INHERITANCE OF ALLOZYMES AND GENETIC VARIATION IN NATURAL POPULATION OF JAPANESE YEW IN PETROV ISLAND, RUSSIA

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ABSTRACT

Fifteen enzyme systems encoded by 22 loci were assayed in Japanese yew (*Taxus cuspidata* Sieb. et Zucc.). Mendelian inheritance was confirmed for allozymes at 10 loci by testing the fit of band-pattern segregation in megagametophytes from heterozygous trees to the expected 1:1 ratio. Linkage relationships were examined for 40 pairs of allozyme loci showing joint segregation. Five pairs of loci appear to be linked: *Adh:Skdh-2* with a recombination frequency (R) = 0.26, *Skdh-2:Idh-1*, with R = 0.32, *Idh-1:Skdh-1*, with R = 0.29, *Skdh-1:Peplgg-3*, with R = 0.23, and *Aat-3:Gpt*, with R = 0.24. The population exhibited high level of genetic variation, with observed and expected heterozygosities estimated for all loci equal to 0.164 and 0.149 respectively. The number of alleles per locus was 1.73 and 45.5 % loci were polymorphic. In the studied population, an excess of heterozygotes was observed compared with the Hardy-Weinberg equilibrium (F = –0.081).

Key words: *Taxus cuspidata*, allozymes, inheritance, linkage, genetic variation.

INTRODUCTION

Japanese yew (*Taxus cuspidata* Sieb. et Zucc.) has a wide geographical distribution and occurs in Japan, the Korea peninsula, north-east China, and in the south Russian Far East including Sakhalin Island and south Kuriles. In Russia, *T. cuspidata* is a rare tree species and it grows in single or small groups of uneven-aged trees on naturally vegetated mixed forests in mountainous regions. Japanese yew form their stands only on some Far East islands including small islands on littoral of the Sea of Japan as Petrov, Naumov and Rimsky-Korsakov Islands (VOROB'EV 1968). At present, *T. cuspidata* is a declining species but neither *in situ* nor *ex situ* conservation strategies have been developed. This paper represents a research initiative in the studying of genetic diversity as one of the first steps on developing of conservation strategies for Japanese yew.

The purpose of the study was to describe 15 enzyme extracted from seeds, inheritance patterns for 22 allozyme loci, linkages among 40 pairs of loci and level of genetic diversity in one of the pure stands of *T. cuspidata* in the Petrov Island.

MATERIALS AND METHODS

Seeds for electrophoresis were collected in 1998 from 42 individual trees of Japanese yew in the Petrov Island of Lazov Natural Reserve. Six megagametophytes per tree were first sampled to define genotypes. Afterwards, additional 18 to 32 megagametophytes per tree were analyzed from 16 highly heterozygous trees that were selected to verify segregation and linkage.

Methods of enzyme extraction followed CHELIAK & PITEL (1994). For electrophoresis, two buffer systems were used: A) stock buffer pH 8.6 – 900 mM tris/500 mM boric acid/20 mM EDTA, gel buffer – dilute 50 ml of stock buffer to 1 liter, electrode buffer – dilute 200 ml of stock buffer to 1 liter (GURIES & LEDIG 1978); B) gel buffer pH 8.0 – 220 mM tris/500 mM tris-HCl (dilute 33 ml of buffer to 1 liter), electrode buffer pH 6.2 – 220 mM tris/86 mM citric acid, adjust to pH 6.2 with 220 mM tris solution (GONCHARENKO *et al.* 1993, POTENKO & VELIKOV 1998).

Gel slices were stained for the activity of 14 different enzymes using recipes described by CHELIAK & PITEL (1984). The enzymes, their abbreviations, enzyme commission codes and buffer systems upon which they were run are listed in Table 1. In addition, poor resolution or enzyme activity was found for alanine aminopeptidase (AAP; E.C. 3.4.11.2), glutamate dehydrogenase (GDH; E.C. 1.4.1.3), formate dehydrogenase (FDH; E.C. 1.2.1.2), glucose-6-phosphate dehydrogenase (G-6-PD; E.C. 1.1.1.49), malic enzyme (ME; E.C. 1.1.1.40), malate dehydrogenase (MDH; E.C. 1.1.1.37) and sorbitol dehydrogenase (SDH; E.C. 1.1.1.14). These seven enzymes were not used in analyses. Mannose-6-phosphate isomerase (MPI; E.C.

Table 1. Enzyme and buffer systems used for electrophoretic analyses of *T. cuspidata*.

Enzyme	Abbreviation	E.C. number	Buffer system
Aspartate aminotransferase	AAT	2.6.1.1	A
Alcohol dehydrogenase	ADH	1.1.1.1	A
Diaphorase	DIA	1.6.4.3	B
Fluorescent esterase	FL-EST	3.1.1.2	A
Fumarase	FUM	4.21.2	B
Glutamate pyruvate transaminase	GPT	2.6.1.2	B
Isocitrate dehydrogenase	IDH	1.1.1.42	B
Leucine aminopeptidase	LAP	3.4.11.1	A
Alanyl-leucine peptidase	PEPAL	3.4.13.-	B
Leucyl-glycyl-glycine peptidase	PEPLGG	3.4.13.-	B
Phosphoglucomutase	PGM	2.7.5.1.	A
6-Phosphogluconate dehydrogenase	6-PGD	1.1.1.44	B
Phosphoglucose isomerase	PGI	5.3.1.9	B
Shikimate dehydrogenase	SKDH	1.1.1.25	B

5.3.1.8) also was excluded from analyses because of very fast migration rate of the enzyme. Non-specific single-banded monomorphic enzyme was observed on all gels stained for NAD-dependent enzymes as ADH, FUM, GDH, SDH and FDH. The enzyme was analyzed as 15th - non-specific NAD-dependent dehydrogenase (NDH).

The zone specifying the most anodally migrating variants was designated as 1, the next as 2, and so on. Within each zone, the most frequent variant was assigned the value of 1.00. Other variants of the zone were described according to their mobility relative to the most frequent variant. Variants lacking stain activity were designated as "Null".

The inheritance of allozyme polymorphism in haploid tissue from heterozygous trees was tested for confirmation with the expected 1:1 ratio, using the chi-square test. As well, the chi-square test was used for the statistical evaluation of linkage relationships. Recombination fraction (R) is calculated by the binomial estimator: $R = r/n$, where r is the number of recombinant types observed and n is the total number of observations. The standard error of this estimate is given by: $[R(1-R)/n]^{1/2}$ (STRAUSS & CONKLE 1986).

Allele frequencies were analysed using the BIOSYS-1 computer program (SWOFFORD & SELANDER 1989). Mean number of alleles per locus (A), percentage of polymorphic loci (P), observed (H_o) and expected heterozygosity (H_e), and Wright's fixation index (F) were computed.

RESULTS AND DISCUSSION

Segregation

Twenty five loci were identified from the 15 enzyme systems analyzed, of which three (*Aat-1*, *Aat-2* and *Lap-2*) could not be scored consistently. Zymogram phenotypes for the remaining 22 allozyme loci are presented in Figure 1. Mendelian inheritance was tested

Table 2. Observed allozyme segregation in megagametophytes of heterozygous trees and χ^2 tests for goodness of fit to 1:1 ratio.

Locus	Alelic combination	Observed segregation	Deviation χ^2 test (1 df)
Aat-3	0.80/1.00	211:182	2.14
Adh	0.90/1.00	13:13	-
	0.94/1.00	78:69	0.55
	0.99/1.00	17:29	3.13
	0.99/1.10	15:9	1.50
	1.00/1.10	106:95	0.60
Lap-1	0.98/1.00	13:12	0.04
	$1.00/^{1.04}/_{1.00}$	68:50	2.75
Skdh-1	1.00/1.10	196:215	0.88
Skdh-2	0.82/1.00	119:93	3.19
	1.00/1.05	88:78	0.60
Fl-Est	0.85/1.00	75:75	-
Gpt	0.94/1.00	98:119	2.03
Idh-1	1.00/1.18	54:66	1.20
Pepal	1.00/Null	91:75	1.54
Peplgg-3	0.57/1.00	152:146	0.12
	0.57/1.13	23:21	0.09
	1.00/1.13	62:64	0.03

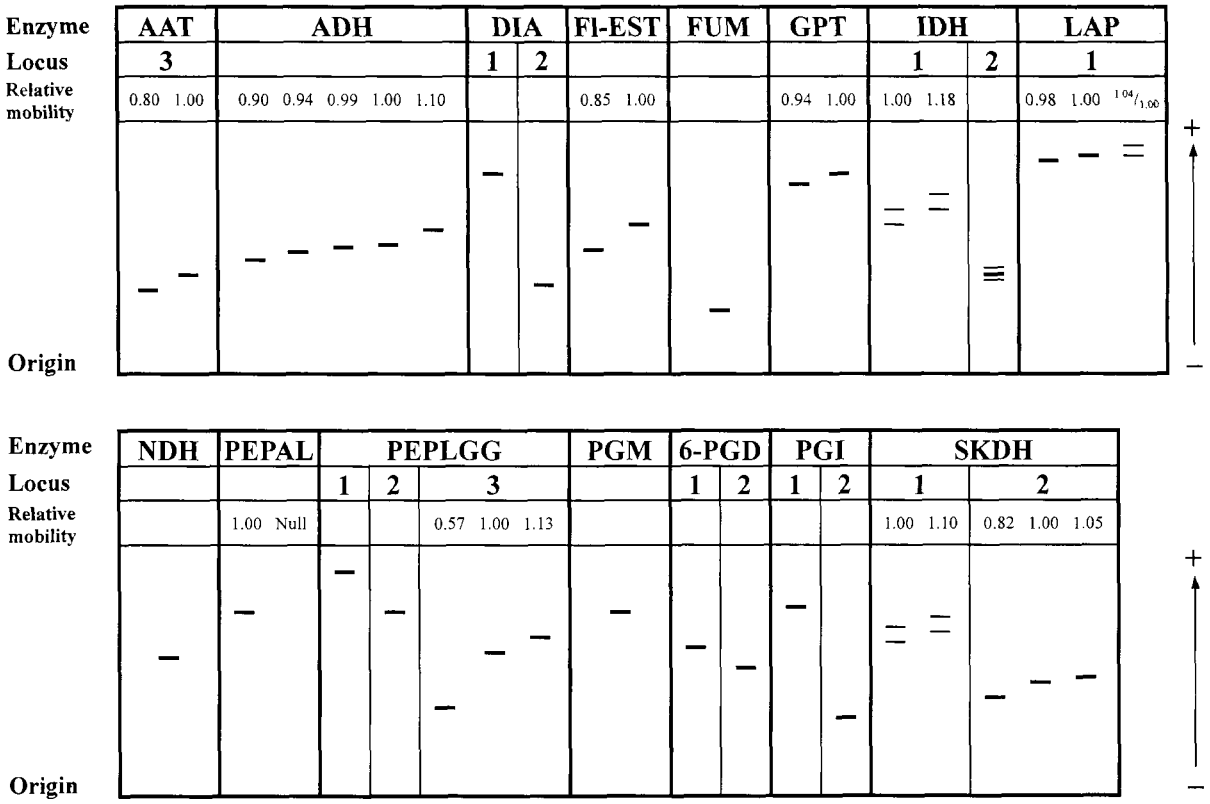

Figure 1. Enzyme phenotypes found in *T. cuspidata*.

for the ten polymorphic allozyme loci. In summary, no significant deviation from the expected 1:1 segregation ratio were observed at any of the loci studied (Table 2), indicating that these allozymes exhibited distinct, co-dominant expression and simple Mendelian segregation in their mode of inheritance. Description of the observed isozyme patterns is presented below.

Monomorphic enzymes

One invariant zone of activity was found on gels stained for fumarase (FUM), non-specific NAD-dependent dehydrogenase (NDH) and phosphoglucomutase (PGM), and two single-banded invariant zones of activity occurred on gels stained for diaphorase (DIA), 6-phosphogluconate dehydrogenase (6-PGD) and phosphoglucose isomerase (PGI).

Polymorphic enzymes

Aspartate aminotransferase (AAT)

There were 3 zones of activity on gels stained for this enzyme. The 2 most anodal zones (*Aat-1* and *Aat-2*) were invariant, but bands at these zones stained faintly or were absent on many gels thus it was excluded from this study. The most cathodal zone *Aat-3* was polymorphic and had two single-banded variants (0.80 and 1.00). Three loci were also reported for AAT of *T. baccata* (LEWANDOWSKI *et al.* 1992, HERTEL 1996) and for many other conifer species (GONCHARENKO *et al.* 1989).

Alcohol dehydrogenase (ADH)

Two zones of activity were evident on gels stained for ADH. The upper invariant zone is *Ndh* that expressed on all gels stained for NAD-depended enzymes. The most cathodal zone (*Adh*) had five variants (0.90, 0.94, 0.99, 1.00, 1.10). A single locus *Adh* was used for analysis genetic diversity of *T. brevifolia* (EL-KASSABY & YANCHUK 1994). A single zone of activity for this enzyme was reported also for many *Pinus* and *Picea* species (GONCHARENKO *et al.* 1989). Contrariwise, two zones of ADH were founded for *T. baccata* (HERTEL 1996).

Fluorescent esterase (FL-EST)

There was 1 variable zone of activity on gels stained for FL-EST with two variants (0.85, 1.00). Other zones of fluorescent activity were diffused and inconsistently

resolved. Two single-banded invariant zones of fluorescent esterase were found for *T. baccata* (LEWANDOWSKI *et al.* 1992). From one to two zones of FL-EST activity was reported for *Calocedrus decurrens* (HARRY 1986), *Picea glauca* (KING & DANCIK 1983), *Pinus attenuata* (STRAUSS & CONKLE 1986), *P. albicaulis* (FURNIER *et al.* 1986), *P. pumila* (GONCHARENKO *et al.* 1993), *Pseudotsuga menziesii* (ADAMS *et al.* 1990) and *Thuja occidentalis* (PERRY & KNOWLES 1989).

Glutamate pyruvate transaminase (GPT)

One zone of activity occurred on gels stained for GPT. In this zone, two variants (0.94, 1.00) were observed. Two zones of activity for this enzyme were reported for *Pinus contorta* (WHEELER & GURIES 1982) and *P. attenuata* (STRAUSS & CONKLE 1986).

Isocitrate dehydrogenase (IDH)

Two zone of activity were evident on gels stained for IDH. The upper, less intensively stained zone (*Idh-1*) had two double-banded variants (1.00, 1.18). The lower zone (*Idh-2*) stained more intensively was invariant. Two zones of IDH activity have been reported for *T. baccata* (LEWANDOWSKI *et al.* 1992, HERTEL 1996) and other conifers (HARRY 1986, MUONA *et al.* 1987, MÜLLER-STARCK & LIU 1988, PERRY & KNOWLES 1989, SCHROEDER 1989, HUSSENDÖRFER *et al.* 1995).

Leucine aminopeptidase (LAP)

There were two zones of activity on gels stained for this enzyme. Two single-banded (0.98, 1.00) and one double-banded (1.04/1.00) variants were observed at the more anodal zone. The second (more cathodal) zone stained much less intensively and was not included in the study.Genetic control of LAP by 2 independent loci has been reported for *T. baccata* (LEWANDOWSKI *et al.* 1992, HERTEL 1996) and for most conifers (GONCHARENKO *et al.* 1989).

Alanyl-leucine peptidase (PEPAL) and Leucyl-glycyl-glycine pepetidase (PEPLGG)

Gels stained for PEPAL had one zone of activity. This zone had one single-banded variant and "Null" variant, i. e. with no enzyme activity.

Three zones of PEPLGG activity were observed on gels. The 2 most anodal zones were invariant for all 42 trees. The most cathodal zone *Peplgg-3* showed three variants (0.57, 1.00, 1.13).

These enzymes were not used in studies of *T. baccata, T. brevifolia* and *T. canadensis* (LEWANDOWSKI *et al.* 1992, HERTEL 1996, EL-KASSABY & YANCHUK 1994, SENNEVILLE *et al.*, 2001). From one to four zones PEP activity was found for different conifers (EL-KASSABY *et al.* 1982, WHEELER & GURIES 1982, STRAUSS & CONKLE 1986).

Shikimate dehydrogenase (SKDH)

Two zones of activity were detected on SKDH gels. The upper, less intensively stained zone (*Skdh-1*) had two double-banded variants (1.00, 1.10). The lower zone (*Skdh-2*) was stained more intensively and had three single-banded variants (0.82, 1.00, 1.05). Two loci of shikimate dehydrogenase were used for analysis genetic diversity of *T. brevifolia* (EL-KASSABY & YANCHUK 1994) and observed for *T. baccata* (HERTEL 1996). In other conifers 2 zones of SKDH activity have usually been reported (HARRY 1986, STRAUSS & CONKLE 1986, GEBUREK & WANG 1990, GONCHARENKO *et al.* 1993, MORGANTE *et al.* 1993, HUANG *et al.* 1994, BERGMANN & HATTEMER 1995).

Table 3. Number of trees analyzed for linkage for each pair of allozyme loci.

Locus	*Aat-3*	*Adh*	*Lap-1*	*Skdh-1*	*Skdh-2*	*Fl-Est*	*Gpt*	*Idh-1*	*Pepal*	*Peplgg-3*
Aat-3	0	4	4	6	7	5	4	1	3	6
Adh		*	2	5	6	0	3	1	2	6
Lap-1			*	4	2	2	0	1	0	2
Skdh-1				*	6	2	3	2	3	7
Skdh-2					*	1	3	1	4	4
Fl-Est						*	1	1	0	3
Gpt							*	1	2	6
Idh-1								*	0	2
Pepal									*	3
Peplgg-3										*

Linkage

Out of the 45 possible two-locus combinations that can be formed from 10 polymorphic loci, 40 pairs of allozyme loci were compared in at least one tree. Of these 40 pairs 32 pairs were analyzed in more than one tree. Table 3 shows the combination of allozyme loci tested and the number of tree studied. The linkage was found between five pair of allozyme loci (Table 4). One slightly linked block was indicated for five allozyme loci, in putative linear order: *Adh*, *Skdh-2*, *Idh-1*, *Skdh-1* and *Peplgg-3*. Pooled estimate of recombinant frequencies for *Adh : Skdh-2*, *Skdh-2 : Idh-1*, *Idh-1 : Skdh-1* and *Skdh-1 : Peplgg-3* were 0.26, 0.32, 0.29 and 0.23, respectively. Also slight linkage was detected between *Aat-3* and *Gpt* with 0.24 recombination frequency.

Only one tree of *T. baccata* was tested for linkage between *Idh-A* (*Idh-1* in this study) and *Skdh* (HERTEL 1996). There was no linkage found between loci in *T. baccata*. Other pair of loci that linked in *T. baccata* was not tested in *T. cuspidata* and conversely. Comparison of linkage relationships in *T. cuspidata* with those reported for other conifer species is problematical because of possibilities that the same zone of isozyme variation may not be coded by the same gene.

Genetic variation

Among the 22 analyzed loci twelve appeared to be monomorphic (*Dia-1*, *Dia-2*, *Fum*, *Idh-2*, *Ndh*, *Peplgg-1*, *Peplgg-2*, *Pgm*, *6-Pgd-1*, *6-Pgd-2*, *Pgi-1* and *Pgi-2*). Allelic frequencies and variability measures of polymorphic loci are presented in Table 5. When only polymorphic loci were analyzed, the expected and observed heterozygosities were 0.328 and 0.360,

Table 4. Significantly linked pairs of allozyme loci in Japanese yew with recombination frequency (*R*), its standard deviation (*SD*) and χ^2 test for linkage.

Pair of allozyme loci	No. linked/ No. studied	Number of trees	Total seeds	Recombination frequency *R* (*SD*)	χ^2 test
Aat-3:Gpt	1 / 4	37	25	0.24 (0.09)	6.76**
Adh:Skdh-2	2 / 6	2	32	0.25 (0.08)	8.00**
		10	32	0.28 (0.08)	6.13*
Idh-1:Skdh-1	1 / 2	15	28	0.29 (0.09)	5.14*
Idh-1:Skdh-2	1 / 1	13	38	0.32 (0.08)	5.16*
Peplgg-3:Skdh-1	2 / 7	9	26	0.31 (0.09)	3.85*
		30	26	0.15 (0.07)	12.46***

Note: Significant levels: * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$.

Table 5. Allele frequencies, indices of genetic variability, and Wright' s fixation index in the population of *T. cuspidata* from Petrov Island.

Locus	Allele*					H_e	H_o	F
	1	2	3	4	5			
Aat-3	0.369	0.631	–	–	–	0.466	0.500	-0.061
Adh	0.012	0.107	0.048	0.655	0.179	0.525	0.619	-0.164
Fl-Est	0.071	0.929	–	–	–	0.133	0.143	-0.064
Gpt	0.122	0.878	–	–	–	0.214	0.244	-0.125
Idh-1	0.952	0.048	–	–	–	0.091	0.095	-0.037
Lap-1	0.012	0.940	0.048	–	–	0.113	0.119	-0.040
Pepal	0.857	0.143	–	–	–	0.245	0.286	-0.153
Peplgg-3	0.298	0.571	0.131	–	–	0.568	0.619	-0.077
Skdh-1	0.571	0.429	–	–	–	0.490	0.524	-0.057
Skdh-2	0.143	0.726	0.131	–	–	0.435	0.452	-0.027
Mean						0.328	0.360	-0.081

* – allele electrophoresis mobility is showed in Figure 1.

Table 6. Genetic variability in species of genus *Taxus*.

Species	*A*	*P*	H_e	H_o	Authors
T. baccata	2.22	66.7	0.279	0.286	LEWANDOWSKI *et al.* 1995
T. brevifolia	1.70	42.3	0.166	0.085	EL-KASSABY & YANCHUK 1994
T. brevifolia	1.50	41.6	0.124	0.122	WHEELER *et al.* 1995
T. canadensis	1.32	26.5	0.098	0.102	SENNEVILLE *et al.* 2001
T. cuspidata	1.73	45.5	0.149	0.164	this study

respectively. The mean number of alleles per locus was 2.6. When all loci were considered, expected and observed heterozygosities were 0.149 and 0.164, respectively (Table 6). The mean number of alleles per locus was 1.73 and 45.5% of the loci were polymorphic (99% criterion).

Compared with other coniferous tree species (HAMRICK *et al.* 1992), *T. cuspidata* have a quite high level of intra-populational genetic variation. Widespread of a progenitor population in Pleistocene and Holocene (GOLUBEVA & KARAULOVA 1983) can explain the high value of genetic variation in Japanese yew. The intensive use of Japanese yew wood by man in the recent period of the Far East land reclamation has resulted in the species becoming rare in Russian Far East forests (SOLODUKHIN 1962).

Parameters of genetic variation for *T. cuspidata* were lower than those for *T. baccata* and higher than those for *T. brevifolia* and *T. canadensis* (Table 6). However, considering the island origin of the studied population, more high level of genetic variation in continental portion of the Japanese yew natural range may be expected. This assumption confirmed by investigations of genetic variation of the internal and coastal populations of *Pinus koraiensis* in the Russian Far East (POTENKO & VELIKOV 1998, POTENKO & VELIKOV 2001). Low level of genetic variation was found in the Petrov Island (P_{99} = 50.0, A = 1.73, H_o = 0.177, H_e = 0.161) in comparison with mean for coastal (P_{99} = 54.4, A = 1.84, H_o = 0.177, H_e = 0.176) and internal (P_{99} = 58.7, A = 1.93, H_o = 0.182, H_e = 0.183) populations of *P. koraiensis*.

Average Wright's fixation index was equal to -0.081 indicating an excess of heterozygotes in studied population of *T. cuspidata*, compared with a population in Hardy-Weinberg equilibrium. A small excess of heterozygotes (-0.025) was also observed in one population of *T. baccata* in Wierzchlas Reserve in Poland (LEWANDOWSKI *et al.* 1995) and some populations of *T. brevifolia* and *T. canadensis* (WHEELER *et al.* 1995, SENNEVILLE *et al.* 2001) that for English yew was explained by selection against homozygotes (LEWANDOWSKI *et al.* 1995) as for most of adult coniferous populations (MUONA 1990).

Generally *T. cuspidata* is considered to be a declining species (OVSJANNIKOV 1924, SOLODUKHIN 1962), but natural regeneration for this species in Petrov Island is abundant. Considering the high level of genetic variation of *T. cuspidata* in the studied population, seeds can be used for Japanese yew reforestation in the south of Russian Far East when seeds from continental populations are not available.

Further studies are needed to expand our knowledge about the genetic variation within and among populations, which will help develop conservation strategies for the studied species.

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