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Genetic and reproductive consequences of forest fragmentation for populations of *Magnolia obovata*

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Abstract In order to evaluate the consequences of forest fragmentation on populations of *Magnolia obovata*, we compared genetic diversity and reproductive characteristics at two nearby sites, one conserved and one fragmented. The genetic diversity between adults trees of the different sites was not significantly different. However, saplings in the conserved site showed a significantly higher genetic diversity than both adult trees in the conserved site and saplings in the fragmented sites; this was found to be the result of the larger gene flow into the conserved site. The density of the adult trees was significantly related to all of the reproductive traits analyzed (fertilization of ovules, insect attack to seeds, ovules that developed into seeds and outcrossing at the stage of seeds) at both sites. At both sites, fertilization of ovules and insect attack on seeds were positively correlated to adult tree density while outcrossing rate was negatively correlated to adult tree density. The fertilization of ovules and outcrossing were more dependent on adult tree density in the fragmented site than in the

conserved site. The probability of ovules developing into outcrossed seeds showed a negative correlation with adult tree density at both sites, indicating the advantage of low density for this species and possibly implying a resilience to habitat fragmentation. A two-generation-analysis did not identify significant differences between sites in terms of the structure of the pollen pool and the number of pollen donors. Although fragmentation affected reproductive characteristics, the effect on seedling establishment and subsequent survival remains to be determined. Proposals for future studies that will assist in the development of management strategies for forests suffering fragmentation are made.

Keywords Fecundity · Forest fragmentation · Fruition · Regeneration · Pollination

Introduction

Forest fragmentation as a result of the extensive anthropogenic use of resources is a threat to biodiversity that may have an influence on forest ecosystems and cause detrimental effects, both genetically as well as ecologically. The level of genetic diversity may decrease in direct response to shrinking populations of trees, followed by further genetic erosion through increases in random genetic drift, inbreeding, and reduced gene flow (Young et al. 1996; Oostermeijer et al. 2003). Fragmented populations may suffer inbreeding depression, increased susceptibility to diseases and pests, the fixation of deleterious alleles, and the loss of self-incompatibility alleles. Within a short period of time, inbreeding may lead to a population with a reduced fitness and lower viability. Reduced genetic diversity may also limit the ability of the population to adapt to environmental changes. Fragmentation may also have ecological consequences for forest ecosystems. For example, changes in the activity, abundance, and species of pollinators and increased distances between trees and populations may result in pollen

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limitation and lower seed set. Shifts in interactions between species following fragmentation may also alter the characteristics of plant reproduction.

Fragmentation negatively affects the reproductive success of plants by reducing performance, ranging from the activity of pollinators (Aizen and Feinsinger 1994a, b; Quesada et al. 2003), pollen deposition (Cunningham 2000; Cascante et al. 2002; Quesada et al. 2003) and seed set (Ghazoul et al. 1998; Fuchs et al. 2003; Quesada et al. 2004) to the regeneration of populations (Benitez-Malvido 1998; Cascante et al. 2002; Benitez-Malvido and Martínez-Ramos 2003). However, several studies have shown that forest fragmentation can have positive (Dick 2001; White et al. 2002; Dick et al. 2003) or neutral (Cascante et al. 2002; Fuchs et al. 2003) effects on a number of traits associated with plant reproduction.

Although the influence of forest fragmentation or deforestation on ecosystems and communities has been analyzed (see studies mentioned above), a relatively smaller number of studies have evaluated the genetic consequences of fragmentation (Aldrich and Hamrick 1998; Aldrich et al. 1998; White et al. 1999; Dayanandan et al. 1999; Collevatti et al. 2001; Dick 2001; Obayashi et al. 2002; White et al. 2002; Fuchs et al. 2003), and the locations of the study sites are biased toward tropical regions (Lowe et al. 2005). These studies, however, have revealed that the effects of habitat fragmentation on the genetic traits of tree populations are more complex than expected (Aldrich and Hamrick 1998; Dick 2001; White et al. 2002; Dick et al. 2003; Lowe et al. 2005).

The objectives of the present research were to assess the consequences of forest fragmentation on the genetic diversity, reproductive characteristics and gene flow of *Magnolia obovata* by comparing fragmented and non-fragmented sites located in a research area of the temperate region where this type of study has only rarely been conducted. The assessment includes an evaluation of the biological conservation value of remnant forest and establishes strategies for sustainable management based on maintaining or even restoring ecological function and genetic resources. In order to assess the consequences of forest fragmentation on the genetic and reproductive traits of *M. obovata*, we compared genetic diversity, distance of pollen movement, and rates of fertilization of ovules, insect attack to seeds, ovules that developed into seeds and outcrossing at the seed stage between conserved and fragmented sites.

Materials and methods

Study species

Magnolia obovata is a common, deciduous tree species growing to 30 m in height in Japanese temperate forests. The standing density of adults of this species is relatively

low – a few trees per hectare. Flowers of *Magnolia* species do not secrete nectar and are primarily pollinated by beetles (Thien 1974; Judd et al. 2002), which are relatively inefficient as pollen vectors (Thien 1974). However, a variety of insects have been observed to visit the flowers of *M. obovata* (Tanaka and Yahara 1988). The flowers are protogynous, with petals closing between the female and male periods (Kikuzawa and Mizui 1990). Although individual flowers last for 3–4 days, a tree flowers for up to 40 days (Kikuzawa and Mizui 1990). The pistil contains 70–150 carpels, and each carpel can bear two seeds. Seeds reach maturity in the autumn and are dispersed by birds.

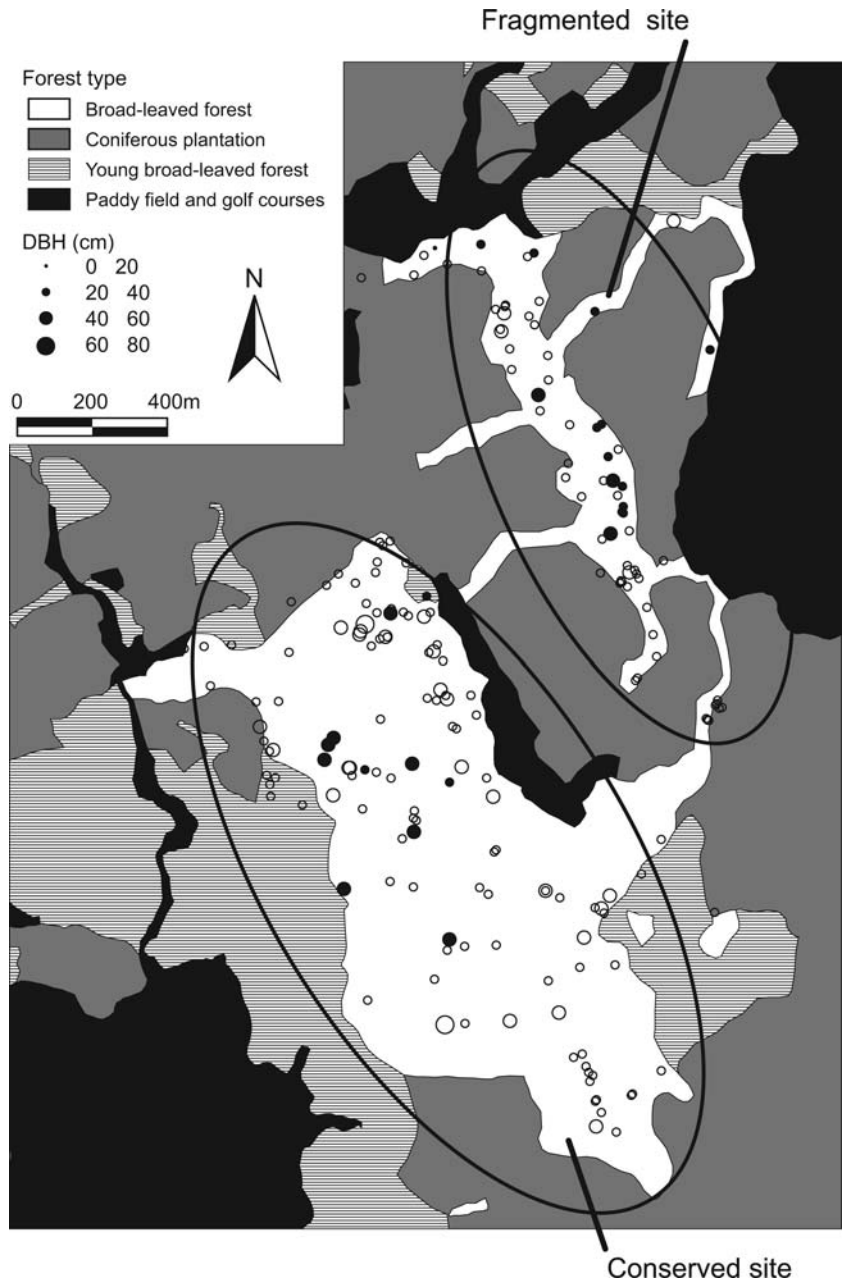
Study area

We compared conserved and fragmented forest sites in the Ogawa Forest Reserve and its surrounding area (36°56'N, 140°35'E), Ibaraki Prefecture, central Japan (Fig. 1). The sites are situated at an altitude of 610–660 m a.s.l. on a quasiplain in the southern part of the Abukuma Mountains. Mean annual air temperature and precipitation at a meteorological station in Ogawa (36°54'N, 140°35'E) during the study period were 10.7°C and 1910 mm, respectively (Moriguchi et al. 2002). The area is covered by a deciduous broad-leaved forest, and the dominant woody species in the canopy are *Quercus serrata*, *Fagus japonica*, and *F. crenata*. Intensive studies of the structure and dynamics of the plant community (Nakashizuka et al. 1992) and on the population dynamics of *Carpinus* (Shibata and Nakashizuka 1995), *Acer* (Tanaka 1995) and *Cornus* (Masaki et al. 1994) have been conducted in the area. The conserved site comprises 98 ha. The fragmented site has an elongated shape (approx. 1500 × 200 m long; 29 ha), is surrounded by a conifer plantation, paddy fields, golf courses, and pasture and is located 500 m to the northeast of the conserved site. The location and size (diameter at breast height; DBH) of all reproductive-age *M. obovata* were determined at both sites (Fig. 1).

Evaluation of genetic diversity

Trees with a DBH of more than 30 cm ($n = 88$ and 29 in the conserved and fragmented sites, respectively), and saplings ($n = 128$ and 92 in the conserved and fragmented sites, respectively) were located and genotyped using 11 microsatellite markers developed for *M. obovata* (Isagi et al. 1999). The height of the saplings ranged from 2.8 to 615 cm, with an average of 70.3 cm. The effective number of alleles (a_e) was calculated for trees and saplings at each site using the formula $a_e = 1/\sum x_i^2$, where x_i is the frequency of the i th allele for each locus (Hedrick 2005). The significance of the difference between the effective number of alleles of adults and saplings and those in the conserved and fragmented sites were compared using paired t -tests.

Fig. 1 Size and location of reproductive *Magnolia obovata* trees at the conserved and fragmented sites. Filled circle Adult tree from which fruit were collected



Pollination, seed set, and self-pollination rates

For the analysis of pollination and seed set, 119 and 136 mature fruit were collected in September 2003 from 14 and 14 adult trees in the conserved and fragmented sites, respectively (Fig. 1). The number of ovules per fruit was estimated by doubling the number of carpels since each carpel of *M. obovata* contains two ovules. The number of fertilized ovules was estimated by summing up the numbers of rotten seeds, seeds attacked by insects, and sound seeds. The germination of the seeds of the 255 fruit from the 28 trees was induced by a continuous temperature fluctuation cycle consisting of 12:12 h at 24°:16°C. The DNA of 414 germinated seedlings was extracted from the root tips (about 5 mm in length), and

genotype analyses at microsatellite loci M6D3, M6D8, M10D3 and M10D8 were conducted to identify the pollination mode (self-pollinated or allogamous).

The relationship between conspecific density and rates of fertilization of ovules, insect attack to seeds, ovules that developed into seeds, and outcrossing at the stage of seeds were evaluated by fitting binomial generalized linear models (GLMs) (no fertilization = 0, fertilization = 1; no insect attack = 0, insect attack = 1; no development of ovule to a seed = 0, development of ovule to a seed = 1; self-pollination = 0, outcrossing = 1) with the logistic link using the R software package (ver. 2.2.1; R Development Core Team 2005). When self-pollination was included in the analysis, the average distance of pollen movement was 20.9 m (Isagi

et al. 2004), and 77% of pollen exchange occurred a radius of 200 m from an adult tree. Based on this result, we evaluated the density of conspecifics around a mother tree by the number of trees within a radius of 200 m. The goodness-of-fit of the models was employed using the deviance chi-square test for the null hypothesis that the parameter estimates do not differ from zero.

Gene flow estimation by the Two-Generation analysis

Gene flow has been quantified by direct and indirect methods. Indirect methods (e.g. Wright 1951; Slatkin 1985) estimate the amount of gene flow per generation based on the contemporary genetic structure and diversity among populations, as this reflects pollen and seed movements over generations. Direct methods based on parentage analyses with highly informative genetic markers, such as microsatellites, can measure current rates of gene flow although such methods require that the genotypes of all offspring and possible parents within the research sites be determined. Where there are large number of possible parents, exclusion of unrelated parents can become ambiguous even with high exclusion probabilities. Hence, direct approaches are only feasible for populations with a relatively small number of individuals. At the conserved and fragmented sites 114 and 70 trees flowered in 2003, respectively, a number that would have rendered direct approaches laborious.

In order to evaluate rates of pollen movement, we employed a two-generation analysis (TwoGener; Smouse et al. 2001). In the TwoGener analysis, genotypes of maternal plants and their offspring are evaluated. Using four microsatellite loci, we determined the genotypes of outcrossed seedlings, of which 53 were from seven seed parents in the conserved site and 88 were from five seed parents in the fragmented site. The TwoGener analysis uses a statistic Φ_{ft} , which is an analog of the F_{ST} coefficient of genetic differentiation among populations (Wright 1951). When the F_{IS} value is more than zero because of a genetic structure within a population, the value Φ_{ft} might be overestimated. Therefore, the value Φ_{ft} was divided by $1 + F_{IS}$ (Austerlitz and Smouse 2001). F_{IS} was calculated with the FSTAT computer program (Goudet 1995). The Φ_{ft} value, which ranges from 0 to 1, represents the genetic differentiation among pollen pools sampled from

different maternal plants. Given the absence of local genetic structure among adult trees, an appropriate distribution of pollen dispersal and equal fecundity of the adult trees, we can estimate the effective number of pollen donors (N_{ep}) with the equation, $N_{ep} = 1/(2\Phi_{ft})$.

Results

Genetic diversity

The effective number of alleles was smaller at the fragmented site than at the conserved one for both adult trees and saplings (Table 1), but the difference was only significant for the saplings ($P = 0.014$). At the conserved site, there were significantly more effective alleles in the saplings than in the adults ($P = 0.0028$).

Pollination, seed set and germination

The fragmented site showed significantly higher ratios than the conserved site in terms of proportions of fertilized ovules, seeds attacked by insects, and ovules that developed into seeds (Table 2). The ratio of outcrossing at the seeds stage was higher at the fragmented site than the conserved site (Table 2), but the difference was not significant ($P = 0.09$).

Adult tree density within 200 m of the seed parents was significantly related to all reproductive traits analyzed at both sites (Fig. 2). The fertilization of ovules and insect attack to seeds were positively related to adult tree density, while outcrossing at the stage of seeds was negatively related to adult tree density (Fig. 2) at both sites. The ratios of ovules that developed into seeds responded oppositely to adult tree density at each site (Fig. 2c). The gradient of the regression curves for the fertilization of ovules and outcrossing were steeper at the fragmented site (Fig. 2a, d), indicating the larger dependence of these traits to adult tree density in the fragmented site.

Pollen pool structure and number of pollen donors

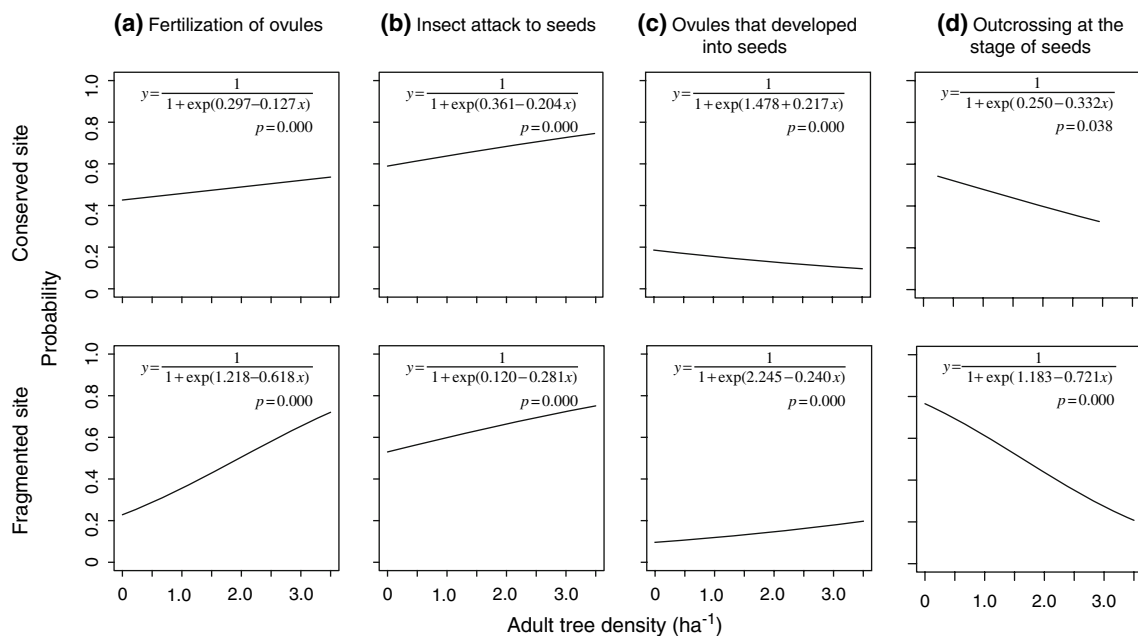
The estimated values of Φ_{FT} were 0.137 and 0.144 for the conserved and fragmented sites, respectively. As

Table 1 Effective number of alleles at 11 microsatellite loci in the conserved and fragmented sites

Maturational stage of <i>Magnolia obovata</i> plants	Site	Microsatellite loci											Average	Difference	
		M6D1	M6D3	M6D4	M6D8	M6D10	M10D3	M10D6	M10D8	M15D5	M17D3	M17D5			
Adult	Conserved	19.26	12.61	7.66	4.61	4.56	12.31	6.26	9.16	2.96	7.99	7.17	8.60	} $p=0.0028$	
	Fragmented	11.55	9.96	9.80	4.46	5.08	10.35	5.98	7.56	3.24	8.03	5.11	7.37		
Sapling	Conserved	22.28	16.49	9.11	5.76	4.54	14.85	6.30	11.79	3.59	10.71	7.19	10.24		} $p=0.014$
	Fragmented	14.81	7.09	8.88	4.50	5.06	11.95	5.53	7.60	2.92	8.58	4.84	7.43		

Table 2 Reproductive performance of *Magnolia obovata* in the conserved and fragmented sites

Traits	Site	Number of ovules/seeds			Ratio of (+)	χ^2	P
		+	-	Total			
Fertilization of ovules	Conserved	12,917	14,029	26,946	0.479	265.42	0.00
	Fragmented	16,649	13,761	30,410	0.547		
	Total	29,566	27,790	57,356	0.515		
Insect attack to seeds	Conserved	8,657	4,260	12,917	0.670	19.59	0.00
	Fragmented	11,560	5,089	16,649	0.694		
	Total	20,217	9,349	29,566	0.684		
Ovules that developed into seeds	Conserved	3,701	23,245	26,946	0.137	45.63	0.00
	Fragmented	4,787	25,623	30,410	0.157		
	Total	8,488	48,868	57,356	0.148		
Outcrossing at the stage of seeds	Conserved	103	92	195	0.528	2.95	0.09
	Fragmented	134	85	219	0.612		
	Total	237	177	414	0.542		

**Fig. 2** Responses of fecundity traits to the density of adult *M. obovata* trees located within 200 m of a seed parent, at the conserved and fragmented sites

indicated by the randomized A & W test (Sork et al. 2005), there was no significant difference between the sites, suggesting equivalence in the amount of gene flow resulting from the movement of pollen. With an assumption of no local genetic structure among adult trees and no inbreeding between adults, the numbers of pollen donors (N_{ep}) were estimated with the equation $N_{ep} = 1/2\Phi_{FT}$ to be 3.6 for the conserved site and 3.5 for the fragmented sites.

Discussion

Genetic diversity

A decrease in the number of trees through deforestation will lead to a decrease in genetic diversity. The effective number of alleles is a more sensitive approach for

detecting the immediate genetic loss following a population bottleneck than heterozygosity, which will be delayed until several generations after the disturbance. Although there were fewer effective alleles for the adult trees at the conserved site, the difference was not significant ($P = 0.145$).

At the conserved site, the effective number of alleles for saplings was significantly ($P = 0.0028$) larger than that for adults. Until the conserved site was designated as a Forest Reserve in 1969, the forest had been subjected to partial cutting for charcoal-burning (Suzuki 2002), and the site presently consists of old growth and 50- to 80-year-old secondary forests. Therefore, the genetic diversity of *M. obovata* in this area likely decreased until 1969 as a consequence of the partial cutting policy, and the genetic diversity of the present adult trees in the conserved site must be lower, thereby reflecting the

cutting in the past. Direct parentage analysis of saplings by means of microsatellite marker analysis revealed substantial gene flow into the conserved site, with 57% of genes of the sapling population derived from outside of the site (Isagi et al. 2000). Hence, the genetic diversity of the population is probably recovering through active gene flow from outside of the site, and the larger effective number of alleles for saplings in the conserved site is likely to reflect the genetic recovery from degradation.

The effective number of alleles for saplings was significantly lower in the fragmented site than in the conserved site. This may reflect the larger gene flow into the conserved site than into the fragmented site that is currently underway, rather than a decrease in the number of effective alleles in the fragmented site itself. Since the TwoGener analysis did not reveal any significant differences in pollen movement between the conserved and fragmented sites, the observed difference in the gene flow seems to be caused by the difference in the number of seeds carried into the sites. The number of seeds carried into the fragmented site could be smaller than that carried into the conserved site because of the weaker attraction of the fragmented site for seed-dispersing birds.

Reproduction processes

In this research, we analyzed and compared processes of reproduction – from fertilization to germination – in *M. obovata* at both conserved and fragmented sites and found complicated responses of the reproductive traits to the processes to fragmentation. Although fertilization success was positively correlated to adult tree density at both the conserved and fragmented sites, the gradient of the curves for the fragmented site was much steeper (Fig. 2a). One possible explanation causing this pattern is that it would reflect a diminished efficiency of pollination at the fragmented site, with higher adult density compensating for the degradation of ecological function.

Because of severe inbreeding depression in *M. obovata* (Ishida et al. 2003), most of saplings were all outcrossed in the conserved site (Isagi et al. 2000), and the proportion of seeds that are out-crossed is important to population process of this species. The ratio of outcrossing at the seed stage was significantly correlated with adult tree density: the ratio of outcrossing was higher at a lower adult tree density. A similar pattern – a higher outcrossing ratio with lower density of plants or a longer distance of pollen movement with lower density – has been reported in earlier studies (e.g. Handel 1983; Fenster 1991; Godt and Hamrick 1993; Schnabel and Hamrick 1995; Kameyama et al. 2001).

Comprehensive evaluation of forest fragmentation

How can we evaluate the overall reproductive consequence of fragmentation based on the different responses of reproductive characteristics at the conserved and fragmented sites?

Fragmentation is thought to have negative ecological and genetic impacts on forest ecosystems. However, the effects of forest fragmentation may be subtle because of the long generation time of trees and the complex interactions of organisms in forest ecosystems. In the present study, we observed a rather complicated reaction to forest fragmentation in terms of genetic and fecundity traits such as effective number of alleles, ratio of fertilization of ovules, insect attack to seeds, ovules that developed into seeds, and outcrossing at the stage of seeds, among others. Because of the severe inbreeding depression in *M. obovata* (Ishida et al. 2003), outcrossed seeds predominantly contribute to the regeneration of the population. The ratio of ovules that developed into outcrossed seeds can be estimated from the product of the ratios of (1) ovules that developed into seeds and (2) outcrossing at the stage of seeds. This ratio showed a negative correlation with adult tree density: reproductive performance was higher with at a lower adult tree density at both the conserved and fragmented sites (Fig. 3). This correlation indicates the advantage of a low density of *M. obovata* trees in forest ecosystems and may imply a resilience to habitat fragmentation. Further comparative studies aimed at examining the correlation for other species growing at higher adult tree densities in natural conditions is required.

To date, most of the research that has been carried out on the effect of forest fragmentation has focused on reproductive traits such as fertilization, seed set and self-pollination rather than on regeneration success and regenerated population viability (Hobbs and Yates 2003; Tomimatsu 2005). The latest stage of the life history of *M. obovata* analyzed in this study was that of seedlings (TwoGener analysis). Although there were changes in reproductive traits related to fragmentation, the TwoGener analysis did not detect any substantial changes in effective pollen movement and number of pollen donors

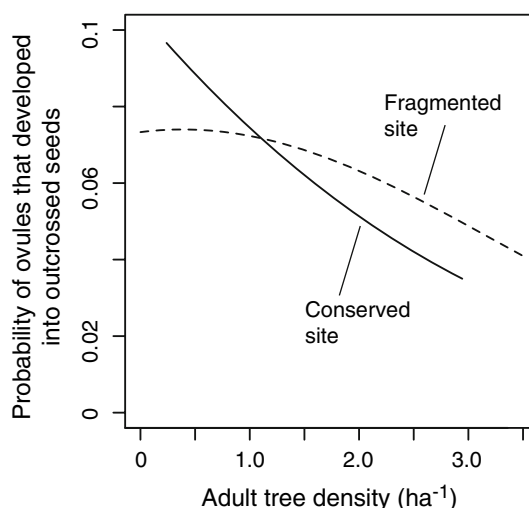


Fig. 3 Relationship between the probability of ovules that developed into outcrossed seeds and adult tree density within 200 m of a seed parent at the conserved and fragmented sites

for the seedlings. However, we have to advise caution with respect to the interpretation of the unchanged traits between the two sites based on the TwoGener analysis. The red seeds of *M. obovata* attract birds. In order to collect seeds before they were consumed by birds, it was necessary to climb and collect fruits high in the canopy. This constraint made it impossible to analyze large numbers of seed parents. There is a possibility that the relatively small number of seed parents used for the TwoGener analysis rendered the analysis incapable of detecting any difference (type II error) between the sites in terms of effective pollen movement, Φ_{FT} , and the number of pollen donors.

Do the present results indicate a resilience of *M. obovata* to the impact of fragmentation? In order to evaluate the effect of fragmentation on forest ecosystems comprehensively, we need to examine the impact of idiosyncratic factors – interactions between species and population processes – as well as issues relating to fecundity. Although any analysis of the consequences of fragmentation on seedling establishment and subsequent survival require longer study periods, they are directly connected to population persistence and are, therefore, important considerations when developing management guidelines for fragmented forests.

The pattern and amount of gene flow in plants are known to vary strikingly between species, years, populations, and even individuals (e.g. Ellstrand and Marshall 1985; Ellstrand et al. 1989; Streiff et al. 1999; Kameyama et al. 2000; Isagi et al. 2004). The flowering and fruiting of *M. obovata* fluctuates among years, and the activity of pollinators may also vary among years in response to weather conditions (Kikuzawa and Mizui 1990). Therefore, the effect of fragmentation needs to be assessed for several years. The size and spatial organization of remnants as well as the intensity and duration of the disturbance might also influence the impact of fragmentation. Additional research sites that are fragmented more severely than the ones studied here might yield more decisive results. Studies on species with a range of life history characteristics might also provide useful information by developing generalized management schemes for fragmented forest ecosystems.

One of the most important interactions to be considered when evaluating forest fragmentation is that between plants and pollinators. Although fragmentation has been regarded as a threat to pollination systems (Kearns et al. 1998; Duncan et al. 2004), unexpected patterns of pollen movement in degraded forests have been reported. Increased gene flow has been observed to occur after forest fragmentation, and pollinator behavior, wind dispersal, and accidental movement of small flying pollinators have been found to change following the opening of the canopy (Dick 2001; White et al. 2002). The complicated responses of fecundity found in the present study are likely to be influenced by the interaction between *M. obovata* and insects, with some of the latter acting as pollinators and others as seed predators. Changes in the composition of the species and

the behavior of the pollinator and/or predator assemblages following forest fragmentation should be an important area of future research.

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