#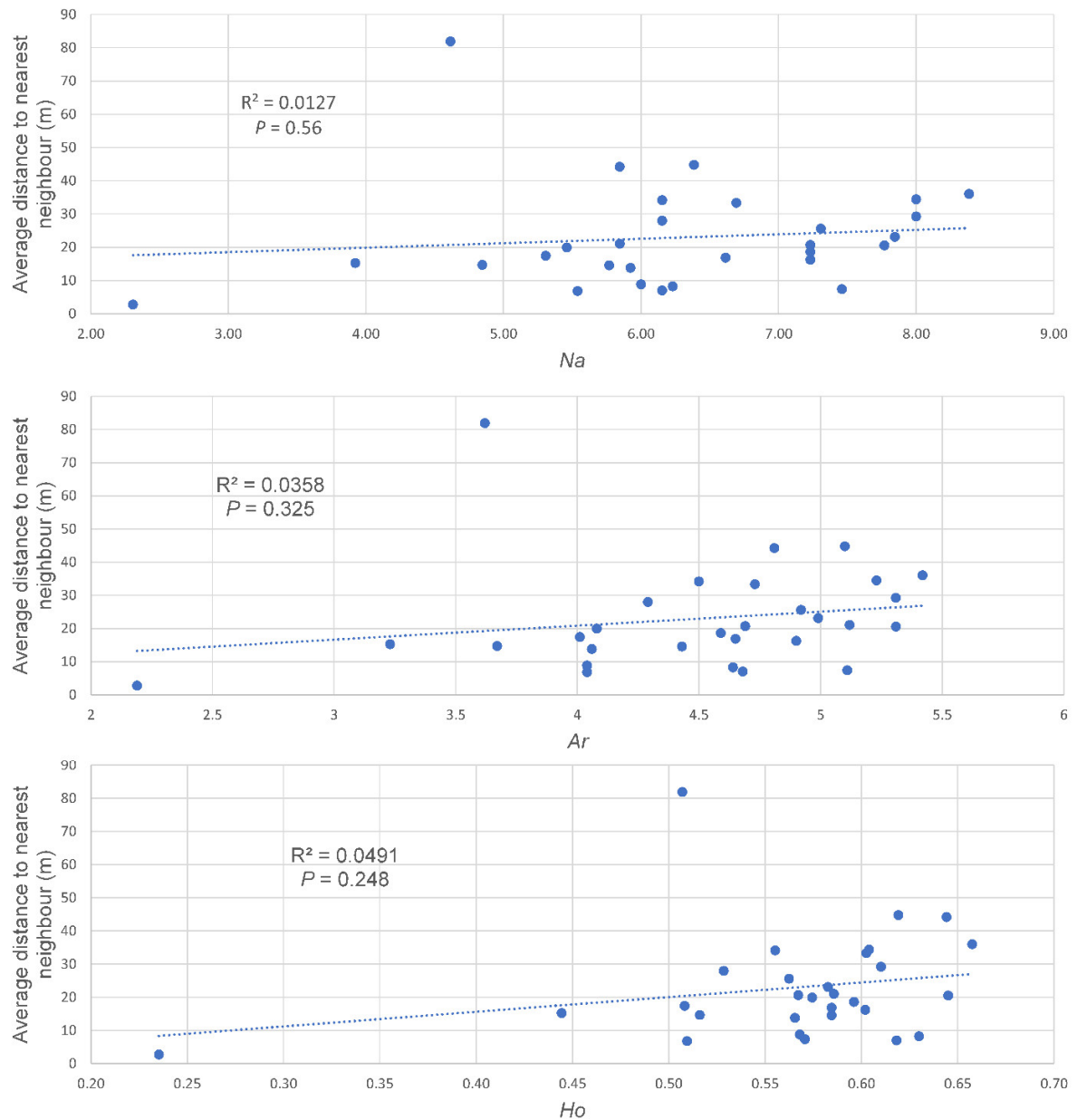 Genetic distinctiveness but low diversity characterizes rear-edge *Thuja standishii* (Gordon) Carr. (Cupressaceae) populations in southwest Japan: Supplementary Materials

Figure S1. Correlation between spatial distancing of samples (m) of the 30 *Thuja standishii* populations versus population-level genetic diversity indices, number of alleles (*Na*), rarefied allelic richness (*Ar*) and observed heterozygosity (*Ho*).
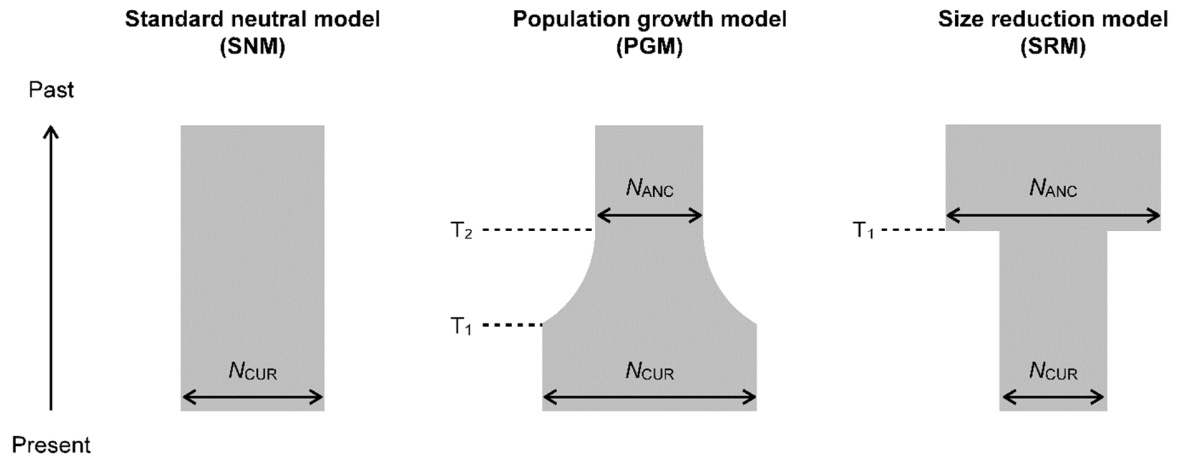
Figure S2 Comparison of the three population size change models used in the ABC based population size change history analysis.
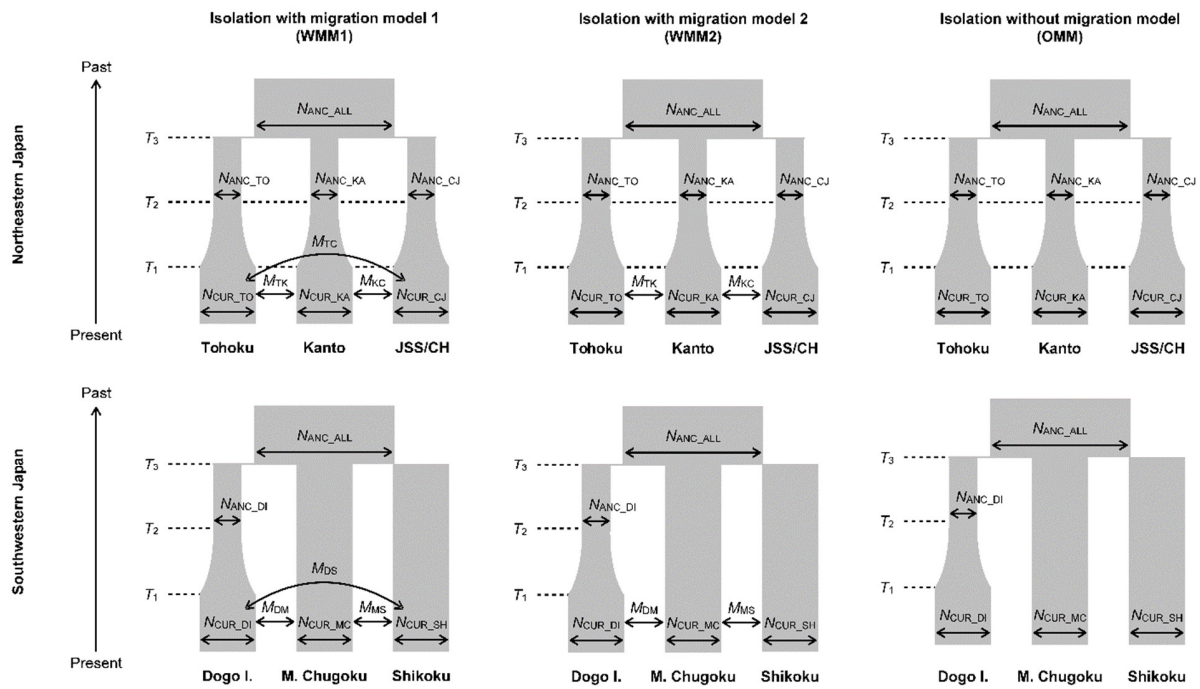
Figure S3 Comparison of the six population divergence models investigated using ABC. JSS/CH stands for Japan Sea side and central Honshu.
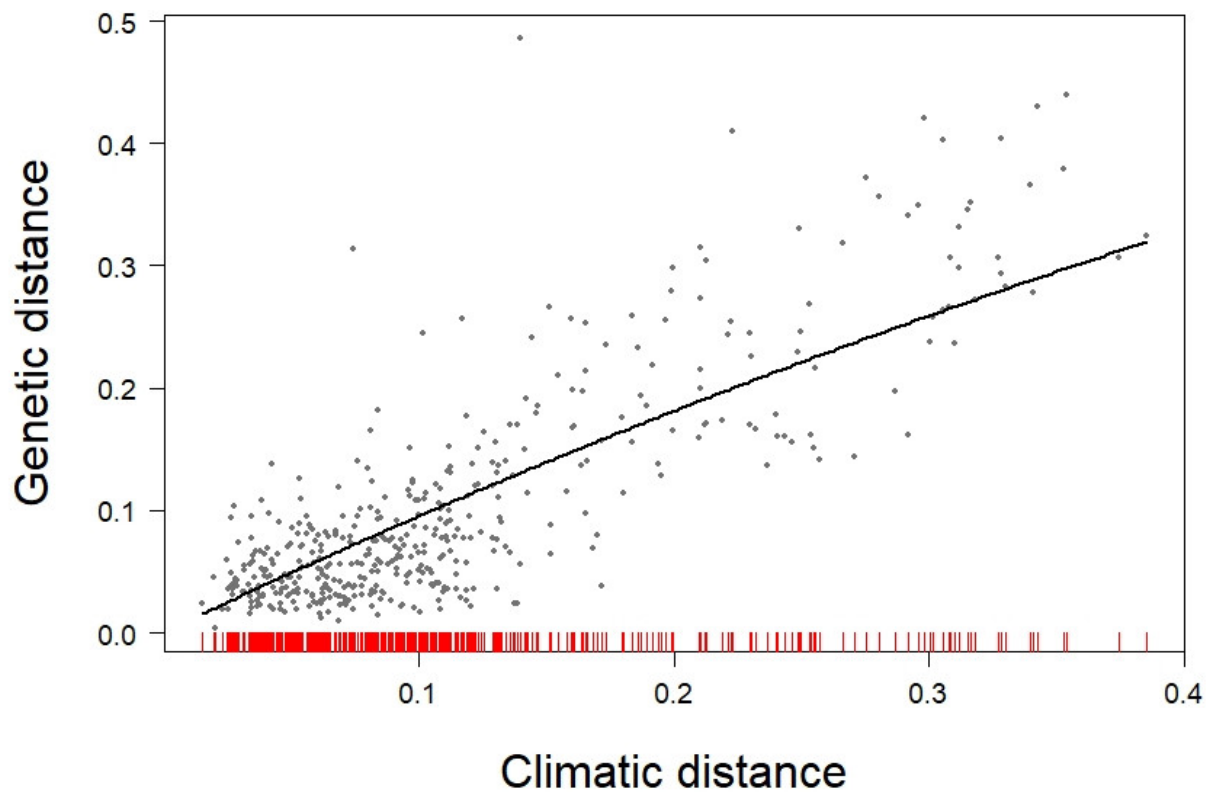
Figure S4 Visualization of the non-linear relationship between climatic distance (the Euclidian distance between the three climate based PC axes) and genetic distance for the 30 populations of *Thuja standishii*. Points correspond to the site pairs. Red tick marks on the x-axis correspond to the spread of the data used in the analysis.
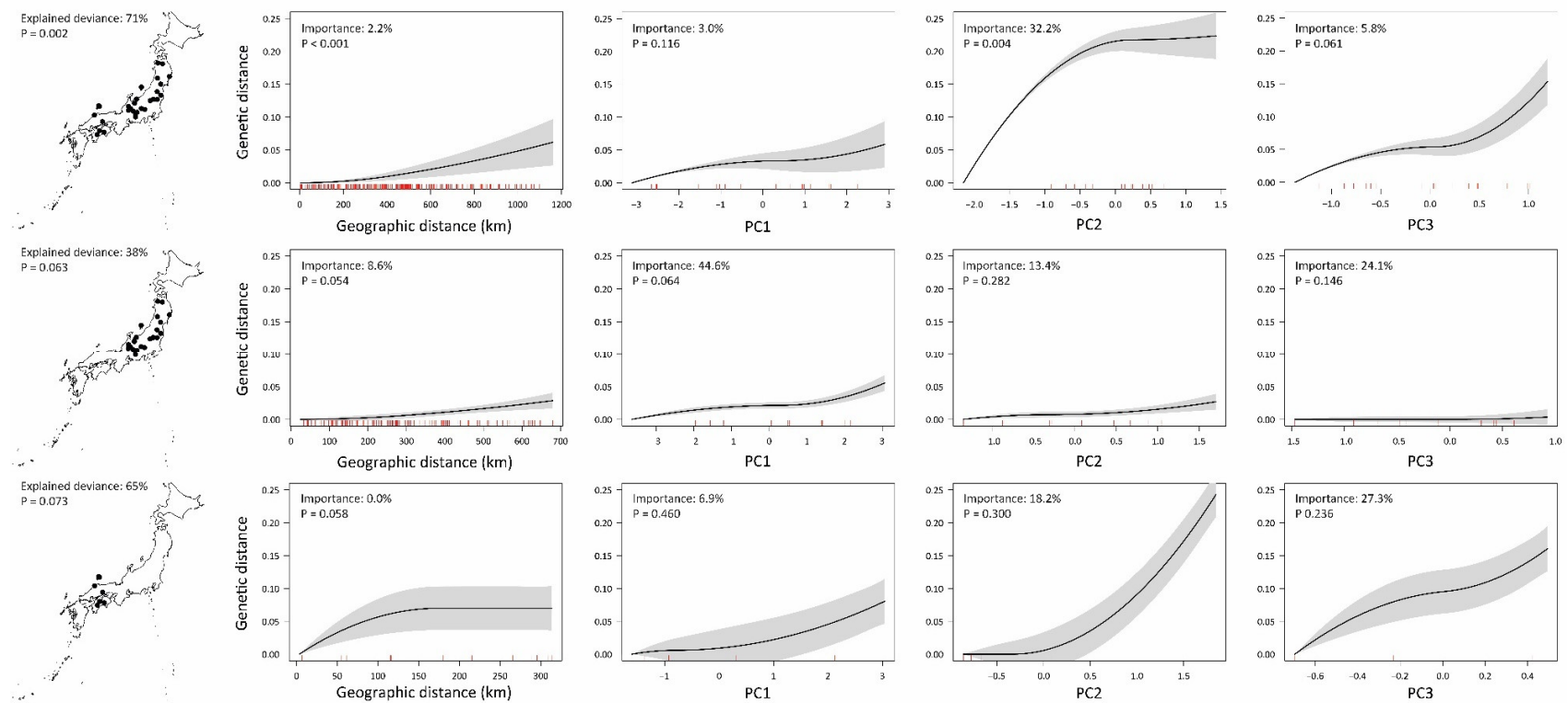
Figure S5 Visualization of the I-splines and their standard errors estimated from 1000 bootstraps of the Generalized Dissimilarity Model for the 30 populations of *Thuja standishii* (top) and the populations in northeastern (middle) and southwestern Japan (bottom). Shown are the I-splines for geographic distance and climatic distance represented by the first three principal components (PC) of a principal component analysis. The amount of deviance in the genetic distance among the populations each model explained is shown in the left-hand panel. The grey area shows one standard deviation of the bootstrapped I-splines. Red tick marks on the x-axis correspond to the spread of the data used in the analysis.
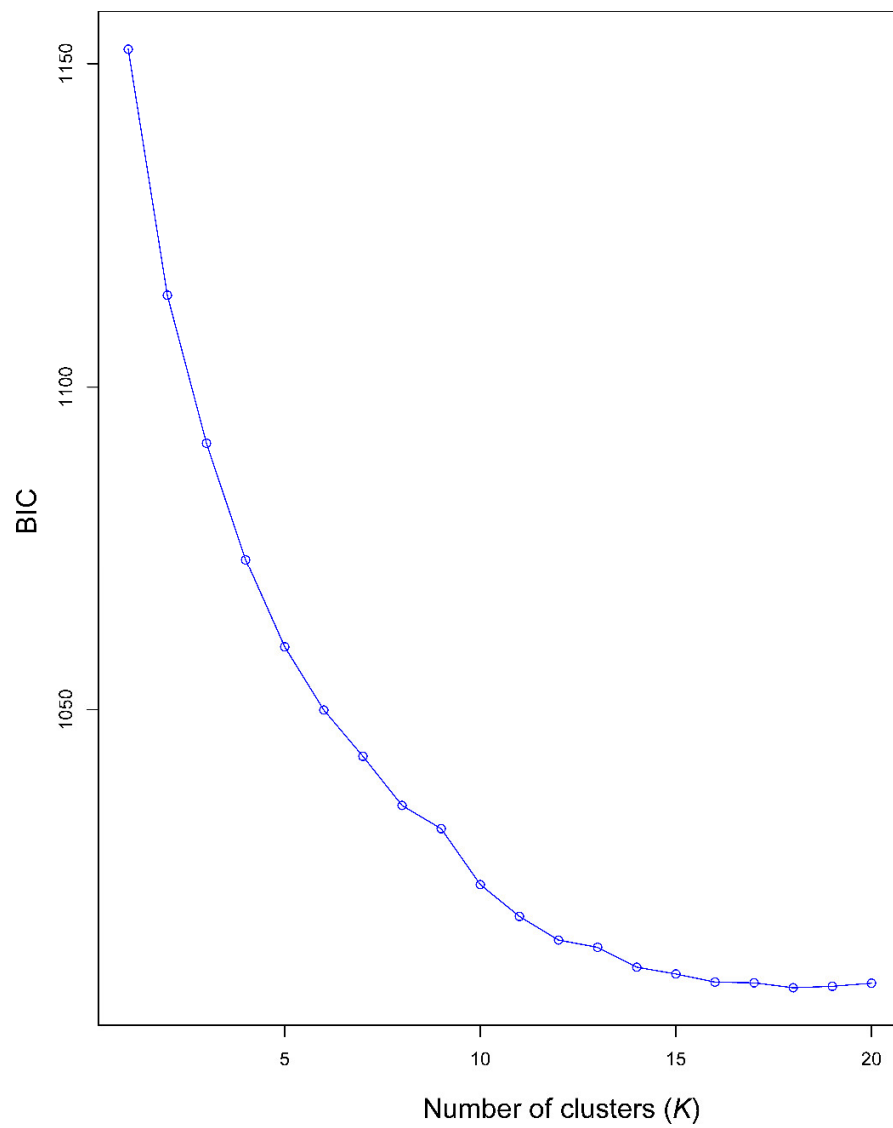
Figure S6 The value of BIC versus the number of genetic clusters (*K*) identified using DAPC analysis.
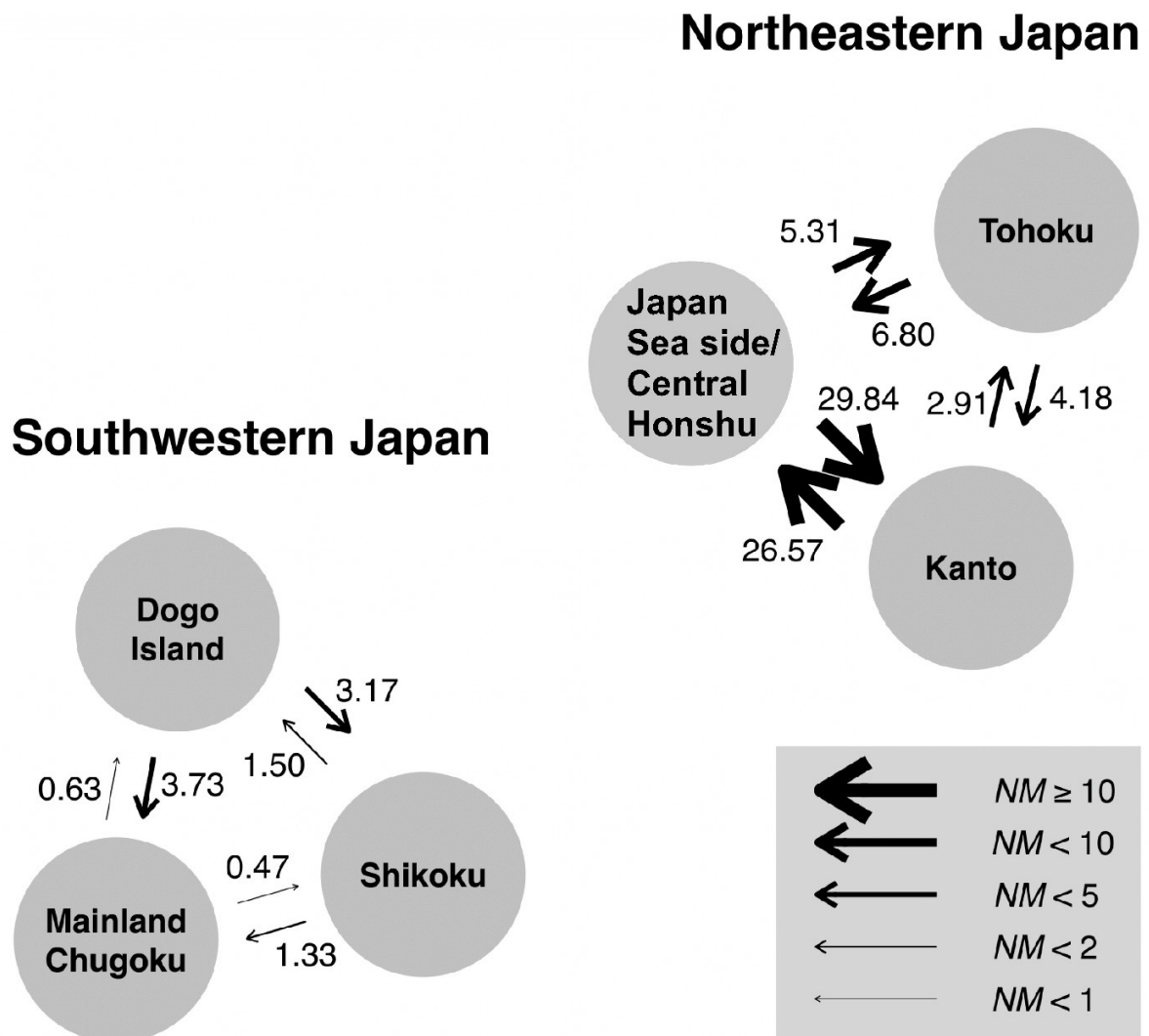
Figure S7 The average number of migrants per generation (*NM*) from the initial divergence of populations from their ancestral population to the present for the northeastern and southwestern Japan regions.
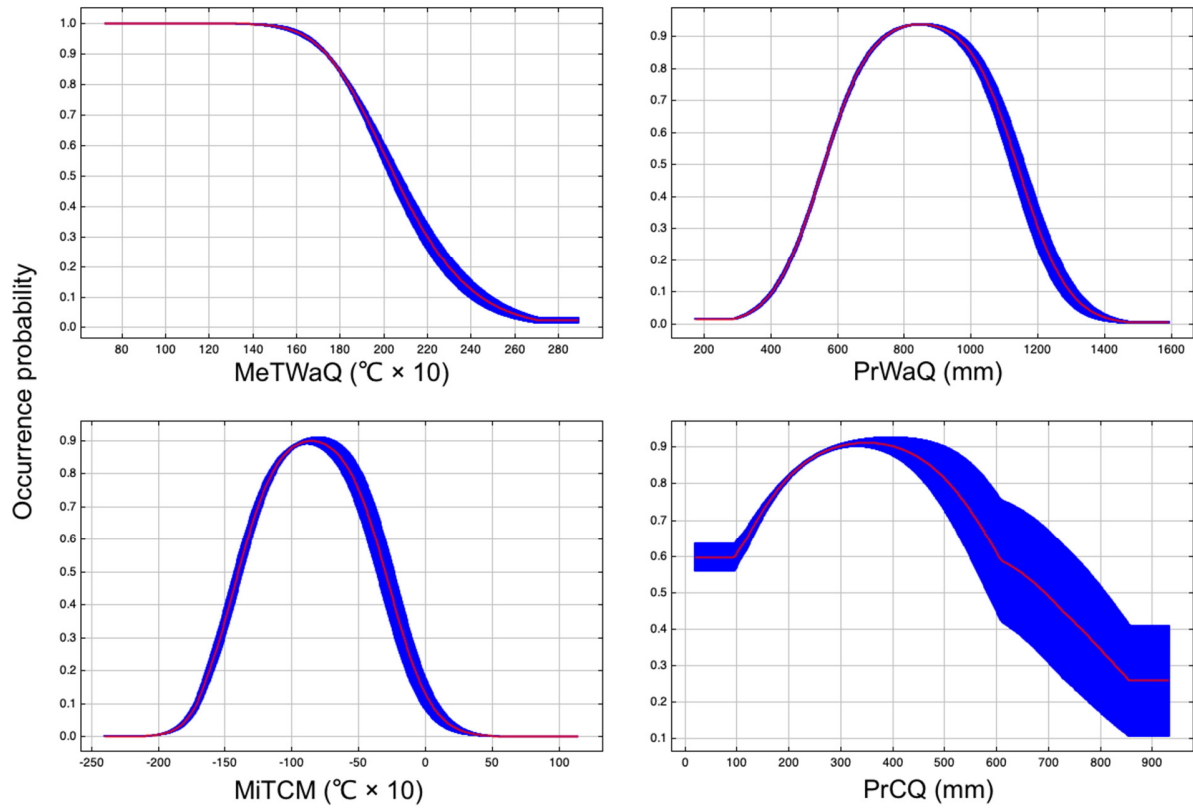
Figure S8 The response of occurrence probability to the four climatic variables used for species distribution modelling of *Thuja standishii*: mean temperature of warmest quarter (MeTWaQ), precipitation of warmest quarter (PrWaQ), minimum temperature of coldest month (MiTCM) and precipitation of the coldest quarter (PrCQ). The curve show how predicted occurrence probability changes as each variable was varied, keeping all other variables at their average value. The curves show the mean response of the 10 replicate model runs (red line) and the mean +/- standard deviation (blue areas).

Table S1 Population-based genetic statistics for the 30 sampled populations of *Thuja standishii* based on thirteen EST microsatellite loci including the number of alleles (*Na*), number of effective alleles (*Ne*), observed (*Ho*) and expected (*He*) heterozygosity, fixation index (*Fis*), rarefied allelic richness (*Ar*), rarefied private alleles (*PAr*) (with the actual number of observed private alleles in brackets) and the probability of re-assignment of individuals to each population based on DAPC analysis. The significance level of deviation from zero of *Fis* is indicated by asterisks (*, $P = 0.01 - 0.05$; **, $P = 0.001 - 0.01$; ***, $P < 0.001$).

No.	Population	Abbrev.	Region	*N*	*Na*	*Ne*	*Ho*	*He*	*Fis*	*Ar* (9 ind.)	*PAr* (9 ind.)	Probability of re-assignment
1	Sakusawa River	SAS	Tohoku	32	6.15	3.06	0.56	0.57	0.039	4.5	0.13 (1)	0.56
2	Lake Towada	TOW	Tohoku	32	5.46	2.84	0.57	0.59	0.036	4.08	0.13 (1)	0.91
3	Mt Goyo	GOY	Tohoku	30	8.00	3.70	0.61	0.62	0.032	5.31	0.15 (3)	0.03
4	Mt Ogoshiki	OGO	Tohoku	30	6.69	3.50	0.60	0.59	0	4.73	0.13 (3)	0.33
5	Mt Ookura	OOK	Tohoku	33	7.85	3.28	0.58	0.60	0.047	4.99	0.11	0.12
6	Nyuugawa	NYUG	Tohoku	10	4.38	2.64	0.58	0.57	0.037	4.24	0.04	0.00
7	Mt Azuma	AZU	Tohoku	32	7.31	3.49	0.56	0.59	0.063*	4.92	0.17 (4)	0.22
8	Mt Otakine	OTA	Tohoku	30	6.62	3.29	0.58	0.60	0.05	4.65	0.12 (2)	0.17
9	Mt Yamizo	YAM	Kanto	34	5.92	2.91	0.57	0.56	0.012	4.06	0.21 (3)	0.15
10	Mt Fuji (Tochigi)	FUJ	Kanto	32	7.23	3.13	0.57	0.58	0.041	4.69	0.12 (1)	0.22
11	Yuno Lake	YUN	Kanto	20	6.38	3.77	0.62	0.65	0.07*	5.1	0.02	0.05
12	Ooyamasawa	OOY	Kanto	36	7.23	2.89	0.60	0.58	-0.005	4.59	0.17 (3)	0.25
13	Mt Kurohime	KUR	Japan Sea side/ Central Honshu	21	6.23	3.16	0.63	0.58	-0.06	4.64	0.19 (1)	0.33
14	Mt Tate	TAT	Japan Sea side/ Central Honshu	27	8.00	3.54	0.60	0.63	0.054*	5.23	0.21 (4)	0.00
15	Sanpouiwa Peak	SAN	Japan Sea side/ Central Honshu	31	8.38	3.92	0.66	0.69	0.057*	5.42	0.11	0.32
16	Nishi Ure Saki	NIS	Japan Sea side/ Central Honshu	31	7.77	3.88	0.65	0.65	0.023	5.31	0.1 (3)	0.16
17	Nyu Dani	NYUD	Japan Sea side/ Central Honshu	29	7.23	3.60	0.60	0.61	0.026	4.9	0.14	0.00
18	Kubota Iwa	KUB	Japan Sea side/ Central Honshu	31	7.46	3.98	0.57	0.65	0.136***	5.11	0.1 (2)	0.00
19	Nagiso Dake	NAG	Japan Sea side/ Central Honshu	16	5.85	3.33	0.64	0.59	-0.069	4.81	0.19 (1)	0.00
20	Shioji Daira	SHIO	Japan Sea side/ Central Honshu	13	5.85	3.73	0.59	0.63	0.107*	5.12	0.14 (1)	0.00
21	Atebi Daira	ATE	Japan Sea side/ Central Honshu	31	6.15	3.03	0.53	0.58	0.099**	4.29	0.06 (1)	0.35
22	Shirakoma Lake	SHIR	Japan Sea side/ Central Honshu	26	6.00	2.59	0.57	0.54	-0.026	4.04	0.12 (1)	0.73
23	Kumi Valley	KUM	Dogo Island	24	5.54	2.63	0.51	0.55	0.095**	4.04	0.03 (1)	0.33
24	Takao	TAK	Dogo Island	20	5.77	3.15	0.58	0.63	0.092*	4.43	0.06 (1)	0.20
25	Washigamine	WAS	Dogo Island	27	6.15	3.66	0.62	0.62	0.028	4.68	0.04	0.41

26	Mt Hanataka	HAN	mainland Chugoku	27	4.62	2.44	0.51	0.53	0.058	3.62	0.03 (1)	0.93
27	Goukei Valley	GOU	mainland Chugoku	17	2.31	1.71	0.24	0.32	0.301***	2.19	0	0.82
28	Monobe Valley	MON	Shikoku	36	3.92	2.19	0.44	0.49	0.105**	3.23	0.08 (1)	0.83
29	Mt Higashiakaishi	HIG	Shikoku	28	5.31	2.86	0.51	0.53	0.059	4.01	0.01	0.86
30	Mt Torigata	TOR	Shikoku	31	4.85	2.40	0.52	0.50	-0.018	3.67	0.11	0.94
			Average	27.23	6.22	3.14	0.57	0.58	0.05	4.49	0.11	0.34

Table S2 For each population where clones were identified, the number of different clones (i.e. identical genotypes) identified, number of samples comprising the clones and number of samples excluded from analyses are shown.

Population	No. of clones	No. of samples comprising clones	No. of samples excluded
Goukei (GOU)	6	13	7
Mt Hanataka (HAN)	1	2	1
Kumi Valley (KUM)	1	2	1
Mt Kurohime (KUR)	3	7	4
Nyuugawa (NYUG)	3	19	16
Mt Otakine (OTA)	1	2	1
Shirakoma Lake (SHIR)	2	4	2
Washigamine (WAS)	1	2	1
Total	18	51	33

Table S3 Prior distributions of structural parameters in each demographic model.

Analysis	Model	Parameter	Distribution
Population size change	SNM	N_{CUR}	Uniform $(10, 5\times10^4)$
	PGM	N_{CUR}[a]	Uniform $(10^4, 2\times10^5)$
		N_{ANC}[a]	Uniform $(10, 3\times10^4)$
		T_1[b]	Log-uniform $(1, 10^3)$
		T_2[b]	Log-uniform $(1, 10^3)$
	SRM	N_{CUR}[c]	Uniform $(10, 3\times10^4)$
		N_{ANC}[c]	Uniform $(10^4, 2\times10^5)$
		T_1	Log-uniform $(1, 10^3)$
Population divergence	WMM1, WMM2, OMM	N_{ANC_ALL}	Uniform $(10, 2\times10^5)$
		T_3	Log-uniform $(10^3, 10^5)$
		M_{XY}[d]	Log-uniform $(10^{-5}, 10^{-3})$

[a] Always $N_{CUR} > N_{ANC}$.
[b] Always $T_1 > T_2$.
[c] Always $N_{CUR} < N_{ANC}$.
[d] M_{XY} is a migration rate between regions X and Y.

Table S4 Summary of BayeScan results for each of three independent runs for all 13 EST microsatellites.

p = the posterior probability for the model including selection; log10(PO) = the logarithm of posterior odds; alpha= a coefficient indicating the strength and direction of selection (+ve indicates diversifying while -ve indicates balancing.

Locus	Run 1			Run 2			Run 3		
	p	log10(PO)	alpha	p	log10(PO)	alpha	p	log10(PO)	alpha
Kurobe_18480	0.00020	-3.69880	-0.00003	0.00040	-3.39768	-0.00006	0.00000	-1000	0.00000
Kurobe_2969	0.00040	-3.39770	0.00001	0.00060	-3.22150	-0.00012	0.00080	-3.09650	-0.00012
Kurobe_23700	0.00000	-1000	0.00000	0.00020	-3.69880	-0.00012	0.00020	-3.69880	-0.00003
Kurobe_44557	0.01840	-1.72700	-0.00858	0.02921	-1.52170	-0.01404	0.03821	-1.40090	-0.01779
Kurobe_15129	0.00140	-2.85320	0.00066	0.00140	-2.85320	0.00048	0.00120	-2.92020	0.00049
Kurobe_16758	0.00040	-3.39770	0.00003	0.00020	-3.69880	-0.00005	0.00080	-3.09650	0.00002
Kurobe_6943	0.00140	-2.85320	0.00051	0.00160	-2.79510	0.00096	0.00120	-2.92020	0.00045
Kurobe_23263	0.00200	-2.69800	-0.00142	0.00140	-2.85320	-0.00089	0.00060	-3.22150	-0.00044
Kurobe_38308	0.00000	-1000	0.00000	0.00120	-2.92020	0.00045	0.00000	-1000	0.00000
Kurobe_41636	0.00040	-3.39770	0.00005	0.00040	-3.39770	0.00002	0.00060	-3.22150	0.00055
Kurobe_42400	0.00020	-3.69880	-0.00001	0.00020	-3.69880	0.00000	0.00000	-1000	0.00000
Kurobe_40825	0.00040	-3.39770	-0.00004	0.00000	-1000	0.00000	0.00020	-3.69880	-0.00003
Kurobe_4219	0.00080	-3.09650	-0.00030	0.00020	-3.69880	-0.00005	0.00040	-3.39770	-0.00014

Table S5 Summary of the results of FreeNa software for the 13 nuclear loci used for genetic analyses.

Locus	Average Estimate of null allele (ENA) frequency per population	*Fst* not using ENA	*Fst* using ENA
Kurobe_18480	0.009	0.097	0.097
Kurobe_2969	0.013	0.096	0.093
Kurobe_23700	0.010	0.080	0.079
Kurobe_44557	0.059	0.068	0.063
Kurobe_15129	0.029	0.132	0.126
Kurobe_16758	0.015	0.093	0.093
Kurobe_6943	0.011	0.131	0.128
Kurobe_23263	0.024	0.068	0.067
Kurobe_38308	0.014	0.102	0.100
Kurobe_41636	0.017	0.045	0.046
Kurobe_42400	0.020	0.061	0.059
Kurobe_40825	0.022	0.073	0.072
Kurobe_4219	0.055	0.089	0.085
Average	0.023	0.087	0.085

Table S6 Results of heterozygosity excess and mode-shift bottleneck tests for each population.

No.	Population	Abbrev.	Region	Mode Shift	Excess Heterozygosity-Wilcoxon signed-rank test
1	Sakusawa River	SAS	Tohoku	normal L-shaped distribution	0.959
2	Lake Towada	TOW	Tohoku	normal L-shaped distribution	0.729
3	Mt Goyo	GOY	Tohoku	normal L-shaped distribution	1.000
4	Mt Ogoshiki	OGO	Tohoku	normal L-shaped distribution	0.945
5	Mt Ookura	OOK	Tohoku	normal L-shaped distribution	0.998
6	Nyuugawa	NYUU	Tohoku	normal L-shaped distribution	0.773
7	Mt Azuma	AZU	Tohoku	normal L-shaped distribution	0.999
8	Mt Otakine	OTA	Tohoku	normal L-shaped distribution	0.772
9	Mt Yamizo	YAM	Kanto	normal L-shaped distribution	0.892
10	Mt Fuji (Tochigi)	FUJ	Kanto	normal L-shaped distribution	0.987
11	Yuno Lake	YUN	Kanto	normal L-shaped distribution	0.683
12	Ooyamasawa	OOY	Kanto	normal L-shaped distribution	0.972
13	Mt Kurohime	KUR	Japan Sea side/ Central Honshu	normal L-shaped distribution	0.993
14	Mt Tate	TAT	Japan Sea side/ Central Honshu	normal L-shaped distribution	0.993
15	Sanpouiwa Peak	SAN	Japan Sea side/ Central Honshu	normal L-shaped distribution	0.944
16	Nishi Ure Saki	NIS	Japan Sea side/ Central Honshu	normal L-shaped distribution	0.993
17	Nyu Dani	NYUD	Japan Sea side/ Central Honshu	normal L-shaped distribution	0.990
18	Kubota Iwa	KUB	Japan Sea side/ Central Honshu	normal L-shaped distribution	0.879
19	Nagiso Dake	NAG	Japan Sea side/ Central Honshu	normal L-shaped distribution	0.863
20	Shioji Daira	SHIO	Japan Sea side/ Central Honshu	normal L-shaped distribution	0.773
21	Atebi Daira	ATE	Japan Sea side/ Central Honshu	normal L-shaped distribution	0.847
22	Shirakoma Lake	SHIR	Japan Sea side/ Central Honshu	normal L-shaped distribution	0.997
23	Kumi Valley	KUM	Dogo Island	normal L-shaped distribution	0.945
24	Takao	TAK	Dogo Island	normal L-shaped distribution	0.752

25	Washigamine	WAS	Dogo Island	normal L-shaped distribution	0.581
26	Mt Hanataka	HAN	mainland Chugoku	normal L-shaped distribution	0.863
27	Goukei Valley	GOU	mainland Chugoku	**shifted mode**	0.080
28	Monobe Valley	MON	Shikoku	normal L-shaped distribution	0.393
29	Mt Higashiakaishi	HIG	Shikoku	normal L-shaped distribution	0.936
30	Mt Torigata	TOR	Shikoku	normal L-shaped distribution	0.878

Table S7 Summary of ABC-RF model comparison.

Region	Proportion of votes						Posterior probability	Classification error rate
	SNM	PGM	SRM	WMM1	WMM2	OMM		
Tohoku	0.135	**0.851**	0.014				0.914	0.330
Kanto	0.123	**0.853**	0.024				0.857	0.335
JSS/CH	0.282	**0.669**	0.049				0.715	0.325
Dogo I	0.324	**0.634**	0.042				0.583	0.337
M Chugoku	**0.598**	0.394	0.008				0.633	0.350
Shikoku	0.451	**0.516**	0.033				0.533	0.334
NE Japan				**0.646**	0.247	0.107	0.699	0.166
SW Japan				**0.559**	0.313	0.128	0.612	0.185

The best model was shown in bold. Note that JSS/CH stands for the Japan Sea side/ Central Honshu

[a] SNM, standard neutral model; PGM, population growth model; SRM, size reduction model

Table S8 Posterior mode and 95% highest posterior density of structural parameters in the best demographic models.

Region	N_{CUR} (×10³)	N_{ANC} (×10³)	T_1 (kya)	T_2 (kya)	N_{ANC_ALL} (×10³)	T_3 (kya)	M_{TK} (×10⁻⁵)	M_{TC} (×10⁻⁵)	M_{KC} (×10⁻⁵)	M_{DM} (×10⁻⁵)	M_{DS} (×10⁻⁵)	M_{MS} (×10⁻⁵)
Tohoku	185 (71.9–200)	9.47 (2.83–25.9)	34.3 (0.230–110)	132 (40.8–150)								
Kanto	176 (73.1–200)	7.04 (2.93–24.1)	16.2 (0.172–87.1)	120 (25.5–150)								
JSS/CH	186 (73.2–199.9)	12.7 (7.84–28.1)	7.40 (1.06–27.1)	127 (71.3–150)								
Dogo I	150 (27.4–196)	11.8 (6.08–27.8)	0.835 (0.151–30.7)	67.5 (3.57–150)								
M Chugoku	4.03 (2.37–7.07)											
Shikoku	11.3 (5.03–25.6)											
NE Japan					1.05 (0.017–16.1)	176 (150–2730)	5.86 (1.03–38.12)	9.53 (1.48–51.67)	53.53 (11.09–99.62)			
SW Japan					15.4 (0.040–159)	250 (153–11412)				15.65 (1.51–59.81)	13.31 (1.84–68.47)	11.73 (1.40–46.99)

Note that JSS/CH stands for the Japan Sea side/ Central Honshu

Table S9 The percent contribution and permutation importance of each of the four bioclimatic variables used for species distribution modelling: mean temperature of warmest quarter (MeTWaQ), precipitation of warmest quarter (PrWaQ), minimum temperature of coldest month (MiTCM) and precipitation of the coldest quarter (PrCQ).

Variables	Percent contribution (%)	Permutation importance (%)
MeTWaQ (℃)	48.7	35.0
PrWaQ (mm)	30.7	32.1
MiTCM (℃)	19.0	29.1
PrCQ (mm)	1.6	3.8