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ORIGINAL ARTICLE

Clonal structure, growth pattern and preemptive space occupancy through sprouting of an invasive tree, *Triadica sebifera*

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ABSTRACT

We investigated clonal structure, growth pattern and colonization through sprouting in the invasive tree species *Triadica sebifera* on Mt. Mikasa, Nara City, Japan. Genets of *T. sebifera* were identified by microsatellite DNA analysis. The age of stems was determined by counting annual growth rings. In a 25 × 60-m plot, we found 214 genets among 315 stems ≥130 cm in height. Of these, 23 genets were multistemmed. Numbers of stems on a genet increased with increasing genet age. The size, shape and number of stems indicated that several genets adopted a guerilla growth strategy in which stems are widely spaced along roots. Only one genet seemed to adopt a phalanx growth strategy characterized by high stem density within limited areas. Older stems occurred only in the vicinity of the parental stems of genets. Young stems occurred not only at the leading edge of the genet but also close to the parental stem. Stem size was negatively correlated with the number of younger stems but positively correlated with that of older stems on the same genet. This suggests that stems were physiologically connected and that translocation of photosynthetic products was directed from old to young stems. Physiological integration may allow small stems to persist under low light availability. These findings indicate that urgent measures are required to control the *T. sebifera* population because the species increases its area of occupancy by vigorous stem sprouting which may compromise the natural forest.

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KEYWORDS
Chinese tallow tree; clonal reproduction; ecological invasion; introduced plant; microsatellite DNA analysis

Introduction

Invasion by introduced plants is one of the most serious threats to native ecosystems (Lake & Leishman 2004). Invasive plants influence the survival and reproduction of indigenous plants and are able to change structure and function of ecosystems (Cameron & LaPoint 1978; Vitousek & Walker 1989; Bruce et al. 1997; Maesako et al. 2007). As an invader that has naturalized in various places in the world, Chinese tallow tree (*Triadica sebifera* (L.) Small) is known. It is a deciduous tree native to China and northern Vietnam (Pattison & Mack 2008) and has naturalized in other parts of Asia (Maesako et al. 2007), Australia (Pattison & Mack 2008) and the USA (Webster et al. 2006). The invasion of *T. sebifera* into natural ecosystems has decreased the richness of native plants (Bruce et al. 1997) and invertebrate fauna (Cameron & LaPoint 1978) and altered ecosystem productivity (Cameron & Spencer 1989).

A single mature tree of *T. sebifera* produces many seeds (Webster et al. 2006) which are spread by birds over several hundred meters (Fukui & Ueda 1999; Okugawa & Nakatsubo 2009). Wide-range seed dispersal that has been emphasized as an attribute contributing to invasion success (Vitousek & Walker 1989; Rejmánek & Richardson 1996; Myers et al. 2004; Trakhtenbrot et al. 2005) would enable *T. sebifera* to be a successful invader. After germination, seedlings of *T. sebifera* grow rapidly under high light conditions (Shimoda et al. 1994), and their roots readily sprout (Siril & Dhar 1997; Webster et al. 2006).

From the viewpoint of colonization, not only by successful spread but also satisfactory survival and growth of established individuals are important. In a post-dispersal phase, maintenance of a plant body and persistence at a newly invaded site are key processes in determining the success of invasion. Plant species with vigorous sprouting ability can be a successful invader because (1) dozens of sprouts emerge per individual (Sakio 2003) and (2) sprouts often have higher survival rates (Sundriyal & Bisht 1988) than seedlings (Koop 1987) probably due to physiological support by translocation of assimilates (Isogami et al. 2011). Not only seed dispersal but also sprouting should be taken into consideration in the population control of invasive species (Takahashi et al. 2008). Although vigorous sprouting of *T. sebifera* likely contributes to its invasion success in diverse regions in the world, detailed quantitative analysis of its vegetative reproduction has not performed yet.

In the present study, we used microsatellite DNA markers to identify genets of *T. sebifera* to reveal the clonal structure of a population. We have four aims. First, we evaluate the sprouting ability of *T. sebifera*. For this purpose, we elucidated the proportion of sprouting genets in the population and the number of stems per a sprouting genet. Second, we assess the dependence on sprouting in the population maintenance of *T. sebifera* by calculating the proportion of stems produced by sprouting genets among all stems in the population. Third, the growing process of sprouting genets is estimated. For this purpose, the relationship between genet age and number of stems of the genet was elucidated. Spatial arrangement of stems within a genet was clarified. Furthermore, factors relating to growth of each stem in a genet were explored. And forth, we discuss

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the role of sprouting for *T. sebifera* to impact on forest ecosystems as an invader.

Materials and methods

Study species and site

T. sebifera is a deciduous tree native to China and northern Vietnam (Pattison & Mack 2008). The seeds of this species are bird-dispersed (Fukui & Ueda 1999). Genets produce new stems asexually via root suckers (Siril & Dhar 1997). *T. sebifera* has a high light demand and grows quickly under well-lit conditions (Shimoda et al. 1994). Its stem often grows 30–80 cm in height per year (Matsuno et al. 1984).

The study was performed on Mt. Mikasa (294 m altitude, 34°41′N, 135°51′E), Nara City, Japan. The Nara Meteorological Station (104 m altitude) recorded an average temperature of 14.9°C and an annual precipitation of 1316.0 mm for the period 1981–2010. The forest is adjacent to Nara Park, where the sacred Sika deer (*Cervus nippon* Temminck) population has been protected and constant to about 1000 since the 1960s (Ohmae et al. 1996). The deer browsing has influenced the vegetation around Mt. Mikasa which is characterized by unpalatable species such as *T. sebifera* (Nanami et al. 1999). *T. sebifera* escapes browsing pressure through chemical defense mechanisms (Takatsuki 1989). The population of *T. sebifera* around Mt. Mikasa originated from roadside trees planted in 1934 (Suganuma 1982).

In 2004, we established a 200 × 400-m plot on the north-west-facing slope of Mt. Mikasa. We mapped all 117 *T. sebifera* trees ≥10 cm in stem diameter at breast height (dbh, 130 cm above ground level) in the plot and recorded their dbh. Fresh leaves for DNA extraction were sampled from all of these trees. In 2009, we established a 25 × 60-m plot in an open-canopy site within the 200 × 400-m plot. The 25 × 60-m plot was located where the stems <10 cm dbh and ≥130 cm in height cannot be found within 20 m from the edge of the plot (Nanami personal observation). In this 25 × 60-m plot, we mapped all 321 stems ≥130 cm in height and sampled fresh leaves. Subsequently, we cut all recorded stems at ground level and sampled their basal cross sections. For each cross section, we measured the minor and major axes to evaluate the size and growth. Annual growth rings were counted in the laboratory to determine age of stems.

DNA analysis

We could use two methods to identify genets of *T. sebifera*, AFLP or microsatellite genotyping. We chose the microsatellite genotyping from the two methods because *T. sebifera* is a tetraploid tree (DeWalt et al. 2006) which is expected to have various genotypes. Microsatellite analysis is an effective procedure for identifying genets of a plant species (e.g. Namroud et al. 2005). In this study, we used six microsatellite loci (DeWalt et al. 2006) to identify genets of *T. sebifera*. Fresh leaves that we had collected were ground to powder in liquid nitrogen with a mortar and pestle. DNA was extracted with a modified cetyl trimethyl ammonium bromide (CTAB) method (Murray & Thompson 1980). Polymerase chain reaction (PCR) amplification was performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The reaction mixture (10.0 µl) consisted of 5.0 µl 2× QIAGEN Multiplex PCR Master Mix (QIAGEN, Tokyo, Japan), 3.0 µl RNase-free water, 0.2 µM primer F, 0.2 µM primer R and 1.0 ng template DNA. The PCR amplification protocol consisted of initial denaturation at 95°C for 15 min, 34 cycles of denaturation at 94°C for 30 s, 57°C annealing temperature for 90 s, extension at 72°C for 60 s and final incubation at 60°C for 30 min. Fragment analysis was performed with an ABI 310 Genetic Analyzer (Applied Biosystems), and genotypes were detected using GeneMapper 3.0 software (Applied Biosystems).

Data analysis

We estimated allele frequencies including null allele of each locus in the Mt. Mikasa population by R statistical computing 2.15.2 using the polysat package following De Silva et al. (2005). For the estimation, we need information of genotype data of a population and selfing rate of the study species. We used information of genotype of the 117 trees ≥ 10 cm dbh in the 200 × 400-m plot. Identical genotypes were not found in the 117 trees. The selfing rate of *T. sebifera* was assumed to be 0.36 (Moriya unpublished data). This value was obtained by artificial self-pollination while preventing the pollen flow from other individuals by bagging.

To assess the power of differentiation of the six simple sequence repeat (SSR) markers, we calculated the probability of identity (P_{ID}), which is the probability that two individuals produced by random mating in a population have the same multilocus genotype (Waits et al. 2001). Because *T. sebifera* is tetraploid (DeWalt et al. 2006), possible combinations of alleles at a locus are large and calculation of P_{ID} is complicated (Narayan et al. 2015). We therefore estimated P_{ID} by using the following Monte Carlo simulation, which was a slightly modified version of the method of Narayan et al. (2015).

First, we simulated a pair of two individuals which had four alleles at *i*th locus. For each individual, four alleles were randomly selected following to the estimated relative frequency of alleles including a null allele. We made 100,000 individual pairs and calculated the proportion of pairs that had the same genotypes as the probability of identity of *i*th locus (P_i). Because we could not detect the presence of null allele and the dosage of each allele by the SSR markers, we distinguished genotypes only from the presence/absence data of alleles (SSR type). Thus, we calculated the value of P_i based on the SSR type of each simulated individual. This would lead to a larger and more conservative P_i value than in the case that we know the dosage and there is no null allele. Simulation was repeated independently for six loci, and P_{ID} was estimated as

$$\prod_{i=1}^{6} P_i$$

Narayan et al. (2015) used a similar method and found that 100,000 simulations provided an accurate enough estimate of P_{ID} for populations of a polyploid tree, *Sequoia sempervirens* (Cupressaceae). The largest P_{ID} calculated was 6.56×10^{-5}. This value indicates that the probability of coincidence of genotypes between different genets was

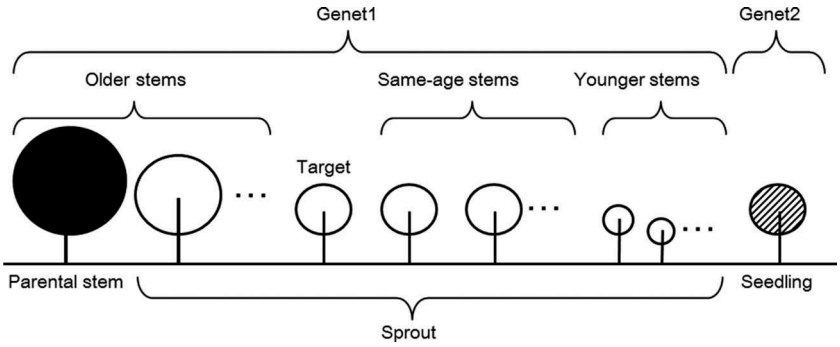

Figure 1. Schematic diagram of generalized linear mixed model (GLMM) to explore factors relating to stem growth of *Triadica sebifera*. The dependent variable is the cross-sectional area of a target stem in 2009. As independent variables, three variables on each stem are used: age (number of annual growth rings indicated as the size of a circle in the figure), the cross-sectional area at 1 year of age and its category. Each stem is categorized into parental stem (indicated as a black circle), sprout (white circle) and seedling (hatched circle). For a multistemmed genet (Genet 1), the oldest stem on the genet is regarded as the parental stem, and other stem is considered to be sprouts. A single-stemmed genet is defined as a seedling (Genet 2). A parental stem and a seedling are considered to develop from a seed, while a sprout has grown up vegetatively from root. The number of younger and older stems than the target stem and the number of stems of the same age as the target on the same genet are also included as the independent variables.

6.56×10^{-5} at most. Thus, stems with identical genotypes were considered members of the same genet.

We calculated allelic variation for 117 trees ≥10 cm dbh in the 200 × 400-m plot and 315 of 321 stems ≥130 cm in height that were successfully genotyped for at least 5 loci in data analysis in the 25 × 60-m plot. We estimated the number of alleles per locus, N_A, observed heterozygosity, H_O and expected heterozygosity, H_E. For calculation, samples with the identical genet were only counted once per population (Pappert et al. 2000).

We scored the observed heterozygosity value for each locus following Brown and Young (2000): thus, the value was the probability that any two alleles drawn at random from a locus were not identical by descent. H_O for phenotypes with one allele, e.g. *AAAA*, is equal to 0. For two alleles (e.g. *A* and *B*), the minimum H_O is 0.50 assuming a genotype of *AAAB* or *ABBB* and the maximum is 0.66 assuming a genotype of *AABB*. For three alleles, e.g. *AABC*, H_O is 0.83, regardless of which allele has two copies. For four alleles, e.g. *ABCD*, it equals 1. We used ATETRA 1.0 software (Van Puyvelde et al. 2010) to calculate expected heterozygosity, H_E.

Parameters of stems and genets

Three variables were measured on each stem: age (number of annual growth rings), cross-sectional area and its category. Each stem was categorized into seedling, parental stem and sprout (Figure 1). The single-stemmed genet was defined as a seedling. For a multistemmed genet, the oldest stem on the genet was regarded as the parental stem, and other stems were considered to be the sprout which has grown up vegetatively from root. The possibility that the parental stem on a genet was outside of the plot would be low. The 117 mature *T. sebifera* trees ≥10 cm dbh in the 200 × 400-m plot didn't have the same genotype with 315 stems in the 25 × 60-m plot and there were no stems <10 cm dbh and ≥130 cm in height within 20 m from the edge of the 25 × 60-m plot (Nanami personal observation). Therefore, regarding the oldest stem in the plot as the parental stem would be valid. Cross-sectional area was calculated from the lengths of major and minor axes assuming an elliptical shape.

We measured three values on each genet: age, number of stems and horizontal extent. We assumed that the age of a genet was equal to the age of the parental stem on the genet. The number of stems on a genet was obtained by counting all stems, including the parental stem and the derived sprouts. To determine the horizontal extent of a genet, we measured distances between all pairs of stems on a genet. Among these, the largest value was used as a measure of genet horizontal extent.

To deduce the growth pattern of genets in the process of aging, we examined the relationships between age and number of stems or horizontal size of genets. To elucidate the strategy of growth, i.e. phalanx or guerilla type (Lovett Doust 1981), of *T. sebifera*, we explored the relationship between horizontal size and number of stems of genets. To infer the process of stem development, the location of stems on a genet by age was investigated.

We used general linear mixed model (GLMM) to explore factors relating to stem growth (Figure 1). The dependent variable was the cross-sectional area of a stem in 2009. Independent variables were age, the cross-sectional area at 1 year of age, the number of younger stems than it on the genet, the number of older stems on the genet, the number of same-age stems on the genet and category (seedling, sprout or parental stem). The random effect is a genet to which a stem belongs. Effective independent variables were selected by a least AIC model. The analysis was performed in R statistical computing 2.15.2 using lmer in the *lme4* package. For the setting of the package, an identity function and a Gaussian distribution were chosen as a link function and an error distribution, respectively.

Results

Of 321 stems, we used 315 stems that were successfully genotyped for at least 5 loci in data analysis in the 25 × 60-m plot. The number of alleles per locus among the 6 microsatellite loci ranged from 4 to 12 and from 3 to 11 among 117 trees ≥10 cm dbh in the 200 × 400-m plot and 315 stems ≥130 cm in height in the 25 × 60-m plot, respectively (Table 1). The expected heterozygosity (H_E) estimated from relative frequencies of alleles ranged from 0.462 to 0.828 in the 117 trees ≥10 cm dbh and from 0.501 to 0.849 in the 315 stems ≥130 cm in height (Table 1).

Table 1. Allelic variation for six microsatellite loci among (a) *Triadica sebifera* trees ≥10 cm in stem diameter at breast height (dbh, 130 cm above ground level) in the 200 × 400-m plot (n = 117), and among (b) stems ≥130 cm in height in the 25 × 60-m plot (n = 315) on Mt. Mikasa, Nara City, Japan.

Locus	N_A	Minimum H_O	Maximum H_O	H_E	n
(a)					
Ts-A10	4	0.443	0.811	0.502	117
Ts-B103	5	0.353	0.782	0.462	117
Ts-B5	12	0.797	0.952	0.828	117
Ts-D101	8	0.593	0.884	0.694	117
Ts-D11	4	0.585	0.936	0.670	117
Ts-D117	8	0.846	0.969	0.751	117
(b)					
Ts-A10	3	0.488	0.642	0.501	315
Ts-B103	4	0.472	0.587	0.531	315
Ts-B5	11	0.880	0.897	0.849	231
Ts-D101	6	0.502	0.578	0.621	310
Ts-D11	3	0.722	0.773	0.666	297
Ts-D117	8	0.792	0.829	0.727	315

N_A: Number of alleles per locus; H_O: minimum and maximum bounds of observed heterozygosity; H_E: estimated heterozygosity; n: the number of stems which was successfully genotyped per locus.

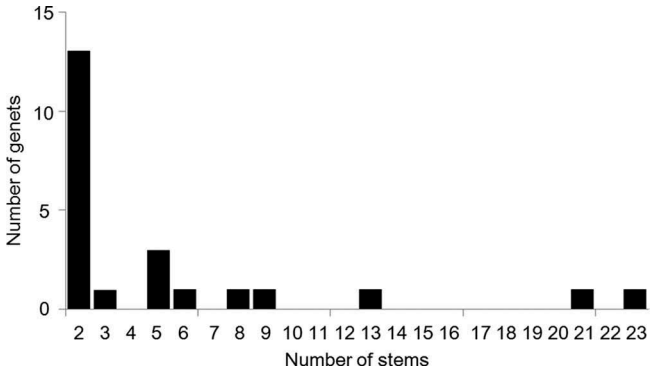

Figure 2. Frequency distribution of number of stems per genet of *Triadica sebifera* in the 25 × 60-m plot on Mt. Mikasa, Nara City, Japan.

We found 214 genets among 315 *T. sebifera* stems in the 25 × 60-m plot. Microsatellite analysis demonstrated that 23 of the 214 genets (10.7%) were multistemmed. Seedlings, parental stems and sprouts were 191 (60.6%), 23 (7.3%) and 101 (32.1%) of 315 stems, respectively. The number of stems on a multistemmed genet ranged from 2 to 23 (Figure 2). Multistemmed genets produced 124 of 315 stems (39.4%) in the *T. sebifera* population. Among multistemmed genets, the age ranged from 4 to 16 years (Figure 3). The number of stems on a genet increased with increasing genet age (Figure 3; Spearman's rank correlation coefficient, r_s = 0.479, P = 0.021, n = 23).

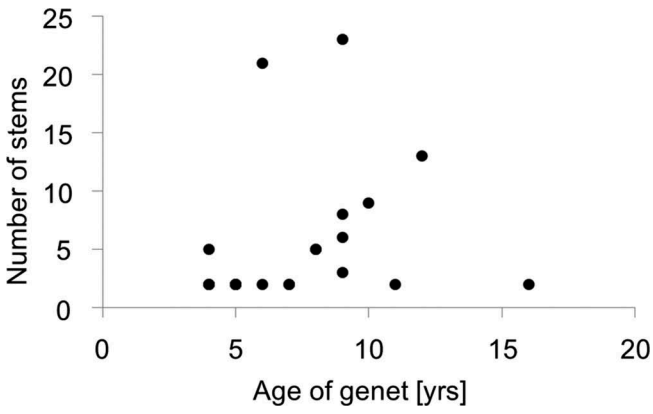

Figure 3. Number of stems on multistemmed genets in relation to the genet age of *Triadica sebifera* in the 25 × 60-m plot on Mt. Mikasa, Nara City, Japan. The number of stems in multistemmed genets increased with increasing genet age. Spearman's rank correlation coefficient, r_s = 0.479, P = 0.021, n = 23.

Most genets appeared to have a long narrow shape; a few genets had spatially restricted distributions within a rounded tract of land (Figure 4). The horizontal extent of genets ranged from 1.9 to 47.8 m. The relationship between age and spread of genets was not significant, because variation of spread of genets was large (Figure 5; Spearman's rank correlation coefficient, r_s = 0.346, P = 0.106, n = 23). The rate of clonal spread, i.e. (horizontal extent of a genet)/(age of the genet), ranged from 0.4 to 9.0 m/year among multistemmed genets and averaged 3.7 m/year. Although spatially large genets tended to have many stems in our study plot (Figure 6; Spearman's rank correlation coefficient, r_s = 0.489, P = 0.018, n = 23), a genet with only 3.6 m spread had 21 stems (Figure 6).

Older stems on a genet occurred only in the vicinity of parental stems (Figure 7). Young stems occurred not only at the leading edge of each genet but also close to the parental stems, where older stems of each genet were also present. Stems distant from parental stems were relatively young among those on each genet.

In the GLMM analysis, six variables were selected as effective predictors for stem size, i.e. age, the cross-sectional area at 1 year of age, the number of younger stems than it on the genet, the number of older stems on the genet, the number of same-age stems on the genet and stem category. The most likely models showed a negative relationship between stem size and numbers of younger stems on the same genet, and a positive relationship between stem size and the other five variables (Table 2).

Discussion

Clonal structure of T. sebifera

The largest probability of identity of a genotype was 6.56×10^{-5}. This value means that the probability of coincidence of genotypes between different genets is 6.56×10^{-5} at most. This low probability guarantees the validity of the judgment of genets.

In previous study, some plant species are known to expand population by vegetative reproduction (Mayes et al. 1998; Suyama et al. 2000; Sakio 2003; Lichstein et al. 2004). However, there are not many studies in which detailed quantitative analysis of the vegetative reproduction has performed. Namroud et al. (2005) reported that less than 25% of genets of *Populus tremuloides*, a clonal plant, were multistemmed. Many genets had small numbers of stems and few genets had large numbers of stems in a *Prunus ssiori* (Nagamitsu et al. 2004) and an *Ilex leucoclada* population (Torimaru & Tomaru 2005). In our study, similar to the previous investigations, multistemmed genets were minority (10.7%) in *T. sebifera*, and the number of stems was small on most multistemmed genets (Figure 2). The *T. sebifera* population has 124 of 315 stems (39.4%) in 23 multistemmed genets. Because the older genets tended to have larger number of stems (Figure 3), multistemmed genets would increase the number of stems with aging. Thus, *T. sebifera* would increase stem number by sprouting after establishment with seeding. The number of stems originated from seedlings was 214 (67.9%) which included

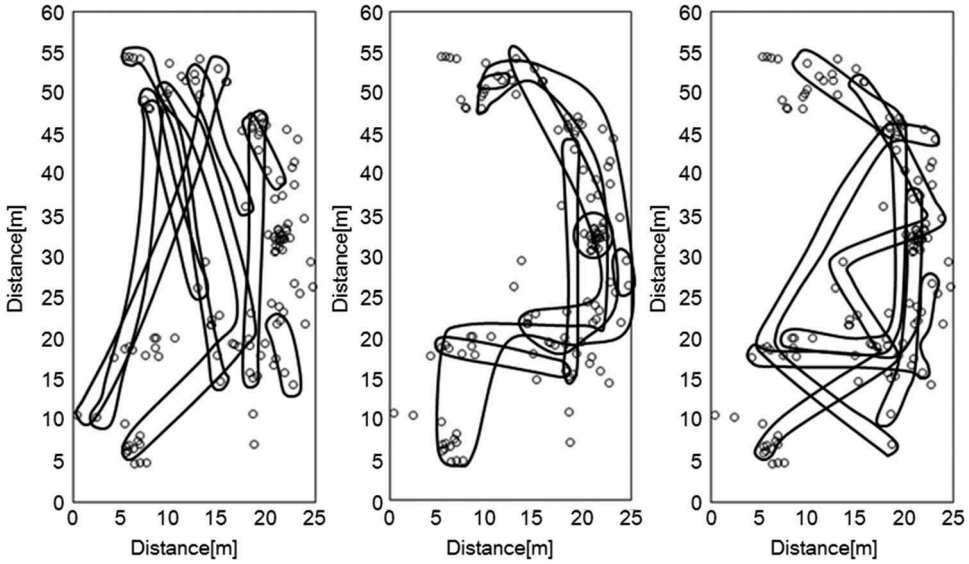

Figure 4. Spatial distribution of genets and stems of *Triadica sebifera* in the 25 × 60-m plot on Mt. Mikasa, Nara City, Japan. Stems (white circle) enclosed by solid lines belong to the same genet. The distribution of 23 genets was drawn after division into 3 mapping plots.

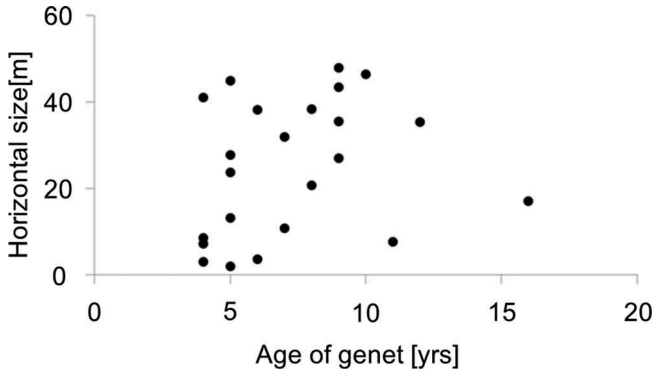

Figure 5. Relationship between age and horizontal extent of genets of *Triadica sebifera* in the 25 × 60-m plot on Mt. Mikasa, Nara City, Japan. Spearman's rank correlation coefficient, $r_s = 0.346$, $P = 0.106$, $n = 23$.

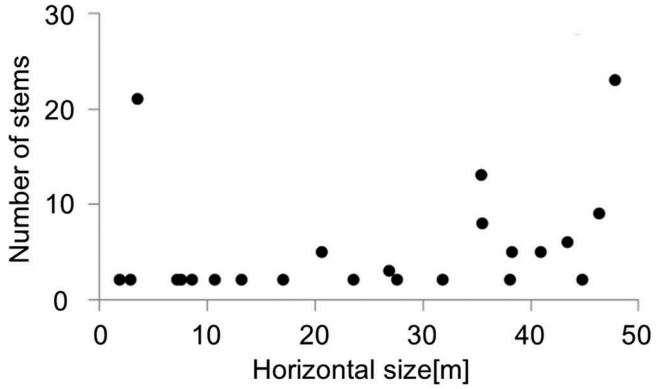

Figure 6. Relationship between horizontal extent and number of stems on genets of *Triadica sebifera* in the 25 × 60-m plot on Mt. Mikasa, Nara City, Japan. Spearman's rank correlation coefficient, $r_s = 0.489$, $P = 0.018$, $n = 23$.

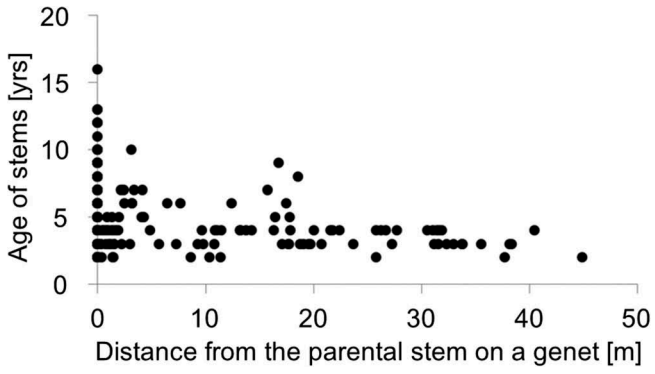

Figure 7. Age of stems on multistemmed genets in relation to distance between each stem and the parental stem on a genet of *Triadica sebifera* in the 25 × 60-m plot on Mt. Mikasa, Nara City, Japan. The oldest and largest stem was identified as the parental stem on a genet.

Table 2. The results of the generalized linear mixed model (GLMM) in multistemmed genets of *Triadica sebifera* in the 25 × 60-m plot on Mt. Mikasa, Nara City, Japan. The dependent variable was stem cross-sectional area in 2009. Independent variables were age, cross-sectional area at 1 year of age, number of younger stems on the genet, number of older stems on the genet, number of same-age stems on the genet and category (seedling, sprout or parental stem). For the category, the reference value was seedling. Effective independent variables were selected by a least AIC model.

Coefficient	Estimate	Standard error	*P* value
Intercept	−4357.390	449.494	<0.001
Age	1155.116	92.815	<0.001
Cross-sectional area at 1 year of age	9.330	2.958	0.002
Number of younger stems on the genet	−130.945	72.836	0.075
Number of older stems on the genet	46.182	77.726	0.554
Number of same-age stems on the genet	26.251	110.776	0.813
Category			
Sprout versus seedling	259.957	671.922	0.700
Parental stem versus seedling	1833.747	775.678	0.020

single-stemmed genets and parental stems of multistemmed genets. On the other hand, the number of stems originated from sprouts was 101 (32.1%). The ability of this invasive species to produce new stems both sexually and asexually may promote its establishment in new habitats and population maintenance.

The shape of most genets looks long and narrow (Figure 4). It is interesting that genets of *T. sebifera* have not radiated in all directions but grown in limited directions. In previous studies, genets of *Sasa senanensis* also grow long and narrow in limited directions (Suyama et al. 2000), and genets of *P. ssiori* radiate in all directions (Nagamitsu et al. 2004). The specific distribution of *T. sebifera* is the same type as *S. senanensis*. It would be one of the features of this species. This feature of *T. sebifera*

would make stems far from other stems on the same genet and from the crown of mother tree. New stems can avoid intraspecific competition at least with other stems on the same genet and mother tree. Horizontal extent varied among genets. The largest, a 9-year-old genet spread over a distance of 47.8 m (Figures 4 and 5). The average rate of clonal spread among multistemmed genets was 3.7 m/year. This rate might be an overestimate due to the methodology for calculating genet age; age was determined by the age of the extant oldest stem on each genet. It is possible that stems older than extant ones had already died and disappeared from the population, resulting in an underestimation of genet age. In the previous studies, Proffitt et al. (2003) reported a rate of 3.1 m/year in the clonal expansion of *Spatina alterniflora*, a perennial grass. *Imperata cylindrica*, a perennial grass, expands at a rate of 10 m/year (Yager et al. 2009). Comparing with the previous studies, the expansion rate estimated in our study would be possible.

Although spatially large genets tended to have many stems in our study plot, several large genets had unexpectedly small numbers of stems for their size (Figure 6). These genets had a guerilla-type strategy of growth (Lovett Doust 1981) that bears widely spaced stems. On the other hand, one genet comprised 21 stems spread over a distance of only 3.62 m. Although the tight-packed genet in the limited area seems to have a phalanx-type strategy of growth (Lovett Doust 1981), it would be rare case for *T. sebifera*. The spatially limited genet with many stems might be physically blocked its clonal spread by stones or roots of other trees. Some old-year genets have few stems within limited space. These genets might have physically split off. The sprouts which were disconnected from parental stems would die and the genets look like small genets.

The spatial distribution of stems on a genet indicates that older stems were present only close to their parental stem, whereas young stems emerged at both the periphery of the genet and close to their parental stem (Figure 7). There might be two types of young stems: those that expand the genet into new areas and those that play a major role to maintain the genet's presence in already colonized areas.

Growth of stems in a genet of T. sebifera

In the *T. sebifera* population we studied, older stems tended to be larger as expected (Table 2). Although stem size of trees is positively correlated with age, variability in size for any given age is usually large (Ågren & Zackrisson 1990). In this study, other determinants of stem size in *T. sebifera* were shown in GLMM analysis (Table 2). First, stem size at 1 year old positively correlated with the present size. Larger stem at initial stage of development would be predominant through asymmetric competition for light because large stems suppress small stems but not vice versa (Ford & Diggle 1981).

Second, composition of stems on a genet had influence on stem size (Table 2). In some clonal plants, ramets on a genet are physiologically connected, and materials are translocated between potentially independent ramets (Slade & Hutchings 1987; Jónsdóttir & Callaghan 1990). Old ramets provide physiological support for young ramets in *Glechoma hederacea* (Slade & Hutchings 1987) and *Carex bigelowii* (Jónsdóttir & Callaghan 1990). In addition, old ramets which provide support were richer than young ramets in resources (Slade & Hutchings 1987). In the *T. sebifera* population, stem size was negatively correlated with the number of younger stems and positively correlated with the number of older stems on the same genet. This indicated that stems which grew large were ones with few younger resource-poor stems and many older resource-rich stems. It suggested that stems were physiologically connected and that photosynthetic products were translocated as shown in previous studies. A stem would get the benefit from supporting by richer stem and would pay the cost to support poorer stems. For young resource-poor stems, the benefit would be larger than the cost. For old resource-rich stems, the cost would be relatively large. Although stem size was positively correlated with the number of same-age stems on the same genet, this correlation was not clear. Because a group of same-age stems would be composed of some resource-poor stems and resource-rich stems, the benefit from supporting would be offset by the cost to support. Despite that *T. sebifera* is a strongly light-demanding species (Shimoda et al. 1994), physiological integration may allow stems in the genet to persist under low light availability.

Third, stems developed as sprout were larger than ones developed as seedling. By getting resources from other stems on the genet, sprouts might grow larger than seedlings which are completely dependent on their own resources.

And fourth, stems developed as parental stems were larger than ones as seedlings. Large and vigorous seedlings would produce sprouts and be parental stems. Alternatively, by getting water and soil nutrient from other sprouts on the genet, parental stems might grow larger than seedlings.

Threat of T. sebifera to natural vegetations

For generalization about invasion of plant species, plant attributes which promote the invasion have been studied. As plant attributes, small seed mass, short germination time and high growth rate of seedlings (Grotkopp & Rejmánek 2007) make a plant a successful invader.

T. sebifera has several attributes to regenerate in the vacant sites. First, bird-dispersed seeds (Fukui & Ueda 1999; Okugawa & Nakatsubo 2009) enable *T. sebifera* to approach to the vacant sites. Second, *T. sebifera* quickly colonizes the vacant sites by fast growth rate (Matsuno et al. 1984) and vigorous sprouting. And third, sprouting allows *T. sebifera* to persist in the colonized sites for a long time. Colonization and persistence of *T. sebifera* in the vacant sites created by deer would hinder the regeneration of native pioneer trees and deteriorate the biodiversity of the forest. The precious ecosystem of the Kasugayama Forest Reserve, an UNESCO World Heritage, is damaged not only by deer, an ungulate herbivore, but also *T. sebifera*, an alien plant. Urgent measures are required to control the *T. sebifera* population for the forest conservation. Understanding the characteristics of sprouting of *T. sebifera* is useful to decrease its impact on local ecosystems in various parts of the world.

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