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RESEARCH ARTICLE

Aegilops tauschii Coss. molecular phylogeny in comparison with proteins electrophoretic polymorphism

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Abstract

In the case of *Aegilops tauschii* the comparison of intraspecies molecular phylogeny based on DNA sequences of nuclear gene *Got2* with electrophoretic polymorphism of allozymes and hystone H1 proteins is actually a comparison of the one detailed phylogenetic tree with a set of low resolution trees. It could help to understand peculiarities of the species evolutionary history and role of cross-pollination in it. Proteins polymorphism patterns rather satisfactory corresponded to *Got2* DNA tree. Electrophoretic polymorphism of essentially polymorphic in *Ae. tauschii* subsp. *strangulata* protein encoding loci considered (*Ak, Est2, Got1, Got3, Hst2, Hst3*) and essentially polymorphic in *Ae. tauschii* subsp. *tauschii* locus *Fdp* displayed correspondence with the molecular phylogenetic tree: each allele was common or predominant on some branches of the tree and rare or absent on the other. In contrast, alleles of *Cat2* locus, $Cat2^{35}$ and $Cat2^{140}$, were "scattered" sporadically through *Ae. tauschii* subsp. *tauschii* branches on phylogenetic tree. Also in *Ae. tauschii* subsp. *tauschii* a set of different extremely rare allozyme alleles, $Acph4^{113}$, $Aco2^{110}$, $Mdh1^{113}$, $Nadhd1^{88}$, was found among three out of four accessions belonging to one of the relict clades of this subspecies on the molecular phylogenetic tree. The data obtained displayed that subsp. *tauschii*, now being relatively less polymorphic than subsp. *strangulata*, in ancient times had good opportunities for genetic exchange between its different phylogenetic lineages, all but one of which are relicts in present time. And the patterns of $Hst3^{977}$ allele occurrences indicated cross-pollination between subsp. *tauschii* and subsp. *strangulata*.

Keywords

Aegilops tauschii, allozymes, DNA sequences, Genetic variation, Histone H1, molecular phylogeny

Introduction

Aegilops tauschii Coss. (syn. *Aegilops squarrosa* auct. non L.) is a diploid, mostly self-pollinating goat-grass (genome DD, 2n = 14) which donated its genome D to common wheat, *Triticum aestivum* L. (genome AABBDD, 2n = 42). *Ae. tauschii* is very important wild relative of common wheat, a donor of agriculturally important genes for its improvement (Kimber and Feldman 1987; Kilian et al. 2011). So, since genetic variability of *Ae. tauschii* is an important natural resource, it has been studied by researches throughout the world since the middle of the previous century. Genetic methods used started from rather simple allozyme technique and up to nowadays methods such as DArT, AFLP, etc. Modern genetic methods revealed rather detailed picture of *Ae. tauschii* genetic differentiation, but they do not explain how all this was formed in the course of the species evolution, i.e. these studies revealed *Ae. tauschii* population genetic structure but not phylogeny. Phylogenetic study was necessary for understanding of *Ae. tauschii* evolutionary history.

It should be mentioned that intraspecies phylogeny is essentially differs from interspecies one. So, in a simple general example of a species presented by one big and practically panmictic population no intraspecies phylogeny could be found. Phylogenetic study will not form "a tree" in this case; a rake-like structure will be formed instead. Of course, it is not a case of *Ae. tauschii*. This species is mostly self-pollinating, it is presented by many local populations, and here we could find very interesting picture of intraspecies divergence (Dudnikov 2021). Nevertheless occasional cross-pollination sometimes happens in *Ae. tauschii* and has an important impact on the species evolution (Dudnikov 2017, 2021). Therefore phylogenies of different parts of *Ae. tauschii* genome could differ.

The usage of molecular-genetic methods (AFLP, SSR, DArT, single nucleotide polymorphisms etc.) providing many genetic markers "scattered" through genome permits to obtain the picture of *Ae. tauschii* population genetic structure. Here recombination occurring in the course of *Ae. tauschii* evolution is being involved. And as it could be expected, different methods gives completely the same picture of *Ae. tauschii* population genetic structure (Lubbers 1991, Pestsova et al. 2000; Saeidi et al. 2008; Takumi et al. 2008, Mizuno et al. 2010, Sohail et al. 2012, Matsuoka et al. 2013, Wang et al. 2013, Su et al. 2020, Zhou et al. 2021). In contrast, the study of *Ae. tauschii* intraspecies phylogeny needs genetic system without recombination. For this purpose non-coding chloroplast DNA sequences were used (Dudnikov 2019). The other study used DNA sequences of nuclear gene *Got2* (Dudnikov 2017, 2021). (Probability of intragenic recombination seems to be negligible in the case of mostly self-pollinating species *Ae. tauschii*.) As it could be expected, the two different methods give different pictures of *Ae. tauschii* intraspecies divergency (Dudnikov 2021). Also the general picture of *Ae. tauschii* molecular phylogeny obtained with molecular-genetic systems with practically no recombination – differ from the picture of the species population genetic structure obtained using molecular genetic systems with recombination being involved. So, population structure presents two

sublineages, western and eastern (to say nothing of *Ae. tauschii* from Henan, China), in TauL1 gene-pool of the species; and two sublineages, western and eastern, in TauL2 gene-pool (Zhou et al. 2021); while molecular phylogeny presents several major lineages (plus some relicts) within TauL2; and only one major lineage and several relicts in TauL1 (Dudnikov 2021).

Ae. tauschii molecular phylogeny based on non-coding cpDNA sequences turned out to be relatively less accurate and reliable in comparison with phylogenetic picture based on DNA sequences of nuclear gene *Got2* (Dudnikov 2019, 2021), and it could be due to the relatively lower mutation rate in cpDNA in comparison with nuclear DNA (Saitou 2018). It would be interesting to compare "*Got2*" phylogenetic tree with other tree(s) based on DNA sequences of nuclear gene(s) located in different parts of *Ae. tauschii* genome. But unfortunately each such a study needs sequencing of more than 300000 b.p. and is rather laborious.

Previously the studies of allozymes geographic occurrence through *Ae. tauschii* range revealed that each allele of each essentially polymorphic enzyme-encoding locus studied has its personal very special geographic pattern of occurrence which distinctly corresponds to environmental conditions, both on geographic map (Dudnikov 2012a, 2014a) and in the space of climatic parameters on the plot of principal component analysis (Dudnikov 2014b). Also it was pointed out that the time span during which seeds retain their germination capacity is in line with allozyme allelic constitution of enzyme-encoding genes in *Ae. tauschii* (Dudnikov 2003a). These mean that allozimes in *Ae. tauschii* are involved in adaptive processes of some kind. Enzyme encoding genes studies in *Fundulus heteroclitus*, *Drosophila melanogaster* and *Colias* spp. butterflies showed that sometimes substitution of a single amino-acid leading to the origin of new allozyme could have an essential adaptive value changing considerably the fitness of organism (Chambers 1988, Powers et al. 1991, Watt 1992, Eanes et al. 1996). But it seemed very unlikely that each such substitution could be adaptive and therefore could be the reason of total correspondence of allozyme variation in *Ae. tauschii* with ecological conditions through the species range. Explanation of spatial patterns of allozyme variation in *Ae. tauschii* has come from the study of *Ae. tauschii* intraspecies phylogeny based on DNA sequences of enzyme-encoding gene *Got2*. It was shown that the shape of *Ae. tauschii* phylogenetic tree is mostly formed by natural selection. Its branches present the species lineages which essentially differ from each other in their ecological "preferences". The tree is a result of competition between different lineages through evolutionary history of *Ae. tauschii* (Dudnikov 2021). Also, investigation of *Got2* DNA sequences revealed that in the case of *Ae. tauschii* the species age is relatively small in comparison with enzyme gene mutation rate, and the probability of the same allozyme (i.e. enzyme with particular electrophoretic mobility) to originate more than once as a result of two or more independent mutations in the course of *Ae. tauschii* evolution is very low. So, it was pointed out that *Got2* enzyme-encoding gene allozyme variation with two alleles, $Got2^{100}$ and $Got2^{105}$, is due to a single unique mutation, the substitution of GA by AT in positions 2415–2416 which led to replacement of

gluetamic acid by isoleucine (Dudnikov 2017). This means that in *Ae. tauschii* allozymes are good and reliable phylogenetic markers, and each allozyme marks its own specific point of intraspecies divergence in the course of *Ae. tauschii* evolutionary history, i.e. a specific part of the phylogenetic tree.

Therefore in *Ae. tauschii* each essentially polymorphic allozymes encoding locus in fact presents a phylogenetic tree. It is a low resolution tree, as usual having just two or three branches, but it is a reliable one. And we have a set of different independent trees of such kind which could be compared with the detailed molecular phylogenetic tree obtained previously using DNA sequences of *Got2* gene.

Histone H1 proteins in *Ae. tauschii* are being encoded by three different loci (Dudnikov et al. 2002). And as well as in the case of allozymes, geographic patterns of histone H1 electrophoretic variants occurrences turned out to be distinctly adaptive (Dudnikov 2012b). Histone H1 encoding genes were not sequenced in *Ae. tauschii*, but the data obtained on *Pisum sativum* L. (Zaytseva et al. 2012) indicate that Histone H1 electrophoretic allele variants could be good phylogenetic markers the same as allozymes.

Comparison of previously obtained detailed *Ae. tauschii* philogenetic tree based on *Got2* DNA sequences with a set of low resolution phylogenies revealed by proteins electriphoretic polymorphisms could help to understand peculiarities of *Ae. tauschii* evolution and the role of cross-pollination in it. The result of such comparison is presented below.

Material and methods

114 *Ae. tauschii* accessions were used for construction of molecular phylogenetic tree based on *Got2* DNA sequences, 57 of ssp. *tauschii* and 57 of ssp. *strangulata* (Suppl. material 1: Table 1s); and all of them except for AL8/78 of ssp. *strangulata* were used for electrophoretic analysis of proteins polymorphism. The sources of the plant material are as follows: (1) N.I. Vavilov All-Russian Institute of Plant Genetic Recourses (VIR), ("k"); (2) Kyoto University ("KU"); (3) IPK Gatersleben ("AE") and (4) the collection of Dudnikov (1998) ("t"). *Ae. tauschii* subdivision into subsp. *tauschii* and subsp.*strangulata* was considered according to Eig (1929), Hammer (1980), Jaaska (1980, 1981), Dudnikov (2021) and Zhou (2021), which means that TauL1 gene-pool belongs to subsp. *tauschii* while TauL2 and TauL3 gene-pools belong to subsp. *strangulata*. Subspecies identification was performed according to Dudnikov (2000).

Got2 DNA sequences of *Ae. tauschii* accession AL8/78 of Armenian origin (Jia et al. 2013) were obtained from GenBank (AOCO010130377, AOCO010130378). *Got2* DNA sequences of all the other 113 accessions were obtained as described in Dudnikov (2021) and the nucleotide sequences were deposited in the DDBJ/EMBL/GenBank database (http://www.ncbi.nlm.nih.gov/, accession numbers KX773890 to KX773948 and MT051581 to MT051634).

Phylogenetic analysis was conducted using MEGA version 6 (Tamura et al. 2013). Maximum likelihood (ML) method was used. Inversion and indels were treated as fifth character state (Simmons and Ochoterena 2000). Statistical bootstrap support of nodes was calculated with 1000 replicates.

Twelve enzyme systems were used for allozyme electrophoretic analysis: aconitate hydratase (ACO, EC 4.2.1.3), acid phosphatase (ACPH, EC 3.1.3.2), adenylate kinase (AK, EC 2.7.4.3), catalase (CAT, EC 1.11.1.6), esterase (EST, EC 3.1.1.2), fructose-1,6-diphosphatase (FDP, EC 3.1.3.11), glutamatic-oxaloacetatic transaminase (GOT, EC 2.6.1.1), glucosephosphate isomerase (GPI, EC 5.3.1.9), malate dehydrogenase (MDH, EC 1.1.1.37), NADH diaphorase (NADHD, EC 1.6.4.3), and phosphoglucomutase (PGM, EC 2.7.5.1). Enzymes extraction, electrophoresis and staining were carried out as in Dudnikov (2014b). Polymorphism of eight essentially polymorphic in *Ae. tauschii* allozyme-encoding loci, *Acph1*, *Got1*, *Got2*, *Got3*, *Est2*, *Ak*, *Fdp*, *Cat2*, and nine low polymorphic loci, *Aco1*, *Aco2*, *Acph4*, *Gpi*, *Mdh1*, *Mdh2*, *Ndhd1, Ndhd2, Pgm*, were considered in the study. Allele $Est2^{null}$ was considered together with $Est2^{100}$ which electrophoretic band is often of low intensity.

Histone H1 proteins extraction, electrophoresis and staining were carried out as in Dudnikov (2012b).

Result

Ae. tauschii ML molecular phylogenetic tree obtained is displayed on Fig. 1. The data on proteins electrophoretic polymorphism are presented in Fig. 1 and Suppl. material 2: Table 2s.

As expected, *Got2* allozyme variation perfectly corresponded to molecular phylogenetic tree based on DNA sequences of the same very locus *Got2*: *Ae. tauschii* accessions from clades "A – I" had $Got2^{105}$ allozyme allele; and accessions from clades "J - M" had $Got2^{100}$ allele (Fig. 1, Table 1). It is known that *Acph1* loci allozyme variation perfectly corresponds to *Ae. tauschii* subdivision into subsp. *tauschii* (TauL1 gene-pool) and subsp. *strangulata* (TauL2 + TauL3 gene-pools) (Dudnikov 1998, 2000, Kirby et al. 2005). So, accessions from clades "A - H" had $Acph1^{95}$ allozyme allele, and accessions from clades "I - M" had $Acph1^{100}$ allele (Fig. 1, Table 1).

All allozyme and histone H1 encoding loci considered which were essentially polymorphic in *Ae. tauschii* subsp. *strangulata* (*Got1, Got3, Est2, Ak, Hst2, Hst3*) had distinctly uneven distribution of occurrences of their alleles among *Ae. tauschii* subsp. *strangulata* clades of *Got2* DNA tree, "A - H", with each allele being predominant or common in some clades and rare or absent in other. So, $Got1^{95}$ and $Got3^{125}$ predominantly occured in clade "C". $Est2^{84}$ predominantly occured in clades "E" and "H". Ak^{92} was rather common in clades "C" and "F". $Hst2^{1026}$ was predominant in clade "F". $Hst2^{988}$ was rather common in clade "D" and was found in this clade only. $Hst3^{977}$ was rather common in clade "F" (Fig. 1, Table 1).

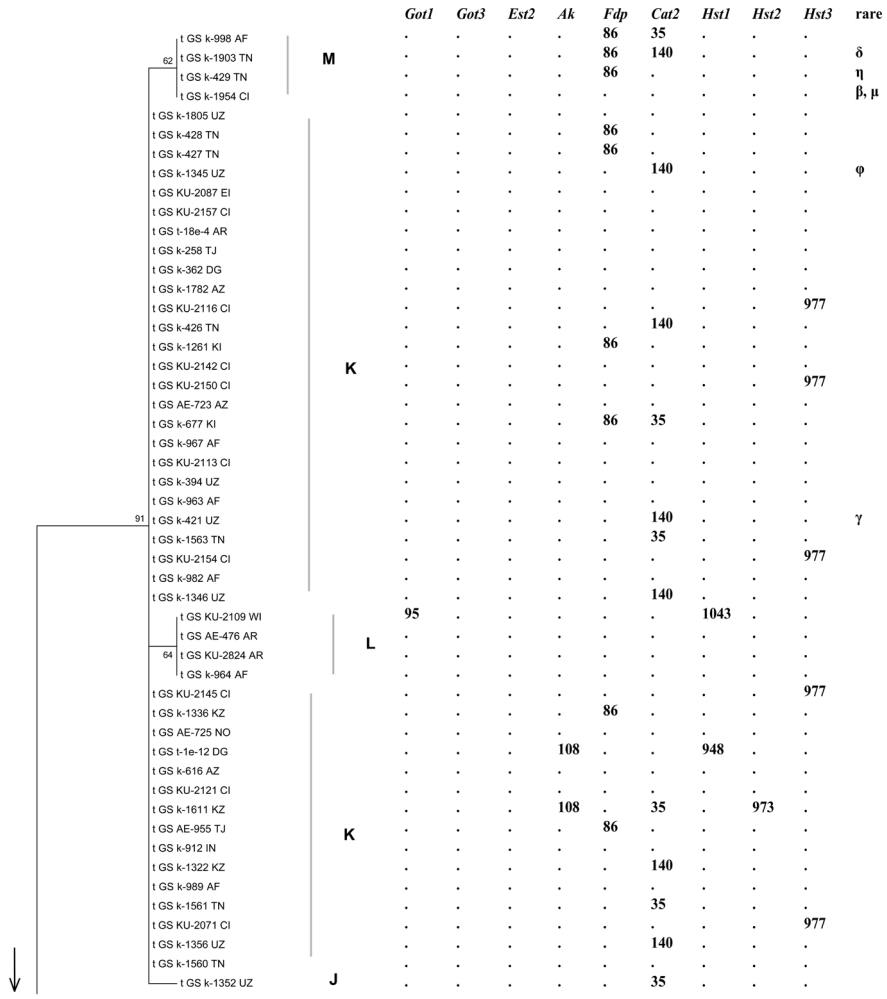

Figure 1. Maximum Likelyhood phylogenetic tree of *Aegilops tauschii* based on *Got2* DNA sequences. Indels were treated as fifth character state. Bootstrap values for 1000 replicates are displayed. Designations before an accession name are as follows: "t" – subsp. *tauschii*, and *Acph1*^{100} allele; "s" – subsp. *strangulata*, and *Acph1*^{95} allele; "GF" – *Got2*^{105} allele (it is peculiar to subsp. *strangulata* and the most ancient relict gene-pool of subsp. *tauschii* (Dudnikov 2017)), "GS" – *Got2*^{100} allele. After an accession name the country of its origin is mentioned: TY – Turkey, NO – North Ossetia, DG – Dagestan, GE – Georgia, AR – Armenia, AZ – Azerbaijan, CI – Continental Iran, WI – Western Precaspian Iran, EI – Eastern Precaspian Iran, TN – Turkmenistan, AF – Afghanistan, PA – Pakistan, IN – India, UZ – Uzbekistan, TJ – Tajikistan, KZ – Kazakhstan, KI – Kirgizstan. Continuing on the next page.

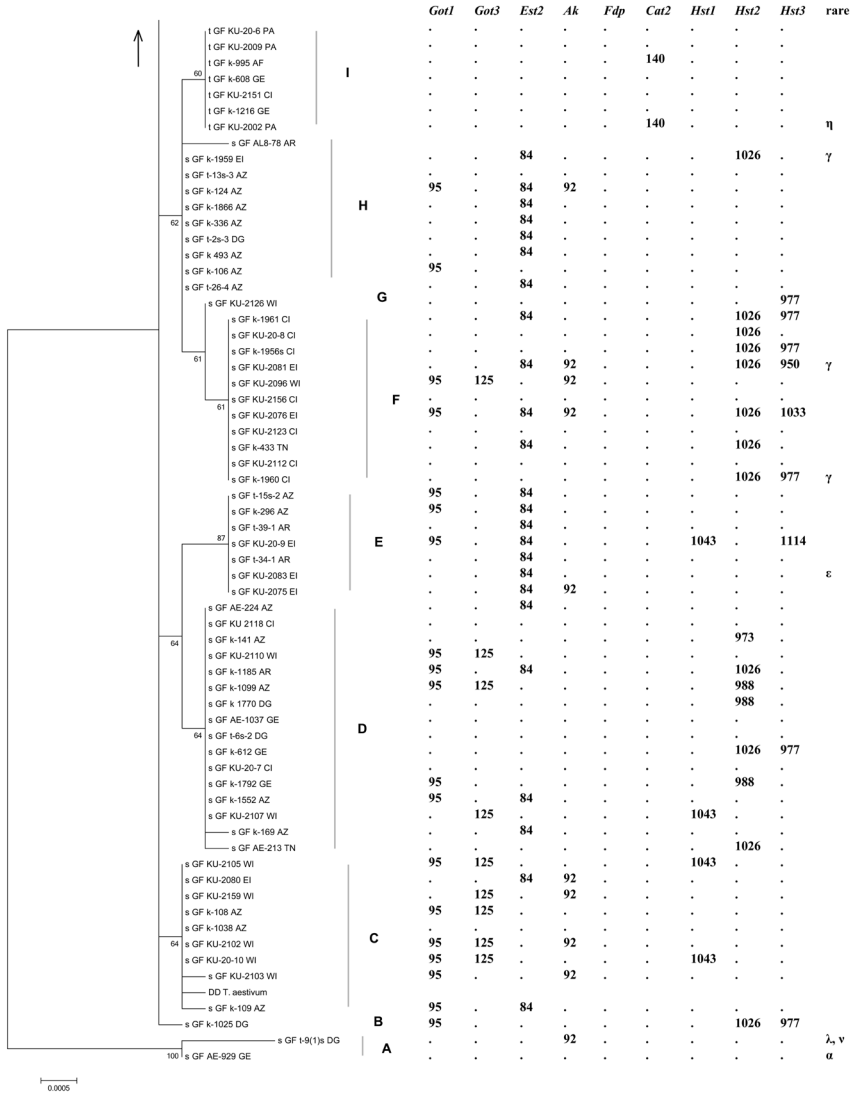

Figure 1. Continuing from the previous page. The subdivision of Precaspian Iran into "Western" and "Eastern" was made according to Dudnikov and Kawahara (2006), with an approximate dividing line going between the towns of Chalus and Babolser, along the 52 meridian. Allelic constitution of essentially polymorphic loci considered other then *Acph1* and *Got2* is displayed to the right side at each accession, with the most common alleles "100" or "1000" being shown as ".". Occurrences of rare allele of low-polymorphic allozyme loci are displayed as follows: "α" – *Aco1*^{107}, "β" – *Aco2*^{110} , "γ" – *Aco2*^{90}, "δ" – *Acph4*^{113}, "ε" – *Gpi*^{45}, "η" – *Mdh1*^{113}, "λ" – *Mdh2*^{90}, "μ" – *Nadhd1*^{88}, "ν" – *Nadhd2*^{92} , "φ" – *Pgm*^{115}.

Table 1. Frequencies of eleven essentially polymorphic loci electrophoretic alleles occurrences among clades of *Ae. tauschii* phylogenetic tree based on *Got2* DNA sequences. The most common allele of each locus, "100" or "1000", is not shown. If a clade contains just one or two *Ae. tauschii* accessions only, the presence or absence of allele is shown as "+" and "-" respectively for each accession

	subsp. *strangulata*								subsp. *tauschii*				
Clade	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**
Number of accessions	**2**	**1**	**9**	**16**	**7**	**11**	**1**	**9**	**7**	**1**	**41**	**4**	**4**
Allele													
Acph1^{95}	+ +	+	1.00	1.00	1.00	1.00	+	1.00	0	-	0	0	0
Got1^{95}	- -	+	0.67	0.31	0.43	0.18	-	0.22	0	-	0	0.25	0
Got2^{105}	+ +	+	1.00	1.00	1.00	1.00	+	1.00	1.00	-	0	0	0
Got3^{125}	- -	-	0.56	0.19	0	0.09	-	0	0	-	0	0	0
Est2^{84}	- -	-	0.22	0.25	1.00	0.36	-	0.78	0	-	0	0	0
Ak^{108}	- -	-	0	0	0	0	-	0	0	-	5	0	0
Ak^{92}	+ -	-	0.44	0	0.14	0.27	-	0.11	0	-	0	0	0
Fdp^{86}	- -	-	0	0	0	0	-	0	0	-	0.15	0	0.75
Cat2^{140}	- -	-	0	0	0	0	-	0	0.29	-	0.15	0	0.25
Cat2^{35}	- -	-	0	0	0	0	-	0	0	+	10	0	0.25
Hst1^{1043}	- -	-	0.22	0.06	0.14	0	-	0	0	-	0	0.25	0
Hst1^{948}	- -	-	0	0	0	0	-	0	0	-	0.02	0	0
Hst2^{1026}	- -	+	0	0.19	0	0.64	-	0.11	0	-	0	0	0
Hst2^{988}	- -	-	0	0.19	0	0	-	0	0	-	0	0	0
Hst2^{973}	- -	-	0	0.06	0	0	-	0	0	-	0.02	0	0
Hst3^{1114}	- -	-	0	0	0.14	0	-	0	0	-	0	0	0
Hst3^{1033}	- -	-	0	0	0	0.09	-	0	0	-	0	0	0
Hst3^{977}	- -	+	0	0.06	0	0.27	+	0	0	-	0.12	0	0
Hst3^{950}	- -	-	0	0	0	0.09	-	0	0	-	0	0	0

Similarly, in *Ae. tauschii* subsp. *tauschii* allele *Fdp*^{86} was found predominantly in clade "M" (Fig. 1, Table 1). In contrast, essentially polymorphic in *Ae. tauschii* subsp. *tauschii Cat2* allozyme encoding locus displayed quite different pattern of its alleles occurrences. *Cat2*^{140} and *Cat2*^{35} occured sporadically through different clades of subsp. *tauschii* on *Got2* DNA based phylogenetic tree (Fig. 1, Table 1).

Also, while considering *Ae. tauschii* subsp. *tauschii* it is interestin to outline that most of rare allozyme alleles found in subsp. *tauschii* belonged to one small relict lineage "M": three out of four accessions from this lineage presented on the tree had different extremely rare allozyme alleles *Acph4*^{113}, *Aco2*^{110}, *Mdh1*^{113}, *Nadhd1*^{88} (Fig. 1).

Among all the loci considered the mentioned above *Hst3* was the only one essentially polymorphic in both subsp. *tauschii* and subsp. *strangulata* with its allele *Hst3*^{977} being presented in both subspecies with the frequency of about 10%.

Discussion

Previously, a comparison of based on nuclear gene *Got2* DNA sequences and cpDNA non-coding sequences molecular phylogenies of *Ae. tauschii* revealed great differences between them indicating effects of cross-pollination in the species evolution and relatively low accuracy of cpDNA based phylogeny as well (Dudnikov 2021).

In the case of different nuclear-based phylogenies being compared, as in the present study, the effects of cross-pollination in *Ae. tauschii* evolution are also obvious, but nevertheless, different nuclear genome based phylogenies correspond rather satisfactory to each other. According to the data we have, *Got2*^{105} allele originated not long before the origin of *Ae. tauschii* subsp. *tauschii* and *Acph1*^{95} originated then at a time of *Ae. tauschii* subsp. *tauschii* appearance. Soon after subsp. *tauschii* segregation, *Got1*^{95} and *Est2*^{84} alleles originated in containing the lineage "C" and the lineages "E + H" parts of the tree, respectively. Later on, *Got3*^{125} originated also in "C" part of the tree; while *Hst2*^{1026} and *Hst2*^{988} – in "F" and "D" parts, respectively (Fig. 1). *Fdp*^{86} originated not recently in the course of *Ae. tauschii* subsp. *tauschii* evolutionary history and corresponds to relict lineage "M" of the subspecies (Fig. 1, Table 1).

Sporadical patterns of *Cat2*^{140} and *Cat2*^{35} occurrence through *Ae. tauschii* subsp. *tauschii* branches of the molecular phyloginetic tree indicate that (1) these alleles originated long ago, probably in some lineage(s) being extinct now; and that (2) different lineages of subsp. *tauschii*, all except lineage "K" being relicts now with fragmentary ranges and practically no contacts with each other, in ancient times had good opportunities to exchange their genetic material. (Occurrence of rare allozyme allele *Mdh1*^{113} in clade "I" and clade "M" al well, also gives evidence of genetic exchanges between these relict clades in the past (Fig. 1, 2))

Example of genetic exchange between subsp. *tauschii* and subs. *strangulata* is displayed by the patterns of *Hst3*^{977} occurrence through the tree. This allele was found in clades "F", "D" of subsp. *strangulata* and in clade "K" of subsp. "*tauschii*" (Fig. 1, Table 1). Accession KU-2109 belonging to relict clade "L" of *Ae. tauschii* subsp. *tauschii* presents another example of genetic exchange between the two subspecies. This accession was collected in western pre-caspian Iran, the region where *Ae. tauschii* subsp. *tauschii* is very rare (Dudnikov and Kawahara 2006) and it has alleles *Got1*^{95} and *Hst1*^{1043} which besides this particular accession of subsp. *tauschii* were found in subsp. *strangulata* only (Suppl. material 1: Table 1s; Suppl. material 2: Table 2s).

Of course, both between subspecies and within subspecies genetic exchanges mentioned should have taken place at some concrete geographic regions. And it should be outlined that occurring sporadically on the phylogenetic tree *Cat2*^{140}, *Cat2*^{35} and *Hst3*^{977} have distinct non-random patterns of occurrence through the range on geographic map. *Cat2*^{140} and *Cat2*^{35} were found only in the eastern part of the range (Dudnikov 2012a), while *Hst3*^{977} occur in the western part of the range and was never found in the east (Dudnikov 2012b). In fact, being involved in rather wide genetic exchange within TauL1, *Cat2* elecrtophoretic polymorphism more correctly brings to light position of clade "I" than *Got2* DNA sequences do. Occurrence of *Cat2*^{140} indicates that clade "I" belong to TauL1, while *Got2* DNA sequences data mistakenly put clade "I" of subsp. *tauschii* (TauL1) together with clades of subsp. *strangulata* TauL2 (Fig. 1)

Occurrence of several extremely rare allozymes in one clade "M" displays that this phylogenetic lineage of *Ae. tauschii* subsp. *tauschii* that in ancient times was presented by one large "wealthy" population, nowadays exist as a set of small isolated local populations which managed to survive in competition with other younger and more successful lineages of this subspecies. In isolated population of small size the impact of genetic drift increases, purifying natural selection becomes not effective, therefore such a population becomes a trap for slightly deleterious alleles, which hardly could be found in large population (Saitou 2018). Examples of alleles or traits found in small isolated populations of *Ae. tauschii* and which slightly deleterious effect is obvious, could be mentioned. So, not only extremely rare allozymes *Aco2*^{110} and *Nadhd1*^{88} were found in accession k-1954 from the relict clade "M" of *Ae. tauschii* subsp. *tauschii* (Fig. 1), but also an extremely rare allele *vrn-D2a* which determines a very rare extremely spring type of growth habit in k-1954 (Dudnikov 2003b). (This effect is known to be a result of deletion in *Vrn-D2* locus (Kippes et al. 2016)). Also, the plants from relict small isolated population t-9^{1}-s of *Ae. tauschii* subsp. *strangulata*, gene-pool TauL3, have extremely rare allozymes *Nadhd2*^{92} and *Mdh2*^{90} (Dudnikov 1998), (Fig. 1). And as well, the seeds of these plants were found to retain their germination capacity during a time span which is about three times shorter than usual for *Ae. tauschii* (Dudnikov 2003a).

Of course, some of alleles which are slightly deleterious for *Ae. tauschii* in wild nature could be useful from applied point of view in *Triticum aestivum* L. breeding programs. So, the usage of *vrn-D2* allele from k-1954 accession (E1 genetic line) of *Ae. tauschii* gave an opportunity to create a very special genetic line of *T. aestivum*, a synthetic *vrn2*-null . This line is being of spring growth habit as a result of having non-functional *vrn-D2*, *vrn-A2* and *vrn-B2* loci (Kippes et al. 2016).

Conclusion

Comparison of the high-resolution phylogenetic tree based on *Got2* DNA sequences with a set of low-resolution trees based on allozymes and histone H1 electrophoretic proteins polymorphism leads to the following conclusions.

(1) Since *Ae. tauschii* is a self-pollinating species, there is a correspondance between different phylogenetic trees, each based on a single locus genetic variation. At the same time, these different trees markedly differs, indicating that cross-pollination do exist in *Ae. tauschii* and plays an important role in the species evolution.

(2) The study displays an example of evolutionary history of a phylogenetic lineage of *Ae. tauschii* subsp. *tauschii*. The lineage "M" in previous time occupied a vast area. Than it was forced out by younger and more successful lineages. A set of small completely isolated populations of lineage "M" managed to survive in different local refugiums; and each these small independent populations became "a trap" for unique genetic variation.

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Supplementary material 1

Table 1s. *Ae. tauschii* accessions

Authors: Alexander J. Dudnikov

Data type: table

Link: http://journal.asu.ru/biol/article/view/14436/12225

Supplementary material 2

Table 2s. Electrophoretic alleles variation of proteins encoding loci among lines of *Ae. tauschii* used for reconstruction of the species phylogeny on the base of *Got2* DNA sequences

Authors: Alexander J. Dudnikov

Data type: table

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