# Microsatellite analysis of the regeneration process of Magnolia obovata Thunb. 

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#### Abstract

We analysed the regeneration process of Magnolia obovata using polymorphic microsatellite markers. Eighty-three adult trees standing in a watershed covering an area of 69 ha, and saplings collected from a smaller research plot ( 6 ha ) located at the centre of the watershed were genotyped using microsatellite markers. Among 91 saplings analysed, 24 ( $26 \%$ ) had both parents, 31 ( $34 \%$ ) had one parent and $36(40 \%)$ had no parent within the watershed. The proportion of genes in saplings inherited from the adults within the watershed was $43 \%$, and therefore $57 \%$ were from outside the site, indicating active gene exchange across the watershed area. Average distance between parents and saplings ( $264.6 \pm 135.3(\mathrm{SD}) \mathrm{m})$ was significantly smaller than that of pairs randomly chosen between adults and saplings ( $436.7 \pm 203.0(\mathrm{SD}) \mathrm{m})$. The distance of pollen movement inferred from the distance between the two parents of each sapling ranged from 3.2 m to 540 m with an average of $131.1 \mathrm{~m} \pm 121.1 \mathrm{~m}(\mathrm{SD})$. Because $34 \%$ ( $=31 / 91$ ) of saplings had only one parent within the watershed, the estimate of average pollen movement must be smaller than the actual one. Longdistance seed dispersal by birds, inbreeding depression and limitation in acceptance of pollen because of the difference of phenology in each individual flower were considered to be the probable causes of large gene exchange across the watershed.


Keywords: gene flow, pollen dispersal, pollination, seed dispersal.

## Introduction

Magnolia obovata is a large, common deciduous tree of temperate forests in Japan reaching 30 m in height. Its large flowers do not secrete nectar, and are primarily pollinated by beetles (Thien, 1974) which are thought to be less efficient than bees. The flowers are protogynous and usually close between the female and male period (Kikuzawa \& Mizui, 1990). Although the flowering period of each flower is 3-4 days, for an individual tree flowering persists for up to 40 days (Kikuzawa \& Mizui, 1990). The standing density of adult trees is relatively low at a few trees per hectare. In temperate forests in Japan, a few dominant tree species often occupy a large proportion of the canopy, e.g. Fagus crenata, and the rest of the canopy is composed of tree species occurring at relatively low density such as Kalopanax pictus, Cornus controversa, Aesculus turbinata, Magnolia obovata, Magnolia salicifolia and Pterocarya rhoifolia.

[^0]Although each species is at a relatively low density, the assemblage of these species is dominant as a whole, and determines the structure and diversity of the forest ecosystem. For such species, it is important to analyse the extent of pollen movement and seed dispersal to elucidate the regeneration process, mechanisms that maintain biological diversity in forest tree communities and also for conservation purposes.

The pattern and degree of gene dispersal can affect the genetic structure of plant populations (e.g. Schaal, 1980; Ellstrand, 1992; Hamrick et al., 1992), and in higher plants, gene dispersal occurs at reproduction through pollen and seeds. Microsatellite loci are ideal for quantifying pollen- or seed-mediated gene transfer in natural plant populations because of their codominant inheritance and high variability. Therefore, they should provide high exclusion probabilities for paternity assignment. We have developed 11 microsatellite marker loci in Magnolia obovata (Isagi et al., 1999) to assign parentage and examine gene transfer of this species. In the present study, we will estimate pollen and seed
dispersal distances and the magnitude of gene transfer in a population where adult tree density was $1.2 \mathrm{ha}^{-1}$.

## Materials and methods

## Field site

Field research was conducted in Ogawa Forest Preserve, Ibaraki Pref., central Japan ( $36^{\circ} 56^{\prime} \mathrm{N}, 140^{\circ} 35^{\prime} \mathrm{E}$ ). The elevation of the research area was $610-660 \mathrm{~m}$ a.s.l. and annual mean air temperature and annual precipitation were $9^{\circ} \mathrm{C}$ and 1800 mm , respectively. Dominant woody species in the canopy were Quercus serrata, Fagus japonica and Fagus crenata, etc. We established two research plots, plots A and B , in the preserve (Fig. 1). Plot A covered the whole watershed area of 69 ha. Within this plot all of the adult trees of $M$. obovata were located and the diameters at breast height (d.b.h.) were measured. The other plot, plot B, occupied an area of 6 ha $(200 \times 300 \mathrm{~m})$, located at the centre of plot A (Fig. 1). In plot B, intensive studies on the


Fig. 1 Map showing the distribution of adults and saplings of Magnolia obovata analysed with lines drawn between parents and their offspring.
plant community structure, community dynamics (Nakashizuka et al., 1992) and population dynamics of various tree species such as Carpinus (Shibata \& Nakashizuka, 1995), Acer (Tanaka, 1995) and Cornus (Masaki et al., 1994) have been made.

## Sampling

Leaf samples from all of the reproductive adult trees (83 trees) of M. obovata in plot A were collected. In plot B, leaf tissue was sampled from 91 saplings. During leaf collection, the position of each tree was mapped. Leaf samples were stored at $-70^{\circ} \mathrm{C}$ prior to DNA extraction.

## DNA extraction and microsatellite analysis

Crude genomic DNA of M. obovata was extracted using the CTAB method (Milligan, 1992). Genotypes of each DNA sample were scored using eight pairs of microsatellite PCR primers developed by Isagi et al. (1999). PCR amplifications were performed, using a thermal cycler (GeneAmp PCR System 9600, ABI), under the following conditions: initial denaturing at $94^{\circ} \mathrm{C}$ for 9 min , then 30 cycles of denaturation at $94^{\circ} \mathrm{C}$ for 30 s , annealing for 30 s , and extension at $72^{\circ} \mathrm{C}$ for 1 min , followed by a final incubation at $72^{\circ} \mathrm{C}$ for 7 min . The volume of the reaction mixture was $10 \mu \mathrm{~L}$ containing 10 ng of DNA from M. obovata, 5 pmol of primers labelled with fluorescent phosphoramidites (TET or 6-FAM), 0.25 U of Taq polymerase (Ampli TaqGold, ABI), $200 \mu \mathrm{M}$ of each dNTP, 1.5 mm of $\mathrm{MgCl}_{2}, 10 \mathrm{~mm}$ of Tris- $\mathrm{HCl}, \mathrm{pH} 8.3$, 50 mm of KCl and $0.001 \%$ of gelatin. The PCR products were resolved on a $5 \%$ denaturing polyacrylamide gel, and the sizes were determined by automated fluorescent scanning detection with the autosequencer ABI377 and GeneScan ${ }^{\text {TM }}$ analysis software (ABI).

## Parentage analysis

Parentage was assigned by comparing alleles between a sapling and candidate parents (Dow \& Ashley, 1996) using genotype data at eight microsatellite loci: M6D1, M6D3, M6D4, M10D3, M10D6, M10D8, M15D5 and M17D5 developed by Isagi et al. (1999). Alleles at every locus of each sapling were compared with those of adult trees, and adults which did not share any alleles at each locus were excluded as candidate parents.

## Results

## Analysis of parentage

The eight microsatellite markers were highly variable, and sufficiently informative to conduct the analysis of

Table 1 Allele frequencies, numbers of heterozygotes and homozygotes, observed and expected heterozygosities at eight microsatellite loci in 83 adults and 91 saplings of Magnolia obovata

| Allele | Frequency (\%) |  | Allele | Frequency (\%) |  | Allele | Frequency (\%) |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Adults | Saplings |  | Adults | Saplings |  | Adults | Saplings |
| Locus M6D4 |  |  | Locus M10D3 |  |  | Locus M10D6 |  |  |
| 146 |  | 1.1 | 209 |  | 0.6 | 271 | 2.4 | 1.7 |
| 148 | 0.6 | 0.6 | 211 | 6.0 | 8.2 | 273 | 21.1 | 28.0 |
| 150 | 13.9 | 14.8 | 215 |  | 1.1 | 275 |  | 1.7 |
| 151 | 1.2 |  | 217 |  | 1.1 | 277 | 12.1 | 6.6 |
| 152 | 15.1 | 15.9 | 219 |  | 2.2 | 279 | 19.3 | 23.6 |
| 154 | 4.2 | 7.1 | 221 | 9.0 | 5.0 | 281 | 16.9 | 9.3 |
| 155 | 0.6 | 0.6 | 223 | 1.8 | 0.6 | 283 | 11.5 | 10.4 |
| 156 | 19.9 | 24.2 | 225 | 4.8 | 2.2 | 285 | 16.3 | 18.1 |
| 158 | 4.2 | 7.7 | 227 | 7.2 | 12.6 | 287 | 0.6 | 0.6 |
| 160 | 2.4 | 3.3 | 229 | 1.8 | 3.9 |  |  |  |
| 161 | 0.6 |  | 232 | 1.2 | 2.2 | No. of alleles | 8 | 9 |
| 162 | 0.6 | 0.6 | 236 | 1.2 | 1.1 | Heterozygotes | 74 | 75 |
| 164 | 1.8 | 1.1 | 238 | 21.1 | 16.5 | Homozygotes | 9 | 16 |
| 165 |  | 0.6 | 240 | 8.4 | 14.8 | $H_{\text {o }}$ | 0.89 | 0.82 |
| 167 | 1.2 | 0.6 | 242 | 3.6 | 1.1 | $H_{\text {e }}$ | 0.84 | 0.81 |
| 171 | 0.6 |  | 244 | 3.6 | 5.5 | Locus M10D8 |  |  |
| 173 | 1.8 | 0.6 | 246 | 1.8 |  | 257 |  | 0.6 |
| 175 |  | 0.6 | 248 | 3.0 | 3.9 | 267 | 0.6 | 1.7 |
| 177 | 0.6 | 1.1 | 250 | 12.7 | 8.8 | 275 | 1.8 |  |
| 179 | 1.8 | 1.7 | 252 | 1.8 | 0.6 | 277 |  | 0.6 |
| 181 | 0.6 | 0.6 | 254 | 1.2 |  | 279 | 16.3 | 14.8 |
| 183 | 2.4 | 0.6 | 258 | 0.6 |  | 281 | 1.2 | 2.8 |
| 185 | 1.2 | 0.6 | 262 | 1.8 | 0.6 | 282 | 15.7 | 25.3 |
| 187 | 0.6 | 3.3 | 264 | 3.0 | 1.7 | 283 | 4.2 | 1.7 |
| 189 | 3.6 | 1.7 | 265 | 4.2 | 6.0 | 284 | 3.0 | 2.2 |
| 191 | 1.8 |  |  |  |  | 285 | 0.6 | 1.1 |
| 193 | 0.6 |  | No. of alleles | 21 | 22 | 286 |  | 0.6 |
| 197 | 3.0 | 2.2 | Heterozygotes | 79 | 88 | 287 | 4.2 | 0.6 |
| 199 | 1.2 |  | Homozygotes | 4 | 3 | 288 | 0.6 |  |
| 201 | 1.8 | 2.8 | $H_{\text {o }}$ | 0.95 | 0.97 | 289 | 10.8 | 8.2 |
| 203 |  | 1.1 | $H_{\text {e }}$ | 0.91 | 0.91 | 290 | 0.6 | 0.6 |
| 205 | 1.8 | 1.1 | Locus M15D5 |  |  | 291 | 4.2 |  |
| 210 | 0.6 |  | 96 | 1.8 | 1.1 | 293 | 6.0 | 9.9 |
| 214 | 1.8 |  | 98 | 4.2 | 4.4 | 295 | 1.2 | 1.1 |
| 216 | 3.0 | 2.8 | 100 | 47.0 | 33.5 | 297 | 13.3 | 8.8 |
| 218 | 1.2 | 0.6 | 102 | 27.1 | 32.4 | 299 | 5.4 | 7.1 |
| 220 | 1.2 |  | 104 | 17.5 | 24.7 | 300 |  | 1.1 |
| 222 | 1.2 | 0.6 | 106 |  | 1.1 | 301 | 4.2 | 3.9 |
| 228 | 0.6 |  | 108 | 2.4 | 2.8 | 302 | 1.8 | 0.6 |
| 235 | 0.6 | 0.6 |  |  |  | 303 | 3.6 | 7.1 |
|  |  |  | No. of alleles | 6 | 7 | 313 | 0.6 |  |
| No. of alleles | 36 | 30 | Heterozygotes | 55 | 64 |  |  |  |
| Heterozygotes | 76 | 80 | Homozygotes | 28 | 27 | No. of alleles | 21 | 21 |
| Homozygotes | 7 | 11 | $H_{\text {o }}$ | 0.66 | 0.70 | Heterozygotes | 76 | 79 |
| $H_{\text {o }}$ | 0.92 | 0.88 | $H_{\text {e }}$ | 0.68 | 0.72 | Homozygotes | 7 | 12 |
| $H_{\text {e }}$ | 0.91 | 0.88 |  |  |  | $H_{\text {o }}$ | 0.92 | 0.87 |
|  |  |  |  |  |  | $H_{\text {e }}$ | 0.91 | 0.88 |

Table 1 (Continued)

| Allele | Frequency (\%) |  | Allele | Frequency (\%) |  | Allele | Frequency (\%) |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Adults | Saplings |  | Adults | Saplings |  | Adults | Saplings |
| Locus M6D1 |  |  | Locus M17D5 |  |  | Locus M6D3 |  |  |
| 130 | 1.2 | 1.1 | 289 | 1.2 |  | 106 | 10.2 | 7.7 |
| 136 | 0.6 |  | 293 | 18.1 | 20.9 | 116 | 4.8 | 2.2 |
| 138 | 1.2 |  | 295 | 3.6 | 3.9 | 118 |  | 1.7 |
| 143 | 3.6 | 4.4 | 297 | 6.6 | 3.9 | 120 | 6.6 | 9.9 |
| 145 | 0.6 |  | 299 | 27.1 | 21.4 | 122 | 4.2 | 5.5 |
| 147 | 4.2 | 1.1 | 301 | 1.8 | 13.2 | 124 | 5.4 | 0.6 |
| 149 | 7.2 | 10.0 | 303 | 12.1 | 11.0 | 126 | 3.0 | 3.3 |
| 151 | 4.8 | 5.6 | 305 | 12.1 | 14.8 | 128 | 6.6 | 11.0 |
| 153 | 1.8 | 4.4 | 307 | 7.2 | 8.2 | 130 | 5.4 | 8.2 |
| 155 | 1.2 | 1.7 | 313 |  | 2.2 | 132 | 4.2 | 6.6 |
| 159 | 4.8 | 3.9 | 317 |  | 0.6 | 134 | 2.4 | 1.7 |
| 161 | 1.8 |  |  |  |  | 136 | 3.0 | 3.3 |
| 167 | 3.0 | 2.8 | No. of alleles | 9 | 10 | 138 | 0.0 | 0.6 |
| 169 | 6.0 | 5.6 | Heterozygotes | 65 | 79 | 141 | 2.4 | 4.4 |
| 171 | 13.9 | 11.7 | Homozygotes | 18 | 12 | 145 | 1.2 |  |
| 173 | 5.4 | 3.3 | $H_{\text {o }}$ | 0.78 | 0.87 | 147 | 0.6 |  |
| 175 | 6.6 | 8.9 | $H_{\text {e }}$ | 0.84 | 0.85 | 150 | 6.6 | 4.4 |
| 177 | 4.8 | 3.9 |  |  |  | 154 | 0.6 | 0.6 |
| 179 | 1.2 | 1.7 |  |  |  | 158 | 0.6 | 0.6 |
| 181 | 0.6 | 0.6 |  |  |  | 160 | 3.0 | 5.0 |
| 183 | 2.4 | 1.7 |  |  |  | 162 | 2.4 | 1.7 |
| 185 | 7.2 | 4.4 |  |  |  | 164 | 0.6 | 3.3 |
| 187 | 1.8 | 2.8 |  |  |  | 166 | 5.4 | 2.8 |
| 189 | 2.4 | 2.2 |  |  |  | 168 | 3.6 | 2.8 |
| 191 | 1.8 | 0.6 |  |  |  | 170 | 7.2 | 7.1 |
| 193 | 1.8 | 6.1 |  |  |  | 172 | 0.6 | 0.6 |
| 195 | 5.4 | 9.4 |  |  |  | 174 | 1.8 |  |
| 197 | 1.2 |  |  |  |  | 178 | 5.4 | 4.4 |
| 199 |  | 1.7 |  |  |  | 180 |  | 0.6 |
| 201 | 0.6 |  |  |  |  | 182 | 0.6 |  |
| 215 | 0.6 | 0.6 |  |  |  | 192 | 0.6 |  |
|  |  |  |  |  |  | 198 | 0.6 |  |
| No. of alleles | 30 | 25 |  |  |  |  |  |  |
| Heterozygotes | 80 | 89 |  |  |  | No. of alleles | 29 | 26 |
| Homozygotes | 3 | 1 |  |  |  | Heterozygotes | 81 | 87 |
| $H_{\text {o }}$ | 0.96 | 0.99 |  |  |  | Homozygotes | 2 | 4 |
| $H_{\text {e }}$ | 0.95 | 0.94 |  |  |  | $H_{\text {o }}$ | 0.98 | 0.96 |
|  |  |  |  |  |  | $H_{\text {e }}$ | 0.95 | 0.94 |

parentage. The number of alleles for each locus ranged from six (M15D5) to 36 (M6D4) with an average of 20.3 for adults and from seven (M15D5) to 30 (M6D4) with an average of 18.8 for saplings (Table 1). The number of alleles unique to adults and saplings was 30 and 19, respectively.

Among 91 saplings found in plot B, 55 had at least one parent (first parent) in plot A whereas 36 had no parents within the watershed. Among the 55 saplings, 24 had the second parent as an exact match, and 31 had only one parent in plot A. Out of the 31 saplings which
had only one parent in plot A, 23 had only one candidate as an exact match, and eight had multiple matches with two candidates for one parent. No sapling seemed to be the product of self-pollination of adults in plot A.

In order to estimate the amount of gene flow into the watershed, it is important to evaluate the amount of cryptic gene flow which reflects the possibility that a sapling identified as having a parent within the research plot actually had the parent outside the plot (Dow \& Ashley, 1996). Using the allele frequencies at the eight

Table 2 Number of saplings having the first and second parents within or outside plot A, and values of cryptic gene flow for Magnolia obovata

|  | First parent | Second parent | Total |
| :--- | :---: | :---: | :---: |
| Saplings having parents <br> within plot A | $55(53.95)$ | $24(23.99)$ | $79(77.94)$ |
| Saplings having parents <br> outside plot A | $67(68.05)$ | $36(36.01)$ | $103(104.06)$ |
| Cryptic gene flow | 1.05 | 0.01 |  |

Figures in parentheses are numbers of saplings corrected for the values of cryptic gene flow. Because the number of saplings analysed was 91, figures in the column 'Total' for either saplings having parents within or outside plot A should sum up to 182 ( $=2$ parents $\times 91$ saplings).
microsatellite loci and the formula of Marshall et al. (1998), the probability of excluding a single randomly chosen unrelated individual in plot A from parentage was determined as 0.999769 for the first parents and 0.999995 for the second parents. Therefore, the probability of excluding correctly all unrelated adults ( 83 trees) within plot A was $0.999769^{83}=0.9810$ for the first parents and $0.999995^{83}=0.9996$ for the second parents. The number of saplings which had the first and second parents in plot A was 55 and 24, respectively (Table 2), so that the amount of cryptic gene flow was estimated as $55(1-0.9810)=1.05$ for the first parent and $24(1-0.9996)=0.01$ for the second parent. Therefore, the total gene flow events from outside plot A into plot B corrected for cryptic gene flow were $68.05+$ $36.01=104.06$ (Table 2). Among 182 possible parents ( $=91$ saplings $\times 2$ ) of saplings in plot B, 104.06 (57\%) were outside plot A , indicating active gene flow across the watershed.

## Distance between parents and saplings

Distance between parents and saplings, which represents either seed dispersal from maternal parents, and pollen movement plus seed dispersal from paternal parents, was large (Fig. 1), ranging from 32.4 m to 563.2 m with an average of $264.6 \mathrm{~m} \pm 135.3 \mathrm{~m}$ (SD) (Fig. 2a). Although the value indicates active pollen and seed dispersal in the research site, the distance is limited to saplings for which parentage has been assigned within plot A. Therefore, it probably represents an underestimate of the true distance because $57 \%$ of the parents of saplings were outside plot A. The distance between random pairs of adults in plot A and saplings in plot B ranged from 10.3 m to 933.8 m with an average of $436.7 \mathrm{~m} \pm 203.0$ (SD) m (Fig. 2b), and was significantly greater than that of the distance between offspring and parent trees ( $U$-test, $P<0.0001$ ) (Fig. 2). This indicates that trees


Fig. 2 Histogram of distances for Magnolia obovata (a) between parents and progeny inferred with microsatellite markers, and (b) between adult trees and saplings randomly chosen in the research site.
at a closer distance contribute more as parents of the saplings.

## Adult d.b.h.

Diameter at breast height (d.b.h.) of adult trees ranged from 5.2 cm to 59.1 cm with an average of 28.3 cm . The average d.b.h. of adult trees that had progeny in plot B was 35.8 cm , and was significantly larger ( $U$-test,


Fig. 3 Diameter at breast height (d.b.h.) of adult Magnolia obovata. (a) d.b.h. of adults having their progeny in plot B. (b) d.b.h. of adults not having their progeny in plot B.
$P<0.0001$ ) than that of adults not having their progeny in plot B ( 24.5 cm ), indicating that adults of larger size contributed more as pollen donors or seed parents to saplings in plot B (Fig. 3).

## Pollen movement

Although we determined two parents of exact match for 24 saplings, it was impossible to distinguish which acted as pollen donor or seed parent of these saplings. Therefore, we can not infer the real distance of seed dispersal by merely determining parentoffspring relationships. However, based on the distance between two parents of exact match, we can infer the extent of pollen movement. The distance of pollen movement ranged from 3.2 m to 540 m with an average of $131.1 \mathrm{~m} \pm 121.1 \mathrm{~m}$ (SD). About $27 \%$ of pollination was carried out between nearest neighbours.

The distances between random pairs of adults in the watershed showed a flat distribution, ranging from 1.3 m to 1543.7 m with an average of 561.5 m (Fig. 4c). The distance between the nearest neighbours for each adult tree within the watershed was low; $93 \%$ of trees had their nearest neighbour within the range of 100 m (Fig. 4a). The average distance of pollen movement was


Fig. 4 Histogram for Magnolia obovata of distances (a) to nearest neighbours for each adult tree, (b) of pollen movement inferred from microsatellite analysis, and (c) between random pairs of adult trees in plot A.
significantly larger ( $U$-test, $P=0.0010$ ) than the average distance between nearest neighbours for each adult tree $(44.1 \pm 37.5(\mathrm{SD}) \mathrm{m})$, and significantly smaller ( $U$-test, $P<0.0001$ ) than that between random pairs of adult trees $(561.5 \pm 352.6$ (SD) m) (Fig. 4c). This indicates that pollination occurs between adults located at closer than the average distance between adult trees within the watershed, but is not always between nearest neighbours (Fig. 5).

The average distance between parents and their offspring ( 264.6 m ; Fig. 2a) was significantly greater ( $U$-test, $P<0.0001$ ) than that of pollen movement ( 131.1 m ; Fig. 4b), reflecting that the former distance consists of pollen movement and seed dispersal.

## Discussion

## Pollen dispersal

Movement of pollen grains and seeds from point sources is known to show a leptokurtic or limited distribution (Sork, 1984; Ellstrand, 1992; Webb, 1998): many are dispersed near the source and there is a long tail of fewer


Fig. 5 Relative positions for Magnolia obovata of pollen donors and seed parents between which pollination occurred. Position 1 indicates that the tree is the nearest to the other parent among adult trees, and position $n$ indicates that there are $n-1$ trees between the two trees.
pollen grains and seeds at greater distances. However, the distribution of distances between parents and offspring inferred for M. obovata with microsatellite markers in the present study was not leptokurtic.

Different pollen vectors and patterns of behaviour remarkably affect pollen dispersal (Schmitt, 1980; Waser, 1982; Hamrick et al., 1992; Webb, 1998). The pollinators for $M$. obovata are primarily beetles (Kikuzawa \& Mizui, 1990), which are thought to be less efficient as pollen vectors (Ramsey, 1988). However, the distance of pollen movement in the present stand was quite large (average 131.1 m with a maximum value of 540 m ) (Fig. 4b). Tanaka \& Yahara (1988) have shown that a variety of insects, other than beetles, i.e. butterflies and bumble bees, pollinated $M$. obovata at a site in central Japan. It is feasible therefore that such pollinators were also effective and account for the long-distance pollen movement in the present stand. About one-third of saplings ( $=31 / 91$ ) found in plot B had only one of two parents within plot A. Hence, the average value of pollen movement must be an underestimate and the maximum distance may be more than 540 m . Chase et al. (1996) analysed the range of pollen dispersal for a tropical tree species, Pithecellobium elegans, using microsatellite markers. They found that average pollen dispersal was 142 m with a maximum value of 350 m . The average distance of pollen movement presently inferred for $M$. obovata is almost equivalent to that for P. elegans. Adult trees of $M$. obovata resemble $P$. elegans in that both species tend to occur at low density. It is
possible that for tree species occurring at low density in natural communities pollination regularly occurs over a wide range. However, long-distance pollen flow for such tree species might be affected by various life cycle characteristics, habitat type, and type of pollen vectors. This will need to be examined in the future.

## Seed dissemination

Seed dispersal characteristics affect the range of seed dissemination (Hamrick \& Murawski, 1990), with short distances occurring for gravity dispersal and longer ones for animal and wind dispersal (Hamrick et al., 1992).

Diaspores of $M$. obovata have a red fleshy edible part, and are dispersed internally by birds. Distance of seed dispersal by birds has been considered to be quite long, for example blue jays carried acorns of Fagus grandifolia up to 4 km from the source (Johnson \& Adkisson, 1985); however, few studies have measured this trait. Instead of direct measurement, the range has been estimated, for example, by determining the home range of birds (e.g. Fukui, 1995). Using appropriate microsatellite markers, and assuming that saplings with no possible parents within the research plot might grow from seeds pollinated outside the research plot and carried in by birds, we can infer the approximate range of seed dispersal by birds. It is notable that $40 \%(=36 /$ 91) of the saplings in plot B had no parents within the 69 ha research site (plot A), and the large proportion of these saplings reflects the active seed dispersal of this species by birds. The range of seed dispersal seems to reach more than several hundred metres and is significantly greater than that reported for seed dispersal by gravity or mammals such as mice and monkeys, whose dispersal ranges are within 100 m from the source (Sork, 1984; Jensen, 1985; Iida, 1996; Yumoto et al., 1998). Dow \& Ashley (1996) analysed acorn dispersal of Quercus macrocarpa using microsatellite markers, and found that seed dispersal of Q. macrocarpa was limited compared with pollen movement; most seed dispersal was less than 30 m whereas pollen dispersal averaged 76.9 m . However, they also stated that long-distance seed dispersal was not so rare as previously estimated: $48 \%$ of the seeds were dispersed secondarily by animals, and among them $16 \%$ were dispersed more than 90 m away from the source. They also estimated the maximum frequency of saplings which had no parents within the research plot (about 5 ha ) at $14 \%$. In contrast, despite the much larger plot size for the present population of M. obovata ( 69 ha ), the proportion of saplings without either parent within the research plot was larger (36/ $91=40 \%$ ).

## Factors that cause large gene transfer for M. obovata

In natural plant populations, it has often been observed that actual gene flow occurs over greater distances than expected allowing for the leptokurtic movement of pollen and seeds. Several factors are thought to account for this discrepancy, namely, the cumulative contribution of pollen by means of leptokurtic but long-tailed distribution (Adams, 1992; Ellstrand, 1992), underestimation of gene dispersal by neglecting carry-over of pollen grains on vectors (Schaal, 1980; Levin, 1981), and inbreeding depression (Waser, 1993).

If a population is genetically structured, and inbreeding or outbreeding depression occurs based on the genetic relatedness of adult trees, some kind of selection on pollen grains could occur. Dow \& Ashley (1996) presumed the existence of a mechanism allowing female flowers of $Q$. macrocarpa to select preferentially pollen from distant sources rather than pollen produced by neighbouring trees. It is known that M. obovata suffers from high inbreeding depression (Ishida \& Nakamura, 1997), and consequently, pollination between less related or spatially distant trees of $M$. obovata might be favoured in spite of the leptokurtic nature of pollen dispersal.

Chase et al. (1996) revealed that most mating events in Pithecellobium elegans were not between the closest neighbours, because of variation in phenology or in flowering behaviour between adult trees. Many tree species show large fluctuations in flowering among years with or without synchronization between trees in a population (Kelly, 1994; Isagi et al., 1997). In the case of episodic flowering without synchronization in a population, only some of the trees in the population can contribute to reproduction in a given year, and this may result in pollination between distant trees. Mating events in $M$. obovata were also not usually between nearest neighbours: more than $70 \%$ of pollination occurred between non-nearest neighbours (Fig. 5), and this might also stem from differences in phenology of individual flowers. Although $M$. obovata has a long flowering period, up to 40 days, the longevity of each protogynous flower is a few days: duration of the consecutive female and male stages is about 1-2 days each. And in most cases, only several or fewer flowers on an adult tree bloom in a given day during the flowering period. Therefore, even within a flowering season, each individual tree may switch among male, female and bisexual phases, and thus not all trees in a population can contribute as pollen donors to flowers in the female stage at the same time.

For M. obovata (i) long-distance seed dissemination by birds (ii) inbreeding depression and (iii) limitation in acceptance of pollen for each tree caused by differences
in flowering period of each individual flower, are likely to cause long-distance gene flow and increase gene exchange between less related or distant trees. Although the present population of M. obovata is in a physically distinct landscape component - a watershed - the amount of gene flow from outside the watershed was sufficient to prevent genetic differentiation by means of genetic drift. This agrees with the fact that most tree species do not exhibit much genetic differentiation among populations; usually more than $90 \%$ of the total genetic variation is found within each population (Ledig, 1986).

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