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PLANT GENETICS

Genetic Relationships among Far Eastern Species of the Family Araliaceae Inferred by RAPD Analysis

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Abstract—A molecular genetic study of Far Eastern species of the family Araliaceae by means of RAPD analysis was conducted. Using 21 primers we assessed variability at 595 loci. Based on matrices of genetic distances *D*, dendrograms of genetic relationships among eleven species of this family were constructed. Our results suggest that *Acanthopanax sessiliflorus* and *Eleutherococcus senticosus* belong to different genera, *Aralia cordata* and *A. continentalis* are different species, and *A. elata* and *A. mandshurica* probably cannot be regarded as distinct species. Genetic similarity of Far Eastern *A. cordata* and American *A. hispida* is shown.

INTRODUCTION

Most members of the family Araliaceae (Juss.) are tropical or subtropical species. They are abundant in tropical regions of America, Australia, Oceania, and especially in East and Southeast Asia [1]. The worldwide diversity of this family is represented by 80 genera comprising about 900 species [2].

The systematics of the family Araliaceae has not been yet satisfactorily developed. Oskol'skii [3] reports eight existing systems of this family. The latest of these is the system by Takhtadzhyan [4], according to which the family Araliacea includes seven tribes: Plerandrea, Schefflereae, Meryteae, Hedereae, Aralieae, Mackinlayeae, and Myodocarpeae. Most Far Eastern Araliaceae species belong to the tribe Schefflereae, and species of the genus *Aralia*, to the tribe Aralieae.

In the Far East, the family is represented by eight species from five genera [5].

The natural range of *Panax ginseng* C.A. Meyer is currently represented by populations inhabiting only the Primorye area. Species of the genus *Panax* are attributed to the tribe Panaceae by most authors [3] and to the tribe Araliae by Takhtadzhyan [4].

Among Far Eastern Araliaceae species, *Kalopanax septemlobus* (Thunb.) Koidz. has the largest range that includes southern Far East, northeastern China, Korea, and Japan.

The range of *Oplopanax elatus* (Nakai) Nakai is limited to southern Primorye and northern Korea.

Acanthopanax sessiliflorus (Rupr. et Maxim.) Seem. or *Eleutherococcus sessiliflorus* (Rupr. et Maxim.) S.Y. Hu inhabits Primorye, southern Khabarovsk krai, southeastern Amur oblast, northern and middle Korea, northeastern China.

The range of *Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim. is larger including additionally central Khabarovsk krai, Sakhalin and Japan (Hokkaido). This species appears similar to *Acanthopanax sessiliflorus* differing from it in habitus, type of inflorescence, and the presence of thorns [5]. Some authors assign the genera *Eleutherococcus* and *Acanthopanax* to the single genus *Acanthopanax* [2, 3, 5, 6], whereas others regard them as distinct genera [7, 8].

The genus *Aralia* L. in the Russian Far East is represented by three species [5]. The range of *A. elata* (Miq.) Seem. is almost as large as that of *Kalopanax septemlobus*. Until recently, another species, *A. mandshurica*, was recognized, which, in contrast to *A. elata*, was thought to be continental [7, 9, 10]. Today the latter two species are regarded as one, *A. elata* [2, 8, 11].

Aralia continentalis Kitag. dwells in southern Primorye, China, and Korea.

Aralia cordata Thunb. occurs in Sakhalin, Kuril islands and Japan. Some authors assign *A. continentalis* to *A. cordata* [12] but many recognize them as separate species [2, 13, 14].

In recent years, approaches involving molecular DNA markers, RAPD analysis among them [15, 16], have been used in population–genetic, phylogenetic, and taxonomic studies. RAPD analysis is widely used for inferring genetic relationships in plants [17–22].

In this study, the results of DNA examination in Far Eastern Araliaceae species by means of RAPD analysis are presented and genetic relationships among them are evaluated.

MATERIALS AND METHODS

Material of the study. We examined 11 species of the family Araliaceae:

(1) *A. elata*; (2) *A. mandshurica*; (3) *A. continentalis*; (4) *A. cordata*; (5) *A. hispida*; (6) *Eleutherococcus senticosus*; (7) *Acanthopanax sessiliflorus* (*Eleuthero-*

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coccus sessiliflorus); (8) *Kalopanax septemlobus;* (9) *Oplopanax elatus*; (10) *Panax ginseng*; and (11) *P. quinquefolius.* Plants of the first nine species were taken from the collections of the Botanical Garden of the Far Eastern Division, Russian Academy of Sciences and Highland–Taiga Station (Far Eastern Division, Russian Academy of Sciences); two *Panax* species were obtained from the collection of the Institute of Biology and Soil Science (Far Eastern Division, Russian Academy of Sciences). All of the species examined occur in Primorye except *A. cordata* introduced from the Sakhalin Island and *A. hispida* and *P. quinquefolius* introduced from the American continent.

DNA isolation. DNA was isolated from 500 mg of dried leaves from five randomly selected plants of each species taken in equal quantities as described in [23]. DNA was analyzed by electrophoresis in 1% agarose gels containing ethidium bromide, in the TBE buffer. The DNA amount was assessed by comparing the intensity of the band with that of bands of phage lambda DNA of the known concentration.

PCR was conducted in a thermal cycler UNO II 48 (Biometra, Germany) with 10-base primers with arbitrary nucleotide sequences (Operon, United States) (Table 1) using the reaction mixture and temperature regime described in [24]. The control probe contained the total amplification mixture without DNA. Each reaction was replicated two to four times.

The PCR products were electrophoretically separated in 1.4% agarose gels containing ethidium bromide and photographed in UV light. To estimate the size of the fragments (amplicons), we used *Eco*RI + *Hin*dIII restriction fragments of the phage lambda DNA (Fermentas, Lithuania). The amplicon designations consist of the name of the corresponding primer and its size in bp.

Statistical treatment of the RAPD data was conducted by means of comparative computer-aided analysis of the RAPD bands of the specimens examined. The negatives of the electrophoregrams obtained were fed into a computer using an Astra 3450 scanner (Umax) and then presented as a binary matrix using the RFLPscanPlus 3.12 procedure. Only the fragments reproduced in the replicate experiments were taken into account whereas intensity polymorphism was not considered. Genetic similarity (dissimilarity) indices, obtained in pairwise comparisons of DNA specimens from different species, were estimated by the following formulas:

$$D = 1 - M_{XY}/N_t, \quad \text{(1) package TREECON [25];}$$

$$D = 1 - 2N_{XY}/N_X + N_Y, \quad \text{(2) package TREECON [26];}$$

$$S = 2N_{XY}/N_X + N_Y, \quad \text{(3) package NTSYS-pc [27];}$$

where D is genetic difference, S is genetic similarity, N_X and N_Y are the numbers of fragments in specimens X and Y, N_{XY} is the number of common fragments shared by specimens X and Y, N_t is the total number of the fragments, and M_{XY} is the total number of the alleles shared by specimens X and Y.

Table 1. Description of the primers

Primer	Nucleotide sequence	Number of fragments
OPA-10	GTGATCGCAG	28
OPA-12	TCGGCGATAG	27
OPA-20	GTTGCGATCC	34
OPB-06	TGCTCTGCCC	25
OPB-12	CCTTGACGCA	31
OPC-02	GTGAGGCGTC	32
OPC-05	GATGACCGCC	21
OPC-08	TGGACCGGTG	24
OPC-14	TGCGTGCTTG	29
OPC-15	GACGGATCAG	31
OPD-02	GGACCCAACC	33
OPD-05	TGAGCGGACA	28
OPD-07	TTGGCACGGG	28
OPD-11	AGCGCCATTG	31
OPD-13	GGGGTGACGA	31
OPD-20	ACCCGGTCAC	31
OPE-11	GAGTCTCAGG	29
OPE-18	GGACTGCAGA	30
OPF-04	GGTGATCAGG	25
OPF-10	GGAAGCTTGG	28
OPF-14	TGCTGCAGGT	19
Total		595

We constructed the dendrograms of the relationships among the species examined on the basis of the genetic similarity (dissimilarity) matrices using the unpaired pair group method with arithmetic averages (UPGMA) [28] (program packages TREECON and NTSYS-pc, version 1.7) and the neighbor joining (NJ) [29] method (TREECON) with bootstrap estimates of branching order reliability [30].

RESULTS AND DISCUSSION

By preliminary screening of 120 commercial primers, 84 primers effective in PCR with ginseng DNA were selected [31]. Some of them were used in RAPD analysis of the 11 species from the family Araliaceae (Table 1). Based on a comparison of the RAPD patterns obtained, variability of 595 loci (on average 28.3 loci per primer) was estimated. The RAPD patterns of the Araliaceae species obtained with primer OPC-15 are presented in Fig. 1. Using the POPGENE program [32], the amplicon frequencies in the patterns were determined and intraspecific genetic polymorphism of the Far Eastern Araliaceae was estimated. Only three of the amplicons (OPA-10-518, OPC-02-788, and OPC-08-1074)

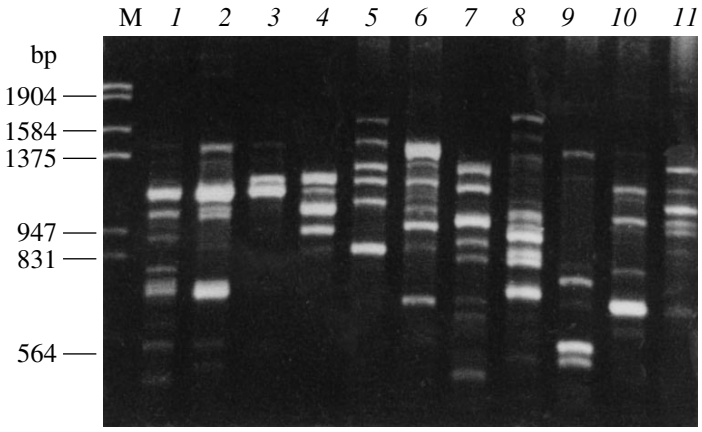

Fig. 1. The DNA amplification products of members of 11 Araliaceae species with primer OPC-15. (*1*) *A. mandsurica*; (*2*) *Aralia elata*; (*3*) *A. continentalis*; (*4*) *A. cordata*; (*5*) *A. hispida*; (*6*) *Eleutherococcus senticosus*; (*7*) *Acanthopanax sessiliflorus*; (*8*) *Kalopanax septemlobus*; (*9*) *Oplopanax elatus*; (*10*) *Panax ginseng*; (*11*) *P. quinquefolius.* M, *Eco*RI/*Hin*dIII restriction fragments of phage λ DNA.

were present in the RAPD patterns of all the species studied whereas other amplicons were shared by several species or occurred only in one of them. Polymorphism in the species of the genus *Aralia* constituted 54.5%. Polymorphism between two morphologically close *Panax* species [21] was substantially lower (25.38%).

These results are reflected in the dendrograms of genetic relationships produced by statistical analysis of the RAPD data using different methods of calculating genetic distances and clustering. Figure 2a presents a tree constructed by UPGMA from the matrix of distances *D* calculated by Eq. (1) (see Materials and Methods). As seen on this tree, the species are grouped in two clusters according to their genetic relatedness. The first cluster includes members of the genera *Aralia, Kalopanax, Oplopanax, Acanthopanax*, and *Eleutherococcus*; the species of the genus *Panax* form the second cluster, which is genetically distant from the first one. All species of the genus *Aralia* form a group with a high bootstrap index (99%) whereas the group *Kalopanax, Acanthopanax, Oplopanax*, and *Eleutherococcus* is characterized by a low bootstrap index (38%).

Figure 2b shows an UPGMA tree constructed using another method of estimating genetic distances (from Eq. (2), see Materials and Methods). Here the species are also divided into two clusters. All species of the genus *Aralia* form a separate cluster. A characteristic feature of this dendrogram is that the species of the genus *Panax* are included in the second cluster as a subgroup together with *Eleutherococcus.* The branching order in this cluster is supported by low bootstrap values.

On the UPGMA genetic similarity dendrogram that is constructed from genetic distances obtained by Eq. (3) (Fig. 2c; see Materials and Methods), the Far Eastern Araliaceae species group in the same clusters as in Fig. 2b.

The species *A. continentalis* and *A. cordata* are in different branches on all of the dendrograms. The genetic similarity between these species is lower than that between the species of the genus *Panax* (Table 2). This indicates that *A. continentalis* and *A. cordata* are different species, which agrees with the view of a number of authors [2, 13, 14]. The genetic similarity between *A. elata* and *A. mandshurica* is considerably higher than that between the other species of the genera *Aralia* and *Panax* (Table 2), which corroborates the opinion of the authors who consider *A. elata* and *A. mandshurica* a single species [2, 8, 11].

The unrooted tree presented in Fig. 3 is obtained by the neighbor joining (NJ) method from the distances calculated by Eq. (2) (see Materials and Methods). The authors of the NJ method think that it is more suitable for constructing phylogenetic trees than, for example, UPGMA [29]. Indeed, branches of the NJ tree are supported by higher bootstrap values as compared to the UPGMA trees (Figs. 2a, 2b). On the NJ tree, the Far Eastern Araliaceae species also form two groups according to their genetic relatedness (Fig. 3). One group consists of all species of the genus *Aralia* while the second includes all remaining species. This phylogenetic dendrogram indicates that *A. cordata* and *A. hispida* are more ancient than the other species of the family Araliaceae. They are also more ancient than the examined *Panax* species, which diverged later. *Aralia elata* is the youngest of the Far Eastern Araliaceae species. Oskol'skii has presented evidence showing that the wood of *A. elata* is more specialized than in the other species, which testifies to the evolutionary advancement of the former [3]. Data on Fig. 3 also suggest that *A. elata* and *A. mandshurica* are species *in statu nascendi.*

Cumulatively, these results show that, in spite of some differences, the dendrograms of genetic relatedness between the Araliaceae species examined share a number of features. For instance, the Far Eastern Araliaceae species mainly cluster into two groups irrespective of the method of obtaining genetic distances and

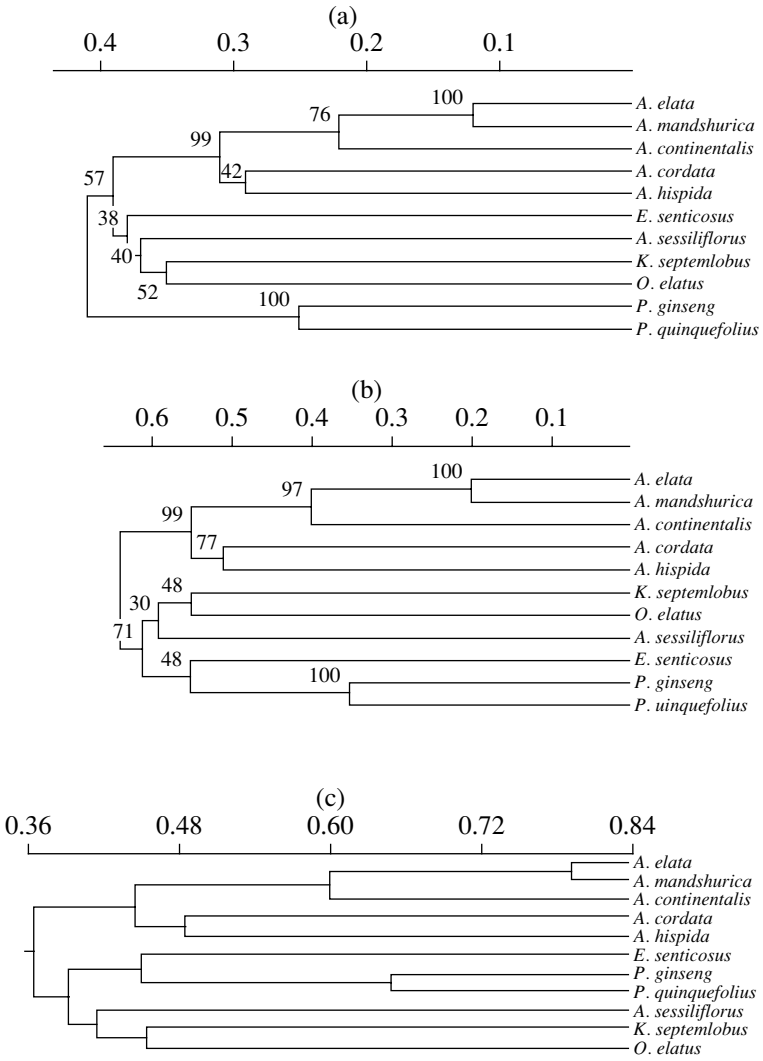

Fig. 2. The UPGMA dendrograms of genetic relationships among the species of the family Araliaceae constructed on the basis of the following formulas (see Materials and Methods): a: (1); b: (2); c: (3).

constructing the trees. One group includes the species of the genus *Aralia* forming a cluster with a specific structure at high bootstrap support. The remaining species form the second cluster, which is supported by lower bootstrap values (Figs. 2b, 2c; Fig. 3). The low bootstrap support may be explained by the fact that the second cluster contains several genera of the family represented by one or two species whereas the first cluster includes the species of the single genus *Aralia*. The same explanation probably accounts for the observed instability of the inner structure of the second cluster, which is manifested mainly by varying position of the ginseng species on the trees.

All the trees obtained also show that the species of the genus *Aralia* in the first cluster are not grouped according to the character of their vital forms. This is in contradiction with the system proposed by Chinese taxonomists who classified the Araliaceae species from China into two subgenera: *Aralia* including trees and shrubs and *Paralia* including perennial grasses [33]. According to our results, the herbaceous species *A. continentalis* groups with the arboreal species *A. elata* and *A. mandshurica* and the herbaceous species *A. cordata* groups with the fruticulose species *A. hispida* (Figs. 2, 3).

Our results demonstrate that American *A. hispida* is genetically closer to Far Eastern species *A. cordata* than to *A. elata* and *A. continentalis* (Figs. 2, 3). The genetic similarity between *A. cordata* and *A. hispida* and between *P. ginseng* and *P. quinquefolius* may be explained by the existence in the ancient time of a close link between Asia and North America through the Beringia, which permitted migration of North American and East Asian plant species. According to Kurentsova [34], members of the genus *Panax* could migrate into America only by this pathway.

As follows from the dendrograms, the species *E. senticosus, A. sessiliflorus, K. septemlobus,* and

Table 2. Matrix of genetic similarity among 11 species of the family Araliaceae

	1	2	3	4	5	6	7	8	9	10	11
1	1.0000										
2	0.7944	1.0000									
3	0.5886	0.6111	1.0000								
4	0.4013	0.4159	0.5159	1.0000							
5	0.4196	0.4587	0.4592	0.4850	1.0000						
6	0.3750	0.4133	0.3448	0.3248	0.3910	1.0000					
7	0.3473	0.3507	0.3738	0.3210	0.3280	0.4370	1.0000				
8	0.4000	0.3719	0.3616	0.3417	0.3506	0.4455	0.4456	1.0000			
9	0.3553	0.3922	0.3195	0.2911	0.3516	0.3937	0.3842	0.4548	1.0000		
10	0.3840	0.3863	0.3562	0.3641	0.3990	0.4665	0.3498	0.4055	0.4221	1.0000	
11	0.3822	0.4000	0.3757	0.3840	0.3627	0.4348	0.3463	0.3714	0.3588	0.6497	1.0000

Note: Species: (1) *Aralia elata*; (2) *A. mandsurica*; (3) *A. continentalis*; (4) *A. cordata*; (5) *A. hispida*; (6) *Eleutherococcus senticosus*; (7) *Acanthopanax sessiliflorus*; (8) *Kalopanax septemlobus*; (9) *Oplopanax elatus*; (10) *Panax ginseng*; (11) *P. quinquefolius*.

O. elatus are genetically less close to one another than the species of the genus *Panax*, which fact probably reflects their affiliation to different species.

The genetic similarity between *Acanthopanax sessiliflorus* and *Eleutherococcus senticosus* is probably lower than the similarity between species within a genus, being comparable to the similarity between species of different genera (Table 2). This confirms the view of the authors who assign *Acanthopanax sessiliflorus* and *Eleutherococcus senticosus* to different genera [7, 8].

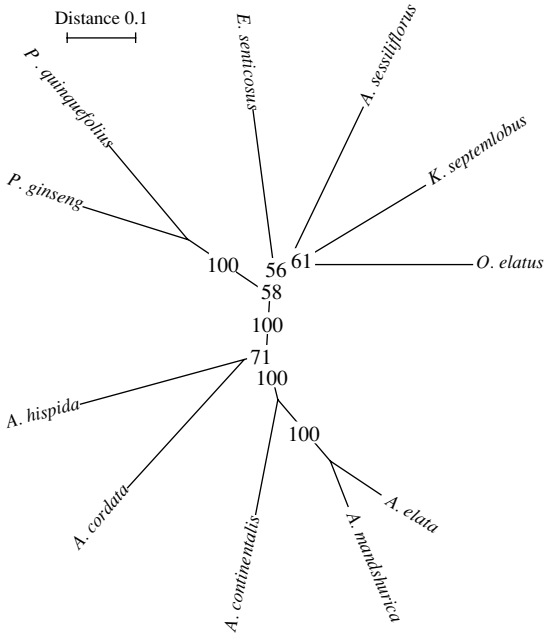

Fig. 3. The NJ dendrogram of phylogenetic relatedness of 11 species of the family Araliaceae constructed on the basis of $D = 1 - 2N_{XY}/N_X + N_Y$.

None of the methods employed classified the ginseng species with aralias (Figs. 2, 3). Consequently, the species of the genus *Panax* cannot belong to the tribe Aralieae as supposed Takhtadzhyan [4]. On our dendrograms, depending on the method of computing *D*, the ginseng species either clustered with *A. sessiliflorus*, *K. septemlobus*, *O. elatus*, and *E. senticosus*, (Figs. 2b, 2c; Fig. 3) or stand separately from all other species (Fig. 2a) "acclaiming" a position in an independent tribe. Interestingly, the tribe Panaceae is present in five out of eight systems of the family Araliaceae given by Oskol'skii [3]. However, the results of our studies do not provide an unambiguous solution to the issue of the taxonomic status of the genus *Panax* among the species of the family Araliaceae.

Thus, we for the first time ever conducted a genetic study of Far Eastern species of the family Araliaceae using molecular DNA markers. The results of this study indicate that *Acanthopanax sessiliflorus* and *Eleutherococcus senticosus* belong to different genera, and *Aralia cordata* and *A. continentalis* are different species. *Aralia elata* and *A. mandshurica* probably cannot be regarded as distinct species. Genetic similarity of Far Eastern *A. cordata* to American *A. hispida* was shown. The examination of a greater number of species from different genera, including those outside of the family, and the employment of other DNA markers will shed light on the issues that were not resolved in this study.

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