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Panax ginseng natural populations: their past, current state and perspectives[1]

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Key words

Panax ginseng; natural population; genetic diversity; mating system; embryology; allozymes; AFLP; SSR

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Abstract

Aim: The mating system of *Panax ginseng*, genetics and ontogenetic structure of its natural populations of Primorye (Russia) were investigated. **Methods:** Genetic diversity was assessed using allozyme and the fluorescently based automated amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) markers. **Results:** Total genetic diversity at species level is low with allozyme assay (0.023), and high with AFLP (0.255) and SSR (0.259) methods. It is observed within populations according to allozyme (>99%), AFLP (>85%), and SSR (>73%) assays. The indices of genetic variability distribution point out the re-colonization of the Sikhote-Alin by ginseng plants from southern refuges during the warming period in the early Holocene. The capability of ginseng plants to cross- and self-pollinate was shown and the assumption that *Panax ginseng* is a facultative apomictic plant was confirmed. The reproductive system of ginseng possesses high plasticity and stability of the fertilization process that help the species to survive in stress conditions. Disturbances caused by external or internal factors can be reduced due to the morphogenetic potential of ginseng ovule or apomictic embryo development. Analysis of life stages structure of ginseng populations demonstrates that all of them are not full-constituents because some life stages are absent or occur rarely. **Conclusion:** In all 3 populations, virgin and young generative individuals are predominant. This means that populations studied are viable and the reintroduction of natural ginseng population is possible yet.

Introduction

Panax ginseng C.A. Meyer is a well-known herb of Oriental traditional medicine, used by people for thousands of years as a valuable remedy for various diseases and a tonic drug for the elderly.

In ancient manuscripts (see review in Zhuravlev *et al*, 1996[1]) ginseng was already described as a rare plant. It was introduced into culture in ancient China and Korea. However, wild-growing ginseng was always considered to be of the most value. It was distributed in the Far Eastern forests on a wide territory, but because of intensive use of wild ginseng roots its area was drastically reduced, particularly in the last century. Indeed, in the first half of XX century wild-growing ginseng plants could be found on the large forestlands of China, Korea and Russian Primorye[2], but nowadays its original distribution area has shrunken to a few habitats in Russia and China. The largest, the Sikhote-Alin population, is located in the southern part of the Sikhote-Alin mountain range. Another population is located in the Nadezhdinsk and Khasan Districts of Primorsky Kray, Russia (Khasan population), and Jilin and Heilongjiang Provinces, China, and a third, the Blue Mountain population, is located in the Spassk District of Primorsky Kray, Russia. Any reports about findings of wild ginseng in China have been rare. In Russia, ginseng was listed in Red Book since 1975 as a threatened species[3]. Nowadays Primorsky Kray of Russia is the only place in the world where natural ginseng populations are still alive and therefore our duty is to conserve this wonderful plant. Ginseng

populations are now close to extinction, and some urgent measures have been suggested to protect natural ginseng resources from exhaustion[4]. Moreover, to change the situation and to work out measures for protection of wild-growing ginseng, in 1997 the Primorye regional administration, Regional Committee of Natural Resources and Institute of Biology and Soil Science of the Russian Academy of Sciences (IBSS) elaborated the "Regional Complex Long-term Program of Restoration (Reintroduction) of Primorye's Ginseng Population up to 2005". This program was based on many years' study of various aspects of ginseng biology conducted in IBSS.

During the program execution, IBSS has been providing centers of reintroduction with scientific justification for the selection and propagation of sampled material and for offspring identification, choice of number and emplacement of centers of reintroduction, search for more intact habitats for reintroduction and detailed recommendations for creation of reintroduced populations. During the program, genetic analysis of extant ginseng populations, studies on their morphological features and mating system were carried out to understand the processes taking place in ginseng populations.

Ginseng is known to be a relic of the Tertiary era[2] and its prosperity in extant habitats depends on its individual ability to adapt to the environment. Estimation of genetic and morphological variability, heterogeneity of populations, and differentiation within and among populations are usually the best way to describe the state of an endangered species[5,6]. At the same time, genetic variation of plant populations is known to be largely determined by evolutionary history and mating system of the species[7]. Therefore, elucidation of these items would be useful for understanding both the current state and perspectives of natural ginseng populations. Moreover detailed study of the ginseng mating system and embryology is urgent for propagation of sampling ginseng plants in reintroduction centers. Significant items of the conservation program are an evaluation of the current state of ginseng populations, including numbers, age composition and viability, and analysis of spatial and temporal variability of coenopopulations. Nowadays ontogenetic analysis of populations is widely used for resource estimation of medicine plants[8,9]. Such investigations should be conducted to forecast the fate of the exhausted natural populations of *Panax ginseng*.

In this paper, we summarize the results of our investigations on the ginseng mating system, genetics and ontogenetic structure of its natural populations for evaluation of its current state and perspectives.

Material and methods

Sampled populations Wild-growing *Panax ginseng* plants were sampled from 3 non-protected natural populations: Khasan (*Kh*), Blue Mountain (*BM*) and Sikhote-Alin (*SA*). Collected living plants were transferred to an open experimental nursery in the natural ginseng habitats (Spassky District of the Primorsky Kray) for further investigations; a part of the plants was also transferred to the laboratory greenhouse.

Sample material Fresh leaves or those frozen in liquid nitrogen were used for allozyme, amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSR) analyses. A total of 206 plants of wild *Panax ginseng* were studied with allozyme analysis (34 from *Kh*, 49 from *BM* and 123 from *SA*). For AFLP assay, 35 *Panax ginseng* plants were studied (10 from *SA*, 14 from *BM*, and 11 from *Kh*), and 57 plants were studied with SSR markers (20 from *SA*, 19 from *BM*, 18 from *Kh*).

For ontogenetic research, wild ginseng plants were pictured and their above-ground organs were collected, dried and studied.

Mating system experiments were conducted in the laboratory greenhouse and the nursery over 4 years. For pollination experiments and embryology studies, flowering 5–25-year-old ginseng plants from natural populations and 5-year-old cultivated plants were used.

Allozyme analysis Enzyme extraction, electrophoresis, enzyme staining and interpretation were carried out as described in Koren *et al*[10]. Enzymes were extracted from fresh leaves or those frozen in liquid nitrogen.

AFLP and SSR analysis Genomic DNA was extracted according to Echt *et al*[11] and then purified by Murray and Thompson[12].

Amplified fragment length polymorphism analysis was conducted according to Vos *et al*[13]. DNA was digested using the restriction endonucleases *Eco*RI and *Mse*I. AFLP primers for pre-amplification contained 2 selective nucleotides. The *Eco*RI primers contained 3 selective nucleotids and 4 selective nucleotids were used for the *Mse*I primers. AFLP adapters and primers were synthesized by "SibEnzim" (Russia). Selectively amplified products were analyzed using the ABI 3100 automated DNA sequencing system (Applied Biosystems, USA). Each of the *Eco*RI-NNN primer types was labeled with FAM fluorescent dye at the 3' end. Size alignment of the AFLP fragments was carried out with ABI GeneScan Analysis Software (Applied Biosystems) with the ABI GeneScan 500 LIZ internal lane

size standard. DNA fragments ranging from 50 to 500 bp in size from the AFLP analysis were scored.

Primers designed to flank 2 microsatellite loci (CT12, CA33) isolated from the ginseng genome were 5'-GAGAG TAACC ACAGG ATAGA GAAA-3' (CT12 F), 5'-CTCCC TTTAC AGGTA GATAG TGAA-3' (CT12 R), and 5'-CGATG TGGAT TTCAA TTTTA AG-3' (CA33 F), 5'-GGTCT ATGAG CCTAG TTTTC ATG-3' (CA33 R)[14]. Reaction mixtures and PCR amplification profiles were carried out according to Qin *et al*[14].

Polymorphism was detected by automated capillary electrophoresis of fluorochrome-labeled polymerase chain reaction (PCR) products. The forward primers were synthesized and labeled with fluorochrome R110 or R6G by "Syntol" (Russia). Duplex PCR products were separated with an ABI 310 DNA sequencer (Applied Biosystems) and the fragments were sized by a ladder labeled with a fluorochrome LIZ. Bands used in scoring ranged in size from 50 to 500 bp.

Data analysis Presence/absence matrices ("1" for present, "0" for absent) for AFLP and SSR markers were generated for all scorable bands for each DNA sample. These matrices were analyzed by POPGENE[15] to estimate polymorphism parameters at both the population and species levels. An exact test for population differentiation[16] and Nei's genetic distance and identity[17,18] were calculated for all pairwise combinations of populations by TFPGA[19]. Parameters of genetic diversity were estimated with the TFPGA[19] program from all loci studied including monomorphic ones. Dendrograms were constructed using UPGMA with TFPGA[19].

Mating system experiments For self-pollination and cross-pollination treatments, flowers were emasculated in buds and pollinated by mature pollen. A single pollen donor per plant was used in the outcross treatments. For agamospermy examination, all flowers were emasculated in buds and isolated from insects, so that seeds could result from agamospermy only. Intact ginseng plants were observed as a control. Fruits of all tested plants were harvested after maturation in the last week of September.

Pollen morphology and embryology Pollen from newly emerged anthers was fixed and uniformly stained with acetocarmine[20] to evaluate pollen size and fertility. Ovules were fixed in a mixture of formaldehyde and acetic acid under vacuum. Fixed ovules were dehydrated, processed through paraffin wax, sectioned at 5–8 μm thickness, stained with haematoxyline/eosin or with haematoxyline/alcian blue and visualized using a Zeiss Axioplan microscope. To study early stages of megagametophyte development we used the clearing technique for angiosperm ovules[21]. Examination of autofluorescence in ovules was carried out with a LSM 510 META confocal laser scanning microscope (Carl Zeiss, Germany) equipped with argon laser. Confocal z-stack images and single plans were obtained after excitation at 488 nm and emission at 522 nm using a 505-nm LP filter. The intensity of the Ar laser was 6%.

Ontogenetic study According to the Rabotnov–Uranov scheme[22], 9 different life stages were classified in the life history of *Panax ginseng*[23]: latent period: seeds (drupes); pregenerative (virgin) period: seedlings, juveniles, immatures, virgin young, virgin adults; generative period: generative young, generative medium, generative mature. Types of natural populations were determined according to Rysin and Kazantseva[24]. Terms for morphology description of plants were used following Serebryakov[25] and ontogenetic terminology[8].

Results

Genetic structure of extant ginseng populations

Electrophoretic analysis of the 25 *Panax ginseng* enzymes revealed gene products from 39 putative loci, only 3 of which were polymorphic. In general, the level of allozyme polymorphism was low for all ginseng populations studied. The lowest level of diversity was observed in *SA* (Table 1). The F-statistics analysis of the natural ginseng population (Table 1) indicates a decrease of total gene diversity in *SA*. F_{IT} (individual inbreeding coefficient for the species) and F_{IS} (individual inbreeding coefficient in respect to a sub-population) values demonstrate a small deficit of heterozygotes in all 3 populations studied, and the highest individual inbreeding coefficient for the species, F_{IT}, is observed in *Kh*, indicating that processes of inbreeding and gene shift are more intense in this population than in 2 others. Proportion of the total diversity among sub-populations (F_{ST}), demonstrates a low level of subdivided ginseng populations, supposing the most part of a total genetic diversity is observed within sub-populations. The most level of subdividing is observed in *SA*, whereas there is no subdividing in *BM*.

All 4 labeled selective primers used for AFLP analysis revealed polymorphism (Table 2). Using DNA from 35 plants, the primers initiated the synthesis of 214 amplicons, of which 202 were polymorphic. It corresponds to a level of polymorphism of 94.39% (Table 3). Of the polymorphic bands, 112 were found in a sample of the *SA*, 167 in the *BM* and 161 in the *Kh* (Table 2). The indices of genetic

Table 1. Parameters of genetic diversity and F-statistics analysis of *Panax ginseng* natural populations based on allozyme data. H_E, the mean expected heterozygosity; H_O, the mean observed heterozygosity; H_T, total genetic diversity; *n*, sample size.

Population	H_O	H_E	H_T	F_{IS}	F_{IT}	F_{ST}
Kh (*n*=34)	0.024	0.027	0.026	0.0018	0.0029	0.0002
BM (*n*=49)	0.021	0.025	0.025	0.0002	0.0003	0.0000
SA (*n*=123)	0.010	0.016	0.022	0.0029	0.0010	0.0019
Mean for 3 populations	0.018	0.023	0.024	0.0010	0.0031	0.0041

Table 2. Primers used to study on a genetic variability of the ginseng natural populations.

	Primer pairs	Number of fragments in populations						Mean for three populations	
		SA		*BM*		*Kh*			
		*T**	*P*	*T*	*P*	*T*	*P*	*T*	*P*
AFLP	Eco(ACT)/Mse(CCTA)	50	28	50	38	50	39	50	50
	Eco(ACA)/Mse(CCGG)	62	26	62	47	62	45	62	58
	Eco(ACA) Mse(CCAC)	35	23	35	28	35	26	35	34
	Eco(ACT)/Mse(CCGA)	67	35	67	54	67	51	67	60
	Total	214	112	214	167	214	161	214	202
SSR	CT12	7	2	7	4	7	1	7	5
	CA33	7	5	7	6	7	4	7	7
	Total	14	7	14	10	14	5	14	12

* - *T*- total number of fragments; *P* - number of polymorphic fragments

Table 3. Genetic variations within and among natural populations of *Panax ginseng* based on AFLP and SSR data. G_{ST}, genetic differentiation among populations; *h*, Nei's gene diversity; *na*, number of alleles per locus; *ne*, effective number of alleles per locus; *p*, percentage of polymorphic loci.

	Populations	*p*	*na*	*ne*	*h*	G_{ST}
AFLP	*SA* (*n*=10)	51.87	1.5187	1.2726	0.1601	
	BM (*n*=14)	78.4	1.7804	1.3966	0.2371	
	Kh (*n*=11)	75.23	1.7523	1.4211	0.2483	
	Total	94.39	1.9439	1.4172	0.2555	0.1484
SSR	*SA* (*n*=20)	50.00	1.5000	1.2713	0.1546	
	BM (*n*=19)	71.43	1.7143	1.4690	0.2787	
	Kh (*n*=18)	35.71	1.3571	1.2319	0.1386	
	Total	85.71	1.8571	1.4417	0.2591	0.2608

variability in the *SA* were the lowest (Table 3).

Both microsatellite loci used in the study were polymorphic when considered for 57 representatives of 3 populations. A total of 14 alleles were detected over SSR primer sets; 7 for CT12 and 7 for CA33 (Table 2). The genetic diversity estimated with SSR markers was high and coincident with that obtained with AFLP markers (Table 3).

Using Nei's genetic distance matrix based on allozyme and SSR data UPGMA dendrograms are constructed. According to both dendrograms, genetic distance between *Kh* and *BM* is very small, whereas distance between these 2 populations and *SA* is much greater (Figure 1).

Mating system and developmental processes taking place in *P*anax *ginseng* Results of the mating system ex-

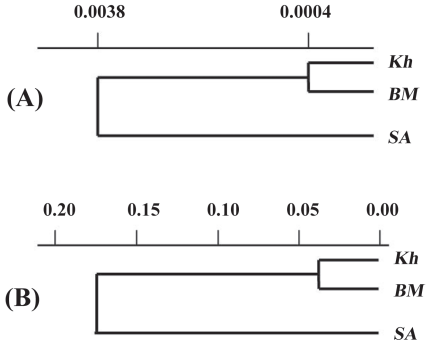

Figure 1. Dendrograms of genetic relationship among *Panax ginseng* populations based on the allozyme (A) and SSR (B) analyses.

periments are summarized in Table 4.

Ginseng pollen is prolate spheroidal in equatorial view and triangular in polar view, with slightly flattened poles. The mean pollen diameter was 27.4029±3.8151 μm. This value is close to the one published for Korean ginseng[26]. Division of the nuclei into pollen grains occurs before the anther undergoes dehiscence. Pollen grains fertility averaged 56%, highlighting disturbances in microsporogenesis.

Most *P*anax *ginseng* ovaries exhibited tetrasporic female gametophyte development. The ovary is 2-locular and each loculus contains 2 ovules. Upper loculus has a reduced embryo sac, but normal development of the upper ovule is rarely observed (Figure 2a). Normal embryo sac

Table 4. Mating system experiments for *Panax ginseng* into nursery and greenhouse.

Treatment	1999		2000	2004		2005		Average	
	*n**	Ss, %	*n*	Ss, %	*n*	Ss, %	*n*	Ss, %	
Intact plants	110	39.6±5.7	264	61.1±5.9	328	66.1±6.8	814	60.2±8.7	56.75±6.8
Autogamy	175	40.9±7.6	116	57.3±5.4	47	55.5±7.7	148	60.3±1.3	53.5±5.5
Outcrossing	559	26.5±4.1	231	---	76	64.1±1.5		---	45.3±2.8
Agamospermy	153	22.9±2.7	283	---	316	11.8±5.9	628	2.6±2.2	9.4±3.6

* -*n*- number of flowers; Ss - seed set; data shown as the mean±SD.

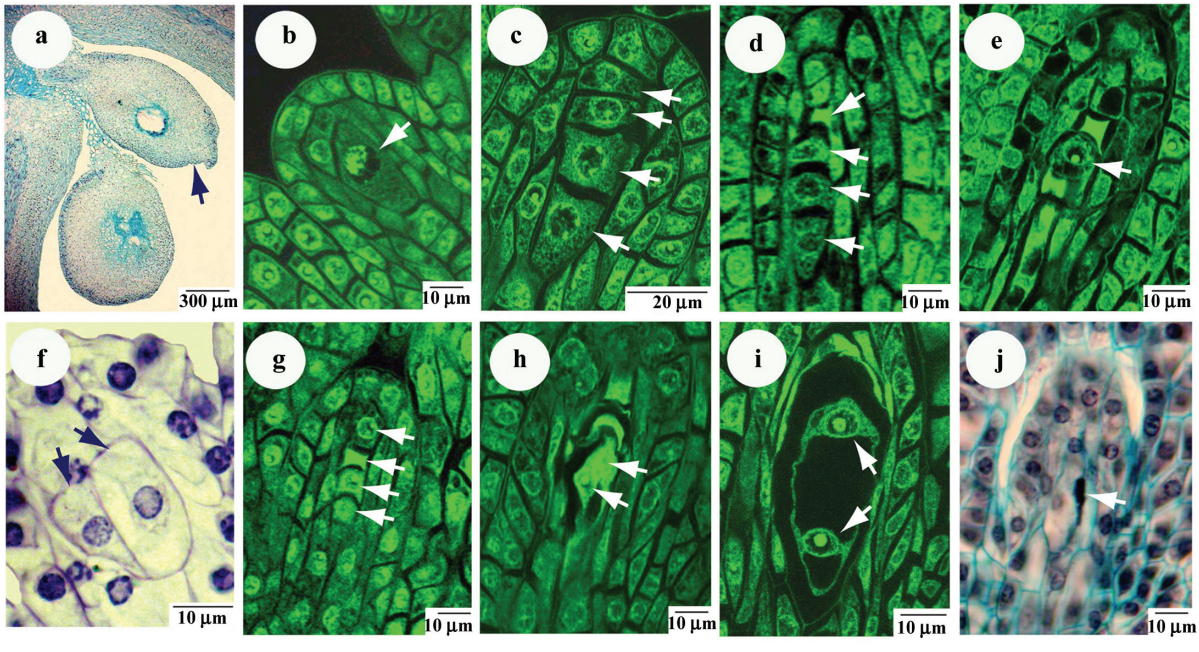

Figure 2. Female megagametophyte development in *Panax ginseng.* (a) Normal development of upper ovule; (b) mother megaspore cell; (c) lineage of 4 megaspores; (d) degeneration of upper megaspore; (e) functional megaspore; (f) couple of mother megaspore cells; (g) disturbance in succession of megaspores degeneration; (h,i) abnormal and normal location of the nucleus at the beginning stages of embryo sac formation, respectively; (j) degeneration of all megaspores.

develops in the lower loculus. Often ginseng plants have formed fruits with one loculus. The differentiation of the archesporium in *P*anax *ginseng* occurs early.

The polygonum-type of embryo sacs is developed in *P*anax *ginseng*'*s* ovules. The lower anatropous, tenuinucellate ovules bears a large central megaspore mother cell (MMC, Figure 2b). Meiotic division of MMC leads to 4 (or sometimes 3) megaspores arranged in a line (Figure 2c). After that, 3 (or 2) megaspores degenerate (Figure 2d) whereas the last one enlarges and becomes a functional megaspore with vacuolated cytoplasm (Figure 2e). Mature female gametophytes usually consist of 7 cells: 3 chalazal antipodals, a single large one with fused central nucleus near the egg apparatus, 2 synergids, and an egg cell at the mycropylar end.

In *P*anax *ginseng*, several deviations take place during megagametophyte development. Absence of the mother megaspore cell or its duplication are often observed (Figure 2f). Disturbances in succession of megaspores degeneration are revealed in Figure 2g. Sometimes there is no visible debris of degenerated megaspores during embryo sac formation. Nuclei are not separated at the beginning stages of embryo sac formation (Figure 2h, 2i). Very often the degeneration of all megaspores in ovule is occurred (Figure 2j) and embryo sac does not form. Megagametophytes development in different ovules of one ovary is asynchronous.

Double fertilization is asynchronous. After fertilization, the synergids degenerate and their debris is observed as 2 darkly stained bodies near the micropyle. The egg cell fertilization precedes the central cell fertilization by premitotic syngamy. The fusion of the first sperm cell with nucleus of the central cell of the embryo sac gives rise to the nuclear endosperm, followed by fertilization of the egg cell by the second sperm cell. The times of pollen tube outgrowth, fertilization and the resting period of the zygote are slightly different to the same data for Chinese ginseng[27]. Most of the normal developing ovules are fertilized at the post pollination stage for about 3 days. Rates of zygotic and parthenogenetic embryo development are different. After cross-pollination, embryos undergo a short period of dormancy.

The unfertilized egg cell and polar nucleus are not degenerated in the 10 days after flower opening.

Ontogenetic structure of natural ginseng populations Life stage structure (ie, relative proportion of each life stage in the studied populations) is shown in Figure 3. These data were used to establish the types of populations and to evaluate their vital force as reflected in the Discussion.

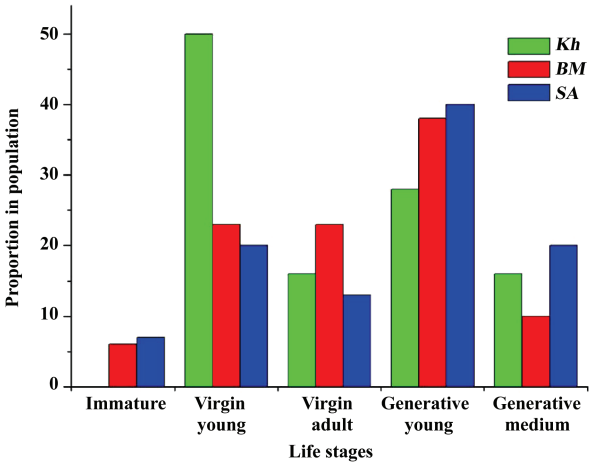

Figure 3. Life stage structure of natural populations of *Panax ginseng*.

Discussion

The evolutionary history of *P*anax *ginseng* is known to influence its genetic diversity[28]. Our data on allozyme polymorphism indicate that Russian's ginseng populations have apparently experienced a severe genetic bottleneck during their evolutionary history. Although *P*anax *ginseng* is restricted to the ancient area where glaciation was absent, there have been several climatic fluctuations that would have affected the distribution of this species. In particular, during the last period of climate cooling (about 18 000 to 20 000 years ago) the territory of Central Sikhote-Alin was occupied with tundra phytocoenosis,[29] which was unfavorable for ginseng. Therefore, the lowest level of variation observed in *SA* could be attributed to the founder effect due to the lack of a Sikhote-Alin ginseng refugium during the recent period of climate cooling. In early Holocene (10 500 to 8000 years ago), intensive warming of the climate promoted the disappearance of permafrost in mountains and the expansion of thermophilic plants from southern refuges to the north[30,31]. Probably, recolonization of Sikhote-Alin by ginseng may have occurred in that period. This assumption is in full agreement with the computation of evolutionary time from the mean Nei's genetic distance between populations on the base of allozyme data. According to Nei's "molecular clock" supposed for allozyme data[32], DN=0.0016 obtained in our study corresponds to approximately 8000 years (considering the mean rate of mutation equal to 10^{-6}).

The results of F-statistics analysis[33] show that genetic diversity is decreased from south-west, *Kh*, towards the north-east, for example *SA* (Table 1). Perhaps, the center of genetic richness and, possibly, the ginseng origin, was

situated south-west of the location of populations studied in the present work. Therefore, study on the genetic diversity of ginseng plants growing outside Russia would be decisive to clear this presumption and improve our understanding of the evolutionary history of *P*anax *ginseng*.

The low level of allozyme polymorphism of natural ginseng populations is in agreement with results obtained recently by other techniques used for evaluating genetic polymorphism of *P*anax *ginseng*[1,34–36]. Allozyme analysis examines only the coding portion of the species DNA and therefore cannot cover the variability of the entire genome. In contrast, PCR-based techniques allow extensive genetic sampling over the entire genome and reveal as a rule the higher genetic variability. Automated capillary electrophoresis of fluorochrome-labeled PCR products is considered as the detecting system with the highest resolving power. The AFLP method[13] has the advantages of PCR technology and the stringency of the restriction fragment length polymorphism (RFLP) technique and therefore AFLP markers are highly reliable and reproducible ones. SSR markers were used in this research because they provide one of the most hypervariable and reproducible marker systems[37].

Exact test of differentiation of ginseng sample studied with SSR markers showed a highly significant difference between *SA* and *BM* and *SA* and *Kh* (P=0.0000), whereas differences in allele frequencies between the *Kh* and *BM* were not significant (P=1.0000). Exact test of population differentiation studied with AFLP markers showed a significant difference between the *Kh* and *BM* (P=0.0077), whereas differences in allele frequencies between the *SA* and *BM* and between *SA* and *Kh* were not significant (P=1.0000 and P=0.4531, respectively). Such genetic patterns demonstrate the discriminative power of SSR markers that exceed this ability of AFLP markers and testify to the heterogenity of the studied *P*anax *ginseng* sub-populations. The AFLP data allows us to classify the *SA* as the youngest among 3 populations under study; this is in full concordance with allozyme data.

Dendrograms constructed using allozyme and SSR markers were similar and showed that *Kh* and *BM* clustered together but *SA* joined them at a certain genetic distance, confirming the genetic remoteness of *SA* from *Kh* and *BM* (Figure 1). For an allozyme-based dendrogram, the mean Nei's genetic distance (DN) between populations was 0.0016, whereas for the dendrogram based on SSR markers, the mean DN between populations was higher and amounted to 0.1258 (Figure 1). With AFLP markers the genetic remoteness of *Kh* plants was shown. It was reported that the type of molecular markers used influenced the patterns of relationships revealed in diversity studies[38,39]. Thus, dendrograms of genetic similarity of sorghum germplasm based on *Pst*I/*Mse*I AFLP markers produced clusters that were different to those based on *Eco*RI/*Mse*I AFLP or SSR markers[39]. The marker sets that adequately cover the entire genome will provide a more precise estimate of genetic relationships[39].

According to AFLP, the index of population differentiation (Gst) was equal to 0.1484 (Table 3), which indicates on approximately 15% of genetic diversity among the ginseng populations. This is closely related to the pattern revealed by the allozyme analysis of wild *P*anax *ginseng* (Fst=0.1896)[40] and wild *P*anax *quinquefolius* (Gst=0.176)[41], and to the pattern obtained by RAPD for cultivated *P*anax *quinquefolius* (Gst=18%)[42]. According to SSR, the proportion of variation found among populations was equal to 0.2608 (Table 3) and was coincident with the population differentiation obtained with RAPDs for *P. ginseng* natural populations, Gst=23.9%[43], and for *P*anax *quinquefolius* natural populations, Gst=28%[43]. By the fluorescently-based automated AFLP method over 40% of the genetic variation was shown to be among populations of wild *P.* stipuleanatus[44].

Allozymes and RAPD assays have provided an average Gst<19% for outbreeding species, 21.2%-24.0% for species with a mixed mating system, and 58.7%-59.6% for inbreeding dicots[45,46]. At the same time, regardless of the breeding system, the total gene diversity at the species level was partitioned primarily between populations (Gst=0.556-0.924), as was shown by RAPD markers for 3 orchid species with different mating systems[47]. Therefore, a species breeding system often plays a central role in determining the distribution of genetic variation within and between populations[45], and other life history traits affect the species population structure too[47]. According to RAPD data, the value of Gst at the species level for *P*anax *ginseng* was 23.9%[43], indicating *P*anax *ginseng* possesses a mixed mating system.

The capability of ginseng to produce seeds via autogamy, outcrossing or agamospermy without pollination was shown early[48]. Because renewal of ginseng population is only possible via seed reproduction, this item required more detail research.

Seed set of autogamy experiments were comparable with controls over 4 years, demonstrating the absence of any self-incompatibility barrier in *P*anax *ginseng*. So, the capability of ginseng plants to cross- and self-pollinate is unlimited, although seed sets in the nursery are larger than

in the greenhouse. Results of agamospermy tests confirm our assumption that *P*anax *ginseng* is a facultative apomictic plant with a diplospory type of agamospermy. This type of apomixis occurs without a reduction in chromosome number due to disturbances in meiosis and formation of diploid megaspores. In this work, we studied ginseng embryology to observe embryo development in different ways of seed formation.

Seed development in angiosperms is provided with 3 steps: the fertilized egg cell develops into the embryo, the fertilized central cell gives rise to the endosperm, and the ovule's integument(s) form the seed coat. During sexual reproduction, these processes are dependent upon double fertilization, but for agamospermy, one or more of these developmental pathways are activated in the absence of fertilization and some anomalies in reproductive structures and embryogenesis are often observed in this case[50.51].

Agamospermy is common among the *Araliaceae* family; for example, it was shown experimentally in *Acanthopanax sessiliflorum, Aralia mandshurica, Eleutherococcus senticosus*[20,52]. A shift to higher levels of inbreeding or asexual reproduction is supposed to be often associated with a processes of rapid colonization and may be caused by selection[53,54]. Agamospermy in *P. ginseng* may be its ancient adaptation that does not play a significant role in seed reproduction nowadays. On the other hand, asexual reproduction may be a relatively recent acquirement of the species due to the low plant density within natural populations.

Gametophytic anomalies such as asynchronization and anomaly in embryological processes in one inflorescence, discords in developmental stages of embryo and endosperm in different ovules indicate the conversion of species into another avenue of seed reproduction[20,55]. These disturbances are considered to increase the evolution potential of species[56].

The reproductive system of ginseng possesses high plasticity and stability of fertilization processes that help it to survive in stress conditions (delay of pollination, unfavorable temperature). Disturbances caused by external or internal factors are reduced due to morphogenetic plasticity of ginseng plants and their capacity to vary the method of embryo development in agreement with these characteristics.

According to the coenopopulation analysis scheme, *BM* and *SA* are characterized as populations of perennials with moderate environmental conditions for seed reproduction (N_3[23]), where the number of virgin plants is approximately equal to generative young and medium individuals. In life history structures of these populations, there are no individuals in the youngest life stages (seedlings and juveniles) and immature and generative mature individuals are rare. The generative young stage is predominant in *BM* and *SA*, whereas the virgin young stage prevails in *Kh*. So, *Kh* can be considered as a population being in unfavorable conditions for seed reproduction (N_4[23]). As a whole, the analysis of life stage structure of ginseng populations revealed that virgin and generative (except generative mature stage) individuals were the most abundant in all habitats studied.

Analysis of life stages structure of studied ginseng populations demonstrates that they can be considered normal, but all of them are not full-constituent because some life stages are absent or occur rarely. This may be connected with ginseng roots being used as a raw material for medicine, when the whole plants that are mainly of the generative mature stage are extracted from populations continuously. Just this stage is lost in all populations studied. Moreover, renewal of ginseng populations happens via seed reproduction only. The pregenerative (virgin) period of *P*anax *ginseng*, (ie, the increase of vegetative weight) is very long; therefore, restoration of the life stage structure of the extant natural ginseng population requires much time. However, because all 3 populations studied are normal (viable), we hope that reintroduction of natural ginseng population is possible yet.

Genetic and population data presented in this paper characterize *Panax ginseng* rather controversially. On one hand, this plant demonstrates a high level of mating system plasticity, multiplicity of ways of reproduction, and an unprecedentedly wide interval of longevity. Such signs are usually inherent in flourishing species. However, this plant is very rare in natural habitats; it possesses only moderate genetic variability and its population structures are not full-constituent for all 3 populations studied. These last signs are indicators of depression. One can suppose that not only overharvesting but some organic defects in the plant body constitution or in its life strategy are responsible for such a complicated situation. The history of the Araliaceae family dates back to ancient times and some later biochemical inventions are absent in body of the ginseng plant. For example, cell wall structure is not sufficiently hard to stop the distribution of fungi or bacterium infection. Such deficiencies can be made up with more effective reproduction only partly since mechanisms of seed spread are restricted also. Some other antagonisms can be laid bare in further analysis. Therefore, only reintroduction oriented to overcome the diversity of shortcomings in ginseng genetics, biochemistry and life style can be successful. To fit these purposes,

the stock material for reintroduction has to have the maximal genetic diversity possible for local population. It may be most important for the current state, the overharvesting and habitat-destroying human activity must be stopped and regulated [57,58].

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Author contribution

Yuri N ZHURAVLEV, Olga G KOREN, and Galina D REUNOVA designed research; Olga G KOREN, Galina D REUNOVA, Tamara I MUZAROK, Tatiyana Yu GORPENCHENKO, Irina L KATS, and Yuliya A KHROLENKO performed research; Yuri N ZHURAVLEV contributed new reagents or analytic tools; Olga G KOREN, Galina D REUNOVA, Tatiyana Yu GORPENCHENKO, and Irina L KATS analyzed data; Yuri N ZHURAVLEV, Galina D REUNOVA, and Olga G KOREN wrote the general parts of paper; Olga G KOREN, and Galina D REUNOVA, Tamara I MUZAROK, Tatiyana Yu GORPENCHENKO, Irina L KATS, and Yuliya A KHROLENKO wrote the related sections of paper.

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