Nordic Journal of Botany 29: 762–772, 2011
doi: 10.1111/j.1756-1051.2011.01277.x,

Subject Editor: Stefan Andersson. Accepted 14 July 2011

Morphological variation in populations of the *Poa orinosa* complex (Poaceae: Poeae) in northeastern China

Marina V. Olonova, Mary E. Barkworth, S. V. Pulkina and Wen-Li Chen

M. V. Olonova and S. V. Pulkina, Biological Inst., Tomsk State Univ., Lenina Street 36, RU-634050 Tomsk, Russia. – M. E. Barkworth (mary.barkworth@usu.edu), Intermountain Herbarium, Utah State Univ., Logan, Utah 84322-5305, USA. – W.-L. Chen, State Key Lab of Systematic and Evolutionary Botany, Inst. of Botany, Chinese Academy of Sciences, 20 Nanxincun, Xiangshan, CN-100093 Beijing, PR China.

We conducted a detailed study of the morphological and cytological variation in seven populations of the *Poa orinosa* complex along an ecological gradient in northeastern China. Three of the populations were at different elevations in wooded habitats; three were in habitats dominated by grasses other than *Poa*; and one was in a shrub–steppe habitat. Plants from two of the wooded sites were diploid; those from the third wooded site were primarily octoploid. Plants from two of the grassy sites were tetraploid; those from the third grassy site were hexaploid. The seventh population, located in a shrub–steppe habitat, was diploid. Twenty-four morphological characters were scored on specimens from each site. Plants from the three wooded sites and the lowest grassy site differed from those from the other three sites in having longer culms, internodes and sheaths. The similarity of the tetraploid plants to the diploid plants suggested that they may be autotetraploids. Similarly, the morphological similarity of the octoploid population to the two diploid populations suggested that it too may be an autopolyploid. The morphological distinction of the hexaploid population suggests that it has incorporated a genome not present in the other populations.

Poa orinosa Keng [≡ *P. versicolor* var. *orinosa* (Keng) Olonova & G. Zhu fide Zhu et al. (2006)] is a member of *Poa* subg. *Stenopoa* (Dumort.) Soreng & L. J. Gillespie that grows over a large area in central and eastern Asia at 30°–40°N (Fig. 1). Research at BM, LE, PE, SU, TK (Thiers continuosly updated) have showed it to be the most variable Chinese taxon in the section, with its variability being greatest where its range overlaps with that of *P. alta* Hitchc., *P. sphondylodes* Trin. s.s. and *P. plurifolia* Keng [= *P. sphondylodes* var. *erikssonii* Meld. fide Zhu et al. (2006)].

The discovery of three diploid populations of *P. orinosa* (Olonova et al. 2008) prompted this study. Before that, the only diploid count in *Poa* subg. *Stenopoa* was on a Himalayan specimen of *P. araratica* Trautv. (Mehra and Sharma 1975), most members of the subgenus being tetraploid or hexaploid (Probatova 1985, 2007). Discovery of the diploid populations led us to conduct an intensive, population-based study of morphological variation along an ecological transect through the diploid populations. Our goal was to develop a better understanding of the extent and probable bases of the morphological variation within and between the populations, information that will aid in understanding evolutionary relationships and evaluating alternative taxonomic treatments of the group. Developing this understanding is of both practical and theoretical significance because subg. *Stenopoa* has a global distribution and its members are often dominant in steppe communities.

Material and methods

Study area

The study area lies about 100 km southwest of Beijing, between 39° 49′–39° 55′N and 115° 30′–115° 38′E in the Baihua Mountain System, which is the northern part of the Taihang Mountain range (Fig. 1). Collections were made at seven sites along a 6 km transect laid out following the protocols of Rusanovich and Skvortsov (1981). The sites included some mid-slope, wooded sites, some grassy sites located above the timberline and one shrub–steppe site (Table 1). At each site, we sampled only representatives of *Poa* subg. *Stenopoa*. The number of specimens collected at each site varied from 11 to 53 (Table 1), depending on the number of plants present. The total number of examined specimens was 151. Each specimen was considered an operational taxonomic unit (OTU) in the analyses.

Cytological analyses

Mitotic chromosome counts were made on up to 25 plants from each site, using root tips from germinated caryopses. The root tips were incubated in a 0.1% solution of colchicine for 2 h and then fixed in a 3:1 solution of ethanol and glacial acetic acid. For further observation, the

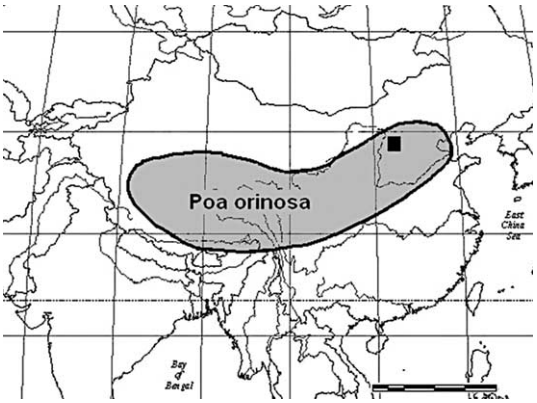

Figure 1. Distribution of *Poa orinosa.* Black square: location of the sampled sites.

chromosomes were stained with hematoxylin (Pausheva 1988). Twenty-five cells were counted for each plant.

Morphological analyses

Examined characters

We scored 24 characters, 21 continuous and 3 two-state categorical, for each OTU (Table 2). These characters reflect the observed variation and include the characters used to distinguish among Chinese members of *Poa* subg. *Stenopoa.* One character, ligule length, was scored both as a continuous character (LIG) and as a five-state categorical character (LGC) (Table 2) because keys vary in their use of the character.

Univariate analyses

We used analysis of variance to determine which of the quantitative characters differed significantly among the sites. The variation of these characters within each site, ploidy level, and plant community was compared using whisker plots. For the binary characters and LGC, we used stacked bar plots to compare the proportion of each character state within the sites, ploidy levels and plant communities.

Multivariate analyses

We conducted several multivariate analyses of the data, using only the continuous characters. We used hierarchical cluster analysis (HCA) and principal component analysis (PCA) to explore the variation among the OTUs without prejudicing the outcome. In these procedures, all the OTUs were treated as coming from the same sample. For the cluster analyses, the data was standardized by mean and standard deviation and the Euclidean distance was used to determine the inter-OTU distances. The results of several different clustering algorithms were examined. In addition, we conducted a minimum spanning tree analysis (MST) to determine each OTUs nearest neighbor.

Discriminant analysis (DA) generates functions that maximize the separation of specified groups. We employed it to determine whether the OTUs from the different 1) sites, 2) ploidy levels, and 3) plant communities could be identified by the continuous characters. The jack-knife classification results were used to evaluate the success of the discriminant functions.

We used Statistica 6 for the analyses of variance; Systat 13 for preparation of the whisker plots, stacked bar plots, and discriminant analyses, and NTSYS (Rohlf 2010) for the cluster analyses, MST, and principal component analysis. Illustrator and Photoshop were used for final preparation of the figures.

Voucher specimens

Representative voucher specimens were prepared from each population and deposited in the herbarium of Tomsk State Univ., Russia.

Results

The seven sites included three dominated by trees (wooded), three dominated by grasses (grassy) and one dominated by a shrub–steppe community consisting of a mixture of woody shrubs and grasses (steppe). Of the wooded sites, two (sites 1, 2) were well below the timberline and the third (site 3) was very close to the timberline. The three grassy sites (sites 4, 5 and 6) and the steppe site (site 7) were above the timberline. Plants from sites 1, 2, 3 and 4 all had exposed upper cauline nodes and, according to existing treatments, belong to *Poa orinosa* [=*P. versicolor* var. *orinosa*]. Plants from sites 5, 6 and 7 all had concealed upper cauline nodes and, according to existing treatments, they belong to *P. plurifolia* [=*P. sphondylodes* var. *erikssonii*].

Cytological analyses

The population samples differed in chromosome number, but all cells within a plant had the same chromosome number. Moreover, with one exception, the chromosome number was constant at each site (Table 1). Plants from the two wooded sites well below timberline were diploids (2n = 14), as were those from the steppe community. Plants from two of the grassy sites, sites 5 and 6, were tetraploids

Table 1. Ecological and cytological characteristics of the sampled populations.

Site	Sample size	Habitat	Somatic chromosome numbers
1	11	wooded community below timberline	14
2	53	wooded community below timberline	14
3	20	wooded community near timberline	56 (17 OTUs), 46, 60, 74 (1 each)
4	21	grassy meadow above timberline	42
5	14	grassy meadow above timberline	28
6	21	grassy meadow above timberline	28
7	11	steppe community above timberline	14

Table 2. Morphological characters used in numerical analyses, their codes and units of measurement. Characters 1–21 were continuous characters; characters 22–25 were categorical characters.

CONTINUOUS: 1. Culm length (CLM, cm); 2. Distance between culm base and top node (BSE, cm); 3. Distance between top node and panicle (PDN, cm); 4. Length of second internode from the base (INT, cm); 5. Length of flag leaf sheath (STH, cm); 6. Length of flag leaf blade (BLL, cm); 7. Width of flag leaf blade (BLW, mm); 8. Length of ligule of flag leaf (LIG, mm); 9. Panicle length (PNL, cm); 10. Panicle width (PNW, cm); 11. Number of branches at lowest panicle node (BRN); 12. Length of longest panicle branch length (BRL, cm); 13. Number of spikelets on longest panicle branch (SPN); 14. Number of florets per spikelet (FRN); 15. Spikelet length (SPL, mm); 16. Length of first glume (G1L, mm); 17. Half width of first glume (G1W, mm); 18. Length of second glume (G2L, mm); 19. Half width of second glume (G2W, mm); 20. Length of lemma (LML, mm); 21. Half lemma width (LMW, mm). DISCONTINUOUS: 22. Ligule length (LGC): 1, ≤1 mm, 2, 1.0–1.5 mm, 3, 1.5–2.0 mm; 4, 2.0–2.5 mm, 5, >2.5 mm); 23. Pubescence of rachilla (RCP: 1 = glabrous; 2 =pubescent); 24. Pubescence between principal and secondary lemma veins (LMP: 1 =glabrous; 2 =pubescent); 25. Pubescence of lemma callus (CLP: 1 =glabrous, 2 =pubescent).

(2n = 28); plants from the third grassy site were hexaploids (2n = 42). Four different chromosome counts were obtained for the 20 plants from site 3, the wooded site located near the timberline. Seventeen plants were octoploids (2n = 56); the other three had 2n = 46, 70 and 74, respectively. For simplicity, the population is referred to below as the octoploid population.

Morphological analyses

Univariate analyses of continuous characters

Analysis of variance demonstrated that the populations differed significantly with respect to eight of the examined quantitative characters (Olonova et al. 2008), four vegetative and four reproductive. Further examination showed that the sites fell into two groups with respect to three of the vegetative characters, CLM, INT and STH (Fig. 2, column 1; see Table 2 for abbreviations). Sites 1, 2, 3 and 4 had high values for these characters and sites 5, 6 and 7 had low values. The other vegetative character in which there were significant differences among the populations, LIG, differed in that one site, site 4, had a markedly lower value than all the other sites. Despite being the most variable in chromosome number, site 3 had no morphological outliers with respect to the eight characters. Site 7 was the only other site for which this was true.

The four reproductive characters that differed significantly among the sites were SPL, G1L, G2L and LML. For all four, sites 1 and 6 had the highest values (Fig. 3, col. 1). Sites 3 and 4 had the lowest values for SPL but sites 3 and 7 had the lowest values for the other three characters.

Differences in these characters among ploidy levels were also striking. The tetraploids had significantly lower values than the other three ploidy levels (Fig. 2, col. 2) for CLM, INT and STH but, for LIG, the hexaploids had the lowest values. The diploids were more variable than the other ploidy levels with respect to all four characters. Among the diploid populations, both CLM and INT had several OTUs with extremely low values. All came from site 7.

All four ploidy levels showed a similar pattern with respect to the reproductive characters (SPL, G1L, G2L and LML; Fig. 3, col. 2), with the octoploid population (3) having the lowest average values and the tetraploid populations (5, 6) the highest average values. There was, however, considerable overlap among OTUs at different ploidy levels.

Comparison of the values for different habitats showed that CLM, INT, and STH were highest in plants from the wooded sites (Fig. 2, col. 3), intermediate in plants from the grassy sites, and lowest in plants from the steppe site. The steppe site was also the least variable, but the number of OTUs involved was also much lower (11 OTUs rather than 74 for wooded sites and 55 for grassy sites). There was little variation in the four reproductive characters (SPL, G1L, G2L, LML) among OTUs from different habitats, the greatest distinction being the tendency of the OTUs from the steppe site to have lower values for G1L and LML (Fig. 3, col. 3).

Univariate analyses of categorical characters

The distribution in character states for LGC and the three binary characters is shown in Fig. 4. At no site was the LGC value uniform. The most uniform sites were sites 1 and 4. At site 1, 82% of the OTUs had an LGC value of 5; at site 4, 86% had an LGC = 5. At sites 5 and 7, the majority (64% and 55%) of the OTUs had LGC = 3; at site 6 the majority (62% had LGC = 4. At site 3, 40% of the OTUs had LGC = 3, 35% had LGC = 4, and 25% had LGC = 5. Site 2 was the only site at which all five LGC states were present, with states 5 and 4 being most prevalent (47% and 28%, respectively).

The sites were less variable with respect to the binary characters. Callus pubescence (CLP) was almost completely uniform, only 2 of the 151 OTUs having hairs on the callus. Both were diploids from site 7. All OTUs from sites 1, 2, 4 and 6 had glabrous rachillas, as did at least 50% of the OTUs from the other sites. The OTUs from sites 4 and 7 were uniform, but different, with respect to LMP, site 4 OTUs being glabrous between the veins and site 7 OTUs pubescent. Of the other OTUs, most OTUs from sites 1, 2, and 3 were glabrous between the veins whereas most from sites 5 and 6 were pubescent.

Data for different ploidy levels (Fig. 4, col. 2) revealed that no ploidy level was completely uniform with respect to ligule length (LGC) or rachilla pubescence (RCP) but the tetra-, hexa- and octoploids were uniform with respect to callus pubescence (CLP), being glabrous. The hexaploid OTUs were also uniform for LMP, being glabrous between the veins. Diploid OTUs were almost all glabrous on the rachilla and the callus. The octoploid OTUs were evenly divided between glabrous and pubescent for RCP, but had a 4:1 ratio for lemma pubescence. The proportion of OTUs with glabrous rachillas decreased with ploidy level: diploids 95%, tetraploids 88%, hexaploids 85% and octoploids 50%.

Rachilla and callus pubescence were the most uniform of the four categorical characters within the different habitats (Fig. 4, col. 3), most OTUs in all three habitats being glabrous for both characters. All steppe OTUs had lemmas that were hairy between the veins; the OTUs in the other two habitats varied in this respect. The steppe OTUs were also more uniform with respect to ligule length, their ligules

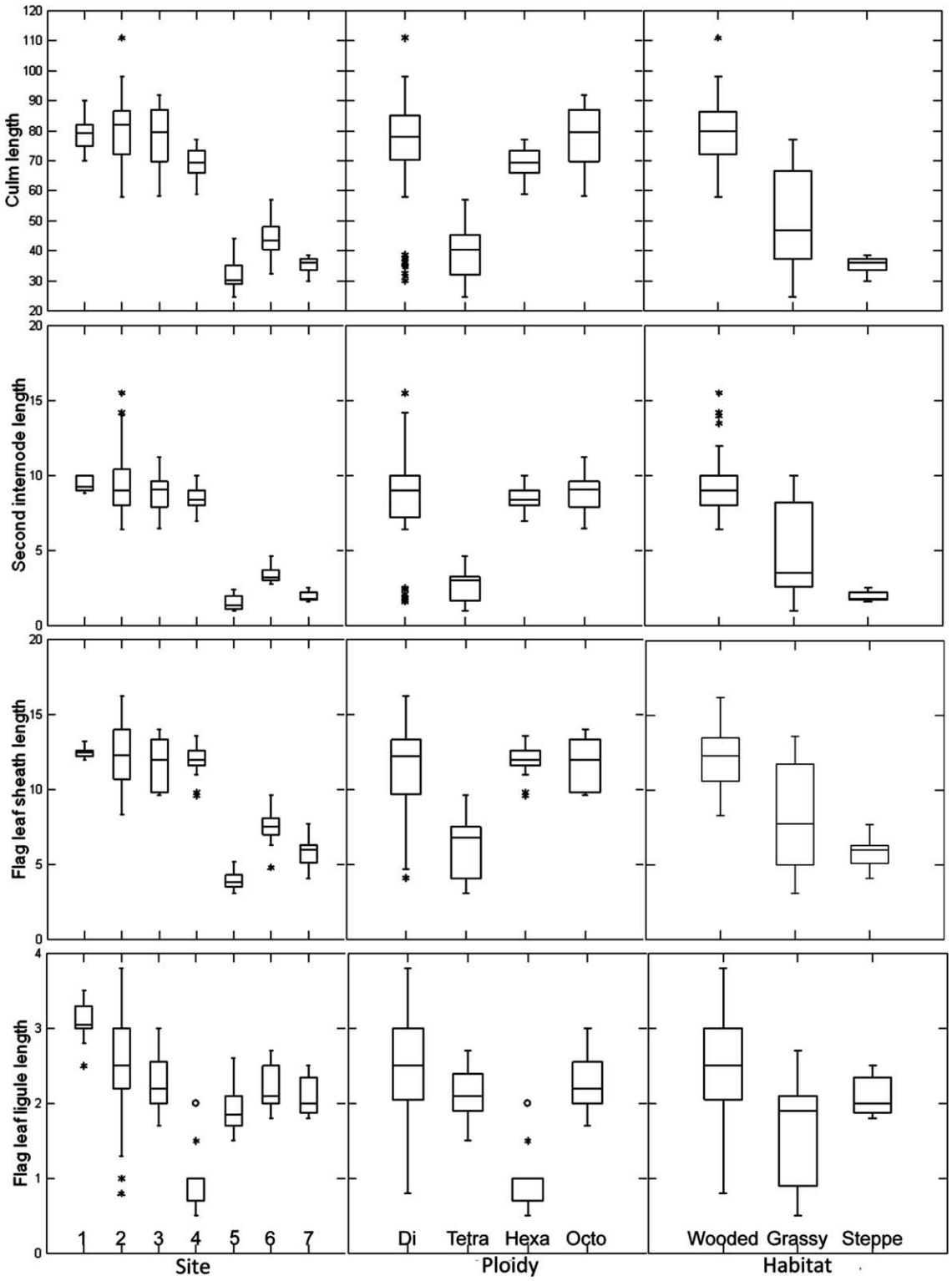

Figure 2. Variation in the continuous vegetative characters by site, ploidy level and habitat. Character codes are given in Table 2. Boxes include 50% of the records; bar is the median value; whiskers extend to the 12th and 87th percentile; asterisks are values between the 6th and 12th or 87th and 92nd percentile; circles are outside these values. Table 1 provides information on sample size and the cytological variation of the 'octoploid' population.

being about equally divided between medium long and long. Those from the grass habitats were about equally divided between very short (LGC = 1), medium long (LGC = 3) and long (LGC = 4) ligules.

Multivariate analyses: cluster analyses

None of the clustering algorithms used were completely successful in recovering the sites, ploidy levels, or plant communities. We present (Fig. 5) the results obtained using

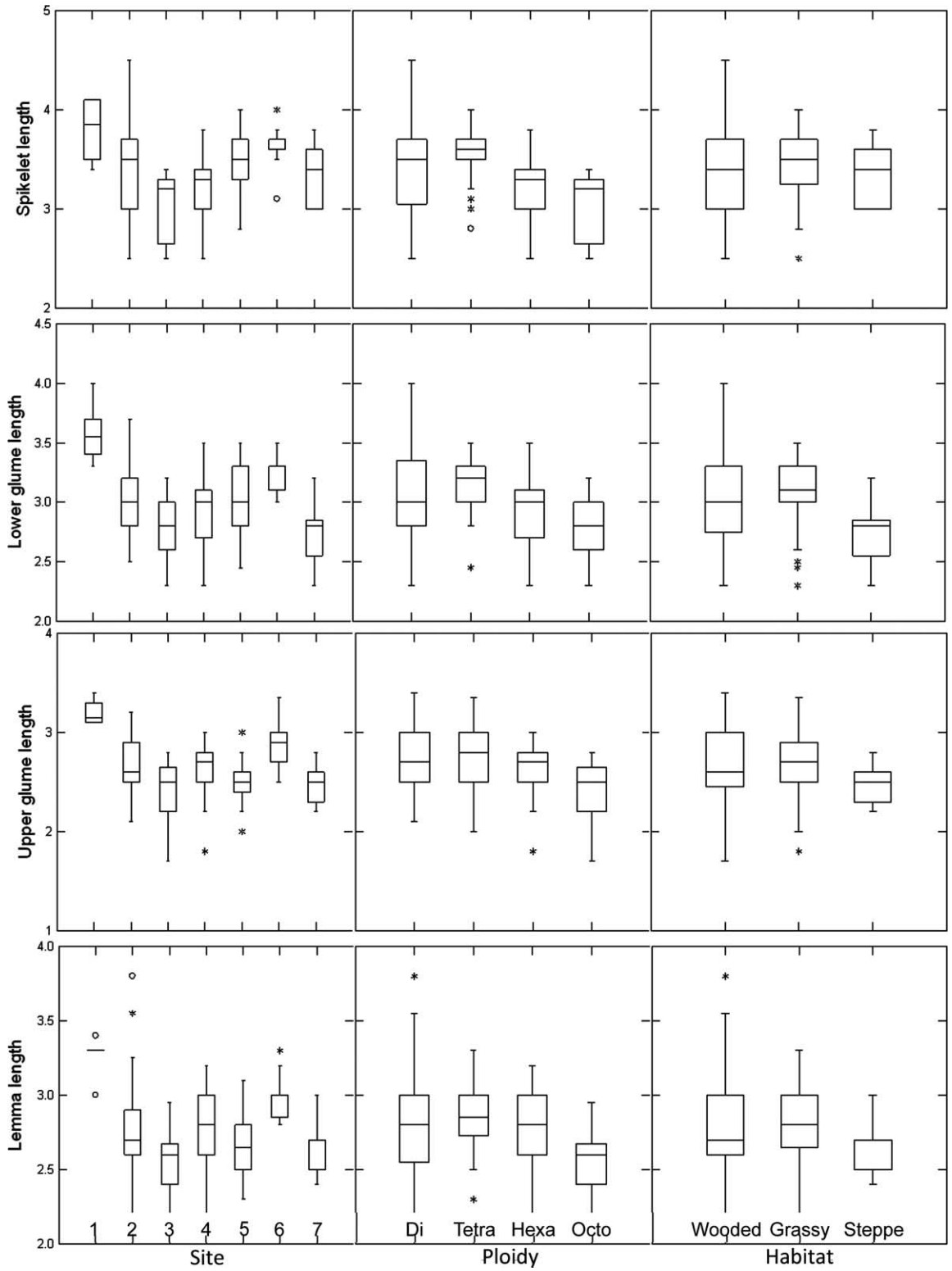

Figure 3. Variation in the continuous reproductive characters by site, ploidy level and habitat. Character codes are given in Table 2. Boxes include 50% of the records; bar is the median value; whiskers extend to the 12th and 87th percentile; asterisks are values between the 6th and 12th or 87th and 92nd percentile; circles are outside these values. Table 1 provides information on sample size and the cytological variation of the 'octoploid' population.

the UPGMA option in NTSYS because it was the most effective in grouping the OTUs into relatively few clusters but even it left some OTUs as outliers of the major clusters. Other linkage algorithms either formed almost no clusters or placed about half the OTUs in a few large clusters and left the others more or less isolated.

The major clusters formed two 'super' clusters (Fig. 5), with clusters A to G containing OTUs from sites 1 through

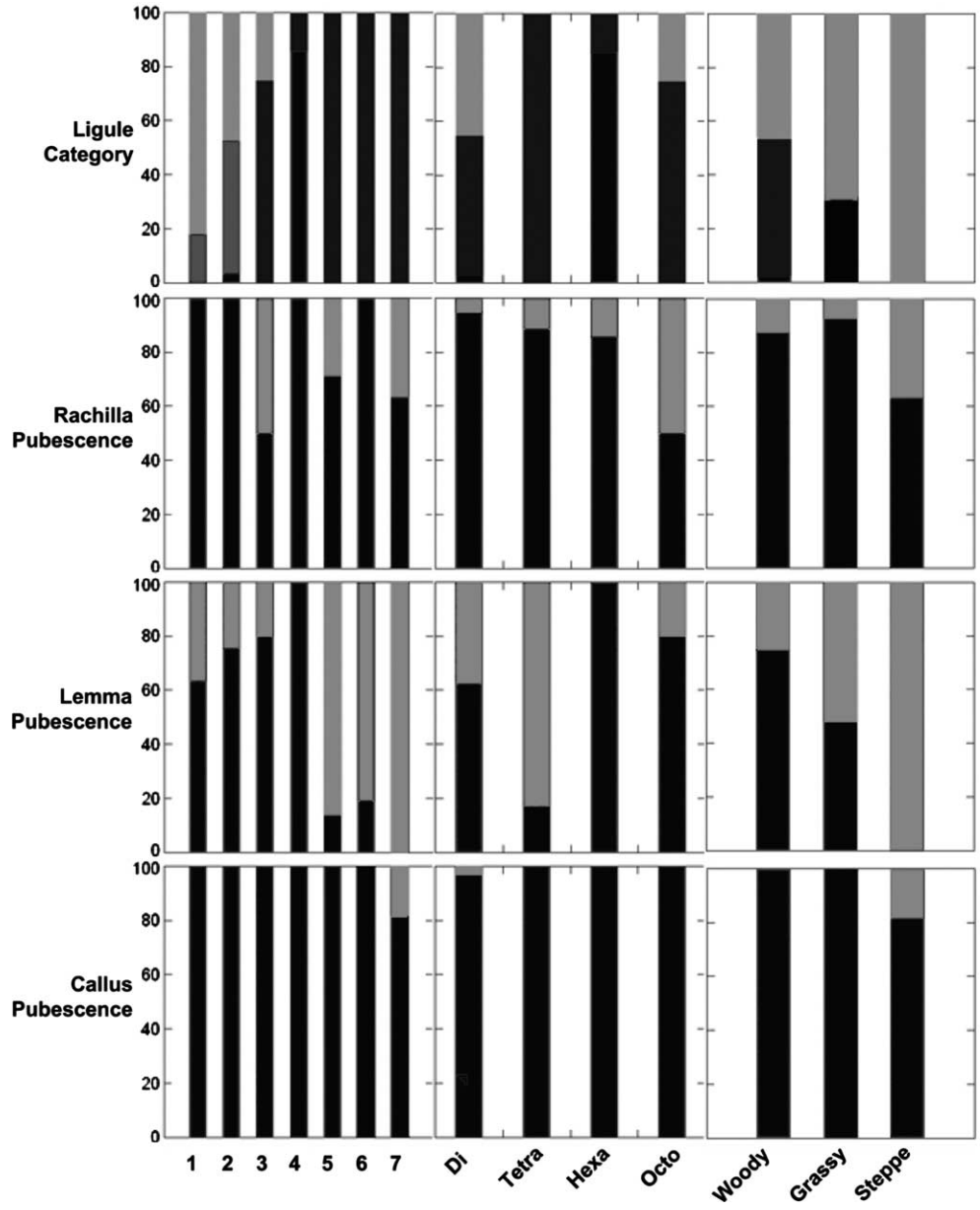

Figure 4. Variation in discontinuous morphological characters by site, ploidy level, and habitat. Character codes and scoring are given in Table 2. Table 1 provides information on sample size and the cytological variation of the 'octoploid' population.

4 and clusters H to J OTUs from sites 5, 6 and 7. Two of the major clusters, C and J, contained only OTUs from a single site (sites 2 and 7, respectively) but cluster C was one of five clusters in which site 2 was represented. Cluster J included all but two site 7 OTUs. All site 6 OTUs were in cluster H, but so were two site 5 and 1 site 7 OTUs. Site 1 OTUs were restricted to clusters A and B, each of which also contained site 2 OTUs.

None of the OTUs from the tetraploid grassy sites (sites 5 and 6) were in a cluster with OTUs from the hexaploid grassy site, site 4. OTUs from site 1, a wooded site, clustered with OTUs from site 2, another wooded site, but not with OTUs from site 3, the wooded site just below the timberline. Its OTUs were clustered with OTUs from sites 2 and 4.

Minimum spanning tree analysis showed that 78.8% of the OTUs had another OTU from the same site as their closest neighbor. Of the remaining 32 links, 7 were to another OTU at the same ploidy level and in a similar habitat, 14 were to another OTU in a similar habitat, but a different ploidy level; 8 were to an OTU in a different habitat but at the same ploidy level. Only three OTUs had as their nearest neighbor an OTU in a population of a different ploidy level and a different habitat.

Multivariate analyses: principal component analysis

The projection of the OTUs onto the first two principal components (Fig. 6A) showed two clouds of points. The lower-left cloud was composed of OTUs from sites 5, 6 and 7. The upper right cloud included all the OTUs from the

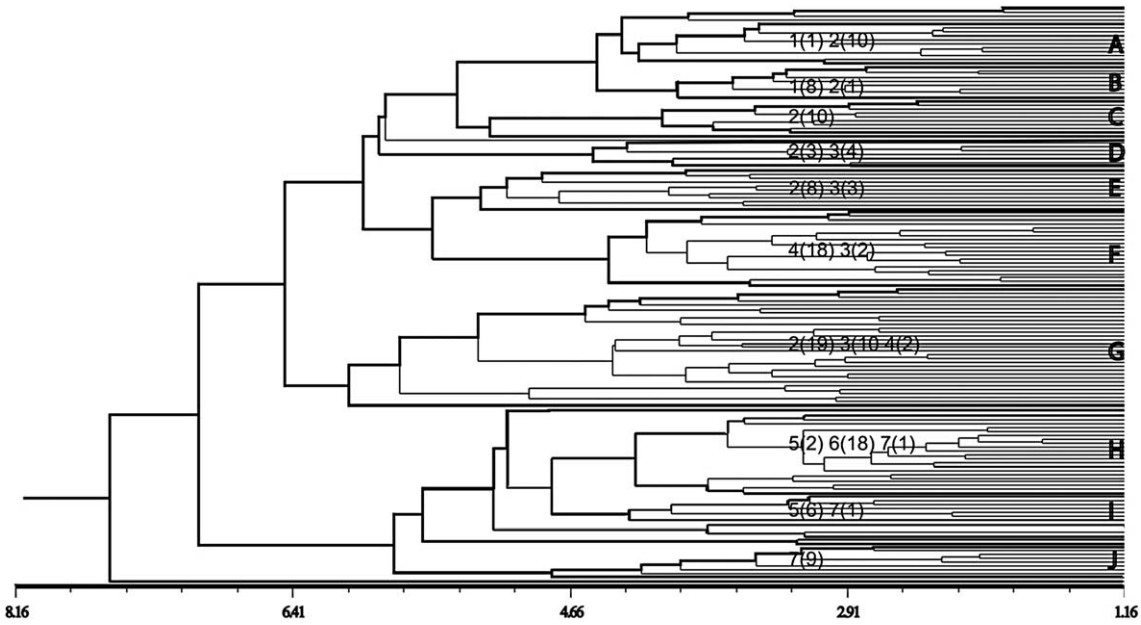

Figure 5. Results of UPMGA cluster analysis of the continuous characters. The numbers within each cluster show how many OTUs from each site were included in that cluster. They do not add to 151 because some OTUs were not included in any of the identified clusters.

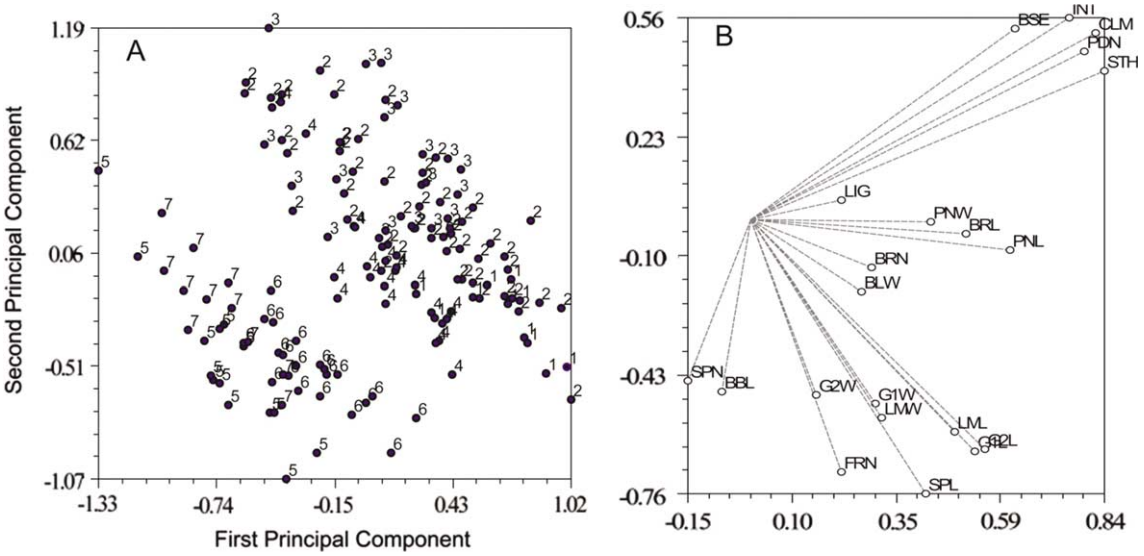

Figure 6. Results of principal component analysis. (A) projection of the OTUs on the first two principal components. These axes accounted for 23.2% and 20.3%, respectively, of the total variation, (B) plot of the eigenvectors on the first two principal components.

other four sites. Site 4 and site 1 OTUs are somewhat localized within the cloud, but site 2 OTUs were scattered throughout the area occupied by the OTUs from sites 1, 3 and 4.

Four of the six characters with the highest loading on the first component (STH, CLM, INT, BSE) were clearly vegetative, and the other two, PDN and PNL are probably under similar genetic control (Table 3). Five of the six characters contributing most to the second component concerned reproductive structures (SPL, FRN, G1L, G2L, LML); the sixth, INT, was vegetative. The six major contributors to the third and fourth components included a mix of vegetative and reproductive characters. LIG contributed little to the first two components but was the top contributor to the third component and third highest contributor to the fourth component.

The plot of the eigenvectors on the first two components (Fig. 6B) showed that the examined characters fell into several clusters. Five vegetative characters, CLM, INI, PDN, BSE and STH formed one cluster. Aspects of panicle size (PNW, BRL, PNL) formed another cluster. Lemma length (LML) clustered with both glume lengths (G1L and G2L), but lemma width (LMW) clustered with the first glume width (G1W) and spikelet length (SPL) whereas the second glume width (G2W) clustered with floret number (FRN). Spikelet number (SPN) was closest to the length of the flag leaf blade (BLL) and branch number (BRN) was closest to the width of the flag leaf blade (BLW).

Table 3. Loadings of the characters on the first four principal components. Characters are listed in descending order of the absolute value of their loading. Characters are abbreviated as in Table 2.

Component 1		Component 2		Component 3		Component 4	
STH	0.842	SPL	–0.757	LIG	0.623	PNW	–0.628
CLM	0.821	FRN	–0.695	PNL	–0.548	SPN	–0.547
PDN	0.793	G1L	–0.639	BRL	–0.510	LIG	–0.522
INT	0.758	G2L	–0.633	BLL	–0.474	BRL	–0.431
BSE	0.629	LML	–0.585	G2W	–0.461	BLW	0.416
PNL	0.616	INT	0.558	SPN	–0.448	LMW	0.313

Multivariate analyses: discriminant analyses

In the site analysis (Fig. 7, top), the discriminant functions correctly classified 83% of the OTUs, but the success rate varied from 100% for OTUs from sites 6 and 7 to 50% for OTUs from site 3. All misclassified site 3 OTUs were placed in site 2. Eight of the nine misclassified site 2 OTUs were placed in site 3; the ninth was placed in site 1. Of the 3 misclassified OTUs from site 5, one was placed in site 6 and the other in site 7 (Table 4).

The first function of the site analysis separated the sites into two clusters, one cluster being composed of sites 1, 2, 3 and 4, the other of sites 5, 6 and 7. The second function resulted in better separation of sites 1–4 with site 4 being the most effectively separated. Characters contributing most to the functions varied depending on whether raw data were used or data standardized by the within group variance. For raw data, the most important characters were G2L, G2W, and LMW (Table 5); for standardized data, they were INT, STH, and PDN. For the second function, G2W, LIG, and LML were the most important for raw data whereas LIG, BSE, and G2W were most important for standardized data.

Discriminant analysis by ploidy level (Fig. 7, lower left) was 81% successful overall, the percentages for the different ploidy levels being 83% for the diploids, 94% for the tetraploids, and 45% for the octoploids (Table 6). Some of the misclassified diploids were placed in each of the other three ploidy levels, with slightly more than half being identified as octoploids. All the misidentified OTUs of higher ploidy levels were identified as diploids. Function 1 separated the diploids and octoploids from the tetraploids and hexaploids. Function 2 separated the tetraploids from the hexaploids. Characters contributing most to function 1 for unstandardized data were G2W, BLW, LIG; for function 2 the most important characters were G2W, G2L, and LIG (Table 7).

Discriminant analysis by habitat resulted in correct classification of all but six OTUs, 5 of which were OTUs from grassy sites. Four of the five were identified as being from the steppe habitat and the fifth from a wooded habitat. The sixth misclassified OTU was from a wooded habitat but was identified as coming from a grassy habitat. OTUs from the wooded habitats had higher values on the first function than those from all the other sites. Values for this function were determined primarily by G2W, LIG, and G2L (raw data) or LIG, PDN, and BSE (standardized data). (Table 7).

Discussion

There have been many studies on the impact of autopolyploidy on plant morphology (Stebbins 1950, Tzvelev 1976) and ecology (Tzvelev 1972, Grant 1981). In general, autopolyploids tend to have longer dimensions than their diploid progenitors (Löve 1953). The consequences of alloploidy are less predictable because of the potential for interaction among the concerned genomes. First generation alloploids may exhibit a combination of intermediate values and extreme values resulting from complementary interactions among the genomes, but natural populations reflect many generations of selection on the original alloploids and

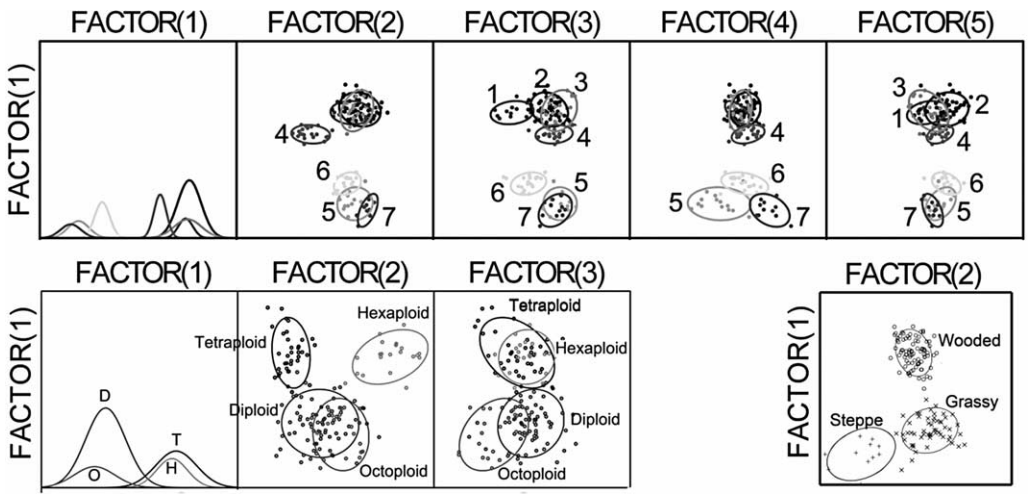

Figure 7. Discriminant analysis of the OTUs by site (top row), ploidy level (bottom row, left side), and habitat (bottom row, right side).

Table 4. Percentage of OTUs correctly classified by discriminant analysis of the sites. Values are from Jackknife classification results. Percentages are rounded to the nearest whole number.

Site	Number of OTUs							% correct
	1	2	3	4	5	6	7	
1	9	2	0	0	0	0	0	82
2	1	43	9	0	0	0	0	81
3	0	10	10	0	0	0	0	50
4	1	0	0	20	0	0	0	95
5	0	0	0	0	11	1	2	79
6	0	0	0	0	0	21	0	100
7	0	0	0	0	0	0	11	100
Overall	11	55	19	20	11	22	13	83

possibly derivatives of backcrosses to the parents or other relatives. Consequently, interpretations of the origin of polyploid populations on the basis of their morphology must be viewed with caution.

The shorter culms, lowest internodes, and sheaths observed in the grassy and steppe sites compared to the wooded sites could reflect environmental selection, pleiotropy, or a combination of the two. The similarity in the pattern of variation among the sites with respect to these characters and with respect to glume and lemma lengths suggests that pleiotropy is involved, at least in part. In woody habitats, competition for light and protection from wind would favor taller plants whereas grassy and steppe habitats, which provide greater light exposure and less protection from wind, may select for shorter structures. Determining the relative importance of these two factors is not possible from our data. Shorter floral parts may be more effective for pollination in woody habitats, but we are not aware of evidence to support or refute such a hypothesis.

Of the seven sites, site 4 is the most distinctive both cytologically and morphologically. Their morphological distinction is noteworthy because both sites 5 and 6 were characterized as being grassy plant communities. The site 4 OTUs came out with plants from the wooded sites on the first axis, but differed from OTUs of all other sites on the second discriminant function to which the reproductive characters contributed most. This suggests the possibility that the third genome present in the population is from a different taxon than that found in the populations at the wooded sites. The characters contributing most to their separation were ligule length, upper glume width, basal internode length, rachilla pubescence, blade width and callus pubescence.

Site 2 was the most morphologically diverse site. This is probably, at least in part, because its sample size was more than twice that of each of the other sites. The steppe OTUs were morphologically similar to those from the two tetraploid grassy sites, but discriminant analysis was able to distinguish them. They differed from the OTUs of all the other sites in having, as a group, shorter culms and internodes, narrower leaf blades, shorter sheaths, longer and more spikelets and longer glumes and lemmas. A few of the OTUs from the steppe site differed from other OTUs at the site and all other OTUs in the study in having lemmas that were pubescent between the veins. This could reflect normal variation in a single gene or introgression from another taxon that is consistently pubescent between the veins.

The similarity of the OTUs from the octoploid wooded site to those from the two diploid wooded sites suggests that they are autopolyploids. On the other hand, the fact that they have, if anything, lower values for all the continuous characters than the diploids, particularly those from site 1, is contrary to what has usually been found in early generation autopolyploids. The OTUs from site 1 have, however, the highest values of all the OTUs for the four reproductive characters in which the sites differ significantly. Why this should be so is not evident. It may be an expression of normal variation within a diploid species in which establishment of somewhat different forms through a

Table 5. Coefficients of the five variables contributing most to the discriminant functions generated by the analysis of data by site. Overall Jackknife classification success 83%. Characters are abbreviated as in Table 2.

	Function 1		Function 2		Function 3		Function 4		Function 5		Function 6	
Unstandardized variables	G2L	−1.36	G2W	−11.77	LMW	8.28	G1W	−1.46	G1W	1.64	G2W	−8.98
	G2W	−1.02	LIG	1.64	LML	−2.27	BLW	−1.46	LML	−1.95	G2W	2.89
	LMW	−0.62	LML	−1.46	G2L	−1.84	G1L	−1.31	G2W	−1.94	BLW	2.19
	SPL	−0.54	SPL	1.36	G1W	−1.76	SPL	1.01	G1W	1.64	LMW	−1.64
	INT	0.43	G1W	1.11	G1L	−1.38	FRN	−0.95	BRN	0.99	LML	−1.55
Standardized by within group variances	INT	0.59	LIG	0.83	CLM	0.82	SPN	0.91	BSE	1.01	STH	−0.57
	STH	0.55	BSE	0.66	LML	0.54	STH	0.62	CLM	0.94	BLW	0.56
	PDN	0.49	G2W	−0.57	LMW	0.50	BRL	−0.51	BRN	0.70	CLM	0.49
	SPN	−0.46	SPL	0.49	G2L	−0.48	FRN	−0.43	BRL	−0.63	G2W	−0.43
	BLL	−0.39	LML and STH	−0.35	BSE	−0.47	G1L	−0.38	LML	−0.47	STH	−0.40

Table 6. Percentage of OTUs correctly classified by discriminant analysis of ploidy levels and habitats. Values are from Jacknife classification results. Percentages are rounded to the nearest whole number.

Ploidy level	Number of OTUs				% correct	Habitat	Number of OTUs			% correct
	Di	Tetr	Hex	Oct			Wood	Grass	Steppe	
Di	62	4	1	8	83	wood	83	1	0	99
Tetr	2	33	0	0	94	grass	1	51	4	91
Hex	2	0	19	0	90	steppe	0	0	11	100
Oct	11	0	0	9	45	overall	84	52	15	96
Overall	77	37	20	17	81					

Table 7. Coefficients of the variables contributing most to the discriminant functions generated by the analysis of data by ploidy level and habitat. Overall Jackknife (JK) classification success is given in the top line. Characters are abbreviated as in Table 2.

	Analysis by ploidy Level (JK = 81%)						Analysis by habitat (JK = 96%)			
	Function 1		Function 2		Function 3		Function 1		Function 2	
Unstandardized variables	G2W	6.96	G2W	9.40	G2W	5.22	G2W	8.25	G2W	3.29
	BLW	1.53	G1W	−2.70	LMW	1.52	LIG	−1.39	BLW	2.33
	G2L	1.11	LIG	−1.33	BLW	−1.33	G2L	1.16	G1W	1.92
	LIG	−1.07	LMW	1.01	G1W	−1.07	G1W	−1.15	SPL	−1.39
	SP;	−1.02	LML	0.96	BRL	−0.94	LMW	0.81	G1L	1.27
Standardized by within group variances	PDN	−0.77	STH	0.90	BRL	−0.67	LIG	−0.89	BLW	0.62
	BSE	−0.66	LIG	−0.71	SPN	0.50	PDN	−0.84	SPN	−0.62
	LIG	−0.57	BSE	−0.48	BSE	−0.46	BSE	−0.53	SPL	−0.57
	BLW	0.46	G2W	0.46	BRN	0.45	SPN	0.48	BSE	−0.52
	SPL	−0.38	BRN	−0.37	BLW	−0.40	G2W	0.43	G1L	0.44

combination of genetic drift and local adaptation is to be expected.

Individuals from the grassy sites, regardless of ploidy level, had shorter ligules, wider glumes and shorter peduncles than those from the wooded sites. The shorter peduncles may reflect adaptation to a better light environment, but it is hard to see any advantage arising from the shorter ligules and wider glumes. Their fixation in such communities may be a result of linkage with advantageous physiological genes or location on a portion of their genome that is conserved.

Our goal in this study was to obtain a better understanding of the complex pattern of variation in the *P. orinosa* complex by focusing on the variation within and among different populations over a 6 km transect. It revealed that plants within the populations were relatively uniform, but that there were sufficient differences among the populations for successful discrimination using the examined morphological characters.

The diploid plants from the steppe habitat were readily distinguished from those of the two diploid wooded habitats. This supports their placement in different taxa. The similarity of the tetraploid plants from the grassy habitats to those in the steppe habitat suggests that they may be autotetraploid derivatives of the steppe habitat diploids. The morphological distinctness of the hexaploid population is most easily explained by invoking the presence of a genome of unknown origin in its chromosome complement. The octoploid population from the wooded site is morphologically similar to the other populations from wooded habitats, both of which were diploid, suggesting that the plants may be autooctoploids.

Using current taxonomic treatments, plants from sites 1–4 would be identified as *Poa orinosa* or its synonym, *P. versicolor* var. *orinosa*, whereas those from sites 5–7 would be identified as *P. plurifolia* or its synonym, *P. sphondylodes* var. *erikssonii*, largely on the basis of the exposure of the top cauline node. Our results suggest that the hexaploid plants should be placed in a third taxon, but doing so before further evaluation of the hypotheses arising from the present study would be premature.

Acknowledgements – We thank Dr R. J. Soreng for many discussions, for making it possible to examine material from BM and SU, and his comment as a reviewer; curators of BM, LE, PE, SU, TK for the loan of specimens; Mr Zh-T. Wang for organizing the expedition for Olonova and Chen; and Dr V. E. Efimov for invaluable help in the statistical analyses. This research was supported by grants from the National Natural Science Foundation of China (no. 3041112011, 30270102), the Russian Fund for Basic Research (no. 1004 00637a) and the Utah Agricultural Research Station, Utah State Univ. It is approved as journal paper no. 8194 of the Utah Agricultural Experiment Station.

References

Grant, V. 1981. Plant speciation, 2nd ed. – Columbia Univ. Press.

Löve, Á. 1953. Subarctic polyploidy. – Hereditas 39: 113–124.

Mehra, P. N. and Sharma, M. L. 1975. Gramineae. – In: Löve, Á. (ed.), IOPB chromosome number reports XLIX. – Taxon 24: 501.

Olonova, M.V. et al. 2008. Towards the study of bluegrass complex *Poa versicolor* Bess. (Poaceae) in northern China.

– Animadvers. Syst. ex Herb. Kryloviano Univ. Tomskensis 99: 16–24, in Russian.
Pausheva, Z. P. 1988. Practical work in plant cytology. – Agropromizdat, in Russian.
Probatova, N. S. 1985. Poaceae. – In: Charkevicz, S. S. (ed.), Vascular plants of the Soviet Far East. Vol. 1. Nauka, pp. 89–382, in Russian.
Probatova, N. S. 2007. Chromosome numbers in family Poaceae and their significance for systematics, phylogeny and phytogeography. – V. L. Komarov Memor. Lect. 55: 9–103, in Russian.
Rohlf, F. J. 2010. NTSYSpc: numerical taxonomy system, ver. 2.21j. – Exeter Software.
Rusanovich, I. I. and Skvortsov, A. K. 1981. Use of discriminant analysis for the discrimination of birch species by their leaf shape. – Zhurn. Obshchey Biol. 42: 762–769, in Russian.
Stebbins, G. L. 1950. Variation and evolution in plants. – Columbia Univ. Press.
Thiers, B. (continuously updated). Index herbariorum: a global directory of public herbaria and associated staff. – New York Bot. Gard. Virt. Herb. – <http://sweetgum.nybg.org/ih/>.
Tzvelev, N. N. 1972. The significance of hybridization processes in the evolution of grasses. – In: Vasilchenko, I. T. (ed.), History of the flora and vegetation of Eurasia. – Nauka, pp. 5–16, in Russian.
Tzvelev, N. N. 1976. Grasses of the USSR. – Nauka, in Russian.
Zhu, G. et al. 2006. *Poa* subg. *Stenopoa* (Dum.) Soreng & L. J. Gillespie. – In: Wu, Z. et al. (eds), Poaceae, Flora of China. Vol. 22. Sci. Press, Missouri Bot. Gard. Press, pp. 295–309.

Appendix 1.

Representative specimens. Site 1: China, northern part of Taihang mountain ridge, Baihua mountain, 39°49′N, 115°35′E, 1790 m a.s.l.; deciduous (mainly birch) forest; 5 Oct 2005; Wang Zh.-T., Chen W.-L., Olonova, M. V. (TK). Site 2: China, northern part of Taihang mountain ridge, Baihua mountain, 39°49′N, 115°35′E, 1800 m a.s.l.; deciduous (birch and larch) forest; 5 Oct 2005; Wang Zh.-T., Chen W.-L., Olonova, M. V. (TK). Site 3: China, northern part of Taihang mountain ridge, Baihua mountain, 39°50′N, 115° 35′E, 1810 m a.s.l.. Deciduous forest near timberline; 5 Oct 2005; Wang Zh.-T., Chen W.-L., Olonova, M. V. (TK). Site 4: China, northern part of Taihang mountain ridge, Baihua mountain, 39°50′N, 115°35′E, 1810 m a.s.l.; grass meadow among bushes above timberline; 5 Oct 2005; Wang Zh.-T., Chen W.-L., Olonova, M. V. (TK). Site 5: China, northern part of Taihang mountain ridge, Baihua mountain, 39°50′N, 115°35′E; 1810 m a.s.l.; grass and forbs meadow above timberline; 5 Oct 2005; Wang Zh.-T., Chen W.-L., Olonova, M. V. (TK). Site 6: China, northern part of Taihang mountain ridge, Baihua mountain, 39°50′N, 115° 35′E; 1810 m a.s.l.; forb (mainly) and grass meadow above timberline; 5 Oct 2005; Wang Zh.-T., Chen W.-L., Olonova, M. V. (TK). Site 7: China, northern part of Taihang mountain ridge, Baihua mountain, 39°50′N, 115° 36′E; 1810 m a.s.l.; steppe community with *Stipa* above timberline; 5 Oct 2005; Wang Zh.-T., Chen W.-L., Olonova, M. V. (TK).