



Original research article

Effects of forest fragmentation on the mating system of a cool-temperate heterodichogamous tree *Acer mono*

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ARTICLE INFO

Article history:

Received 26 January 2015

Received in revised form 9 April 2015

Accepted 9 April 2015

Available online 22 April 2015

Keywords:

Acer mono

Heterodichogamy

Mating system

Pollination

Microsatellites

Forest fragmentation

ABSTRACT

Pollination is a key process for reproduction and gene flow in flowering plants. Anthropogenic habitat fragmentation, however, can disrupt plant–pollinator interactions, and may have a negative impact on the reproductive success and population viability of entomophilous plants. Heterodichogamous plants containing protandrous and protogynous individuals within a population may be susceptible to habitat fragmentation due to a lack of available mating partners. In this study, we investigated the effects of forest fragmentation on the mating system in the heterodichogamous plant *Acer mono*, a major constituent of cool-temperate deciduous forests in Japan. Microsatellite analysis was applied to 212 adult trees and 17 seed families from continuous and fragmented forests. Dispersal kernel modeling using the neighborhood model indicated that pollen dispersal of *A. mono* was highly fat-tailed. The estimated parameters of the model suggested that the siring success of a pollen donor increased approximately fivefold, with a 100 cm increase in its diameter at breast height (DBH), and that disassortative mating was five times more frequent than assortative mating. The mating system parameters of each mother tree, outcrossing rate (t_m), biparental inbreeding ($t_m - t_s$), and paternity correlation (r_{pm}) varied among sites and conditions, depending on the local density of potential pollen donors. Whereas *A. mono* was effectively outcrossed ($t_m = 0.901$, $t_m - t_s = 0.052$, and the number of effective sires was $1/r_{pm} = 14.93$) in the continuous forest, clumped trees within the fragmented forest showed increased biparental inbreeding and reduced pollen pool genetic diversity ($t_m = 0.959$, $t_m - t_s = 0.245$, $1/r_{pm} = 1.742$) as a result of localized mating combined with spatial genetic structures. In contrast, the isolated trees had a higher selfing rate, but the pollen pool diversity was maintained ($t_m = 0.801$, $t_m - t_s = 0.022$, and $1/r_{pm} = 15.63$) due to frequent long-distance pollination. These results suggest that although pollen limitation following habitat fragmentation could result in negative genetic consequences, enhanced long-distance pollination across a fragmented landscape could partly compensate for this limitation depending on the degree of forest fragmentation.

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1. Introduction

Habitat fragmentation due to anthropogenic modification constitutes a major threat to biodiversity (Wilcox and Murphy, 1985). It is a complex process that not only affects the physical environment but also has impacts on the biological processes required for the persistence of a species (Murcia, 1995; Saunders et al., 1991). Pollination is a key process for seed reproduction in flowering plants and a major component of gene flow. For animal-pollinated species, the negative impacts

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of fragmentation are expected to be pronounced because these plant–animal interactions are susceptible to anthropogenic habitat disturbances (Aizen and Feinsinger, 2002; Hadley and Betts, 2012; Harris and Johnson, 2004; Winfree et al., 2009). A lack of effective plant pollination may therefore not only reduce reproductive success, but could also negatively affect the genetic diversity of the plant populations over time.

Over the last two decades, studies have investigated the effects of habitat fragmentation on the pollination and reproduction of animal-pollinated plants (reviewed by Aguilar et al., 2006; Eckert et al., 2010; Hadley and Betts, 2012). The use of genetic markers, which enables estimates of mating system parameters, has contributed to understanding mating-system responses in fragmented landscapes (Coates et al., 2007), and has confirmed that habitat fragmentation has had negative impacts on reproduction through pollen limitation and/or increased selfing (Aguilar et al., 2008; Eckert et al., 2010; Ghazoul, 2005; Hadley and Betts, 2012). However, the susceptibility of plant reproduction to fragmentation varies greatly, and under some circumstances, fragmentation has also facilitated outbreeding (Bacles et al., 2005; Dick et al., 2003; White et al., 2002). These differential responses have not been fully explored, but they have been attributed to a function of the plant breeding system, the life history, the pollination vector, and pollinator specialization (Aguilar et al., 2006; Harris and Johnson, 2004).

The plant breeding system determines the dependence of its reproductive success on the availability of pollinators (Aizen and Feinsinger, 2002; Bond, 1994). Self-compatibility may also be an important predictor, as self-incompatible plants are considered to be more susceptible to fragmentation than self-compatible plants because they obligately require cross-pollination for seed reproduction (Aguilar et al., 2006). However, plants exhibit a wide array of breeding systems ranging from complete selfing to outcrossing (Goodwillie et al., 2005; Lande and Schemske, 1985), and include complex breeding systems such as heterostyly and heterodichogamy (Barrett, 2002). Moreover, the role played by the variation of the plant breeding system in its susceptibility to fragmentation has not yet been fully explored.

Maples (genus *Acer*, family Sapindaceae), a major constituent of boreal forests in the Northern Hemisphere, are known to exhibit labile complex sex expression, including dioecy, heterodichogamy, and duodichogamy (Gleiser and Verdú, 2005). Heterodichogamy is a complex breeding system in which both protandrous and protogynous individuals coexist in a population (Renner, 2001). Although it has been reported for only 17 genera in 11 families of angiosperms (Renner, 2001), almost half of the *Acer* species show heterodichogamy (Gleiser and Verdú, 2005). Heterodichogamy in *Acer* is particularly referred to as heterodichogamous androdioecy because it occasionally includes a low proportion of male individuals.

We previously investigated the mating system of the heterodichogamous maple *Acer mono*, and revealed that heterodichogamy in this maple effectively avoided selfing and promoted mating between protandrous and protogynous individuals (disassortative mating) without obligate compatibility systems (Kikuchi et al., 2009). Seed reproduction in this mating system is presumed to depend on the availability of potential mating partners and thus may be susceptible to habitat fragmentation.

Our aim was to investigate the effects of fragmentation on the pollination process and the reproduction of the heterodichogamous maple *A. mono*. The effects of fragmentation on the seed reproduction of this maple have already been examined by Shibata et al. (2009), who observed increased rates of empty and immature seeds with increased isolation of the seed parents. Therefore in this study, we focused on the quality of seeds; i.e., the effects of fragmentation on mating systems from a genetic perspective. It was hypothesized that, although heterodichogamy would avoid selfing in *A. mono*, habitat fragmentation would have significant negative impacts on the reproduction of this maple, because a decline of compatible partners in forest fragments would reduce effective outcross pollination, and subsequently the genetic variation of pollen pools of its progenies.

In this study we conducted microsatellite genetic analyses on the adults and their progenies in continuous and fragmented populations of *A. mono*. We particularly examined the genetic structure of adult individuals, the mating patterns, and pollen dispersal kernels. The mating system parameters in the progeny populations were also analyzed. Paternity analyses and estimation of pollen dispersal kernels provided accurate estimates of contemporary pollen transfer. Multilocus estimation based on the mixed mating model (Shaw et al., 1981) and the correlated mating model (Ritland, 2002) are robust and informative tools to evaluate the mating systems in plant populations, including selfing rates, biparental inbreeding, and pollen pool diversity. Moreover, these mating-system parameters can be applied directly to conservation because they influence population genetic diversity over time (Breed et al., 2013; Neel et al., 2001). The genetic structure of the adult populations was not the main focus of this study, but it was an important determinant of the impacts on seed quality.

2. Materials and methods

2.1. Field data and sample collection

This study was conducted at two sites (OGW and FRG) in a deciduous broad-leaved forest (36°56–57′N, 140°35′E, 610–660 m a.s.l.) in the North Kanto region of Japan (Fig. 1). The OGW consists of a 12-ha (400 × 300 m) rectangular site located in a 98-ha continuous forest (the Ogawa Forest Reserve, Ibaraki Prefecture). The FRG is a forest fragment located nearby (in Fukushima Prefecture) interspersed with artificial conifer (*Cryptomeria japonica* and *Chamaecyparis obtusa*) plantations. It comprises about 29 ha, and consists of thin strips, 5–200 m in width and 500–1500 m in length, which are located mainly along the ridges and rivers. These forest sites are dominated by deciduous broad-leaved trees, including

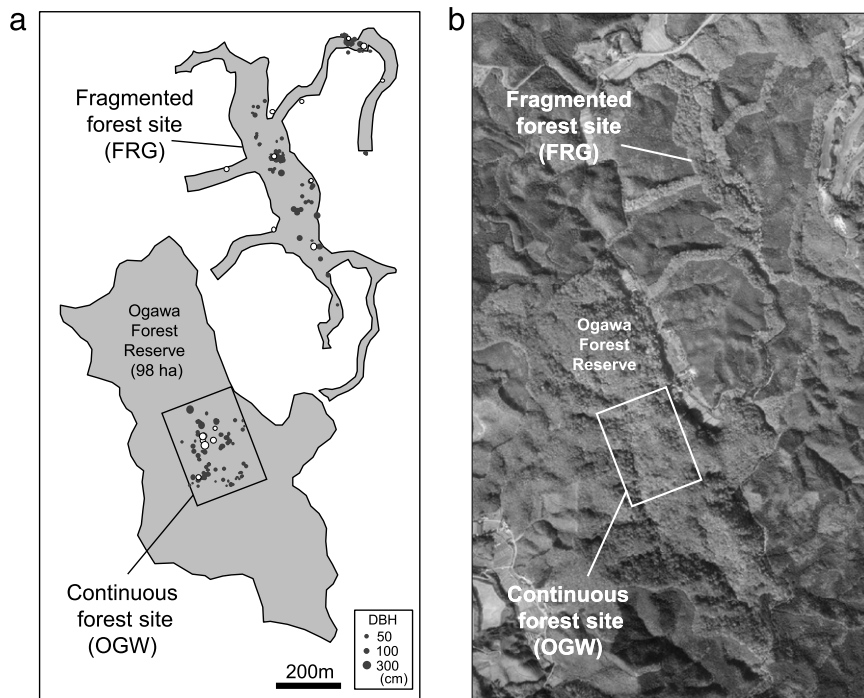


Fig. 1. (a) Study areas and location of the adult trees of *Acer mono*. Open circles indicate the maternal trees from which seeds were sampled. (b) Aerial photograph showing the study sites, taken by the Geographical Survey Institute (Photograph number: NJ-54-23-7) on November 5 1998.

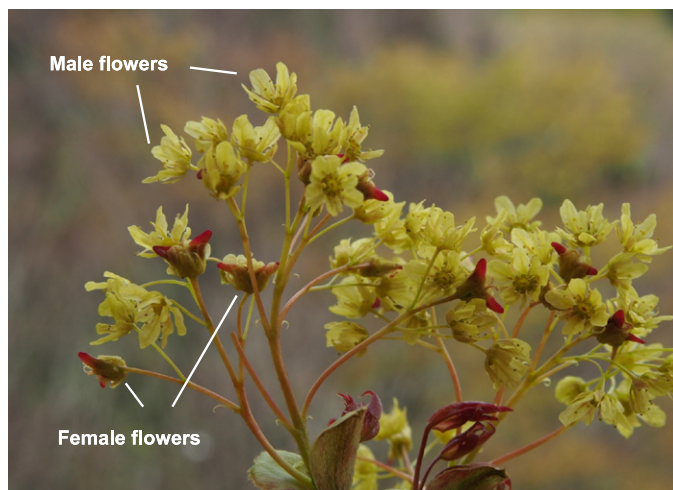


Fig. 2. Cymes of *A. mono*. This individual is protogynous, with female flowers growing wings and male flowers in bloom.

Fagus crenata, *Fagus japonica*, *Quercus serrata*, and *Castanea crenata*. They were once a single continuous forest prior to their fragmentation caused by the establishment of plantations during 1972–1995 (Miyamoto et al., 2011).

A. mono Maxim. is a deciduous tree that grows to a height of 20 m and is a major constituent of cool-temperate deciduous forests in Japan. It is classified into several subspecies and forms. The taxon used for this research was *A. mono* Maxim. var. *marmoratum* (Nichols.) Hara f. *dissectum* (Wesmael) Rehder (hereafter simply denoted as *A. mono*). Flowers are produced in cymes during April to May (Fig. 2). Two types of hermaphroditic individual (protogynous and protandrous) and a small proportion of males coexist within a population (Shibata et al., 2009). The flower-visiting insects observed (Matsui, 1991;

Shibata et al., 2009) included flies (*Calliphora*, *Lucilia*, *Fannia*, and *Helina*), hover flies (*Episyrphus*, *Eristalis*, and *Syrphus*), and bees (Anthophoridae, Halictidae, and Andrenidae). Occasionally, the flowers were visited by honey bees (Shibata et al., 2009). Fruits are winged, two-seeded samaras, which usually fall by November.

From 2003 to 2005, all *A. mono* individuals with a diameter at breast height (DBH) > 5 cm were tagged and mapped, and the leaves were collected. During April–May, flowering and the sexual types (male, protandrous, and protogynous) of these individuals were checked if inflorescences were present. Six and eleven flowering trees were selected as the mother trees at the OGW and FRG sites, respectively, from which mature samaras were collected from October to November. All the fresh samples (leaves and samaras) were stored at -20°C until use.

2.2. Genetic analysis

DNA was extracted from the leaves using the CTAB method described by Murray and Thompson (1980). DNA from the seeds was extracted using a DNeasy Plant Mini Kit (Qiagen, Venlo, The Netherlands) after removing the pericarps and seed coats.

Genetic polymorphisms were analyzed at six microsatellite loci, including four markers developed for *A. mono* (Kikuchi et al., 2008), one developed for *Acer pseudoplatanus* L. (Pandey et al., 2004) and one for *Acer capillipes* Maxim. (Terui et al., 2006). The polymerase chain reaction (PCR) amplifications of the microsatellite loci were performed in a 5 μL reaction mixture consisting of ~ 1 ng of template DNA, 0.2 μM of each primer, and $1 \times$ Multiplex PCR Master Mix (Qiagen). The PCR conditions included an initial denaturation step of 5 min at 95°C , followed by 35 cycles of 30 s at 95°C , 90 s at an annealing temperature of 60°C , and 30 s at 72°C , followed by a final extension step of 5 min at 72°C . The PCR products were electrophoresed and the alleles were scored using an ABI 3130 sequencer (Applied Biosystems, Foster City, CA, USA) and GeneMapper software version 4.1 (Applied Biosystems).

2.3. Statistical analyses

2.3.1. Spatial genetic structure of adult populations

The genetic variation of each adult population was evaluated based on the total number of alleles (A), the observed heterozygosity (H_O), the unbiased expected heterozygosity (H_E), and the inbreeding coefficient F_{IS} using the GenALEX 6.5 software (Peakall and Smouse, 2006, 2012). Because an uneven number of samples was obtained from each population, allelic richness corrected for the sampling bias (A_R) was calculated using the rarefaction method (Hurlbert, 1971) implemented in the HP-Rare software (Kalinowski, 2005).

Because the genetic composition of neighboring trees could have a large impact on the seed quality, the spatial genetic structure (SGS) of each adult population was evaluated by autocorrelation analysis using SPAGeDi 1.2 (Hardy and Vekemans, 2002). Kinship coefficients (F_{ij}) were calculated according to Loiselle et al. (1995), and the significance of the values was verified after 1000 permutations for 10 automatically defined distance classes, so that each class included an even number of individual pairs. Because the kinship coefficient represents the relative value, which depends on the observed allele frequencies in a population, the reference allele frequency incorporated all the adults from two populations. The kinship values were regressed against the logarithm of the spatial distance over a distance range of 0–400 m. Standard errors of regression slopes (b log) were obtained by jackknifing over loci. The magnitude of the SGS was estimated using the Sp statistic (Vekemans and Hardy, 2004), given by $Sp = -b \log / (1 - F_{(1)})$, where $F_{(1)}$ is the average kinship coefficient between individuals of the first distance class.

2.3.2. Mating system parameters

A , H_O , H_E , F_{IS} , and A_R were calculated for each seed family. Moreover, three mating system parameters were estimated; the multilocus outcrossing rate (t_m), biparental inbreeding ($t_m - t_s$), and a multilocus paternity correlation within trees (r_{pm}) were calculated using the MLTR software (Ritland, 2002). The paternity correlation (r_{pm}) indicated the probability that any two randomly chosen seeds were sired by the same father, and therefore its inverse ($N_p = 1/r_{pm}$) was referred to as the effective number of pollen donors. The standard errors of these parameters were obtained after 1000 bootstraps. The mating parameters were regressed against ecological parameters, such as the local densities of the adults (i.e., the number of other flowering trees within a 100 m radius (N_{100}) and distances to the nearest flowering tree (D_N)).

The genetic structure of the pollen pool was assessed using the two-generation approach (Smouse et al., 2001). Pairwise values of genetic differentiation among the pollen pools (Φ_{it}) were computed for each pair of seed families using the TwoGener method (Austerlitz and Smouse, 2001, 2002) implemented in the POLDISP software (Robledo-Arnuncio et al., 2007) and regressed against the distance between the mother trees.

2.3.3. Paternity and pollen dispersal

To assess the effect of fragmentation on mating patterns, the paternity of collected seeds was determined using the maximum-likelihood methods using the Cervus 3.0 software (Kalinowski et al., 2007). Following Kikuchi et al. (2009), the

paternity of a seed was assigned to the individual with the highest LOD (logarithm of the odds) score and with a delta value (the difference in LOD scores between the most likely parent and the second most likely parent) exceeding a certain confidence level: here we used both the 80 and 95% confidence limits. If none of the candidates in the plots had positive LOD scores, the seeds were scored as having a pollen parent outside of these populations.

The average pollen dispersal curve was estimated using the neighborhood model (Burczyk et al., 2002) implemented in the NM+ program (Chybicki and Burczyk, 2010). Based on the neighborhood model, assuming that a seed of a mother tree was pollinated via self-pollination with a probability of s , pollen from outside the neighborhood with a probability of m and cross-pollinated within the neighborhood with a probability of $1 - s - m$, the probability that a seed i , from a mother tree j , contains the genotype g_{ij} is given as Eq. (1).

$$P(g_{ij}|M_j) = sP(g_{ij}|M_j, M_j) + mP(g_{ij}|M_j, B) + (1 - s - m) \sum_k \Phi_{jk} P(g_{ij}|M_j, F_{jk}) \quad (1)$$

where $P(g_{ij}|M_j, M_j)$, $P(g_{ij}|M_j, B)$, and $P(g_{ij}|M_j, F_{jk})$ are the probabilities that a seed has the genotype g_{ij} when a mother tree of the genotype M_j is self-pollinated, pollinated by an unknown tree outside of the neighborhood (B), and pollinated by a tree within the neighborhood having genotype F_{jk} , respectively. The parameter Φ_{jk} represents the relative mating success of the k th flowering tree within the neighborhood and is expressed as $\Phi_{jk} = \pi_{jk} / \sum_{k \in N} \pi_{jk}$, where π_{jk} is a function of factors affecting the reproductive success of the k th pollen parent in the seeds of the j th mother, such as the mating distance and the phenotypic trait relating to fecundity. In this study, two models were explored. The effects of tree size T , measured as the DBH, were incorporated into the model as well as the mating distance as $\pi_{jk} = p(d_{jk}) \exp(\gamma_1 T)$, where γ_1 is a selection gradient for the covariate T , and $p(d_{jk})$ is the probability density function (i.e., dispersal kernel) of the mating distance (d) between the i th mother and the k th pollen parent. Because our previous study suggested a leptokurtic pollen dispersal curve for this maple, we applied an exponential-power function with scale (a) and shape (b) parameters and having a mean dispersal distance δ . By pooling the data over all seeds from the 17 mother trees, the maximum-likelihood estimates of the parameters (s , m , γ_1 , b , and δ) were obtained using the Newton–Raphson algorithm implemented in NM+ (Chybicki and Burczyk, 2010).

In the second model, following a previous study (Kikuchi et al., 2009) that suggested disassortative mating in heterodichogamous *A. mono*, the effects of flowering type were added as $\pi_{jk} = p(d_{jk}) \exp(\gamma_1 T + \gamma_2 \times Ft_{jk})$, where Ft_{jk} is flowering type assigned to 0 or 1 when the flowering types of the j th mother and the k th pollen parent were the same or the reciprocal. When the flowering type of a pollen parent was unknown, Ft_{jk} was applied to values of 0.5.

3. Results

3.1. Population structure

The OGW and the FRG contained 78 and 134 individuals of *A. mono* with a DBH > 5 cm, respectively. Flowering was observed in a total of 143 individuals, which included 86 protandrous and 57 protogynous individuals, but no males. Both populations displayed unimodal size distributions that peaked at a DBH of 60–80 cm, with means of 87.5 ± 55.9 cm in OGW and 77.5 ± 37.2 cm in FRG. The adult tree density, as indicated by N_{100} for each seed parent, was 25.8 ± 2.8 on average at OGW. In contrast, the mother trees at FRG showed variable N_{100} values with an average of 15.5 ± 20.0 trees, including five clumped trees (with a mean N_{100} of 32.3 ± 18.8) and six isolated trees (with a mean N_{100} of 1.5 ± 2.3). Hereafter, they are termed “clumped” or “isolated” trees, respectively.

3.2. Genetic variation and structures in the adult populations

A total of 138 alleles were detected in 212 adult *A. mono* trees from 6 loci, and the mean polymorphic information content (PIC) was 0.8706. The non-exclusion probability of second parents was 0.00015, which provided sufficient detective power for paternity analyses. An average number of alleles per locus of 17.5 alleles was detected at OGW (78 trees, 12 ha) and 20.17 alleles at FRG (134 trees, 29 ha). However, the number of alleles after rarefaction and the level of heterozygosity did not differ between the sites (Table 1). Based on spatial autocorrelation analysis, a significant SGS was detected at both sites (Fig. 3). The partial regression slopes of kinship coefficients on spatial distance were -0.02219 at OGW and -0.02284 at FRG. The kinship values at the shortest distance class were $F_{(1)} = 0.0368$ and 0.0605 at OGW and FRG, respectively. As a result, the neighborhood size Nb was almost equal between the sites (40.94 vs. 39.68), suggesting that the two populations did not differ in the intensity of SGS.

3.3. Mating system parameters of pollen pools

Seeds from 17 mother trees contained 146 alleles, including 18 unique alleles not found in the adult populations. Table 1 shows the indices of the genetic variation of seeds from each maternal tree. The FRG clumped trees had a lower allelic richness than the FRG isolated trees and the OGW trees, as suggested by the genetic diversity indices, A_R (Kruskal–Wallis test, $p = 0.007$) and N_A ($p = 0.012$). Moreover, the fixation index F was highest for the FRG isolated trees ($p = 0.037$).

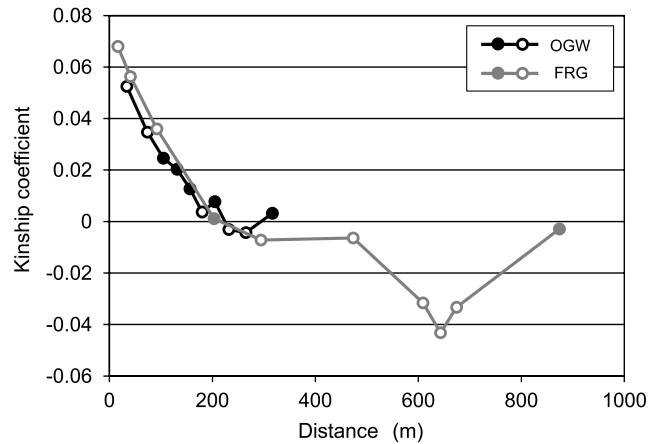


Fig. 3. Comparison of the spatial genetic structure (SGS) of adult populations at the OGW and FRG sites. Kinship coefficients for each distance class were plotted against the mean distance between individuals. Open dots indicate values that lie below or above the 95% confidence interval based on permutation tests.

Table 1

Genetic diversity and fixation indices for adult trees and seeds, as well as mating system parameters within each seed family. Indices of genetic diversity include N_A (number of alleles per locus), A_R (number of alleles after rarefaction), H_O (observed heterozygosity), and H_E (unbiased expected heterozygosity), and were given for adult trees from each site and for seeds from each of the mother trees, along with the sample size (n) and fixation index (F). The standard errors are given in parentheses. The flowering type of each mother tree is also shown (PA: protandry, PG: protogyny).

	Mother tree ID	Flowering type	n	N_A	A_R	H_O	H_E	F
OGW adults			78	17.50 (2.49)	12.96	0.90 (0.02)	0.87 (0.02)	-0.03 (0.02)
OGW seeds	3123	PA	37	8.17 (0.48)	7.23	0.89 (0.06)	0.73 (0.04)	-0.22 (0.05)
	4762	PG	410	18.00 (2.7)	8.85	0.78 (0.01)	0.72 (0.03)	-0.10 (0.03)
	5153	PG	31	8.67 (0.71)	7.98	0.83 (0.05)	0.75 (0.02)	-0.11 (0.04)
	5249	PG	149	12.83 (1.47)	7.02	0.79 (0.04)	0.68 (0.04)	-0.17 (0.07)
	6487	PG	210	14.83 (1.58)	7.44	0.73 (0.01)	0.63 (0.03)	-0.18 (0.05)
	6587	PA	131	12.00 (1.06)	6.91	0.7 (0.02)	0.63 (0.02)	-0.11 (0.05)
FRG adults			134	20.17 (3.66)	12.34	0.89 (0.02)	0.87 (0.01)	-0.03 (0.02)
FRG seeds								
Clumped trees	PA884	PG	54	7.67 (0.76)	5.89	0.67 (0.08)	0.61 (0.06)	-0.11 (0.07)
	PE32	PA	85	7.83 (0.7)	5.00	0.82 (0.05)	0.68 (0.03)	-0.19 (0.03)
	PE38	PG	91	9.17 (0.7)	5.91	0.79 (0.01)	0.68 (0.01)	-0.15 (0.01)
	PE4	PG	24	7.17 (0.65)	7.17	0.76 (0.05)	0.66 (0.04)	-0.15 (0.05)
	PE5	PA	37	5.17 (0.48)	4.54	0.70 (0.05)	0.61 (0.04)	-0.14 (0.02)
Isolated trees	PA993	PG	105	16.17 (1.89)	9.90	0.83 (0.02)	0.72 (0.03)	-0.16 (0.04)
	PA995	PA	80	14.67 (1.43)	9.21	0.72 (0.02)	0.68 (0.03)	-0.06 (0.04)
	PA996	PG	61	13.00 (1.53)	9.21	0.78 (0.02)	0.73 (0.01)	-0.08 (0.02)
	PE14	PG	33	9.83 (1.08)	8.54	0.74 (0.04)	0.67 (0.04)	-0.11 (0.03)
	PE18	PA	92	9.83 (1.11)	6.53	0.67 (0.05)	0.64 (0.05)	-0.05 (0.03)
	PE25	PA	70	12.5 (1.31)	9.10	0.80 (0.04)	0.74 (0.01)	-0.08 (0.05)

The mating-system parameters in each maternal tree are shown in Table 2. When pooled for each condition, the outcrossing rate t_m was lower in the isolated trees ($t_m = 0.801$) than in the clumped trees at FRG ($t_m = 0.959$) and OGW ($t_m = 0.901$). Biparental inbreeding ($t_m - t_s$) was highest in the clumped trees (0.245) and low for the isolated trees at FRG (0.022) and the maternal trees at OGW (0.052). Paternity correlation within the samples (r_{pm}) was also highest for the clumped trees (0.574), followed by the isolated trees at FRG (0.064) and the OGW trees (0.067). Thus, the effective numbers of pollen donors were 1.742, 15.63, and 14.93 for the FRG clumped trees, FRG isolated trees, and OGW trees, respectively.

The mating system parameters for each maternal tree showed close relationships with the distribution of pollen donors. As shown in Fig. 4, t_m was significantly correlated ($r = 0.600$, $p = 0.011$) with the number of other flowering trees within a 100 m radius (N_{100}), and was marginally negatively correlated ($r = -0.464$, $p = 0.0608$) with the distance to the nearest neighbor (D_N). Similarly, $t_m - t_s$ was strongly correlated with N_{100} ($r = 0.718$, $p = 0.0012$), while r_{pm} was marginally correlated with N_{100} ($r = 0.426$, $p = 0.0883$) and negatively correlated with D_N ($r = -0.591$, $p = 0.0125$).

The differentiation of pollen pools (Φ_{ft}) was 0.0912 among all seed families. Pollen pool differentiation among the FRG isolated trees (0.0556) was comparable to that detected at OGW (0.0419), whereas high differentiation was observed among the FRG clumped trees (0.1803). Notably, pollen pool differentiation was much higher among the protandrous mother trees

Table 2

The mating system parameters for each seed family with the standard deviations in parentheses. The multilocus outcrossing rate (t_m), biparental inbreeding ($t_m - t_s$) and multilocus paternity correlations (r_{pm}) are shown.

	Mother tree ID	t_m	$t_m - t_s$	r_{pm}
OGW seeds	3123	0.95 (0.09)	0.02 (0.07)	0.31 (0.09)
	4762	0.90 (0.02)	0.00 (0.01)	−0.04 (0.00)
	5153	1.20 (0.00)	0.31 (0.03)	0.17 (0.09)
	5249	0.99 (0.08)	0.10 (0.07)	0.14 (0.02)
	6487	0.82 (0.03)	0.02 (0.01)	0.12 (0.02)
	6587	0.91 (0.02)	0.22 (0.03)	0.22 (0.05)
FRG seeds				
Clumped trees	PA884	1.20 (0.00)	0.47 (0.03)	0.41 (0.07)
	PE32	0.99 (0.11)	0.17 (0.10)	0.72 (0.06)
	PE38	1.20 (0.00)	0.50 (0.02)	0.51 (0.06)
	PE4	1.20 (0.00)	0.48 (0.04)	0.51 (0.12)
	PE5	0.70 (0.08)	0.28 (0.04)	0.51 (0.12)
Isolated trees	PA993	0.96 (0.03)	0.05 (0.03)	−0.11 (0.11)
	PA995	0.75 (0.05)	0.05 (0.02)	−0.03 (0.23)
	PA996	0.77 (0.05)	0.05 (0.03)	0.06 (0.11)
	PE14	0.82 (0.07)	0.10 (0.05)	0.10 (0.18)
	PE18	0.55 (0.05)	−0.10 (0.02)	0.23 (0.06)
	PE25	0.96 (0.05)	0.07 (0.05)	0.09 (0.02)

(0.136) than among the protogynous trees (0.070). When pairwise Φ_{it} was plotted against the distance between the parental trees (Fig. 5), significant SGS in the pollen pools was detected only at OGW ($r = 0.833$, $p = 0.00012$). The figure also showed that the pollen pools were rather similar among the isolated mother trees.

3.4. Paternity and pollen dispersal curves

Using a confidence threshold of 95%, the paternity of 775 seeds (45.6%) was assigned to a candidate within each of the plots. From these, 138 seeds (8.1%) were the result of selfing. The frequency of unambiguous disassortative mating was approximately threefold higher than assortative mating (442 vs. 158 seeds). However, when a confidence limit of 80% was applied, paternity was determined for 935 offspring (55.0%). The proportion of selfed seeds was 11.4% (193 seeds), which was almost equal to the selfing rate estimated using the MLTR software ($s = 0.885$). Disassortative mating was still about threefold higher than assortative mating (520 vs. 179 seeds). Hereafter, we refer to the results in which a confidence level of 80% was applied.

The mating patterns at each site and their conditions are shown in Fig. 6. At the OGW site, the selfing rate was 9.8%, and the distribution of outcrossing mating distance followed a gradual, unimodal distribution with a peak at 30–60 m distance. However, mating patterns at the FRG site were markedly different; the mating distance of the clumped trees followed an L-shaped distribution, in which >50% of the seeds were sired by a pollen donor <30 m from the maternal trees. In contrast, long-distance pollen dispersal of >200 m occurred frequently at the isolated maternal trees. Moreover, the selfing ratio differed considerably between conditions; it decreased to 4.0% in the FRG clumped trees but increased to 19.4% in the FRG isolated trees.

The parameters of the pollen dispersal curve estimated by NM+ are listed in Table A1 (see Appendix A). The selfing and migration rates estimated almost matched the results of the paternity tests using the 80% threshold level. The shape parameter b was <1 ($b = 0.283$), suggesting a fat-tailed dispersal curve of pollen. Note that a more leptokurtic dispersal curve ($b = 0.207$) with a shorter average dispersal distance ($\delta = 134$ m) was estimated for the protandrous mother trees than for the protogynous trees ($b = 0.251$, $\delta = 811$ m). The selection gradient γ_1 for a covariate of DBH was consistently 0.016–0.017 in all the models tested. The selection gradient γ_2 , which represented the contribution of the reciprocal flowering morph to the mating success, was 1.56 for the protogynous mother trees and 1.75 for the protandrous trees.

4. Discussion

4.1. The mating system of *A. mono*

The application of the neighborhood model produced a fat-tailed kernel of pollen dispersal in *A. mono*, with a low value of the estimated shape-parameter ($b = 0.28$). Although a similar pattern had been reported from anemophilous and entomophilous plants (e.g., Albaladejo et al., 2012; Goto et al., 2006; Matter et al., 2013; Nakanishi et al., 2012; Robledo-Arnuncio and Gil, 2005), our results suggested a highly leptokurtic distribution compared with other entomophilous genera such as *Castanopsis* ($b = 0.537$; Nakanishi et al., 2012) and *Ranunculus* ($b = 0.71$; Matter et al., 2013). The strongly leptokurtic

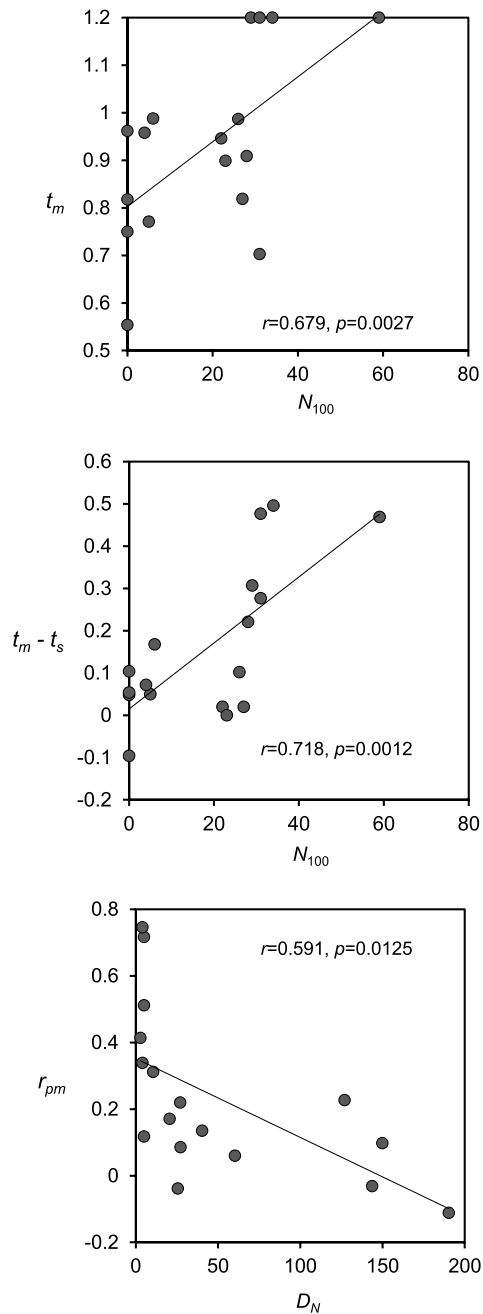


Fig. 4. Relationships between the mating system parameters (t_m , $t_m - t_s$, and r_{pm}) and mate densities, as indicated by N_{100} (the number of other flowering trees within a 100 m radius) or D_N (distance to the nearest neighbor).

pollen dispersal in *A. mono*, which implies that a considerable amount of long-distance dispersal occurred whereas short-distance pollen transfer predominated might be attributable to a wide variety of the pollinator fauna. Although the visitors to *A. mono* flowers, such as small insects such as flies, hover flies and solitary bees are generally habitat-dependent and have narrow foraging ranges (Steffan-Dewenter et al., 2002), occasional visits by honey bees (Shibata et al., 2009) might contribute to long-distance pollen dispersal because they have a large foraging range (Visscher and Seeley, 1982).

The neighborhood model can incorporate the effects of the ecological traits of pollen donors on the mating success (Burczyk et al., 2006). The selection gradient γ_1 in this study described the effects of tree size and resulted in similar values (0.0162 to 0.0169) in all three models examined, which suggested a 5.05 ($=e^{1.62}$)–5.42 ($=e^{1.69}$) fold increase in the relative mating success Φ , with a 100-cm increase in their DBH.

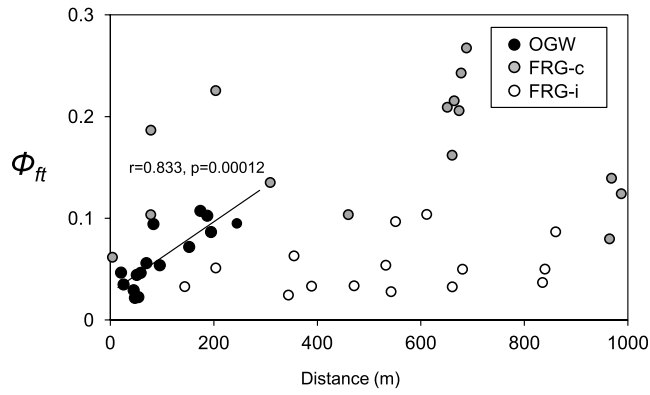


Fig. 5. Relationship between pairwise Φ_{ft} (differentiation among the pollen pools) and geographical distance among the OGW trees, among the FRG clumped trees (FRG-c), and among the FRG isolated trees (FRG-i).

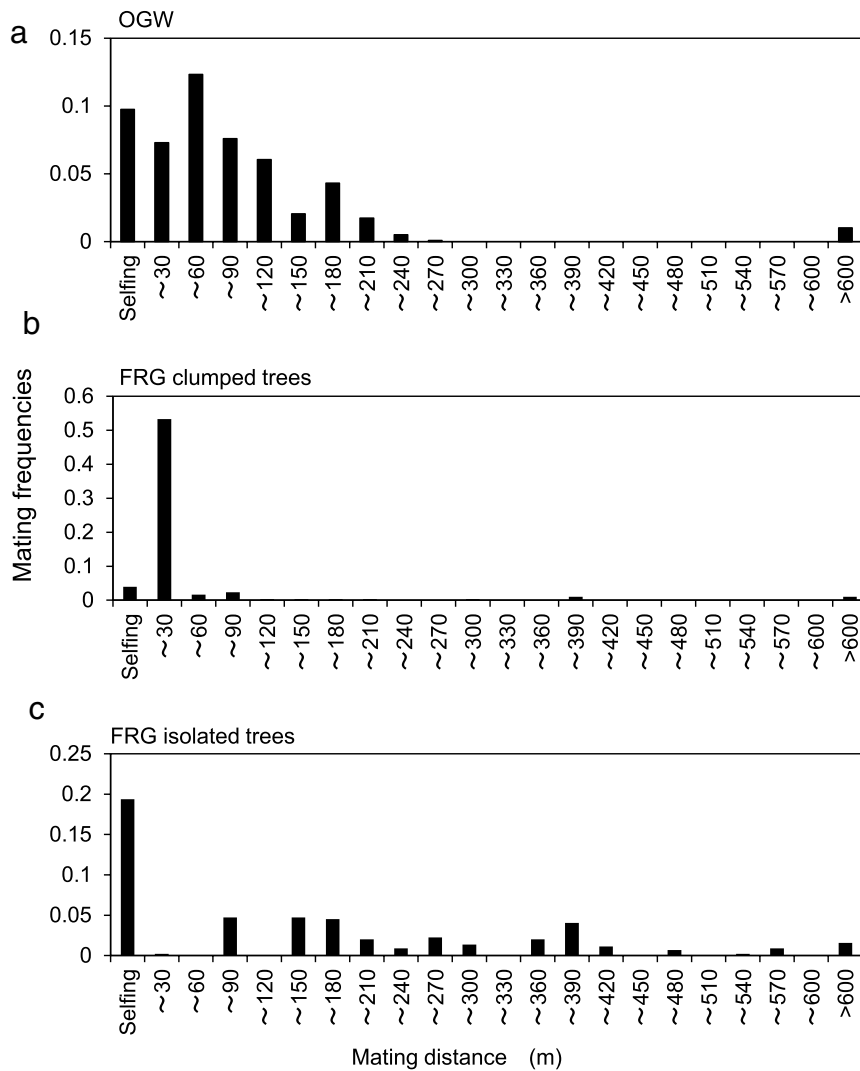


Fig. 6. Mating distance frequencies based on paternity tests for the seeds from OGW (a), FRG clumped trees (b), and FRG isolated trees (c).

Similarly, a preference for disassortative mating was inferred from the selection gradient γ_2 . It also showed similar values (1.56 and 1.75) for each sex expression and predicted that relative mating success of a pollen parent increased by

4.76 – ($=e^{1.56}$) and 5.75 – ($=e^{1.75}$) fold when the flowering types of a mother and a pollen parent were the reciprocal than when they were the same. These results suggested that disassortative mating would occur about five times more frequently than assortative mating in this *A. mono* population.

Darwin's hypothesis that heterodichogamy is as an outcrossing mechanism (Darwin, 1877) has been of interest in reproductive and evolutionary biology. However, the hypothesis regarding disassortative mating was not vigorously tested until Bai et al. (2006, 2007) were able to confirm highly disassortative mating in the heterodichogamous walnut *Juglans mandshurica*. In this study, the neighborhood model unambiguously evaluated the level of disassortative mating by removing other influential factors such as tree size and distance.

4.2. The effects of fragmentation on the mating systems

Shibata et al. (2009) explored the effects of forest fragmentation on the reproduction of *A. mono* from the perspective of seed quantity related to the spatial isolation of the mother trees. Isolation had been suggested to increase the rates of empty and immature seeds, but a decrease in the numbers of predated and decayed seeds offset this rate by an increase in sound seeds. The use of genetic markers enabled us to examine the qualitative aspects of seed reproduction, including the selfing ratio and the genetic diversity of the pollen pool. Our results indicated both negative and positive impacts of forest fragmentation on seed quality.

The negative impacts included an increased selfing rate in the isolated maternal trees, which was twice as high as in the continuous forest. Moreover, the clumped trees showed an increase in biparental inbreeding and a decrease in the pollen pool diversity. In contrast, positive effects were observed in the higher outcrossing rate in the clumped trees, and the pollen pool genetic diversity in the isolated trees as indicated by the number of sires (N_p) being comparable to that in the continuous forest. The primary factor giving rise to these differences was undoubtedly the spatial pattern of the reproductive trees, since the mating system parameters (t_m , $t_m - t_s$, r_{pm}) were all correlated with the local density of the neighboring flowering trees (Fig. 4). These relationships had been examined in previous studies (Fernández-M and Sork, 2005; Murawski and Hamrick, 1991; Restoux et al., 2008; Robledo-Arnuncio et al., 2004; Ward et al., 2005). However, a negative correlation between r_{pm} and the local density has not been reported before, and was contradictory to the trend demonstrated by Coates et al. (2007). These results suggested that the spatial isolation of mother plants increased inbreeding, decreased biparental inbreeding, and increased the pollen pool genetic diversity of *A. mono*.

The local tree density may affect seed reproduction by altering the abundance and behavior of the pollinators (Hadley and Betts, 2012). The leptokurtic pollen dispersal suggested the presence of a proximity advantage (Lourmas et al., 2007; Robledo-Arnuncio et al., 2004; Robledo-Arnuncio and Gil, 2005), whereby short-distance pollen transfer would be preferred in the presence of neighboring compatible pollen donors. However, a strong probability of long-distance pollen dispersal remained. The isolation of trees would therefore facilitate the effective pollination from distant trees (Hadley and Betts, 2012, Kwak et al., 1998), although this would be accompanied by reduced seed production or increased selfing due to a lack of available outcross pollen. Previous studies also observed extensive pollination in fragmented landscapes (e.g., Dick et al., 2003; Kamm et al., 2009; Mathiasen et al., 2007; Nason and Hamrick, 1997; White et al., 2002), and concluded that the potential for long-distance pollen transfer of a plant relied on the foraging range and behavior of its pollinators (Hadley and Betts, 2012; Harris and Johnson, 2004).

The SGS of the adult populations was required to form our conclusions. The kin structure detected in both of the populations can be mainly attributed to restricted seed dispersal (Johnson, 1988), as suggested in a previous study of the genus *Acer* (Young et al., 1993). Although it seems that forest fragmentation had no effect on the intensity of SGS, this may be because the time elapsed since fragmentation (ca. 20–40 yrs) was short. The SGS of the populations, with or without fragmentation, was considered to play a significant role in determining the heterogeneous mating systems. In the presence of a significant SGS, local pollen movements result in mating between relatives, whereas even a small amount of long-distance pollination contributed to the pollen pool.

The susceptibility of plant reproduction to habitat fragmentation is determined by a complex of factors including the ecological characteristics of plants and their pollinators, and the interactions between them (reviewed by Hadley and Betts, 2012). However, Aguilar et al. (2006) highlighted the impacts of the ability to self-fertilize on a plants reproductive susceptibility to fragmentation. Results from this meta-analysis suggested that only the self-compatibility of species significantly affected the differences in the effects of fragmentation. Generally, the seed reproduction of self-incompatible plants was more vulnerable to fragmentation (Larson and Barrett, 2000), whereas in self-compatible plants, a shortage of outcross pollen was compensated for through selfing (Eckert et al., 2010). Heterodichogamy in *Acer* may represent an intermediate state between complete outcrossing and self-compatible species; although *A. mono* is self-compatible, heterodichogamy effectively reduces the potential for self-fertilization. The chance of selfing appears to be restricted to the short hermaphroditic periods between the male and female stages. Therefore, we consider that heterodichogamy played an important role in the results obtained in this study, which was an intermediate between the self-incompatible and self-compatible plants.

The reproductive susceptibility of entomophilous plants to fragmentation inevitably depends on pollinator availability (Hadley and Betts, 2012). As stated above, *A. mono* flowers are visited mainly by small insects such as flies and solitary bees, and rarely by honeybees. Although qualifying pollinator abundance and behavior is difficult, it is generally considered that these small pollinator insects are more sensitive to fragmentation. A comparison with previous studies of plant species with

different types of pollinator would be useful, provided they were conducted on a comparable spatial scale. Two studies have been performed at the same forest sites, which explored the effects of fragmentation on the mating systems of the deciduous trees *Kalopanax septemlobus* (Fujimori et al., 2006) and *Magnolia obovata* (Isagi et al., 2007). *K. septemlobus* did not exhibit a decline in the seed set, suggesting the high flight capacity of its main pollinators, bumble bees. In *M. obovata*, however, the outcrossing rate was negatively correlated with the local tree density. The isolation of the *M. obovata* trees was thought to enhance the among-tree movement of pollinators, such as flower beetles, which had been identified as effective outcrossing pollinators (Matsuki et al., 2008). These comparisons suggested that the mating system of *A. mono* might be rather susceptible to fragmentation.

4.3. The effects of fragmentation on population sustainability

Changes in plant mating systems in subdivided populations can result in negative impacts on progeny fitness, and subsequently may negatively affect the persistence of plant populations over time (Coates et al., 2007). Decreased offspring fitness through diminished seed reproduction and increased inbreeding depression, resulting from limited outcrossed pollen due to habitat fragmentation, was hypothesized to reduce population viability (Lennartsson, 2002; Rathcke and Jules, 1993). Moreover, limited gene flow across fragmented landscapes can lead to a loss of population genetic diversity, which lowers the population viability in later generations (Aguilar et al., 2008; Honnay et al., 2005; Vranckx et al., 2012).

However, the evidence suggested that fragmentation would have only a limited impact on the sustainability of *A. mono* populations. A previous study suggested that the decline in seed production due to pollen limitation was offset by a decrease in density-dependent mortality, such as seed predation (Shibata et al., 2009). Also, although inbreeding depression in self-fertilized seeds may be present in *A. mono*, as suggested by Gabriel (1967) for the congener *A. saccharum*, the mostly negative F_{IS} estimates of the progenies did not provide evidence of the accumulation of inbreeding. Moreover, evidence of a genetic rescue effect exists for some species (Aguilar et al., 2006; Brown and Kodric-Brown, 1977; Winfree et al., 2009). Our results revealed that pollen pool diversity in the isolated seed parents remained unchanged, with low differentiation, which suggested that enhanced long-distance pollen transfer, even if in small amounts, was able to prevent the progenies of isolated trees from being genetically isolated.

However, note that our results only apply to relatively small temporal and spatial scales of habitat fragmentation. A population genetic study of *A. saccharum* (Young et al., 1993) focused on habitat fragmentation occurring over a distance of 400 m and a duration of 100 years, and identified slightly lower population genetic diversity in the fragmented forests. Although this represents a complex result of the ecological and demographic processes in the long term, this classic example implied a threshold distance beyond which genetic isolation occurs in *Acer*. The effects of fragmentation on plant–pollinator interactions is scale- and landscape-dependent (García and Chacoff, 2007; Steffan-Dewenter et al., 2002), whereas gene flow via seed dispersal in *A. mono* may be more susceptible to habitat fragmentation (Young et al., 1993). Detecting these “fragment boundaries” (Kramer et al., 2008) may be challenging and should be investigated in future research.

Acknowledgments

We thank Y. Matsuki and R. Tateno for their assistance in the field, and E. Ihara, C. Amagai, Y. Kawamata, and A. Hisamatsu for their assistance in the laboratory experiments. We are also grateful for the helpful comments by K. Niiyama and H. Yoshimaru. Grants in support of this work were partly provided by the research project “Integrated research on elucidation of ecological order in the agriculture, forestry and fishery systems and optimum control (Bio-cosmos plan)” of the Ministry of Agriculture, Forestry and Fisheries and by the research project “Evaluation of sustainable forest use options and their perspectives (Project 2-2)” of the Research Institute for Humanity and Nature, Japan.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.gecco.2015.04.005>.

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