



Expansion of an invasive species, *Ailanthus altissima*, at a regional scale in Japan

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Abstract

Ailanthus altissima, which is recognized as an invasive tree in the Western world, has been widely observed in Japan. To investigate how *A. altissima* expanded within-population and to new populations within a region, 446 *A. altissima* trees were sampled from three separate sites (A, B, and C) including 35 distantly positioned patches, with three chloroplast DNA markers and nine nuclear microsatellite markers. We detected 2, 2, and 3 chloroplast haplotypes in sites A, B, and C, respectively. In addition, 271, 40, and 41 nuclear genotypes were detected in sites A, B, and C, respectively. The clonal richness value was 0.85, 0.78, and 0.53 in sites A, B, and C, respectively. Most trees with the same genotypes were distributed in the same patch, indicating that range expansion by asexual reproduction was limited to a maximum of 45 meters. According to autocorrelation analysis, the extent of nonrandom spatial genetic structure was approximately 0-2 km in sites A and C. KINGROUP analyses showed that 812, 74, and 111 nuclear genotype pairs were detected to have kinship in sites A, B, and C, respectively. Most nuclear genotype pairs were detected within the same patches or sites. These results indicate that the number of *A. altissima* trees gradually increased from seeds, some of which were produced by trees within sites, meaning that this species could regenerate naturally. This shows the need for the future management of *A. altissima* as an invasive species in Japan.

Key words : *Ailanthus altissima*, alien tree, chloroplast DNA, invasive tree, nuclear SSR, sexual and asexual reproduction

INTRODUCTION

Ailanthus altissima (Mill.) Swingle (Simaroubaceae) is a deciduous and dioecious tree native to East China and North Vietnam (Kowarik and Säumel 2007). It rapidly grows (Little 1974) and can reproduce both sexually, i.e., by seed dispersal, and asexually, i.e., by adventitious roots (Little 1974, Kowarik 1995, Kowarik and Säumel 2007). *A. altissima* has invasively expanded in Europe and North America, where it was introduced in the 1700's (Kowarik and Säumel 2007). *A. altissima* was first introduced into Japan in the late 1800's. Recently, this species has spread widely throughout Japan (Shimizu 2003, Kurokochi et al.

2013). The establishment of an invasive woody species might alter ecosystem functioning, consequently having a major impact on native ecosystems (Vitousek 1990, Parker et al. 1999). The invasion of native ecosystems by non-native species is accelerating as the world's human population multiplies and the transport of goods ever more rapidly increases on a global scale (Pimentel 2002). Invasive species and their effects on ecosystems are a cause of biodiversity loss worldwide (Pimentel 2002). Because the invasive expansion of *A. altissima* is possible in Japan as well as in other countries in Europe and North

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America, it is necessary to understand the range expansion of this species in Japan.

Kolar and Lodge (2001) indicated that invasiveness of a plant species is related to characteristics such as taxon, the history of invasion, and asexual reproduction. While an accurate history of the expansion of an introduced species is difficult to obtain, the range expansion of some invasive plants has been demonstrated through population-genetic analysis (Bossdorf et al. 2005, Rosenthal et al. 2008, Pairon et al. 2010). For *A. altissima*, through the genetic analysis of five nuclear microsatellite markers, Aldrich et al. (2010) suggested that the dispersal of *A. altissima* is caused by the movement of seed via traffic along road and railway systems connecting cities in the Eastern part of the U.S. In our previous study, we focused on the history of *A. altissima* in Japan by analyzing 64 Japanese populations of *A. altissima* with a total of ca. 2400 bp of chloroplast DNA sequencing (Kurokochi et al. 2013), showing that most *A. altissima* trees in Japan harbor one of two genetically dissimilar chloroplast haplotypes that are distributed throughout Japan. On the other hand, Liao et al. (2014) reported 12 cp-haplotypes from 440 individuals of 44 populations throughout China after chloroplast DNA sequencing (a total of approximately 1600 bp sequencing). These results indicate that the *A. altissima* populations in Japan have been initially derived from only a few geographically distinct regions in China and have widely spread throughout Japan. Determining the geographic pattern of range expansion of *A. altissima* in each location of Japan from the limited number of introductions is an additional challenge. Considering that *A. altissima* has abilities of both sexual and asexual reproduction, *A. altissima* populations may expand through a combination of both kinds of reproduction. However, the actual condition of *A. altissima*'s expansion within population and to new populations within a region remains unclear.

It is known that the use of microsatellite markers for polymorphism analysis is quite effective for reproductive analyses in a wide range of organisms, from fungi to higher plants and animals. Nuclear microsatellite (nSSR) markers with high polymorphism can contribute to the analysis of parenthood, kinship, and population differentiation (Kurokochi and Hogetsu 2014). The capability of asexual reproduction of woody species via adventitious roots could form large genets with a lot of ramets (Gyokusen et al. 1991, Kowarik 1995, Ogawa and Hokusima 1996, Kurokochi et al. 2010), and genets can be readily detected with high polymorphism nuclear DNA markers (Jung et al. 2009, Kurokochi et al. 2010).

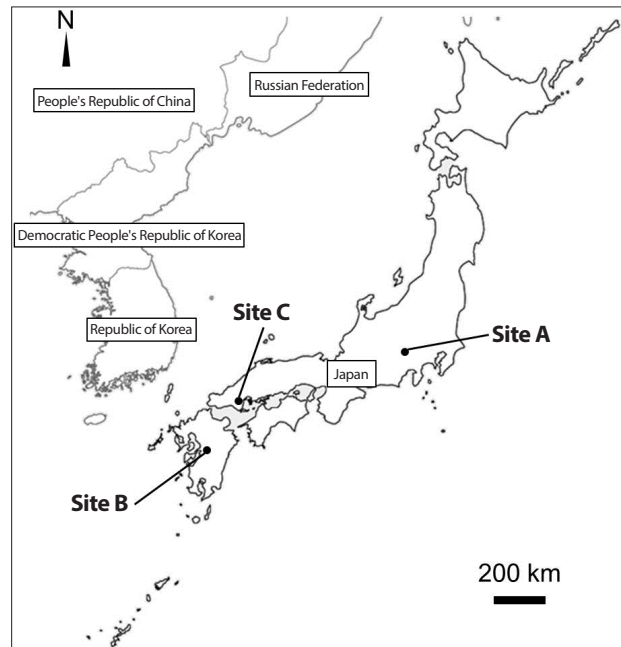


Fig. 1. Locations of sites A, B, and C.

In this study, to gain a better understanding of the method of range expansion of *A. altissima* on a regional scale in Japan, we investigated the genetic relationships among *A. altissima* trees by simultaneously evaluating the polymorphism of chloroplast and nuclear DNA markers.

MATERIALS AND METHODS

Site, patch, and sample collection

We collected *A. altissima* at three sites (A, B and C) in Japan. These sites were located in the center of Yamanashi prefecture, the center of Kumamoto prefecture, and the southwest of Yamaguchi prefecture, respectively (Fig. 1 and Table 1).

There were many *A. altissima* patches of various sizes in the sites. In this study, a patch was defined as one *A. altissima* tree or one continuous stretch of *A. altissima* trees. The long sides of patches were shorter than 100 m. The distances between patches were more than 1 km. In total, 21, 6, and 8 patches were selected in sites A, B, and C, respectively (Fig. 2 and Table 2).

After *A. altissima* patches were haphazardly chosen from sites, *A. altissima* trees were chosen from each patch by the following rules: (1) the diameter at breast height (DBH) of the chosen trees was more than 10 cm and (2)

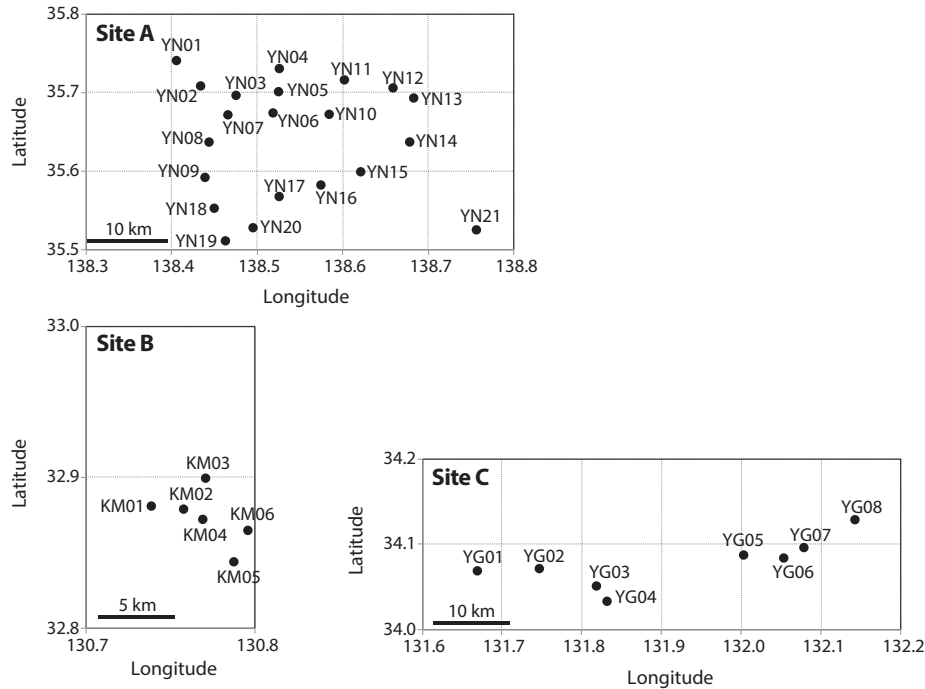


Fig. 2. Distributions of 21, 6, and 8 patches in sites A, B, and C, respectively. Black filled circles represent patches. The names of patches are the same as in Table 2.

the distance between the chosen trees (with DBH > 10 cm) was more than 5 m. A few leaflets were sampled from the chosen trees, whose stand positions were recorded with a hand GPS (Garmin, Chicago, IL, US), and leaflets were dried separately with silica gel in plastic bags, and stored at room temperature for later use.

DNA extraction and microsatellite analysis

DNA was extracted from each leaflet using a modified CTAB method (Kurokochi et al. 2013). Three chloroplast DNA (cpDNA) markers (Aacp01, Aacp02, and Aacp03; Kurokochi et al. in press) and nine nuclear SSR (nSSR) markers (Aa22, Aa68, Aa69, Aa75, Aa76, Aa79, Aa80, Aa82, and Aa92; Dallas et al. 2005) were used. All loci from the cpDNA and nSSR markers were amplified by polymerase chain reaction (PCR) with the Type-IT kit (Qiagen, Hilden, Germany). PCR products were electrophoresed using an

ABI 3130 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA), and their fragment sizes were analyzed with a GeneMapper software (Applied Biosystems).

Statistical analysis

Marker resolution, genotyping, and clonality

Chloroplast haplotypes (cp-haplotypes) of each tree were determined using the three cpDNA markers. For the nine nSSR markers, the number of alleles (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O), and combined non-exclusion probabilities of identity and sib-identity were evaluated with CERVUS 3.0.3 (Marshall et al. 1998). Deviation from the Hardy–Weinberg equilibrium (HWE) was calculated using 1,000 permutations in FSTAT ver. 2.9.3.2 (Goudet 2001). In the analysis, trees of the same cp-haplotype and n-genotype belonged to the

Table 1. Site information and genetic indices of each site

Site	Lat	Long	N	MLG	Hap1	Hap2	Hap3	R	A_R (n = 29)	H_E	F_{IS}
Site A	35°38'24.8"	138°32'21.6"	318	271	91	0	180	0.85	13	0.84	0.211*
Site B	32°52'24.4"	130°46'11.7"	51	40	20	0	20	0.78	10.5	0.81	0.154*
Site C	34°04'40.0"	131°55'04.0"	77	41	14	12	15	0.53	9.7	0.78	0.306*

Lat, latitude; Long, longitude; N, number of analyzed samples; MLG, number of multilocus genotypes; Hap1 to Hap3, chloroplast haplotypes 1 to 3; R, clonal richness; A_R , allelic richness; H_E , expected heterozygosity; F_{IS} , inbreeding coefficient; * $P < 0.01$.

same haplotype group and genet, respectively. Clonal richness (R), a measure of the proportion of unique genotypes in the population, was calculated as $R = (G - 1)/(N - 1)$, where G denotes the number of genotypes and N the total number of genotyped samples (Dorken and Eckert 2001). In a monoclonal stand R is '0' and if R is '1' then every sample has a unique genotype.

For the following statistical analyses, in cases where one n-genotype was detected for multiple trees, we selected one tree as a representative of that n-genotype.

Genetic diversity for each site and genetic differentiation among sites

To understand the genetic diversity of each site, allelic

richness (El Mousadik and Petit 1996), Nei's unbiased expected H_E (Nei's H_E) (Nei 1987), and inbreeding coefficients (F_{IS}) of the *A. altissima* patches in sites A, B, and C were calculated with FSTAT ver. 2.9.3.2, using the nSSR markers. The significance of F_{IS} deviation from zero was tested by 1000 random permutations using FSTAT ver. 2.9.3.2.

In order to detect genetic differentiation among sites, pairwise F_{ST} values were calculated with the Arlequin 2.0 software (Schneider et al. 2000) with 1,000 permutations, using the nSSR markers.

Genetic similarity among trees

In order to detect genetic correlation with geographic

Table 2. Patch information and detected multi-locus genotypes including the distribution of chloroplast haplotypes

Site	Patch	Lat	Long	N	MLG	Hap1	Hap2	Hap3	R
Site A	YN01	35°44'26.8"	138°24'21.3"	23	21	0	0	21	0.91
	YN02	35°42'30.5"	138°26'02.7"	9	9	9	0	0	1.00
	YN03	35°41'47.4"	138°28'31.6"	16	16	16	0	0	1.00
	YN04	35°43'50.1"	138°31'33.6"	24	21	0	0	21	0.87
	YN05	35°42'4.3"	138°31'30.2"	16	13	8	0	5	0.80
	YN06	35°40'26.9"	138°31'07.0"	17	17	4	0	13	1.00
	YN07	35°40'18.2"	138°27'57.4"	22	21	3	0	18	0.95
	YN08	35°38'13.4"	138°26'38.1"	23	15	5	0	10	0.64
	YN09	35°35'31.6"	138°26'21.2"	19	13	13	0	0	0.67
	YN10	35°40'20.6"	138°35'03.1"	15	14	14	0	0	0.93
	YN11	35°42'58.3"	138°36'07.1"	3	1	0	0	1	0.00
	YN12	35°42'21.4"	138°39'31.4"	22	17	0	0	17	0.76
	YN13	35°41'35.4"	138°40'58.3"	15	9	6	0	3	0.57
	YN14	35°38'13.9"	138°40'41.6"	23	22	0	0	22	0.95
	YN15	35°35'57.4"	138°37'15.7"	23	21	0	0	21	0.91
	YN16	35°34'56.9"	138°34'28.3"	3	3	1	0	2	1.00
	YN17	35°34'4.1"	138°31'32.8"	2	2	2	0	0	1.00
	YN18	35°33'10.4"	138°26'59.9"	6	6	0	0	6	1.00
	YN19	35°30'41"	138°27'47.1"	6	2	2	0	0	0.20
	YN20	35°31'41.2"	138°29'43.1"	8	8	4	0	4	1.00
	YN21	35°31'31.4"	138°45'21.9"	23	20	4	0	16	0.86
Site B	KM01	32°52'51.7"	130°44'19.4"	10	6	1	0	5	0.56
	KM02	32°52'44.4"	130°45'28.0"	13	9	5	0	4	0.67
	KM03	32°53'58.1"	130°46'14.7"	4	4	1	0	3	1.00
	KM04	32°52'20"	130°46'08.7"	11	9	3	0	6	0.80
	KM05	32°50'38.8"	130°47'14.7"	10	9	8	0	1	0.89
	KM06	32°51'53.7"	130°47'44.5"	3	3	2	0	1	1.00
Site C	YG01	34°4'8.5"	131°40'08.6"	4	1	0	0	1	0.00
	YG02	34°4'18"	131°44'48.1"	2	2	0	0	2	1.00
	YG03	34°3'4.7"	131°49'05.9"	3	1	1	0	0	0.00
	YG04	34°2'0.2"	131°49'53.7"	26	19	12	7	0	0.72
	YG05	34°5'15.2"	132°00'10.2"	7	3	0	3	0	0.33
	YG06	34°5'2.6"	132°03'11.0"	5	1	0	0	1	0.00
	YG07	34°5'46.6"	132°04'42.0"	27	13	1	2	10	0.46
	YG08	34°7'44.1"	132°08'32.2"	3	1	0	0	1	0.00

Lat, Long, N, MLG, Hap1 to 3, and R: same as for Table 1.



distance among trees, autocorrelation analysis was performed with SPAGeDi (Hardy and Vekemans 2002), using the nSSR data set. Genetic correlation was calculated by Pairwise FRATERNITY coefficients.

For the detection of genetic relationship among trees, the presence of potential kinship among sampled trees was tested using the nSSR markers at a significance level of 0.01 with KINGROUP ver. 2 (Konovalov et al. 2004) using 10,000,000 permutations following the protocol of Kurokochi and Hogetsu (2014). N-genotype pairs with detected full-sibling and half-sibling relationships were considered as having kinship.

RESULTS AND DISCUSSION

Marker resolution, genotyping, and clonality

In total, 318, 51, and 77 samples from sites A, B, and C, respectively, were analyzed. Two, 2, and 3, cp-haplotypes were detected in sites A, B, and C, respectively. In addition, 271, 40, and 41 n-genotypes were detected in sites A, B, and C, respectively. Trees with the same n-genotype always had the same cp-haplotype. Cp-haplotypes were shared between patches within sites whereas no n-genotype was found in more than one patch. The clonal richness statistics were 0.85, 0.78, and 0.53 at sites A, B, and C, respectively (Table 1). Using 271, 40, and 41 n-genotypes detected in sites A, B, and C, respectively, the values of N_A , H_E , and H_O of each nSSR marker were calculated (Table 3). The mean of N_A , H_E , and H_O were 22.1, 0.843, and 0.650, respectively. The combined non-exclusion probabilities of identity and sib-identity were both <0.001.

Table 3. Summary of genetic diversity based on nine nuclear microsatellite markers of *Ailanthus altissima*

Locus name	NA	H_E	H_O	P-HWE
Aa22	15	0.73	0.53	*
Aa68	30	0.94	0.58	*
Aa69	29	0.90	0.75	*
Aa75	20	0.86	0.76	*
Aa76	11	0.70	0.65	*
Aa79	26	0.86	0.56	*
Aa80	27	0.85	0.60	*
Aa82	19	0.88	0.70	*
Aa92	22	0.87	0.72	*
Average	22.1	0.843	0.650	*

NA, the number of alleles per locus; H_E , expected heterozygosity; H_O , observed heterozygosity; P-HWE, deviation from Hardy-Weinberg equilibrium; * $P < 0.01$; combined nonexclusion probabilities of identity and sib identity were both <0.001.

In our previous study on a countrywide scale, we observed that some *A. altissima* populations contained plural cp-haplotypes, whereas others were composed of only one cp-haplotype with no significant genetic differentiation of cp-haplotypes by distance (Kurokochi et al. in press). In the present study, 19 patches had only one cp-haplotype and the other patches had more than one (Table 2). Given that the cp-haplotype is derived from the mother tree, these distributions indicate that more than one seed source was present around the sites and that the *A. altissima* trees in the present study sites were derived from those sources.

Kowarik and Säumel (2007) indicated that *A. altissima* can disperse themselves by clonal ramets, although usually less than 50 m from the parent tree. In this study, all trees with the same n-genotype were located in the same patch (Table 2). Our results suggest that *A. altissima* expanded by active asexual reproduction but the range expansion by asexual reproduction was limited to a maximum of about 45 m.

Genetic diversity for each site and differentiation among sites

A_R and H_E of site A were highest and F_{IS} of site C was the largest. The values of F_{IS} were significantly greater than zero at all sites (Table 1). Because a positive value of F_{IS} is associated with homozygote excess, mating between relatives within sites may affect these significant deviations, which is similar to that in *Robinia pseudoacacia*, an invasive tree species in Japan (Kurokochi and Hogetsu 2014). Or, it is also possible that F_{IS} values at the site level are generated by there being gene frequency differences among patches due to the Wahlund effect, which could give rise to F_{IS} values of the magnitude observed. In addition, pairwise F_{ST} values were 0.206, 0.046, and 0.214 between sites A-B, B-C, and C-A, respectively, and those values were significantly larger than zero ($P < 0.05$). These results indicate that there are genetic differentiations among the sites.

Genetic relationship among trees

A. altissima produces many seeds; for instance, a matured *A. altissima* tree can produce a maximum of 325,000 samaras per year (Bory and Clair-Maczulajty 1980). *A. altissima* is typical of wind-dispersed trees (Kowarik and Säumel 2008), and its seeds can be dispersed and established to up to more than 100 m (Landenberger et al. 2007). In addition, 75% of seedlings were established up

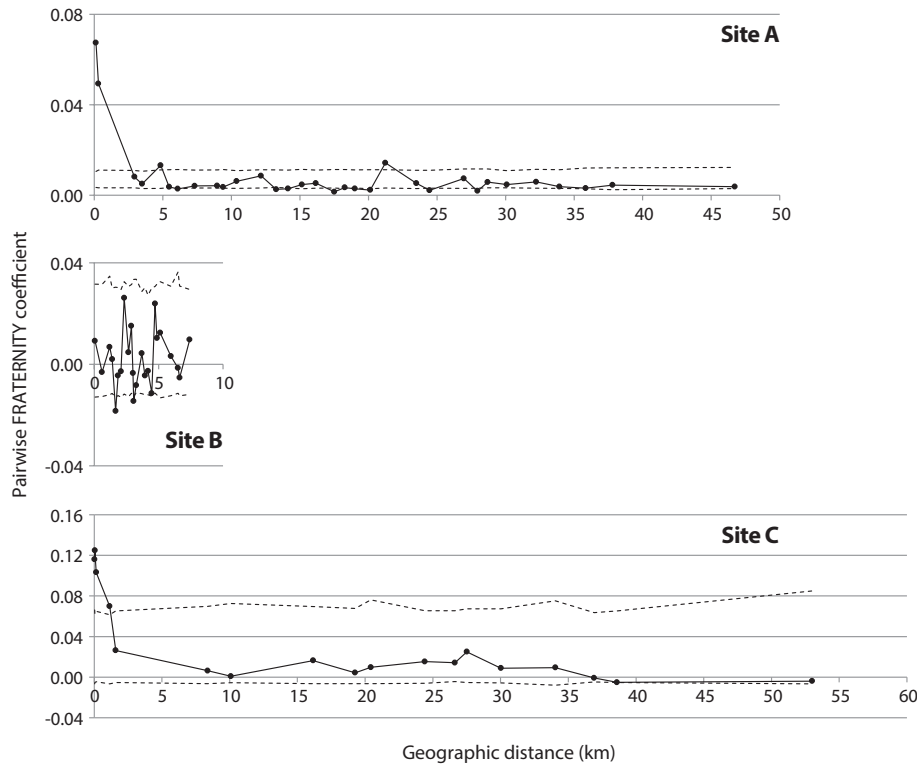


Fig. 3. Result of autocorrelation analysis among 271, 40, and 41 genotypes of *Ailanthus altissima* detected from sites A, B, and C, respectively. Dotted lines denote 95 % significance level.

to 65 m away from the parent tree (Cho and Lee 2003). According to the autocorrelation analysis, nonrandom spatial genetic structures were strong within 1 km in sites A and C (Fig. 3), indicating that *A. altissima* trees could have gradually been established through seeds within a region, some of which were produced from trees within the sites. In contrast, a nonrandom spatial genetic structure was not detected in site B, of which area was narrower than those of sites A and C (Fig. 3). However, in this study, it was still unclear that the result of site B came from small sample size or local conditions.

KINGROUP analyses showed that 812, 74, and 111 n-genotype pairs had kinship in sites A, B, and C, respectively. Most of those pairs (782 of 812, 45 of 74, and 78 of 111 n-genotype pairs) were detected within the same patches or sites (Table 4). These results also supported the autocorrelation analysis, suggesting that the dispersed seeds were mostly established within several hundred meters from mother trees and contributed to the expansion of *A. altissima* trees on a fine scale and that some of them could become founders of new *A. altissima* populations distant from mother trees. On the other hand, some n-genotype pairs having kinship were detected among trees in different sites (Table 4) and this result seemed to have

some relation with the results of autocorrelation analysis in site A, i.e., nonrandom spatial genetic structures were detected approximately in 5 and 22 km (Fig. 3). It might be possible that dispersed seeds had traveled more than several hundred kilometers with the movement of traffic along road and railway systems like in connecting cities in the Eastern U.S. (Aldrich et al. 2010), but it was very unlikely with consideration of the previous report about natural seed dispersal (Cho and Lee 2003). Or, the curious pairs might have occurred incidentally. It is worth noting that the absence of geographic genetic structure has been previously reported on a broad (countrywide and/or landscape) scale for several invasive woody plants (Dunphy and Hamrick 2005, Aldrich et al. 2010, Le Roux et al. 2011), and this tendency has also been previously reported for *A. altissima* in the U.S. (Aldrich et al. 2010) and Japan (Kurokochi et al. 2013). Moreover, *A. altissima* trees derived from only a few genetically confined populations appear to have been introduced into Japan (Kurokochi et al. 2013). In view of these previous studies, some trees established in a geographically distant location may share similar alleles. Such sharing may also account for the anomalous n-genotype pairs.

When cp-haplotypes of each tree were subjected to

KINGROUP analyses, most n-genotype pairs showing kinship were detected among trees belonging to the same cp-haplotypes and only a few of these pairs showed different cp-haplotypes (Appendix 1). Given that some n-genotype kinship pairs showed different cp-haplotypes, it is possible that two trees derived from different intercrossed cp-haplotype groups. Therefore, one reason why most of these n-genotype pairs were detected among trees belonging to the same cp-haplotypes despite the presence of several cp-haplotypes in the same patches may be due

to the low number of initial trees in a location and/or short history of *A. altissima* establishment in each patch.

Range expansion of *Ailanthus altissima* in Japan

The mechanism of the range expansion of *A. altissima* trees at various locations in Japan was inferred by considering the active planting history of *A. altissima* in the past in Japan (Uehara 1959, Shimizu 2003), and findings of our previous (Kurokochi et al. 2013, Kurokochi et al. in press), and present study.

First, some *A. altissima* trees derived from genetically limited populations were introduced to Japan (Kurokochi et al. 2013, Kurokochi et al. in press). Second, seedlings and/or seeds derived from the part of the introduced populations were planted in various locations (Uehara 1959, Shimizu 2003). At the same time, the planted trees grew, matured, and dispersed seeds to the surrounding area mostly within several hundred meters and a few more than several kilometers (Fig 3). Third, a few dispersed seeds succeeded in being established at some distance from the planted trees (Table 4). Fourth, the initially established trees grew, expanded to a maximum several dozen meters through asexual reproduction (Table 2), and dispersed their seeds in the local vicinity (Table 4 and Fig. 3), leading to the establishment of seedlings. As a consequence, *A. altissima* populations developed at various locations in Japan.

Table 4. Kinship pairs of nuclear genotypes

Site	Patch	No. of pairs connected by kinship				
		O+A+B+C	O	A	B	C
Site A	YN01	116	91	21	1	3
	YN02	41	8	33	0	0
	YN03	30	14	16	0	0
	YN04	45	30	14	1	0
	YN05	28	6	21	0	1
	YN06	58	40	12	0	6
	YN07	62	50	12	0	0
	YN08	30	10	15	3	2
	YN09	45	26	19	0	0
	YN10	51	37	13	1	0
	YN11	1	0	1	0	0
	YN12	39	27	8	3	1
	YN13	21	12	8	0	1
	YN14	62	38	22	0	2
	YN15	54	42	12	0	0
	YN16	3	0	3	0	0
	YN17	2	0	2	0	0
	YN18	4	1	3	0	0
	YN19	23	1	22	0	0
	YN20	63	22	41	0	0
	YN21	34	17	12	4	1
Site B	KM01	8	1	1	6	0
	KM02	20	3	5	10	2
	KM03	8	0	0	7	1
	KM04	13	2	3	5	3
	KM05	20	1	4	9	6
	KM06	5	0	0	1	4
Site C	YG01	0	0	0	0	0
	YG02	8	1	0	1	6
	YG03	0	0	0	0	0
	YG04	68	44	9	12	3
	YG05	6	2	1	1	2
	YG06	0	0	0	0	0
	YG07	29	11	7	2	9
	YG08	0	0	0	0	0

O, number of nuclear genotype pairs connected by kinship within each patch; A, number of nuclear genotype pairs connected by kinship with patches in site A; B, number of nuclear genotype pairs connected by kinship with patches in site B; C, number of nuclear genotype pairs connected by kinship with patches in site C.

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Appendix 1. Numbers of nuclear genotype pairs connected by kinship in pairs of patches

Patch	YN01	YN02	YN03	YN04	YN05	YN06	YN07	YN08	YN09	YN10	YN11	YN12	YN13	YN14	YN15	YN16	YN17	YN18	YN19	YN20	YN21	KM01	KM02	KM03	KM04	KM05	KM06	YG01	YG02	YG03	YG04	YG05	YG06	YG07	YG08		
YN01	91	6(6)	4	3	7(2)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
YN02	8																																				
YN03	6(6)	14	1	1	1(1)	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
YN04	4		30																																		
YN05		1	1	6(1)	2	2	40(1)	3(1)	1	1(1)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
YN06																																					
YN07	3																																				
YN08	7(2)																																				
YN09		1	2	3	3	9	10(2)	26	37																												
YN10																																					
YN11																																					
YN12																																					
YN13																																					
YN14																																					
YN15																																					
YN16																																					
YN17																																					
YN18	1																																				
YN19																																					
YN20																																					
YN21																																					
KM01																																					
KM02																																					
KM03																																					
KM04																																					
KM05	1(1)																																				
KM06																																					
YG01																																					
YG02																																					
YG03																																					
YG04	2(2)																																				
YG05																																					
YG06																																					
YG07	1(1)																																				
YG08																																					

Patch, same as Table 1; in parentheses, number of nuclear genotype pairs connected by kinship detected among different chloroplast (cp)-haplotypes; before parentheses, number of nuclear genotype pairs connected by kinship.