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ORIGINAL ARTICLE

Genetic diversity and reproductive traits in triploid and tetraploid populations of *Gladiolus tenuis* (Iridaceae)

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Abstract Most perennial herbaceous plants are able to reproduce vegetatively as well as sexually. Sometimes, such plants may lose the capacity for sexual reproduction. We have studied the case of sterility in triploid populations ($2n = 3x = 45$) of *Gladiolus tenuis* M.Bieb. in a considerable part of its area of distribution. Initially, we recorded the presence of a large clone of *G. tenuis* to the east of the Volga River, as a result of isozyme analysis. We also used AFLP fingerprinting to genotype 55 samples from 10 populations of *G. tenuis* and one population of the closely related *G. imbricutus* L. This analysis revealed an extremely low genetic diversity in sterile triploid populations of *G. tenuis* and a rather high genetic diversity in fertile tetraploid populations ($2n = 4x = 60$) over most of the area of this species. Genetic distances between fertile and sterile populations of *G. tenuis* were similar to those between different species of gladioli. It appears that a single sterile genotype has spread vegetatively over 800 km, propagating by daughter corms. The study of the reproductive features of *G. tenuis* suggests that the cause of sterility may be self-incompatibility between individuals of the clone.

Keywords AFLP fingerprinting · Clonal plant · Genotypic diversity · *Gladiolus tenuis* · Sterility

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Introduction

Most perennial herbs and some woody plants can reproduce vegetatively. Vegetative reproduction allows the plants to quickly occupy new areas and does not depend on the availability of pollinators or conditions for the maturation and germination of seeds and spores. The plant structures responsible for vegetative propagation often contain a greater supply of nutrients than that of the seeds and spores. This gives a significant competitive advantage to young plants of vegetative origin in plant communities. In arctic-alpine conditions or on the edge of areas where seed propagation is hindered, the benefits of vegetative propagation are most evident in species naturally growing in this habitat and in plants growing at the limits of their distribution (Dorken and Eckert 2001; Eckert 2002).

Most clonal plants can maintain sufficiently high levels of genetic variability because they reproduce sexually from time to time (Ellstrand and Roose 1987; Hamrick and Godt 1989). However, there are some cases of complete loss of sexual reproduction, leading to formation of clones of various sizes (Dorken and Eckert 2001; Hollingsworth and Bailey 2000; Lynch et al. 1998; Peterson et al. 2010). Odd polyploidy, aneuploidy, hybridization, and other causes leading to lack of seed reproduction may contribute to the formation of sterile clones. A remarkable example is the king's lomatia (*Lomatia tasmanica* V.M.Curtis)—locally endemic to Tasmania and represented by a single clone (genetically identical specimens). This species is triploid and characterized by a complete lack of seed reproduction and genetic diversity (Lynch et al. 1998). A sterile "megaclone" was detected in Belgian Gagea (*Gagea spathacea* Salisb.) in Central Europe (Pfeiffer et al. 2012). The authors consider the high ploidy and the putative hybrid origin as the reasons for the sterility of *G. spathacea*. The sterility of the flowering rush (*Butomus umbellatus* L.),

introduced to America (Eckert 2002; Eckert et al. 2003), and *Sedum bulbiferum* Makino in Japan (Tsujimura and Ishida 2008) are associated with anorthoploidy. There are also many examples of asexual reproduction and the formation of clones in populations from peripheral parts of a species distribution area. Clones were detected in the northern populations of *Decodon verticillatus* (L.) Elliott (Dorken and Eckert 2001), in regional populations of *Gagea bohemica* (Zauschn.) Schult. & Schult.f. (Peterson et al. 2010) and in populations of *Saxifraga cernua* L. in different parts of its range (Gabrielsen and Brochmann 1998; Kapralov et al. 2006).

The genus *Gladiolus* includes species of different ploidal levels, all of them able to reproduce vegetatively. Two closely related species of this genus, *G. tenuis* M.Bieb. and *G. imbricatus* L., growing in the European part of Russia, are sometimes combined into a single species (Mayewsky 1964, 2006). The main part of the distribution area of *G. tenuis* covers the Crimea, the Caucasus, and some other southern regions of the European part of Russia. The Southern Urals and surrounding areas lay at its eastern limit. Because of the evidence for vegetative reproduction by small corm lets produced as offsets by the parent corms in the Southern Urals populations, we assumed that seed reproduction on the edge of the area may have been disturbed, resulting in the formation of clones. Using isozyme analysis, we studied several populations in the Orenburg Region and Southern Bashkortostan and found that they all belong to a single clone. Chromosome counts revealed that the clonal plants are all triploids ($2n = 3x = 45$). Fertile plants from the central part of the area of distribution of the species are, however, tetraploids ($2n = 4x = 60$).

Until recently isozyme analysis was useful in the study of clonal plants (Lynch et al. 1998; Tsujimura and Ishida 2008). In recent years, AFLP analysis has been applied to such studies. This technique generates highly polymorphic "fingerprints," which permits identification of genets (Mueller and Wolfenbarger 1999). Also, unlike isozyme analysis, AFLP allows genetic analysis of polyploids. In this paper, we have used both types of analysis in the study of clonal *Gladiolus*. The aim of our study was to identify the genetic structure of populations and assess the reproductive system of *G. tenuis* in different parts of its distribution, to determine the boundaries of the putative vegetative clone and to compare its level of genetic variation with that of the fertile plants.

Materials and methods

Studied species

***Gladiolus tenuis* M.Bieb.** (≡*Gladiolus communis var. tenuis* (M.Bieb.) Wahlb.) is a herbaceous perennial plant with an underground corm. It is propagated by seeds as well as vegetatively by daughter corms, which develop at the base of the parent plant. The corm is replaced annually. The main distribution area of *G. tenuis* covers Central and Eastern Europe, the southern part of the East European Plain, the Crimea, the Caucasus, and northwest Kazakhstan (Mugojar Mountains). In most of its range, *G. tenuis* is tetraploid ($2n = 4x = 60$), but on the eastern boundary of the area, in the Southern Urals, triploids ($2n = 3x = 45$) were found.

In carrying out the AFLP fingerprinting, the closely related species *Gladiolus imbricatus L.* was used as an outgroup. Synonyms are *Gladiolus apterus* Klokov., *Gladiolus rossicus* Pers., *Gladiolus galiciensis* Schultes ex Besser., *Gladiolus neglectus* Schultes (Govaerts and Barker 2014). It differs from the *G. tenuis* in the large number of flowers in the inflorescence, winged seeds, and an absence of daughter corms. It is a European species, native to Central and Eastern Europe, including the European part of Russia, the Baltic States, and the Carpathians. The chromosome number of *G. imbricatus* is $2n = 4x = 60$ (Fedorov 1969).

Study sites and sample collection

Plant material was collected from ten populations of *G. tenuis* from Orenburg, Volgograd, and Samara Regions, the Republic of Tatarstan, and the Republic of Bashkortostan. One population of *G. imbricatus* from the Republic of Belarus (Table 1; Fig. 1) was sampled for an out-group comparison in AFLP analysis. In all populations of *G. tenuis*, except population 3, there were more then 15–20 individuals. Only two individuals were in population 3, so we could not include this population in the isozyme analysis and the evaluation of reproductive system. In all populations, plants were sampled over a distance of at least 4 m. For isozyme and DNA analyses, we collected corms and leaves of gladioli. Extracts for isozyme analysis were prepared from fresh bulbs. The bulbs and leaves for AFLP fingerprinting were stored at −80 °C until DNA extraction.

Isozyme extraction and electrophoresis

To prevent degradation of isozymes, the extraction and subsequent operations were carried out at a temperature of 0–5° C. Plant material (about 100–150 mg) was homogenized with 0.8 ml of extraction buffer (Tris–HCl pH 7.5 with 1 % β-mercaptoethanol) at a temperature of 0–5 °C. Extracts were stored at −80 °C. Samples were electrophoretically separated in 6.4 % polyacrylamide gel in a vertical chamber with Tris–EDTA–borate buffer system (pH 8.0), as described previously (Semerikov et al. 2002). Populations 1 and 2 (Table 1) of *G. tenuis* were tested for 13 enzyme systems. Some of them appeared monomorphic or

Table 1 Sampling locations and population identities of *G. tenuis* and *G. imbricatus*

Population	Location	Coordinates latitude/ longitude
G. tenuis		
1	Volgograd Reg., Chernishkovsky District, to the east of Morskoy Farm	48.0982 42.6834
2	Volgograd Reg., Chernishkovsky District, the vicinity of Tormosin Farm	48.1670 42.6750
3	Samara Reg., Shigonsky Distr., Usa River floodplain	53.4608 48.5832
4	Republic of Tatarstan, Spassky District, Yasachka River	54.9315 49.2944
5	Republic of Bashkortostan, Tuymazinsky Distr., near Kostiantynivka, Seraphimovskoe swamp	54.4171 53.7336
6	Republic of Bashkortostan, Tuymazinsky Distr., Kandrykul Lake	54.5091 54.0194
7	Republic of Bashkortostan, Zianchurinsky District, to the east of Petrovskoe	51.8565 56.6310
8	Orenburg Reg., Kuvandyksky Distr., 5 km from the Dubinovka Station, floodplain of Blagoderka River	51.5103 56.7973
9	Orenburg Reg., Kuvandyksky Distr., 1 km from Rysaevo Station	51.3789 57.5294
10	Orenburg Reg., Gaysky Distr., vicinity of Guberlya Station	51.2857 58.1581
G. imbricatus		
11	Republic of Belarus, Minsk Distr., Zhukovka	54.1849 27.5081

not clearly identifiable on gels under histochemical staining. Six polymorphic enzyme systems, alcohol dehydrogenase (ADH), 6-phosphogluconate dehydrogenase (6PGD), shikimate dehydrogenase (SKDH), phosphoglucoisomerase (PGI), glutamate oxaloacetate transaminase (GOT), and phosphoglucomutase (PGM) were used for the subsequent analysis. Due to polyploidy of *G. tenuis*, the bands could not be assigned to specific loci, being probably represented by product combinations from different loci. As a consequence, each isozyme showed a specific banding pattern and was recorded as a phenotype. The presence of identical band profiles in all isozyme systems, we interpreted as a single genotype (a clone). Genotypic diversity was evaluated using a modified Simpson index as suggested for clonal plants by Ellstrand and Roose (1987): $D = D = 1 - \{[\Sigma n_i(n_i - 1)]/[N(N - 1)]\}$. Here n_i means the number of plants identified in the sample from the i-th isozyme phenotype (and accordingly, the genotype); N stands for the total number of plants that were analyzed; the diversity index D varies from 0 in a population, consisting of a single genotype, to 1 in populations where each individual represents a unique genotype. Higher values of D correspond to greater clonal diversity within a population.

DNA isolation and AFLP fingerprinting

Genomic DNA was isolated using the CTAB method (Devey et al. 1996) from frozen (−80 °C) leaves and corms. AFLP analysis was carried out according to the standard type protocol (Vos et al. 1995) with modifications described in Samils et al. (2001) using a 3130 Genetic Analyzer (Applied Biosystems, USA) with fluorescently labeled *Eco*RI primers. In order to identify a combination of selective primers, 12 pairs of primers were tested. Five combinations with clear amplification profiles and an optimal number of fragments (*Eco*RIAGC^{Ned} + *Mse*CTT, *Eco*RIACT^{Fam} + *Mse*CAA, *Eco*RIACG^{Joi} + *Mse*CAA, *Eco*RIAGC^{Ned} + *Mse*CAA, *Eco*RIACG^{Joi} + *Mse*CCTC.) were selected for further analyses. Fluorescently labeled products of each selective PCR were linked to the molecular weight standard GeneScan ROXTM 500 (Applied Biosystems, USA) prior to loading into the Analyzer. The chromatograms obtained were analyzed using the GeneMapper® version 4.0 program (Applied Biosystems, USA). The fragment lengths obtained were verified manually. Only loci that demonstrated unambiguous interpretation were accepted for the analysis, monomorphic loci were excluded. AFLP typing of the fragments was represented as a matrix of the band's "presence" or "absence" with 1 or 0 coding, respectively.

Data analysis

We accounted for the following parameters of intrapopulation variability: the percentage of polymorphous loci (P), unbiased expected heterozygosity (Uh_e), and Nei's genetic distances (D) (Nei 1978) calculated from the allele frequencies of AFLP loci. All the parameters were analyzed using the GenALEx version 6 (Peakall and Smouse 2006), taking into account the Hardy–Weinberg equilibrium. The gene flow was calculated as follows: $Nm = 0.25 \times (1 - Fst)/Fst$ based on Wright's statistics (Slatkin and Barton 1989). Analysis of molecular variance (AMOVA) (Weir and Cockerham 1984) was carried out according to the following hierarchy levels: between species, between groups of populations, between populations and within populations.

The ordination of the studied samples with the method of principal coordinates based on genetic distances was estimated using GenALEx. An alternative analysis of the population structure and assessment of the probability of hybrid nature of species was carried out using the Bayes' algorithm based on the Hardy–Weinberg equilibrium model in STRUCTURE 2.2 (Pritchard et al. 2000; Falush et al. 2007). This approach allows estimation of the probability of dividing

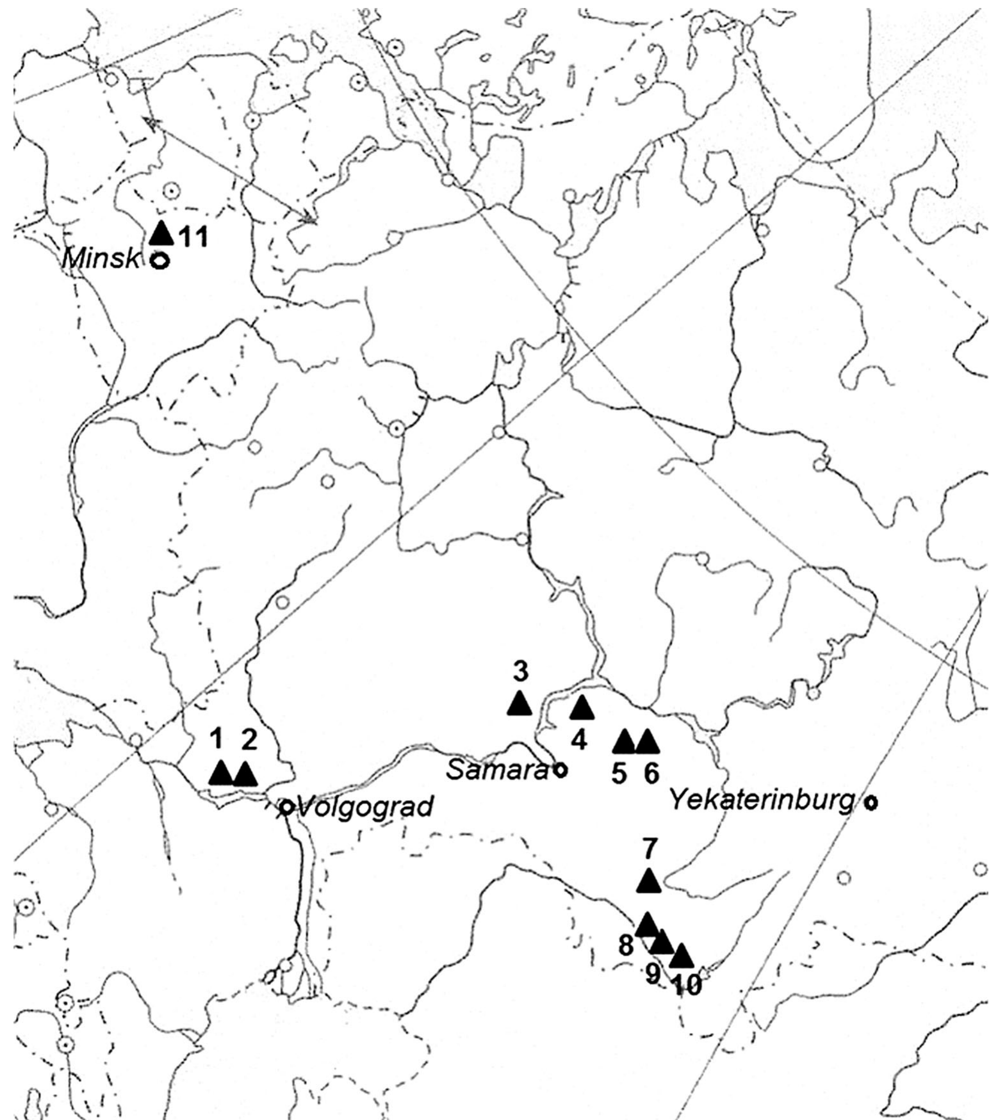

Fig. 1 Locations of populations of *Gladiolus tenuis* and *G. imbricatus* as given in Table 1

specimens into an optimal number of groups, K value, at which the logarithm of the posterior probability function ($\ln P(D)$) reaches a plateau. The analyses were repeated five times for each of the five (2–6) K values tested. The admixture model was used, which takes into account the possible mixed origin of populations based on the independent frequencies of alleles between the clusters. The analysis was carried out with one million iterations. The burn-in point of the Markov chain was preliminarily chosen after 100,000 iterations.

Evaluation of the reproductive system in *Gladiolus tenuis*

Inflorescences of *G. tenuis* were fixed in alcohol–acetic acid fixative (3:1) and stored in 70 % alcohol. Male fertility was estimated by pollen stainability in acetocarmine (Jensen 1962), determined in a sample of 200 grains from each plant. The presence of pollination was evaluated by germination of pollen grains on the stigma, the pollen tube growth in the pistil, and pollen tube penetration into the ovule. These cytological features were evaluated under a fluorescence microscope (Leica DM 2500) after maceration of the pistils in 10 % KOH and staining of them with fluorochrome aniline blue. In each of nine populations (1, 2, 4–10), we have studied 10 plants.

An assessment of reproductive features was made for all populations of *G. tenuis* except population No. 3 (Table 1; Fig. 1), due to the small number of flowering individuals in it.

Results

Isozyme analysis

Isozyme analysis of tetraploid ($2n = 4x = 60$) populations (No. 1 and No. 2) revealed a high genotypic variability.

Table 2 Genotypic diversity in populations of *G. tenuis* based on the results of isozyme analysis

Population	*N*	*G*	*D*
1	11	10	0.98
2	21	12	0.91
4	14	1	0
5	24	1	0
6	24	1	0
7	20	1	0
8	15	1	0
9	16	1	0
10	26	1	0

N number of samples, *G* number of multilocus genotypes, *D* Simpson's index

Simpson's index of genotypic diversity (*D*) is 0.91 and 0.98, respectively (Table 2). To the east of the Volga River, all the populations are triploid ($2n = 3x = 45$), monoclonal ($D = 0$), and represented by the same multilocus genotype.

AFLP fingerprinting

The use of five combinations of primers recovered 401 loci, according to which the studied samples were genotyped. The number of polymorphic loci by a primer pair varied from 37 to 146 (Table 3).

On average, for the populations of *G. tenuis,* the percentage of polymorphic loci in the investigated samples (Table 4) varied from 5.24 % (No. 1 and No. 7) to 18.45 % (No. 2). The only studied population of *G. imbricatus* was characterized by the highest level of polymorphism (38.4 % of polymorphic loci). The average polymorphism for groups of tetraploid populations of *G. tenuis* was 13.88 %, while for seven triploid populations it was 8.59 %.

Unbiased expected heterozygosity (Uh_e) in tetraploid populations of *G. tenuis* ranged from 0.029 (No. 3) to 0.085 (No. 2). In triploid populations, it was lower, from 0.028 (No. 7) to 0.047 (No. 5). According to the results of AMOVA, the proportion of variability attributable to species differences (Storz 2005) between the two studied species of gladioli is 68.29 % and between two groups of *G.tenuis*, tetraploid fertile and triploid sterile populations, it is 66.36 % (Table 5). The share of the differences among populations dispersion of *G. tenuis* is only 4.85 %. The results of the principal coordinates analysis of the sample are shown in Fig. 2. The first three principal coordinates describe 46.60, 14.24, and 3.57 % of the total variability. The analysis shows clear separation of three groups of specimens: the population of *G. imbricatus,* the three tetraploid (No. 1, No. 2, and No. 3), and the seven triploid populations of *G. tenuis*. The greatest values of Nei's distance (D) were shown between the populations of *G. tenuis* and *G. imbricatus* (0.271–0.352) (Table 6). The distance between the tetraploid populations of *G. tenuis* ranged from 0.045 to 0.130. The triploid populations of *G. tenuis* appear to be closely genetically related to one another (*D* ranges from 0.002 to 0.010), except for population No. 7, the distance to which is somewhat greater and varies from 0.029 to 0.036. Mean Nei's genetic distances (*D*) between the different ploidy groups of populations of *G. tenuis* and *G. imbricatus* are shown in Table 7. As it is clear from the table, the distance between triploid and tetraploid groups of populations of *G. tenuis* is rather large and amounts to half the distance to the outgroup species.

Bayesian analysis with STRUCTURE 2.2 under the admixture model revealed the maximum value of the logarithm of posterior probability function for $K = 3$ (three

Table 3 Number of polymorphic loci in populations of *G. tenuis* and *G. imbricatus*

Combinations of primers	Number of polymorphic loci
*Eco*RIAGC^{Ned} + *Mse*CTT	89
*Eco*RIACT^{Fam} + *Mse*CAA	146
*Eco*RIACG^{Joi} + *Mse*CAA	37
*Eco*RIAGC^{Ned} + *Mse*CAA	86
*Eco*RIACG^{Joi} + *Mse*CCTC	43
Total	401

Table 4 AFLP genetic diversity in populations of *Gladiolus tenuis* and *G. imbricatus*

Population	*N*	%P	Uh_e
G. tenuis, $2n = 4x$			
1	3	17.96	0.076 (0.008)
2	3	18.45	0.085 (0.009)
3	2	5.24	0.029 (0.006)
G. tenuis, $2n = 3x$			
4	6	8.73	0.037 (0.006)
5	7	11.72	0.047 (0.007)
6	6	10.97	0.044 (0.007)
7	2	5.24	0.028 (0.006)
8	4	7.23	0.033 (0.006)
9	3	6.73	0.030 (0.006)
10	9	9.48	0.039 (0.006)
G. imbricatus, $2n = 4x$			
11	10	38.40	0.130 (0.009)

Standard errors are given in brackets

N sample size, *P* percentage of polymorphic loci, Uh_e unbiased expected heterozygosity

Table 5 Results of molecular variance analysis (AMOVA) for populations of gladioli

	df	SS	% of total variance	Φst	*P*
One group: 11 populations					
Among populations	11	1434.03	64.13	0.641	0.001
Within populations	43	620.19	35.87	0.359	0.001
Two groups: *G.tenuis* and *G.imbricatus*					
Among groups	1	889.70	68.29	0.683	0.001
Among populations	10	544.34	12.23	0.122	0.001
Within populations	43	620.19	19.48	0.195	0.001
Two groups of *G.tenuis*: tetraploid and triploid populations					
Among groups	1	351.97	66.36	0.664	0.001
Among populations	8	155.18	4.85	0.049	0.001
Within populations	35	386.27	28.79	0.288	0.001

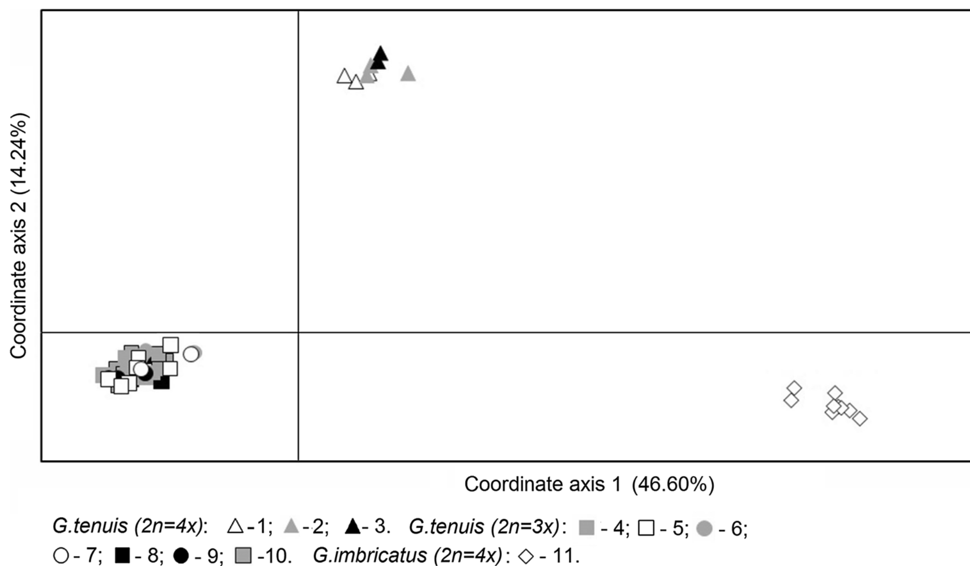

Fig. 2 Principal coordinates analysis of AFLP data

Table 6 Nei's genetic distances, estimated from allele frequencies of AFLP loci between populations of *Gladiolus tenuis* and *G. imbricatus*

1	2	3	4	5	6	7	8	9	10	11	Population
0.000											1
0.045	0.000										2
0.130	0.090	0.000									3
0.182	0.151	0.224	0.000								4
0.169	0.143	0.214	0.005	0.000							5
0.165	0.140	0.214	0.008	0.007	0.000						6
0.192	0.164	0.224	0.029	0.036	0.036	0.000					7
0.181	0.155	0.230	0.009	0.009	0.010	0.033	0.000				8
0.188	0.161	0.230	0.007	0.007	0.008	0.033	0.010	0.000			9
0.173	0.144	0.218	0.002	0.002	0.005	0.031	0.005	0.006	0.000		10
0.293	0.271	0.341	0.337	0.322	0.322	0.337	0.327	0.352	0.326	0.000	11

groups of genotypes) in all the program runs. This means that the sample may be divided with maximum posterior probability into three genetic clusters (Fig. 3).

The first cluster corresponds to the tetraploid populations of *G. tenuis*: No. 1, No. 2, and No. 3. The second cluster is represented by triploid populations of *G. tenuis*:

Table 7 Nei's genetic distances between the groups of populations of *Gladiolus tenuis* and *G. imbricatus*

G. imbricatus, $2n = 4x$	*G. tenuis*, $2n = 3x$	*G. tenuis*, $2n = 4x$	
0.000			*G. imbricatus*
0.314	0.000		*G. tenuis*, $2n = 3x$
0.249	0.143	0.000	*G. tenuis*, $2n = 4x$

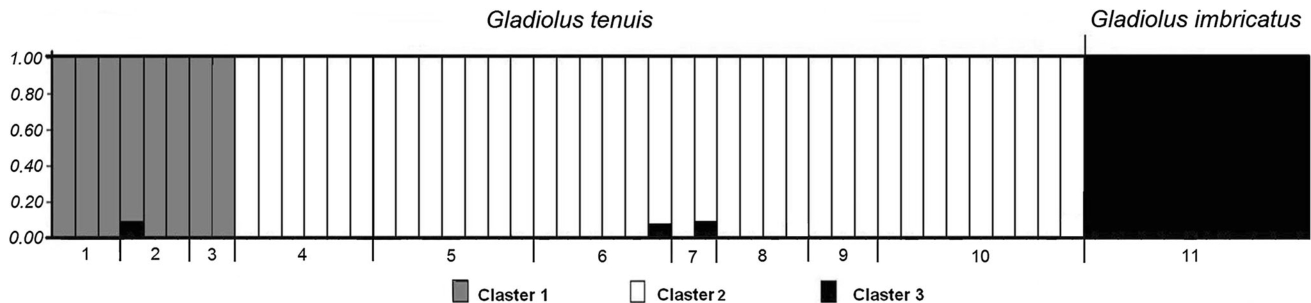

Fig. 3 Posterior probabilities of allocation of 55 samples of gladioli to one of the three clusters assessed by STRUCTURE from the AFLP data

Table 8 Pollen fertility and the presence of pollination in tetraploid and triploid populations of *G. tenuis*

Population	Pollen fertility, % ±SD	The surface condition of the stigma, the presence/absence of pollen on the stigma and pollen tube growth in pistils
G. tenuis, $2n = 4x$		
1	96.3 ± 3.7	The surface of the stigma was well developed, many pollen grains landed on the stigma surface, and most of them (>30) grew into the tissue of the stigma; in the column of the pistil, a massive growth of pollen tubes was observed. The tubes penetrated into the fertile ovules. Structural abnormalities were not found
2	97.1 ± 3.1	
G. tenuis, clon $2n = 3x$		
4	56.9 ± 19.4	The surface of the stigma was poorly developed, buds began to degenerate before pollination
5	62.6 ± 26.9	In populations, No. 4–6 pollen only a few pollen grains landed on the stigma surface and not more than 2–3 of them grew. But growth of pollen tubes in the pistil column had stopped
6	68.5 ± 15.9	
7	61.8 ± 7.9	In populations, No. 7–10 pollen grains on the stigma were nearly absent. Occasionally some germinated pollen grains were observed; the pollen tube did not grow into the stigma. Pollen tubes were not found growing in the pistil column
8	51.2 ± 8.5	
9	45.8 ± 6.5	
10	57.7 ± 6.6	

No. 4–10; and the third one is composed by the samples from the population of *G. imbricatus*.

Reproductive features of *Gladiolus tenuis*

Some reproductive features of *G. tenuis* are presented in Table 8.

Discussion

The genus *Gladiolus* L. comprises 260–270 species, most of which originated in South Africa, and only 10 in Eurasia. Most of the South African species are diploids; the European species are polyploids (Goldblatt et al. 1993). As has already been noted above, *G. tenuis* is tetraploid in most of its area of distribution, as well is *G. imbricatus*. The studied tetraploid populations of *G. tenuis* combine vegetative and seed modes of reproduction, as follows from the repetition of isozyme and AFLP profiles among population samples. At the same time, infrapopulation genotypic diversity is rather high in these populations. The plants of the tetraploid populations invariably demonstrate high fertility of pollen and the presence of pollen tube growth in pistil.

On the contrary, samples studied from the eight triploid ($2n = 3x = 45$) populations of *G. tenuis* to the east of the Volga River possess the same isozyme genotype and

probably represent a single clone. In these populations, we showed abnormalities in both the female and male reproductive systems. Namely, the level of pollen fertility is significantly lower than in the tetraploid populations (Table 8) and ovules usually degenerate at different stages of development. All these irregularities lead to the complete absence of seed reproduction in triploid populations. Reproduction of these plants can presumably occur only vegetatively, by means of daughter corms.

It is known that triploidy may not necessarily lead to complete sterility. According to the literature, triploids may vary as to pollen fertility. In triploid *Datura stramonium* L. (Solanaceae), pollen fertility determined by acetocarmine staining was 50–60 %, although only 15 % of them were able to germinate (Satina and Blakeslee 1937). In triploid *Eupatorium macrocephalum* Less. (syn. *Campuloclinium macrocephalum*, Asteraceae), fertility of pollen ranging from 46.64 to 54.83 % (Farco and Dematteis 2014). At the same time, some triploids, e.g., accessions of the *Musa acuminata* Colla (Musaceae) and plants of *Taraxacum* section *Ruderalia* have completely sterile pollen (Adeleke et al. 2004; Meirmans et al. 2006). As to seed propagation, it rarely occurs in triploid flowering rush *Butomus umbellatus* L. (Butomaceae) (Eckert et al. 2000) and breadfruit *Artocarpus altilis* (Parkinson) Fosberg (Moraceae) (Ragone 2001). Genetic diversity in populations of triploid *Tulipa riparia* indicates the haphazard occurrence of seed reproduction, though crossbreeding may probably happen only between different clones. Low pollen germination on stigmas and arrest of pollen tube growth in pistil columns indicate self-incompatibility of the triploid clone of *G. tenuis,* which could be the cause of its sterility.

The clone size and possible mechanisms of dispersal

The sterile triploid clone of *G. tenuis* is found over a distance of about 800 km from population No. 4 in Spassky District of the Tatarstan Republic in the North to population No. 10 in the Southeast of the Orenburg Region. Such a wide distribution is not unusual in clonal plants. For example, the clone of *Gagea spathacea* (Liliaceae) has spread over great distances in Europe (Pfeiffer et al. 2012), propagating by means of small underground bulbils. Pfeiffer et al. (2012) found that all the samples collected from locations up to 1500 km apart were genetically identical as to their AFLP profiles and DNA sequences.

There are two possible complementary mechanisms for spatial expansion of the *G. tenuis* vegetative propagules. The hydrochory is one of them, enabling the transfer of daughter corms by streams of water during heavy rains or floods. Another way of distribution of vegetative propagules depends on the activity and migrations of animals. Small mammals like field voles and zokors collect bulbs and corms of *Tulipa, Gagea,* and *Crocus* (Sludskiy 1978) and enable their local dispersal. It seems probable that the daughter corms of the gladioli can also be dispersed by the local activity of small mammals. Propagules can also spread over greater distances on the fur of larger mammals, as was shown for subsidiary bulbs of *Gagea spathacea* dispersed by boars (Pfeiffer et al. 2011). Boars could play a similar role in the distribution of daughter corms of *Gladiolus*, which may adhere to their fur. In late Pleistocene to early Holocene periods, other large mammals such as mammoths, whose fossils are known from late Pleistocene sediments between the Volga River and the Ural River, as well as in the Ural River valley (Smirnova 2004), could have participated in spreading the clone of *G. tenuis*.

Comparison of the results obtained by isozyme and AFLP analyses

The samples from the seven populations of *G. tenuis* collected to the east of the Volga River appeared to be completely identical in nine isozyme polymorphic loci. However, as was previously mentioned, isozyme analysis is not very sensitive. Besides, this method does not allow analysis of the allelic composition in polyploids and, consequently, the genetic structure of a population. AFLP method allows analysis of polyploids, as ploidy does not affect the interpretation of the results. AFLP data often show greater polymorphism in clonal plant populations compared to isozyme markers. AFLP analysis can identify a large number of highly reproducible markers, overcoming the problems inherent in both isozyme and RAPD methods, and is, therefore, particularly suitable for obtaining individual “fingerprints.” However, AFLP fingerprints may not be absolutely identical even between two replicates of the same plant or a genet.

Therefore, a certain threshold of similarity between clones needs to be established for a particular study. To calculate this, various factors such as the biological features of a species and the sensitivity of the method may be taken into account. In various studies, thresholds of similarity for clonal populations are in the range of 0.95–1.00, most often being set to 0.98 (Arens et al. 1998; Douhovnikoff and Dodd 2003). Perhaps, this is the reason for differences identified in our samples from the group of triploid populations, where genetic distances range from 0.002 to 0.010. Population No. 7 from Southwest Bashkortostan seems to be an exception, since its distance from other populations of the clone varies from 0.029 to 0.036. This may be due to a somatic mutation that occurred in this population. Pfeiffer et al. (2012) explain small differences in the AFLP profile and sequences of several samples from the giant clone of *Gagea spathacea* by somatic mutations.

Genetic differentiation between populations and species in *Gladiolus*

Bayesian analyses in STRUCTURE and principal component analysis all suggest considerable differentiation of the sterile triploid population from the fertile tetraploid. The distances between the three sexual and the seven clonal populations of *G. tenuis* are similar to those between them and *G. imbricatus* (i.e., out-group). The gene flow between populations of *G. tenuis* is estimated as very low, about 0.116 or one-tenth individual per generation, and probably only reflects the existing gene exchange between sexual populations. Bayesian analyses in STRUCTURE 2.2 in all the runs under the admixture model revealed three genetic groups ($K = 3$) with maximum posterior probability. These three identified clusters clearly correspond to the two groups of *G. tenuis* (sexual and clonal) and to *G. imbricatus.G. tenuis* and *G. imbricatus* have a number of morphological differences and are regarded as two separate species in the World Checklist (Govaerts and Barker 2014). However, some authors do not recognize *G. tenuis* as an independent species lumping it with *G. imbricatus* (Mayewsky 1964, 2003; Komarov 1935). According to our results, the recognition of these species is correct, as there are significant genetic differences between them.

Conclusions

It appears that *Gladiolus tenuis* represents quite an unusual phenomenon, the transition of some populations of the species to a triploid level accompanied by a complete loss of sexual propagation and the formation of an extensively distributed (about 800 km) vegetative clone. High genetic similarity of the clonal populations leads us to propose that the transition from tetraploid to triploid level occurred only once. Since then, the triploid sterile genotype seems to have spread widely via vegetative propagation. Genetic differences between the sexual tetraploid and the clonal triploid populations of *G. tenuis* are quite significant and comparable to those between the two closely related species *G. tenuis* and *G. imbricatus*. The genome reorganization that occurred in the triploid may be the reason for such a difference. More research is needed to understand the origin of the triploid clone of *G. tenuis* and the mechanisms of its spread and survival.

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