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RESEARCH PAPER

Genetic variation and population history of three related fir species *Abies sachalinensis*, *A. nephrolepis* and *A. gracilis* (Pinaceae) revealed by nuclear microsatellites

Vladimir L. Semerikov* & Svetlana A. Semerikova

Vladimir L. Semerikov *
e-mail: semerikov@ipae.uran.ru

Svetlana A. Semerikova
e-mail: s.a.semerikova@ipae.uran.ru

Institute of Plant and Animal Ecology, Ural Branch RAS, Yekaterinburg, Russia

* corresponding author

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ABSTRACT

Seventeen nuclear microsatellite loci (nSSR) were used to study the genetic diversity and historical demography of three Northeast Asian fir species: insular Sakhalin fir *Abies sachalinensis* (Fr. Schmidt) Mast., continental *A. nephrolepis* (Trautv.) Maxim. and Kamchatka endemic *A. gracilis* Kom. Bayesian clustering, performed using the STRUCTURE software separated the considered species from each other and divided *A. sachalinensis* populations into several groups located from south to north. According to the results of ABC analysis, the ancestors of *A. nephrolepis* and the ancestors of southern populations of *A. sachalinensis* split about 600 ka BP. Populations of *A. sachalinensis* of northern Sakhalin originated from *A. nephrolepis* about 300 ka BP. Populations of central Sakhalin were formed, probably, as a result of mixing of populations of northern and southern Sakhalin during the late glacial (about 18 ka BP). The origin of the *A. gracilis* is inferred as a result of separation from populations of northern Sakhalin, probably before the last glacial maximum.

Keywords: *Abies sachalinensis*, *A. nephrolepis*, *A. gracilis*, population structure, demographic inferences, nSSR

РЕЗЮМЕ

Семериков В.Л., Семерикова С.А. Генетическая изменчивость и популяционная история трех родственных видов пихт *Abies sachalinensis*, *A. nephrolepis* и *A. gracilis* (Pinaceae) по данным ядерных микросателлитных локусов. Семнадцать ядерных микросателлитных локусов (nSSR) были использованы для изучения генетического разнообразия и исторической демографии трех видов пихт Северо-Восточной Азии: островного вида пихты сахалинской *Abies sachalinensis* (Fr. Schmidt) Mast., континентальной пихты белокорой *A. nephrolepis* (Trautv.) Maxim. и эндемичной камчатской пихты *A. gracilis* Kom. Байесовская кластеризация, выполненная с помощью программы STRUCTURE, отделила рассматриваемые виды друг от друга и разделила популяции *A. sachalinensis* на несколько групп, расположенных с юга на север. По результатам ABC анализа предки *A. nephrolepis* и предки южных популяций *A. sachalinensis* разделились около 600 тыс. л.н., популяции *A. sachalinensis* северного Сахалина возникли в результате отделения от *A. nephrolepis* около 300 тыс. л.н. и популяции центрального Сахалина – вероятно, в результате смешения популяций северного и южного Сахалина в позднеледниковье, около (18 тыс. л.н.). Происхождение пихты камчатской *A. gracilis* реконструируется как результат отделения от популяций северного Сахалина, вероятно до последнего ледникового максимума.

Ключевые слова: *Abies sachalinensis*, *A. nephrolepis*, *A. gracilis*, популяционная структура, демографические оценки, nSSR

Electronic Appendix: http://www.geobotanica.ru/bp/2023_12_02/BP_2023_12_2_semerikov_e_suppl.pdf

Three of the four fir species of the Russian Far East, *Abies sachalinensis* (Fr. Schmidt) Mast., *A. nephrolepis* (Trautv.) Maxim. and *A. gracilis* Kom., belong to the section *Balsamea* (Farjon & Rushforth 1989) and are genetically close to each other (Semerikova 2016, Semerikova et al. 2018). The first two species take a significant part in the composition of coniferous forests of the Northeast Asia, which determines their ecological and economic value, and *A. gracilis* is the endangered endemic species, forming the only population on an area of 22 hectares in the southeast of Kamchatka (Turkov & Shamshin 1963, Naumenko 1981, Neshataeva & Fet 1994).

Global warming characteristic of the modern climatic epoch threatens boreal forests of the Northern Hemisphere due to increased frequency of droughts, competition from more thermophilic vegetation, insect and pathogen invasions. An important condition for the adaptation of taiga conifers to changing environment is the magnitude and structure of genetic variability. The genetic variability of the considered species has been previously investigated using different type of markers – allozymes (Nagasaka et al. 1997, Semerikova et al. 2011), AFLP (Semerikova et al. 2011), ISSR (Woo et al. 2008), mitochondrial markers (Semerikova et al. 2011, Jiang et al., 2011, Kwak et al., 2020, Semerikov et al. 2022) and chloroplast microsatellites (Semerikova & Semerikov 2007, Semerikova et al. 2012). Recently, highly variable microsatellite loci (nSSR) have been used for this purpose (Hong et al. 2011, Hong et al. 2016, Kwak et al. 2020, Kitamura et al. 2020), but these studies were limited to the Hokkaido and the southern Korean Peninsula only.

Abies sachalinensis is a morphologically highly variable species (Krylov et al. 1986, Farjon & Filer 2013). Its study based on mitochondrial DNA markers, allozymes and AFLP (Semerikova et al. 2011) revealed differentiation of populations of southern Sakhalin and Kuriles with respect

to northern Sakhalin, and similarity of northern Sakhalin populations with continental *A. nephrolepis*. The transition by nuclear markers from southern Sakhalin fir populations to northern populations was gradual, which was interpreted as a result of colonization of northern Sakhalin by *A. nephrolepis* during the Pleistocene glacial phases and subsequent invasion of the more thermophilic southern forms of *A. sachalinensis* to northern Sakhalin during the interglacial periods (Semerikova et al. 2011).

Apparently, the alternation of such counter migrations, as well as local adaptation to the latitudinal gradient of climatic conditions, formed a gradual genetic transition from southern to northern Sakhalin. However, the timing of these processes remains uncertain. Genetic differentiation south – north largely agrees with earlier observations of differentiation of *A. sachalinensis* by morphology of cone bract scales (Bukhteeva 1963). Trees with long bracts much larger than the seed scales, regarded as *A. sachalinensis* var. *mayriana* Miyabe et Kudo. (Farjon 2001), dominate in southern Sakhalin and are gradually replaced to the north by trees with shorter bract scales slightly longer than the length of the seed scales (*A. sachalinensis* var. *sachalinensis*), which brings them closer to continental *A. nephrolepis*, which has bract scales shorter than the seed scales (Orlova 2003).

The study of genetic variability of *A. nephrolepis* in the Russian part of the range revealed the absence of mitochondrial DNA polymorphism and somewhat reduced intrapopulation variability compared to *A. sachalinensis* and weak interpopulation differentiation by nuclear markers (Semerikova et al. 2018). In connection with this the study of the magnitude and structure of *A. nephrolepis* variability using more informative nSSR markers is of interest.

Comparison of genetic variability of Kamchatka fir with related species and study of its phylogenetic position in the system of the genus *Abies* confirmed the species rank of *A. gracilis* (Semerikova et al. 2011, 2018), although some researchers still treat it as a variety of the Sakhalin fir – *A. sachalinensis* var. *gracilis* (Kom.) Farjon (Farjon 2001, Farjon & Filer 2013). Study of genetic diversity of Kamchatka fir revealed reduced variability compared to *A. nephrolepis* and *A. sachalinensis* by AFLP (Semerikova et al. 2011) and a very low diversity of cpSSR haplotypes, inferior even to *A. semenovii* B. Fedtsch. (Semerikova et al. 2012), which corresponds to the small population size of *A. gracilis* and is obviously caused by significant population reductions – both modern and past, and likely caused by the last glacial maximum (LGM) or volcanic eruptions. The origin of fir in Kamchatka remains not fully elucidated. Paleobotanical data indicate the distribution of forests with fir in Kamchatka in the Pliocene (Skiba 1975), but in the Pleistocene, the finds of fir pollen become sporadic and, probably, fir was periodically completely extinct there (Egorova 2008) and was repopulated from outside Kamchatka during favorable climatic periods.

Comparison of the three species in phylogenetic studies revealed greater similarity between *A. sachalinensis* and *A. gracilis* in chloroplast DNA (Bayesian Probability 100 %), lack of topology resolution for these species in the nuclear DNA tree, and, in contrast, greater relatedness of *A. nephrolepis* and *A. gracilis* (bootstrap support 100 %) in mitochondrial DNA (Semerikova et al. 2018, Semerikov et al. 2022). This uncertainty, in case of *A. gracilis*, is presumably related to "incomplete lineage sorting", as well as hybrid processes in the complex of these species.

The present paper is aimed at studying the magnitude and structure of genetic variability, as well as the historical demography of a complex of related firs of the Far East. Its aims are to investigate the origin of the species under consideration and the main population groups previously identified using allozymes, AFLP, and mitochondrial DNA (Semerikova et al. 2011), to identify migration pathways, to estimate the age of major events, and other demographic parameters. In this study, we used samples collected from the nearly entire range of *A. sachalinensis*, *A. nephrolepis* and *A. gracilis* and genotyped using a set of nuclear microsatellite loci (nSSR) used in the study of *A. sibirica* Ledeb. and *A. semenovii* (Semerikov & Semerikova 2023), which allows comparison of the species studied. We use the Approximate Bayesian Computation (ABC) approach to analyze demographic models and estimate their parameters.

MATERIAL AND METHODS

Samples used

Ten population samples (188 trees) of *A. sachalinensis*, four samples (94 trees) of *A. nephrolepis* collected in all major parts of their ranges, and one sample of *A. gracilis* (32 trees) were analyzed (Table 1, Fig. 1). Most of them have been previously used in the study of variability of allozyme loci, chloroplast microsatellites, AFLP and mitochondrial DNA (Semerikova et al. 2011, 2012).

Laboratory analysis

Seventeen SSR loci were used (Semerikov & Semerikova 2023), including ten loci: Ak174, Ak246, Ak5, Ak247, Ak87, Ak176, Ak182, Ak240, Ak264, Ak252, developed for *A. koreana* E.H. Wilson and *A. nephrolepis* (Hong et al. 2016), four loci: As13, As21, As20, As08, for *A. sachalinensis* (Lian et al. 2007) and three loci: As_404145, As_2288295, As_43741, for *A. sibirica* (Nikhaenko 2020). Detailed descriptions of the loci, PCR conditions, and subsequent fragment analysis are provided in Semerikov & Semerikova (2023). Fragments were separated on a Nanofor 05 automatic sequencer (Institute of Analytical Instrumentation, Russian Academy of Sciences, Russia) at the Center for Collective Use "Modern Technologies for Ecological Research" of the IPAE, Ural Branch of RAS.

Data analysis

Chromatograms were converted into genotypic data using GeneMapper 3.5 software (Applied Biosystems). Genotypic data were analyzed using GenAlEx 6.5 software (Peakall & Smouse 2012). Deviation from Hardy-Weinberg equilibrium was assessed using GENEPOP (Rousset 2008). The presence of null alleles was tested according the method of Van Oosterhout et al. (2004). Bayesian clustering of genotypes was performed using STRUCTURE 2.3.4 software (Pritchard et al. 2000) under the "admixture" model assumption. For each K ten runs of the program

were used, each with 100,000 steps as burning and 500,000 steps to accumulate data. The number of K clusters was considered from 1 to 10. Data from several runs were combined by the Clumpak program (Kopelman et al. 2015). The most likely number of clusters in STRUCTURE analysis based on ΔK (Evanno et al. 2005) and Ln P(D) (Pritchard et al. 2000) approaches was estimated using STRUCTURE HARVESTER (Earl & VonHoldt 2012). PCoA ordination of *Abies* populations was performed based on genetic distances of M. Nei (1978) using GenAlEx 6.5.

Historical demography analysis

Demographic modeling of fir species was carried out using the Approximate Bayesian Computation (ABC) method implemented in the DIYABC program (Cornuet et al. 2014). For this purpose, we used obtained data on 17 nuclear microsatellite loci and data on two chloroplast DNA microsatellite loci Pt30204 and Pt71936 (Semerikova & Semerikov 2007), which were interpreted as microsatellites of the human Y chromosome. Six groups of relatively "pure" populations according to STRUCTURE data (at K = 6, see Results) were considered: group N1 – Kurils and Hokkaido (populations no. 8, 9, 10), group N2 – southern Sakhalin (populations no. 6, 7); group N3 – central Sakhalin (no. 2, 3); group N4 – northern Sakhalin (no. 1), group N5 – *A. nephrolepis* (no. 11–14) and group N6 – *A. gracilis* (no. 15). Populations with pronounced admixture of other clusters were excluded.

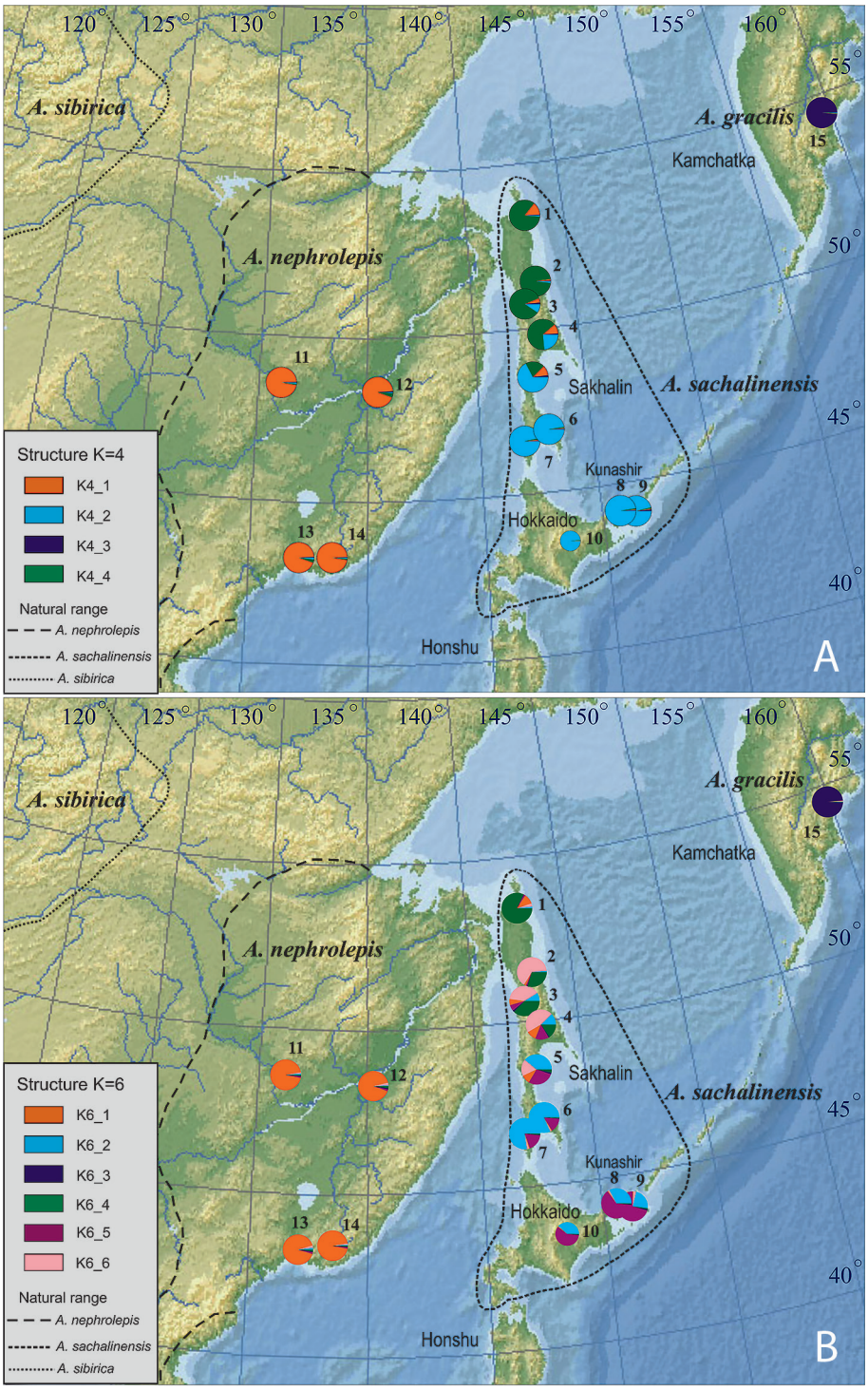

Figure 1 Ареалы *Abies sachalinensis* (Fr. Schmidt) Mast., *A. nephrolepis* (Trautv.) Maxim. и *A. gracilis* Kom. Частоты кластеров STRUCTURE в исследованных популяциях показаны круговыми диаграммами при K = 4 (A) и K = 6 (B)

Five alternative scenarios were used (Fig. S3, Supplementary materials):

Scenario 1: at time t5, group N2 (*A. sachalinensis* in southern Sakhalin) and group N5 (*A. nephrolepis*) split apart; at time t4, group N1 (Kurils and Hokkaido) separated from group N2 (southern Sakhalin); at time t3 N4 (northern Sakhalin) and N5 (*A. nephrolepis*) split apart, at time t2 group N6 (*A. gracilis*) separated from group N5 (*A. nephrolepis*); at time t1, the populations of central Sakhalin (group N3) formed as a result of admixture of group N4 (northern Sakhalin) and group N2 (southern Sakhalin).

Scenario 2 differs from the first one in that group N4 was formed at time t3 not by separation from group N5 (*A. nephrolepis*), but by mixing the genes of this group and group N2.

Scenario 3 differs from the first one in that the formation of group N4 occurred at time t3 by separating from group N2.

Table 1. Characteristics of the studied populations of *A. sachalinensis, A. nephrolepis* and *A. gracilis*. Sample size *n*, diversity measures: number of alleles N_a, observed heterozygosity H_o, expected heterozygosity H_e, coefficient inbreeding *F* and Hardy-Weinberg test *P*-value under H1= heterozygote deficiency are given.

No.	Population name	Latitude	Longitude	*n*	N_a	H_o	H_e	*F*	*P*
Abies sachalinensis									
1	Oha	53°34´	142°56´	21	8.5	0.686	0.726	0.045	<0.0001
2	Nogliki	51°50´	143°00´	23	8.9	0.689	0.775	0.111	<0.0001
3	Tymovskoye	50°44´	142°43´	21	9.9	0.718	0.769	0.066	<0.0001
4	Vaida	49°42´	142°40´	23	10.7	0.745	0.786	0.056	0.0001
5	Makarov	48°39´	142°51´	16	10.7	0.777	0.800	0.029	0.0006
6	Ohotskoye	46°50´	143°10´	20	10.5	0.758	0.776	0.023	0.0073
7	Nevelsk	46°40´	141°52´	24	11.0	0.803	0.787	-0.030	0.2491
8	Tretyakovo	44°00´	145°39´	23	11.1	0.732	0.783	0.056	<0.0001
9	Kurilskoe	44°01´	145°52´	16	9.6	0.734	0.769	0.047	<0.0001
10	Hokkaido	43°39´	143°10´	1	1.8	0.824	0.412		-
Mean				**18.8**	**9.3**	**0.747**	**0.738**	**0.045**	**<0.0001**
Abies nephrolepis									
11	Obluchie	49°00´	131°05´	24	7.6	0.650	0.673	0.029	0.0001
12	Bychiha	48°18´	134°45´	23	7.8	0.718	0.698	-0.026	0.6099
13	Vladivostok	43°16´	132°03´	24	8.3	0.669	0.696	0.041	0.0797
14	Falasa	43°08´	132°48´	23	7.9	0.655	0.698	0.072	<0.0001
Mean				**23.5**	**7.9**	**0.673**	**0.691**	**0.029**	**<0.0001**
Abies gracilis									
15	Kamchatka	54°07´	159°59´	32	2.7	0.424	0.422	-0.013	0.9954

Scenario 4 differs from the first one in that group N6 (*A. gracilis*) separated at time t2 not from group N5 (*A. nephrolepis*), but from group N1 (Kurils and Hokkaido).

Scenario 5 differs from the first one in that group N6 (*A. gracilis*) separated during t2 not from group N5 (*A. nephrolepis*), but from group N4 (northern Sakhalin).

The uncertain position of *A. gracilis* in the nuclear phylogenetic trees (Semerikova et al. 2018) necessiteted consideration of several variants of its origin. Its separation from the Kurils – Hokkaido populations is possible in case of Sakhalin fir migration to Kamchatka along the Kuril Islands. Separation of *A. gracilis* from *A. nephrolepis* is possible during migrations around the Sea of Okhotsk. Separation from northern Sakhalin populations is also possible due to a drop of sea level and exposure of the Sea of Okhotsk shelf around northern Sakhalin during glacial periods (Pletnev 2004).

The hybrid origin of central Sakhalin populations (group N2) was accepted for all three scenarios, by PCoA results (Fig. 2), where these populations occupy an intermediate position between northern and southern Sakhalin, and because these populations also contain an admixture of corresponding clusters according to STRUCTURE (Fig. 1B). For northern Sakhalin (group N3), three variants of its origin are possible: separation from *A. nephrolepis*, separation from southern Sakhalin populations and hybrid origin.

The effective population size priors of the populations considered were assumed to be uniformly distributed from 10 to 60,000 individuals. The prior distributions of time parameters were: 10 <t1<2000, 10<t2<5000, 10<t3<5000, 10<t4<5000, 10<t5<20000 under conditions t5>t1, t5>t2, t5>t3, t5>t4, t4>t1, t3>t1. The mutation rate priors were chosen as $5{\cdot}10^{-5} – 5{\cdot}10^{-4}$ generation^{-1} for nSSR and $10^{-4} – 10^{-3}$ generation^{-1} for cpSSR. The following "summary statistics" were selected: for each population, the mean number of alleles per locus, the mean diversity of genes per locus, the mean dispersion of allele size per locus, and for each pair of populations, the mean number of alleles per locus, the mean diversity of genes per locus, the mean dispersion of allele size per locus, F_{st}, distance by shared alleles, distance $(\delta\mu)^2$.

RESULTS

Variability of nSSR loci within populations

In *A. sachalinensis*, 325 alleles in 17 loci were found among 188 trees in 10 populations. The total number of alleles per locus ranged from 4 (As_228) to 35 (Ak174) with an average value of 19.1 (Table 2). The average number of alleles (N_a) in the population ranged from 2.6 (As_228) to 15.4 (Ak5), and

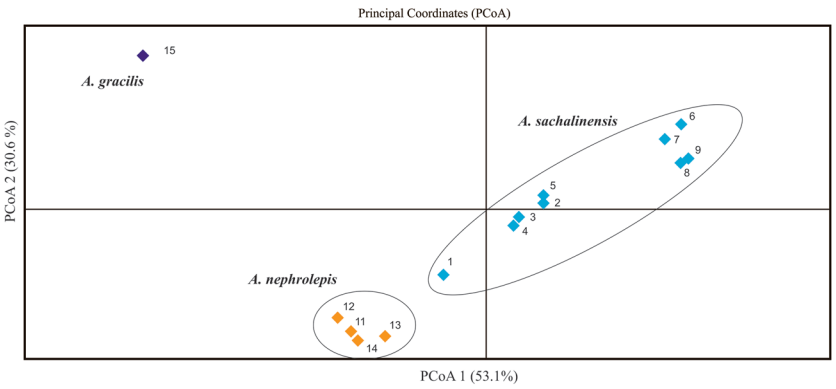

Figure 2 Scatter-plot of studied populations on the plane of the first two PCoA coordinates, based on nSSRs and genetic distances (Nei 1976). Populations are marked with the color corresponding to the predominant cluster at K=3

Table 2. Diversity measures over loci in *Abies sachalinensis, A. nephrolepis* and *A. gracilis*. Fixation indexes F_{is}, F_{it}, F_{st}, sample size N, number of alleles N_a, total number of alleles N_{tot}, observed H_o and expected heterozygosity H_e and Hardy-Weinberg test P-value.

Locus	F_{is}	F_{it}	F_{st}	N	N_a	N_{to_t}	H_o	H_e	P-value
Abies sachalinensis									
Ak174	-0.086	0.008	0.086[a]	18.7	15.4	35	0.937	0.863	0.4372
Ak246	-0.100	0.012	0.101[a]	18.8	9.3	22	0.842	0.766	0.5806
Ak5	-0.071	0.014	0.080[a]	18.8	16.8	39	0.934	0.872	0.3443
Ak247	-0.075	-0.009	0.062[a]	18.8	8.6	15	0.825	0.767	0.6407
Ak87	-0.026	0.067	0.090[a]	18.8	12.5	24	0.859	0.837	0.0048
Ak176	-0.065	0.015	0.075[a]	18.6	9.7	20	0.854	0.802	0.2956
Ak182	-0.046	0.032	0.075[a]	18.6	7.3	15	0.770	0.736	0.1787
Ak240	-0.021	0.073	0.092[a]	18.0	6.7	13	0.728	0.713	0.011
Ak264	0.174	0.340	0.201[a]	18.8	4.0	6	0.423	0.512	0.0002
Ak252	0.056	0.141	0.089[a]	18.3	11.6	28	0.744	0.789	<0.0001
As13	-0.096	-0.008	0.081[a]	18.8	10.1	17	0.901	0.822	0.4578
As21	0.208	0.283	0.095[a]	18.2	12.3	29	0.663	0.837	<0.0001
As_404145	-0.075	0.041	0.108[a]	18.8	3.4	7	0.539	0.501	0.882
As20	0.095	0.171	0.084[a]	18.6	11.5	21	0.753	0.832	<0.0001
As_2288295	-0.056	0.025	0.077	18.8	2.6	4	0.420	0.398	0.259
As_43741	0.000	0.093	0.093[a]	18.4	10.8	19	0.812	0.811	<0.0001
As08	0.007	0.109	0.103[a]	18.5	5.3	11	0.688	0.692	<0.0001
Mean	**-0.010**	**0.083**	**0.094[a]**	**18.6**	**9.3**	**19.1**	**0.747**	**0.738**	**<0.0001**
SE	**0.022**	**0.024**	**0.007**	**0.495**	**0.390**	**9.84**	**0.016**	**0.014**	
Abies nephrolepis									
Ak174	0.018	0.036	0.018	23.5	16	28	0.872	0.888	0.2114
Ak246	-0.001	0.028	0.029[a]	23.5	8	13	0.789	0.788	0.3097
Ak5	-0.012	0.016	0.028[a]	23.5	14	22	0.903	0.892	0.5687
Ak247	0.075	0.097	0.024	23.5	7.5	10	0.726	0.785	0.0004
Ak87	-0.047	-0.029	0.017	23.5	13.3	21	0.895	0.855	0.6846
Ak176	0.023	0.049	0.027	23.0	7.5	12	0.738	0.755	0.1176
Ak182	-0.051	-0.024	0.026[a]	23.5	5.0	8	0.628	0.597	0.5262
Ak240	-0.011	0.012	0.022	23.3	7.3	10	0.730	0.722	0.0991
Ak264	0.092	0.124	0.035[a]	23.5	2.0	3	0.106	0.117	0.2965
Ak252	0.099	0.113	0.016	23.5	8.0	12	0.712	0.791	0.0518
As13	-0.025	0.008	0.032[a]	23.5	8.8	14	0.830	0.810	0.4793
As21	0.178	0.198	0.024	23.5	11.8	18	0.649	0.789	0.0000
As_404145	0.066	0.083	0.018	23.5	3.0	3	0.350	0.375	0.2668
As20	0.001	0.027	0.026[a]	23.5	10.3	15	0.841	0.842	0.2657
As_2288295	0.004	0.028	0.024	23.5	2.3	3	0.468	0.470	0.3251
As_43741	0.078	0.147	0.075[a]	23.3	6.0	10	0.629	0.682	0.0158
As08	0.033	0.058	0.027[a]	23.5	3.8	6	0.575	0.594	0.0113
Mean	**0.030**	**0.057**	**0.028[a]**	**23.4**	**7.9**	**12.2**	**0.673**	**0.691**	**<0.0001**
SE	**0.060**	**0.062**	**0.013**	**0.141**	**4.133**	**7.040**	**0.209**	**0.207**	
Abies gracilis									
Ak174	-0.270	–	–	32	2	2	0.625	0.492	0.9662
Ak246	-0.106	–	–	32	3	3	0.219	0.198	1.0000
Ak5	-0.281	–	–	32	3	3	0.563	0.439	0.9791
Ak247	-0.037	–	–	32	3	3	0.531	0.512	0.6135
Ak87	-0.123	–	–	32	2	2	0.469	0.417	0.8516
Ak176	0.088	–	–	31	2	2	0.452	0.495	0.4121
Ak182	0.024	–	–	32	4	4	0.531	0.544	0.3710
Ak240	0.607	–	–	11	2	2	0.182	0.463	0.0588
Ak264	-0.067	–	–	32	2	2	0.125	0.117	1.0000
Ak252	0.295	–	–	32	3	3	0.344	0.488	0.0536
As13	0.217	–	–	32	3	3	0.344	0.439	0.1092
As21	0.054	–	–	32	2	2	0.469	0.496	0.4837
As_404145	-0.467	–	–	32	4	4	0.813	0.554	0.9992
As_2288295	0.259	–	–	31	3	3	0.387	0.522	0.0825
As_43741	-0.085	–	–	32	2	2	0.156	0.144	1.0000
Mean	**0.007**	**–**	**–**	**30.5**	**2.7**	**2.7**	**0.414**	**0.421**	**0.2007**
SE	**0.266**	**–**	**–**	**5.4**	**0.7**	**0.7**	**0.191**	**0.145**	

[a] *Fst* significant with P<0.01.

averaged 9.3 per locus. The genetic diversity (H_e) averaged across populations and loci was 0.738 (Table 2). There was no significant excess of heterozygotes at any locus or population, but heterozygote deficiency was detected at eight loci using the "global" test (Rousset 2008) and H1 = heterozygote deficiency. The presence of null alleles assessed using the method of Van Oosterhout et al. (2004), was detected at eight loci, including As21 in 7 populations, As20 in 4 populations, As252 and As_437 at three populations each, and at the As08 and Ak264 loci, two populations each, and Ak87 and Ak240 at one population each (Table 2).

In 94 trees of *A. nephrolepis*, 208 alleles were detected in four populations. The number of alleles per locus ranged from 3 (Ak264 and As_228) to 28 (Ak174), averaging 12.2 per locus. The average number of alleles (N_a) in the population ranged from 2.2 (As_228) to 16.0 (Ak5), averaging 7.9

per locus. The genetic diversity (H_e) averaged across populations and loci was 0.691 (Table 2). There was no significant excess of heterozygotes at any locus or population, but heterozygote deficiency was detected at four loci using the "global" test and H1 = heterozygote deficiency. The presence of null alleles was detected at three loci Ak174, Ak247 and As21 in one population each.

Forty alleles were detected in 32 trees of *A. gracilis*. The number of alleles per locus ranged from 2 to 4 averaging 2.7 per locus. The genetic diversity (H_e) averaged across loci was 0.422. No significant excess or significant deficiency of heterozygotes was observed at any locus using the "global" test. The presence of null alleles was detected at As_437 locus.

Spatial structure of variability

The level of interpopulation F_{st} differentiation varied in *A. sachalinensis* between loci from 7.5 % (Ak176, Ak182) to 20.1 % (Ak264) with an average value of 9.4 %. In *A. nephrolepis*, F_{st} ranged from 1.6 % in Ak252 to 7.5 % in As_43741 with an average of 2.8 % (Table 2).

PCoA based on M. Nei's genetic distances arranged the *A. sachalinensis* populations linearly over the scatter-plot towards the population group *A. nephrolepis* as a function of geographic latitude (Fig. 2). The northernmost Sakhalin population (no. 1) turned out to be genetically closest to *A. nephrolepis*, the populations of southern Sakhalin and the Kuril Islands being the most distant.

Bayesian clustering performed using the STRUCTURE software separated species and geographic groups of populations (Figs 1, S2, Supplementary materials).

At K=2, clustering patterns among the ten runs were not stable, and the *A. gracilis* genotypes formed a common cluster with either the *A. nephrolepis* populations or the southern Sakhalin populations.

At K=3, the three selected clusters correspond to three species. The genotypes of *A. gracilis* formed a specific cluster (dark purple). In populations of northern and central Sakhalin, the *A. sachalinensis* cluster (blue) is dominant, but there is an admixture of *A. nephrolepis* cluster (orange) (Fig. S2, Supplementary materials).

The most probable K number calculated using the ΔK approach of Evanno et al. (2005) was 4 (Fig S1, Supplementary materials). Compared to K=3, a green cluster (K4_4) appeared in northern and central Sakhalin (Figs 1A, S2, Supplementary materials).

At K=5, the cluster characteristic of the Kurils and Hokkaido (dark crimson) separated from southern Sakhalin (blue), but the clustering was unstable.

At K=6, a second peak appears on the ΔK plot (Fig. S1a, Supplementary materials) and a plateau on the LnP(D) curve (Fig. S1b, Supplementary materials). At this K value, the pink cluster (K6_6) characteristic of central Sakhalin separates from the northern Sakhalin green cluster (K6_4) (Fig. 1B, S2, Supplementary materials).

ABC study of historical demography

Based on the results of the comparison of five scenarios (Fig. 3, S3, Supplementary materials), the fifth scenario was chosen, which had the maximum value of the posterior probability (Fig. S4, Supplementary materials). According to this scenario, the age estimate (t1) of the formation of the central Sakhalin population group as a result of mixing the genetic pools of the southern Sakhalin and the northern Sakhalin population groups was 18.4 ka (hereinafter, the mode of the posterior distribution is used as an estimate), assuming a generation time of 100 years. The proportion of the northern Sakhalin group in this admixture (r1) was 0.8. Age of separation of northern Sakhalin group from continental *A. nephrolepis* (t3) was estimated as 292 ka, age of separation of *A. gracilis* from populations of northern Sakhalin (t2) was 37.5 ka, age of separation of southern Sakhalin and Hokkaido–Kurils populations was 118 ka and the age of separation of *A. nephrolepis* and *A. sachalinensis* (t5) was 588 ka. The effective population size of Hokkaido–Kurils, southern Sakhalin, central Sakhalin and northern Sakhalin groups of *A. sachalinensis* and *A. nephrolepis* was 51, 38, 12, 15 and 40 thousand individuals, respectively, and of *A. gracilis* 270 individuals (Table S1, Supplementary materials).

DISCUSSION

Fir diversity under maritime vs continental climate

The data based on nSSR markers significantly clarify and complement the information on genetic variability and biogeography of Northeast Asian firs obtained earlier using allozymes, AFLP, cpSSR, and mtDNA markers (Semerikova et al. 2011). The new data mainly confirm the previously identified trends of interspecies differences in the value of genetic diversity (Semerikova et al. 2011, 2012). The studied species differ markedly in the level of intrapopulation variability (Table 1). The number of alleles and expected heterozygosity H_e in any of the populations of *A. sachalinensis* (except the Hokkaido population represented by one tree) was greater than in any of the populations of *A. nephrolepis*, and in *A. nephrolepis* greater than in *A. sibirica* studied using approximately the same set of microsatellite loci (Semerikov & Semerikova 2023). In *A. gracilis*, represented by an isolated small population, the variability (H_e = 0.422) is less than in any population of *A. sibirica* (mean 0.569), but higher than in the Tien Shan endemic *A. semenovii* (H_e = 0.252)

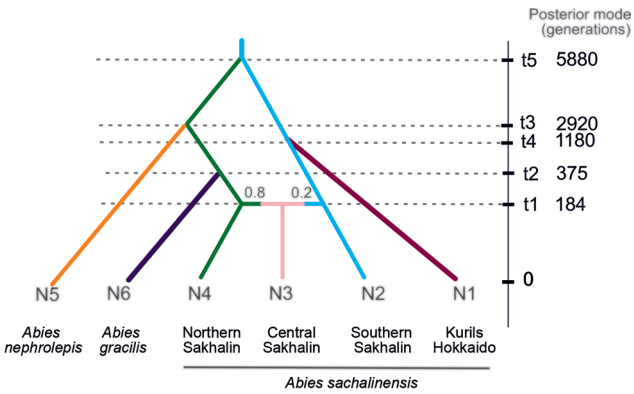

Figure 3 The most likely demographic scenario for the three species of *Abies*, analyzed by ABC. N1 – N6 are the effective population sizes of the five considered population groups, r1 is the contribution of northern Sakhalin to the gene pool of central Sakhalin populations. Modes of posterior distributions of parameters t1, t2, t3, t4, t5 are given. Population groups are marked with the color corresponding to the predominant cluster at K=6

(Semerikov & Semerikova 2023) despite the larger area of the latter – 3470 hectares vs 22 hectares (*A. gracilis*).

The low effective population size of *A. gracilis* of 270 individuals, estimated using ABC, with a census population of about 30,000 trees, and the almost complete absence of variability of cpSSR loci, highly variable in other fir species, indicates a population bottleneck that has occurred relatively recently (Semerikova et al. 2012). The dependence of variability in other species on distance from the ocean is obvious and is related to the fir's increased demands for air humidity and the lack of sharp temperature contrasts compare to other boreal trees. Accordingly, fir species experienced the deepest population depressions and the associated loss of genetic diversity in the continental interior, where climate continentality only increased during the glacial phases, (Semerikova et al. 2011, 2012).

Differentiation and demographic history of the Sakhalin fir

Abies sachalinensis populations are significantly more differentiated (F_{st} = 9 %) than *A. nephrolepis* populations (F_{st} = 2.8 %). On the PCoA plot (Fig. 2), Sakhalin fir populations are located linearly, from populations of southern Sakhalin and Kurils to a cluster of populations of northern Sakhalin (population no. 1, Oha), towards *A. nephrolepis*.

This pattern is quite consistent with the allozyme, AFLP, mitochondrial DNA (Semerikova et al. 2011) and can be explained by the hybrid origin of the central Sakhalin populations as a result of mixing *A. nephrolepis* and southern forms of *A. sachalinensis*. It has been suggested (Semerikova et al. 2011) that during the last glaciation, the cold resistant *A. nephrolepis* penetrated Sakhalin, which at that time was connected to the continent through the northern part of the island due to the sea level drop. Later, after the end of glaciation, the thermophilic and fast-growing southern forms of *A. sachalinensis* spread to northern Sakhalin, forming a hybrid complex.

However, this study shows that the colonization of Sakhalin by *A. nephrolepis* occurred long before the last glaciation (about 300 thousand years ago), probably due to Pleistocene periodic climate and sea level fluctuations (Pletnev 2004). At the same time, the estimate of the age of hybrid formation of the central Sakhalin populations, 18 thousand years, is quite consistent with the LGM.

The zones of predominant distribution on Sakhalin of the northern Sakhalin (green) and southern Sakhalin (blue) clusters at K = 4 (Fig. 1A) correspond well to the location of the boundary between "circumboreal" and "East Asian" floristic regions, the so-called Schmidt's line, which separates the northeast of Sakhalin from the southwest (Krestov et al. 2004). This coincidence probably reflects the presence in Sakhalin fir of certain complexes of genes determining adaptation to the two types of environmental conditions.

The DIYABC software, which implements simulations of demographic models, cannot account the continuous gene flow between the considered population groups, although the existence of such flow is evident given the STRUCTURE results. Thus, a significant presence of the *A. nephrolepis* cluster (orange) is noticeable in northern and central Sakhalin (Fig. 1B), and clusters of southern Sakhalin (blue), Kuril Islands, and Hokkaido (dark cherry) are found in central Sakhalin. Morphological data also indirectly show modern gene flow. Thus, according to the observation of Urusov (1988), fir trees with bract scale morphology characteristic of *A. sachalinensis* are found near town Sovetskaya Gavan, in the north of the Sikhote-Alin Mts, which makes the existence of a gene flow from Sakhalin to the continent quite probable. Ignoring gene flow in the ABC analysis can significantly bias estimates of the separation age of populations, since gene flow between populations reduces the differences between them. Thus, the ABC estimate of the separation age of *A. sachalinensis* and *A. nephrolepis* (588 ka) is lower than the estimate made in the phylogenetic study based on chloroplast DNA (795 ka) and even lower than the estimate from nuclear DNA sequences – 1318 ka (Semerikova et al. 2018), i.e., probably underestimated.

In the southern part of the *A. sachalinensis* range, STRUCTURE at K= 6 reveals two clusters (Fig. 1B), one of which (blue, K6_2) is dominant in southern Sakhalin, and the other (dark crimson, K6_5) in the Kurils and Hokkaido. The time of separation of these two population groups is estimated at 118 ka, which corresponds to the last interglacial (MIS 5). Perhaps the marine transgression at that time was the cause of their isolation. Although the straits between Sakhalin, Kurils, and Hokkaido disappeared during the subsequent glacial intervals (Igarashi & Zharov 2011), this did not lead to the erasure of genetic differences between the populations of these territories.

Obviously, estimates made on the basis of the ABC approach should be treated with caution, since any model inevitably contains a significant simplification. Nevertheless, the results obtained clarify the ideas about the history of the fir forests on Sakhalin. In particular, based on paleontological data, it was assumed that fir was absent on northern Sakhalin during the LGM (Igarashi & Zharov 2011), which contradicts the estimate of the age of the northern Sakhalin population. Based on this estimate, it can be assumed that fir has persisted in most of Sakhalin including the northern part of the island during several glacial cycles.

In contrast to *A. sachalinensis*, the continental species *A. nephrolepis* has reduced intrapopulation diversity and low differentiation (Table 1,2, Fig. 2), indicate a reduced, compared to *A. sachalinensis*, effective population size (Table S1, Supplementary materials) and a recent, probably postglacial, distribution in the study area from one refugium.

Origin of *A. gracilis*

ABC analysis suggests the origin of *A. gracilis* as a result of separation from northern Sakhalin population, which is closer to *A. nephrolepis* and not to the southern form of *A. sachalinensis*, which contradicts the phylogeny by chloroplast DNA, but is consistent with morphological data (Orlova 2003, Orlova & Firsov 2004) and mtDNA data (Semerikov et al. 2022). The age of this event is estimated at ~ 37.5 ka (95 % interval 11–353 ka BP, Table S1, Supplementary materials), i.e. it apparently occurred before or around the LGM. The most probable periods close to this estimate, when fir from the continent could have reached

Kamchatka, are the relatively warm epoch MIS 3 (57–29 ka) preceding the LGM, or the Last Interglacial MIS 5 (130–71 ka) with a warmer than modern climate and expansion of tree ranges northward in Northeast Asia. According to pollen data at this time, taiga forests with spruce and possibly fir predominated in Chukotka and in the basin of the river Penzhina (Giterman 1985), and spruce-fir forests were widespread in the Kamchatka itself (Skiba 1975).

The origin of the Kamchatka fir from the Northern Sakhalin fir explains the uncertainty of the phylogenetic position of this taxon. Previously, it was found that the northern Sakhalin populations have a mitochondrial haplotype that brings it closer to *A. nephrolepis*, but not to typical Sakhalin fir of southern Sakhalin (Semerikova et al. 2011), which was used for phylogenetic analysis (Semerikova et al. 2018, Semerikov et al. 2022). At the same time, chloroplast DNA, according to cpSSR data, is almost identical in all fir populations on Sakhalin, but differs from chloroplast DNA of *A. nephrolepis* (Semerikova et al. 2011). Consequently, if Kamchatka fir originates from populations of northern Sakhalin, it should be closer to *A. nephrolepis* by mitochondrial DNA and to the South Sakhalin *A. sachalinensis* by chloroplast DNA.

The existing grove of Kamchatka fir is a relic of a more extensive distribution in the past. The relic character is confirmed by the uniqueness of the plant community in the "fir grove" (Neshataeva & Fet 1994), as well as the composition of bryophytes, which includes a number of species rare for Kamchatka (Kuzmina & Neshataeva 2011). The genetic individuality of the Kamchatka fir, indicated in particular by nuclear microsatellites, confirms the species status of *A. gracilis* and requires comprehensive protection measures and scientific attention.

CONCLUSIONS

In this paper, the genetic variability of three fir species of the section *Balsamea*, *A. nephrolepis*, *A. sachalinensis*, and *A. gracilis*, was studied for the first time using nuclear microsatellite loci throughout most of their range. ABC modeling of the population history of the species under consideration allowed us to select the most reasonable demographic scenario and obtain estimates of model parameters, including the age of individual events, effective population size, etc. The Middle Pleistocene age of the separation of populations of northern Sakhalin from *A. nephrolepis* inhabiting the continental part of the Far East was established. The origin of the Kamchatka fir as a result of separation from populations of the northern Sakhalin has been revealed.

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