *Erigeron canadensis*. Successful invasion of this species is most likely due to modification variability. The differences in haplotypes in various populations indicate the spreading of the species eastwards along the Trans-Siberian Railway. The sites of formation of populations resistant to abiotic environmental factors act as invasion foci.

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