1 **Title**: Spatial variation in bird pollination and its mitigating effects on the

2 genetic diversity of pollen pools accepted by *Camellia japonica* trees within

3 a population at a landscape level

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12

1   **ABSTRACT**

2

3   Bird pollination can vary spatially in response to spatial fluctuations in

4   flowering even within plant populations. In this study, we examined the

5   hypothesis that the spatial variation in bird pollination may induce mitigating

6   effects which maintains or increases genetic diversity of pollen pools at local

7   sites with low flowering densities. To test this hypothesis, we analyzed the

8   landscape-level genetic effects within a population of *Camellia japonica* on

9   the pollen pools accepted by individuals in two reproductive years by using

10   genotypes at eight microsatellite loci of 1323 seeds from 19 seed parents.

11   Regression analyses using the quadratic models of correlated paternity

12   between pollen pools against spatial distances between the seed-parent pairs

13   revealed not only local pollination but also some amount of long-distance

14   pollen dispersal. The genetic diversity of pollen pools accepted by seed

15   parents tended to be negatively related to the densities of flowering

16   individuals near the seed parents during winter (when the effective pollination

17   of *C. japonica* is mediated mostly by *Zosterops japonica*). We show that the

18   low density of flowering individuals may induce the expansion of the

foraging areas of *Z. japonica* and consequently increase the genetic diversity

of pollen pools. This spatial variation in bird pollination may induce the

mitigating effects on the *C. japonica* population. The comparisons between

the two study years indicate that the overall pattern of bird pollination and the

genetic effects described here, including the mitigating effects, may be stable

over time.

INTRODUCTION

Local flowering density (defined in this study as the number of flowers produced per flowering individual and the density of flowering individuals at a site) may affect the patterns of animal pollination. Considering that optimally foraging pollinators are expected to minimize energetic costs during foraging bouts by moving preferentially between nearby flowers (Krauss *et al*., 2017), high flowering densities per individual would induce short-distance pollination. Furthermore, high densities of flowering individuals may induce animal pollinators to forage more locally, thereby reducing pollen dispersal distances (Hardy, 2009). On the contrary, low flowering densities should increase the distances of animal pollination. Therefore, the distance might negatively depend on the flowering density. In this study, we describe such relationship as "negative density-dependence of pollination distance". According to the model $N_{ep} = 8\delta^2 d$ (Austerlitz and Smouse, 2001), where N_{ep}, δ, and d are the effective number of pollen parents, mean distance of pollen dispersal, and density of reproductive individuals, respectively, N_{ep} theoretically decrease with decreasing d if δ is assumed to

1   be constant (Austerlitz and Smouse, 2001). However, the negative density-

2   dependence of pollination distance, which increases δ as d decreases as

3   observed in some animal-pollination in forest tree species (Stacy *et al*., 1996;

4   White *et al*., 2002; Fukue *et al*., 2007), could mitigate the effect of decreasing

5   d, thus maintaining or increasing both N_{ep} and the consequent genetic

6   diversity of pollen pools (mitigation effect). Therefore, if flowering density

7   spatially vary within a population, pollen dispersal patterns may vary among

8   seed parents that accept pollen depending on local flowering density even

9   within continuous populations at the landscape level. Owing to the spatial

10   variation in pollen dispersal, the genetic diversity of pollen pools accepted by

11   each seed parent might also spatially vary even within populations. The

12   consequent mitigation effect on the genetic diversity of pollen pools could

13   contribute to the preservation of genetic diversity within populations by

14   maintaining or increasing the genetic diversity of offspring from seed parents

15   at local sites with low flowering density. However, such spatial variations in

16   pollen dispersal and the consequent genetic diversity of pollen pools within

17   continuous populations have not been fully elucidated.

18   The effects of flowering density on pollination depend on the

pollination vectors (Breed *et al*., 2015a). Although the reduced density of

small-insect pollinated species may led to provision of low pollen diversity,

the bird-pollinated tree species may not suffer from the effects of decreased

density owing to the ability to sample larger and more diverse pollination

neighborhoods (Breed *et a*l., 2015a). Such mitigation effect probably due to

the negative density-dependence of bird-pollination has been indicated by

previous studies on forest tree species (Abe *et al*., 2011; Breed *et al*., 2015a,

b). Bird pollination can be characterized by high mobility and high energy

needs, which may induce birds to visit several widely scattered nectar sources

(Stiles, 1978). Owing to the negative density-dependence of pollination

distance based on the ability of birds to track fluctuations in flowering better

than insects (Stiles, 1978), low flowering density should substantially

promote pollen dispersal via birds. Therefore, bird-pollination patterns may

vary spatially in response to the spatial fluctuations of flowering even within

a population.

Although the optimal foraging behavior of nectar- or pollen-feeding

animals has been thought to result in short-distance pollen dispersal, as well

as frequent selfing, biparental inbreeding, and highly correlated paternity

1 within maternal sibships (Bezemer *et al*., 2016; Krauss *et al*., 2017), Krauss

2 *et al*. (2017) predicted that high mobility, limited grooming, and aggression

3 are likely to be key behavioral characteristics of birds that facilitate pollen

4 carryover and promote high mate diversity in plant species. Therefore, these

5 behavioral characteristics as well as the effect of flowering density should be

6 examined to elucidate the genetic effect of bird pollination. Furthermore, an

7 examination on the stability of pollination patterns across different

8 reproductive years is essential for the exact characterization of the pollination

9 pattern.

10 *Camellia japonica* blossoms in winter when pollinators other than

11 birds are usually scarce because the low temperatures restrict the activities of

12 insect pollinators (Fang *et al.*, 2012). *C. japonica* strongly depends on the bird

13 species *Zosterops japonica* (Japanese white-eye) for pollination and the bird

14 strongly depends on *C. japonica* for food resources in winter (Kunitake *et al.*,

15 2004). Furthermore, the flowering density of the species may vary spatially

16 even within a population at a land scape level. Therefore, an examination of

17 the relationship between flowering density during winter and genetic

18 diversity of pollen pools in such populations of *C. japonica* could reveal the

1   effect of flowering density on bird pollination. Previous studies on *C.*

2   *japonica* populations have shown that the high mobility and effectiveness of

3   *Zosterops japonica* as a pollinator may strongly contribute to the maintenance

4   of pollen genetic diversity (Abe *et al*., 2011, 2013). However, these studies

5   have only been conducted at small scales (0.3–1.0 ha plots), and spatial

6   variation in pollination and its consequent genetic effects on pollen pools

7   within a population have yet to be examined.

8   In this study, we examined the overall patterns of bird pollination in

9   *Camellia japonica* and their stability across different reproductive years

10   within an intact and continuous forest at a landscape scale. We also examined

11   the hypothesis that the spatial variation in bird pollination, in response to the

12   spatial fluctuations of flowering, may induce mitigating effects which

13   maintains or increases genetic diversity of pollen pools at local sites with low

14   flowering densities within a population.

15

16   **MATERIALS AND METHODS**

17

18   *Study site*

1

2　The study site was in the Tatera Forest Reserve (34° 9' N, 129° 13' E) on the

3　South Island of Tsushima, which belongs to Nagasaki Prefecture in Japan and

4　is located between the Japanese Archipelago and Korean Peninsula. The

5　reserve is protected as a national natural monument, covers approximately

6　100 ha, and is situated on the north-facing slope of Mt. Tatera (560 m above

7　sea level). There has been no human interference in the reserve for several

8　centuries, and an old-growth, evergreen, broad-leaved forest is well

9　developed (Itow, 1991). The vegetation features of the forest have been

10　described by Manabe *et al.* (2000), who investigated all tree stems with a

11　diameter at breast height (DBH) ≥ 5 cm in a 4 ha plot established within the

12　forest. The plot contained a total of 45 species and 4570 living stems ≥ 5 cm

13　DBH, with a total basal area of 63.9 m^2 ha^{-1}. The dominant species in terms

14　of stem density was *Distylium racemosum* (Hamamelidaceae) at 410.0 stems

15　ha^{-1}, but *Castanopsis sieboldii* (Fagaceae) was dominant in terms of basal

16　area at 24.9 m^2 ha^{-1}. *Camellia japonica* was only one bird-pollinated tree

17　species of the major species in the plot and had the fifth largest basal area (2.4

18　m^2 ha^{-1}) and third highest density (143.3 stems ha^{-1}).

1

2 *Study species*

3

4 *Camellia japonica*, which is an evergreen broad-leaved tree of the family

5 Theaceae, is distributed throughout Japan, Korea, and China. The species

6 commonly dominates the subcanopy layer in warm-temperate, evergreen,

7 broad-leaved forests in Japan. It produces hermaphrodite flowers from

8 November to April (Kunitake *et al.*, 2004). Its pollen is mainly dispersed by

9 Japanese white-eye birds (*Zosterops japonica*; Kunitake *et al*., 2004), and its

10 seeds are dispersed primarily by gravity and secondarily (in some places) by

11 mice (Abe *et al*., 2006). This species is self-incompatible (Wendel and Parks,

12 1984), and its flowers do not set fruits without pollinator visitation (Kunitake

13 *et al*., 2004). Kunitake *et al*. (2004) found that fruit set in flowers visited by

14 birds was sixfold greater than that in flowers without bird visitation and the

15 level of fruit set was saturated by only five visitations of *Z. japonica*.

16 Furthermore, most visitors to the flowers were *Z. japonica* individuals in the

17 field observation (Kunitake *et al*., 2004). Therefore, pollination by *Z.*

18 *japonica* should be important for the seed production and the contribution by

1   pollinators other than *Z. japonica* may be minor.

2

3   *Field methods*

4

5   In September 2006, we sampled leaves and seeds from 16 *Camellia japonica*

6   seed parents: 3 seed parents at each of 4 sites (s1, s2, s3, and s4) and 4 seed

7   parents at another site (s5) in the 100 ha old-growth, evergreen, broad-leaved

8   forest (Figure 1, Table 1). In September 2008, we sampled seeds from 16 seed

9   parents: 13 seed parents that were also in the previous set (1 seed parent at s5

10   and 3 seed parents each at s1, s2, s3, and s4) and other 3 seed parents at s5.

11   In addition to seeds, leaves were collected from the three seed parents at s5.

12   In total, we sampled leaves and 1323 seeds from 19 seed parents. The number

13   of fruits sampled from each seed parent ranged from 4–11, and the number of

14   seeds sampled from each fruit ranged from 1–13.

15   The locations of all seed parents were determined by hand-held GPS

16   units (eTrex Vista C or Colorado 300, Garmin, Kansas, USA). The mean

17   distances between pairs of the 16 seed parents sampled in 2006, 16 seed

18   parents sampled in 2008, and 13 seed parents sampled in both years were

1 442.9 ± 270.8 (standard deviation), 448.1 ± 276.6, and 362.1 ± 227.4 m,

2 respectively, and those within sites were 33.3 ± 20.6, 31.1 ± 18.4, and 28.8 ±

3 16.4 m, respectively.

4   To quantify the density of flowering individuals at each site over the

5 entire flowering period and particularly during winter, we monitored the

6 flowering of 14 of the 16 seed parents sampled in 2006 (s1-1, s1-2, s1-3, s2-

7 1, s2-2, s2-3, s3-1, s3-2, s3-3, s4-1, s4-2, s4-3, s5-1, and s5-2; see Table 1)

8 and all other *Camellia japonica* individuals within 10 m radii from the

9 respective seed parents. In surveys lasting one to 3 days, we recorded the

10 flowering status (flowering or nonflowering) for each of these individuals 9

11 times from November 2007 to June 2008 (except for seed parent s2-2, which

12 was not surveyed on the first occasion). Furthermore, to estimate the

13 flowering amplitude of each of these individuals at each site over the entire

14 flowering period, we counted fallen flowers under its crown at each survey,

15 removed them from the site, and summed the numbers of flowers that had

16 fallen from each individual recorded in all surveys. If a fallen flower was

17 located under overlapping crowns of multiple *C. japonica* individuals, we

1   allocated the flower to one of the individuals, the stem of which was nearest

2   to the flower.

3

4   *DNA extraction and microsatellite genotyping*

5

6   Genomic DNA was extracted from sampled leaves and seeds by using the

7   hexadecyltrimethylammonium bromide method (Murray and Thompson,

8   1980) with minor modifications. Eight expressed sequence tag microsatellite

9   loci, namely, MSE0030, MSE0045, MSE0049, MSE0051, MSE0062,

10   MSE0078, MSE0079, and MSE0087, which were originally developed for

11   *Camellia sinensis* (Taniguchi *et al*., 2012) were selected for genotyping adults

12   and seeds. Genotyping at the microsatellite loci was performed by capillary

13   electrophoresis using a 3100 Genetic Analyzer and GeneScan software

14   (Applied Biosystems, California, USA).

15

16   *Estimation of polymorphism at microsatellite loci*

17

18   Polymorphisms at the 8 microsatellite loci were characterized by calculating

1   the following standard population genetic parameters on the basis of the

2   genotypes of all 19 sampled seed parents: observed number of alleles (N_O),

3   effective number of alleles (N_E), observed heterozygosity (H_O), and gene

4   diversity (H_E) for each locus and all 8 loci.

5

6   *Analysis of mating systems*

7

8   We estimated mating systems by using MLTR software version 3.4 (Ritland,

9   2002) as follows. We calculated single- and multilocus estimates of

10   outcrossing rates (t_s and t_m, respectively) and estimated the level of biparental

11   inbreeding from the difference between multilocus and mean single-locus

12   rates ($t_m - t_s$). We also calculated the multilocus estimates of the correlation

13   of paternity (r_p), which is the probability that two maternal-sibs are outcrossed

14   full-sibs sharing the same pollen parent and is the inverse of the effective

15   number of pollen parents (N_{ep}; Smouse and Robledo-Arnuncio, 2005). The

16   above parameters were calculated separately for each of the two reproductive

17   years covered in the study by using the Newton–Raphson method

18   incorporated in the software constraining allele frequencies of pollen to equal

15

1   to those of ovule (default settings) on the basis of the genotypes of seeds and

2   seed parents. Their standard deviations were estimated by bootstrap

3   resampling 1000 times, and all 16 seed parents sampled in each year were

4   included in these analyses.

5

6   *Analysis of correlated paternity of pollen pools between seed-parent pairs*

7   *and the overall patterns of pollen dispersal*

8

9   Correlated paternity, i.e., the probability of offspring sired by the same

10   paternal parent in different maternal families, can be used to investigate

11   biological factors determining effective pollination (Hardy *et al*., 2004;

12   Oddou-Muratorio *et al*., 2006) and has been expressed as a function of pollen

13   dispersal distribution (Austerlitz and Smouse, 2001; Robledo-Arnuncio *et al*.,

14   2006). To examine the overall pollen dispersal pattern within a population of

15   *Camellia japonica* in an indirect manner, the spatial pattern of correlated

16   paternity was analyzed as follows.

17   First, the correlated paternity of pollen pools was estimated between

18   all seed-parent pairs in each of the two reproductive years from the obtained

1   genotypes of seed parents and seeds by using the POLDISP software package

2   (Robledo-Arnuncio *et al*., 2007). Then, to examine the similarity of pollen

3   dispersal patterns between the two years, we calculated the Spearman's rank

4   correlation coefficient between the years for the correlated paternity values

5   of pollen pools accepted by each seed-parent pair for the 13 seed parents

6   sampled in both years by using R ver. 3.3.3 (R Development Core Team,

7   2012). Furthermore, we used quadratic models to examine the regressions of

8   the correlated paternity of pollen pools between seed-parent pairs against the

9   spatial distances between the seed-parent pairs as follows.

10           $C_{\mathrm{p}} = a + b\,D + c\,D^2,$

11   where C_{p} is the correlated paternity, D is the spatial distance between seed-

12   parent pairs, a is the parameter for intercept, b is the parameter for coefficient

13   in linear term, and c is the parameter for coefficient in quadratic term. We

14   examined the quadratic terms to assess whether the correlated paternity

15   monotonically decreased as the distance between seed-parent pairs increased

16   and whether the correlated paternity reached a global minimum value at

17   intermediate distances. This analysis was conducted for each of the two

18   reproductive years, including all 16 seed parents sampled in each year by

1   using R ver. 3.3.3 (R Development Core Team, 2012).

2   We used the KINDIST model (Robledo-Arnuncio *et al*., 2006)

3   implemented in the POLDISP software package to infer the pollen dispersal

4   kernel (the probability density that pollen lands at a given position away from

5   a source individual), on the basis of the exponential power function, for each

6   of the two reproductive years (Supplementary Method S1 for more detail).

7

8   *Two-generation analysis*

9

10   Two-generation (Two-Gener) analysis (Smouse *et al*., 2001) can quantify the

11   spatial genetic differentiation (Φ_{FT}) among the pollen pools sampled by seed

12   parents via the analysis of molecular variance (AMOVA; Excoffier *et al*.,

13   1992) of pollen haplotypes. Modified versions of the analysis (Irwin *et al*.,

14   2003) can also quantify interannual genetic differentiation among the pollen

15   pools sampled by each seed parent (Φ_{YF}) and global pollen pools averaged

16   for all seed parents (Φ_{YG}). Φ_{FT}, which is analogous to Wright's F_{ST} value and

17   an inverse function of mean pollination distance (Smouse *et al*., 2001),

18   reflects landscape-level gene movement via pollen. Φ_{YF} and Φ_{YG} reflect the

1 interannual variation in the contributions of pollen donors to the pollen pools

2 accepted by each seed parent and all seed parents, respectively. To examine

3 patterns of pollen dispersal for each year and the interannual variation of

4 pollination at a landscape level within a population, we conducted Two-Gener

5 analyses on the basis of the genotypes of seeds and seed parents by using the

6 three methods described in the Supplementary Method 2. The GENEALEX

7 6.3 software package (Peakall and Smouse, 2006) was used for all Two-Gener

8 analyses to estimate the Φ statistics. We also used Φ_{FT} values to calculate the

9 effective number of pollen parents (N_{ep}; Smouse et al., 2001) from the

10 equation $N_{ep} = 1 / 2\,\Phi_{FT}$.

11

12 *Analysis of the genetic diversity of pollen pools*

13

14 To test a hypothesis about the mitigating effects of bird pollination within a

15 population, we examined the effect of the local density of flowering

16 individuals on the genetic diversity of pollen pools accepted by seed parents

17 as follows. Considering that *Camellia japonica* strongly depends on effective

18 pollination by *Zosterops japonica* and that birds strongly depend on *C.*

1   *japonica* for food resources during winter (Kunitake *et al*., 2004), flowering

2   density during winter should strongly affect pollination dynamics. Thus, we

3   used the density of flowering individuals only during winter (from December

4   20–21, 2007, to February 22–23, 2008) in the following analyses.

5   First, we estimated genetic diversity of pollen pools as follows. The

6   paternal alleles of each seed were identified by subtracting the maternal

7   alleles from the seed genotype (see Supplementary Method 3). By using the

8   frequencies of paternal alleles in pollen pools, we calculated the gene

9   diversity (H_E) of accepted pollen pools for each seed parent in each of the two

10   reproductive years.

11   Second, to examine whether the levels of genetic diversity of pollen

12   pools accepted by each seed parent tended to be consistent between the two

13   years, we examined Spearman's rank correlation coefficient between the two

14   years for the H_E values of pollen pools accepted by each seed parent. This

15   analysis was conducted for the 13 seed parents sampled in both years using R

16   ver. 3.3.3 (R Development Core Team, 2012).

17   Third, we used linear regression to analyze the effect of density of

18   flowering individuals near respective seed parents on the genetic diversity of

1   pollen pools accepted by seed parents. The response variable was pollen pool

2   H_E value (estimated from seeds sampled in 2008), and the explanatory

3   variable was the density of individuals flowering during winter (from

4   December 20–21, 2007, to February 22–23, 2008) within a 10 m radius of the

5   seed parent. Given that the densities of flowering individuals within 10 m of

6   s5-5, s5-6, and s5-7 were not observed, we used the values for s5-1, s5-1, and

7   s5-2 as proxies because these were relatively close to s5-5 (6.0 m), s5-6 (2.6

8   m), and s5-7 (9.7 m), respectively. R ver. 3.3.3 (R Development Core Team,

9   2012) was also used for this analysis.

10

11   **RESULTS**

12

13   *Flowering of seed parents and neighboring individuals*

14

15   In the surveys of flowering, we found 109 flowering *Camellia japonica*

16   individuals in total at the five sites (including the 14 seed parents and other

17   95 individuals within the 10m radii from the seed parents), which had at least

18   one flower either on or under their crowns. The total flowering period of the

1   109 individuals lasted more than 6 months (Supplementary Figure S1). The

2   total number of flowers that fell from each tree over the entire flowering

3   period varied among individuals within the s1, s2, s3, and s4 sites (mean ±

4   standard deviation; 199.6 ± 254.1, 157.8 ± 249.7, 280.2 ± 493.8, and 349.1 ±

5   284.4, respectively; Supplementary Figure S2). At s5, it remained

6   consistently low (26.1 ± 36.8). The densities of flowering individuals over the

7   entire period were as follows: s1, 308 ha^{-1}; s2, 127 ha^{-1}; s3, 149 ha^{-1}; s4, 212

8   ha^{-1}; and s5, 684 ha^{-1}. Although the density of flowering individuals over the

9   entire period was highest at the highest altitude site, namely, s5, the density

10   during winter (when pollination of *Camellia japonica* may be mediated

11   mostly by *Zosterops japonica*) tended to be higher at lower altitude sites

12   (Table 2). Our linear regression analysis revealed that altitudes have a

13   significantly negative effect on the density of flowering individuals during

14   winter ($t = -2.710$, $P < 0.05$). At the lowest altitude site, namely, s1, the local

15   density of flowering individuals during winter was highest (202 ha^{-1}). By

16   contrast, at the highest altitude site, namely, s5, the density during winter was

17   lowest (48 ha^{-1}).

18

1   *Polymorphism at the eight microsatellite loci*

2

3   The 8 loci were highly polymorphic in the 19 sampled seed parents

4   (Supplementary Table S1). In total, 64 alleles were detected at the 8 loci (3 to

5   16 alleles per locus, with a mean of 8.0). The effective number of alleles (N_E)

6   per locus ranged from 2.0 to 11.1 with a mean of 5.1. The observed

7   heterozygosity (H_O) per locus ranged from 0.368 to 0.895 with a mean of

8   0.750 and gene diversity (H_E) per locus ranged from 0.511 to 0.935 with a

9   mean of 0.763.

10

11   *Mating systems*

12

13   The estimated multilocus outcrossing rates (t_m) in 2006 and 2008 were 0.966

14   ± 0.017 (standard deviation) and 0.985 ± 0.008, respectively (Table 3), and

15   the estimated levels of biparental inbreeding ($t_m - t_s$) in 2006 and 2008 were

16   0.072 ± 0.025 and 0.063 ± 0.015, respectively. The estimated multilocus

17   correlation of paternity within maternal sibs (r_p) was 0.193 ± 0.039 in 2006

18   and 0.108 ± 0.023 in 2008. The estimated effective number of pollen parents

1   (calculated as the inverse of r_p) was 5.2 in 2006 and 9.3 in 2008.

2

3   *Correlated paternity of pollen pools between seed-parent pairs and the*

4   *overall patterns of pollen dispersal*

5

6   The correlated paternity of pollen pools between the seed-parent pairs

7   examined ranged from -0.168 to 0.136 (mean ± standard deviation, -0.011 ±

8   0.052) and from -0.101 to 0.122 (-0.007 ± 0.035) in 2006 and 2008,

9   respectively. A significantly positive Spearman's rank correlation coefficient

10   existed between the two reproductive years for the correlated paternity values

11   of pollen pools accepted by each seed-parent pair ($r = 0.602$, $P < 0.001$, Figure

12   2).

13   Polynomial regressions using quadratic models significantly

14   explained the relationships between the correlated paternity of pollen pools

15   and spatial distance for seed-parent pairs in both years (2006: $F = 30.9$, $P <$

16   0.001; 2008: $F = 35.7$, $P < 0.001$; Figure 3). For both years, both linear (2006:

17   $t = -7.469$, $P < 0.001$; 2008: $t = -7.370$, $P < 0.001$) and quadratic terms (2006:

18   $t = 6.174$, $P < 0.001$; 2008: $t = 5.514$, $P < 0.001$) were significant (Table 4).

1   The significance of quadratic terms indicated that the expected correlated

2   paternity did not monotonically decrease in the long ranges as the distance

3   between seed-parent pairs increased, reaching global minimum values of

4   -0.037 at a distance of 610.2 m in 2006 and -0.026 at 681.8 m in 2008.

5   Therefore, the correlated paternity decreased monotonically to the minimum

6   values as the distances between seed-parent pairs increased within the ranges

7   of 0 to 610.2 m in 2006 and 0 to 681.8 m in 2008 but increased beyond those

8   ranges.

9   The Spearman's rank correlation coefficients between the correlated

10   paternity and distance between seed-parent pairs were -0.360 in 2006 ($P <$

11   0.001) and -0.392 in 2008 ($P < 0.001$). According to the KINDIST model,

12   by assuming an exponential power function as the dispersal kernel, the

13   estimated scale parameter (a), shape parameter (b), and average pollination

14   distance (δ) in 2006 were 0.000036, 0.156, and 1487.4 m, respectively (model

15   least-squared residual: 5.249; Fig. 4). The estimates of a, b, and δ in 2008

16   were 0.000190, 0.168, and 1476.9 m (model least-squared residual: 4.556),

17   respectively. The dispersal kernels in the two years were very similar (Fig. 4).

1

2 *Genetic differentiation of pollen pools among seed parents and between years*

3

4 Two-Gener analysis based on model 1 (see Supplementary Method S2)

5 revealed the significant but low genetic differentiation of accepted pollen

6 pools among seed parents in both years (Φ_{FT} = 0.105 and 0.070, respectively;

7 $P < 0.005$). The analysis based on model 2 revealed the significant but low

8 genetic differentiation of accepted pollen pools among seed parents (Φ_{FT} =

9 0.061, $P < 0.005$) and between the two years nested within seed parents (Φ_{YF}

10 = 0.029, $P < 0.005$). The analysis based on model 3 showed no significant

11 genetic differentiation of the global pollen pool averaged for all seed parents

12 between the two years (global Φ_{YG} = −0.003). However, it indicated

13 significant but low genetic differentiation among seed parents nested within

14 years (Φ_{FT} = 0.087, $P < 0.005$). The effective number of pollen parents (N_{ep})

15 based on model 1 was 4.8 for 2006 and 7.1 for 2008 (estimated from Φ_{FT}

16 values), whereas the N_{ep} value based on model 2 was 8.2.

1

2   *Genetic diversity of pollen pools*

3

4   The H_E values of pollen pools accepted by each seed parent ranged from 0.450

5   to 0.736 in 2006 (mean ± standard deviation, 0.658 ± 0.069) and from 0.593

6   to 0.760 in 2008 (0.697 ± 0.049). A significantly positive Spearman's rank

7   correlation coefficient existed between the two reproductive years of the H_E

8   values of pollen pools accepted by each seed parent ($r = 0.683$, $P < 0.05$;

9   Supplementary Figure S3). The H_E values of pollen pools tended to be

10   negatively related to the density of flowering individuals near respective seed

11   parents during winter (Fig. 5; linear regression analysis, $F = 4.8$, $P < 0.05$).

12

13   **DISCUSSION**

14

15   *Overall patterns of Camellia japonica gene dispersal via bird pollination*

16

17   Despite the high density of *Camellia japonica* reproductive individuals in our

18   study, our estimates of multilocus selfing rate ($1 - t_m$), biparental inbreeding

27

1   level ($t_m - t_s$), and multilocus correlation of paternity within maternal sibships

2   (r_p) were not high when compared to reported estimates of those for several

3   other bird-pollinated forest tree species (Breed *et al.*, 2015a, b; Bezemer *et*

4   *al.*, 2016) and various forest tree species pollinated by animals with high

5   mobility (Breed *et al.*, 2015a) (Supplementary Table S2).

6   Our estimates for the effective numbers of pollen parents (N_{ep}, 4.8-

7   9.3) are close to previous estimates for animal-pollinated forest tree species

8   [$7.1 \leq$ mean $N_{ep} \leq 10.6$, calculated with 10 studies on the animal pollinated

9   species reviewed by Smouse and Sork (2004)] and is substantially lower than

10   the estimates for wind-pollinate tree species [mean $N_{ep} > 17.1$, calculated with

11   12 studies on the wind pollinated species reviewed by Smouse and Sork

12   (2004)]. This result is not surprising because N_{ep} is inherently lower for

13   animal-pollinated species than wind-pollinated species (Smouse and Sork,

14   2004). However, the N_{ep} values we found were also lower than the previous

15   estimates for forest tree species mainly pollinated by birds [mean $N_{ep} = 13.6$,

16   calculated with 12 studies on bird pollinated tree species reviewed by Krauss

17   *et al.*, (2017)]. Thus, we show that the overall pattern of bird pollination in

18   this population leads to the lower diversity of pollen parents within maternal

1 sibships than either wind-pollinated tree species or average bird-pollinated

2 tree species, similar to that for pollination by animals other than birds.

3 Deviation of the bird pollination from the optimal foraging behavior would

4 induce higher diversity of pollen parents than forest tree species pollinated by

5 animals other than birds (Krauss *et al*., 2017). However, in contrast to the

6 other bird pollinated tree species, our results of low N_{ep} might indicate the

7 optimal foraging behavior of birds in *C. japonica* population.

8 　　We found that the correlated paternity between pollen pools

9 decreased monotonically as the distances between seed-parent pairs increased

10 within a certain distance range (from 0 to 610.2 m and from 0 to 681.8 m in

11 2006 and 2008, respectively) but increased beyond this range. Different seed

12 parents located near to each other may be frequently pollinated by the same

13 pollen parents because of limited pollen dispersal (probably by optimal

14 foraging behaviors of birds); therefore, correlated paternity may depend on

15 the distance between the seed-parent pair at local scales, particularly within

16 ca. 200 m (see Fig. 3). However, the increase in correlated paternity that we

17 found with the increased distance of the seed-parent pair suggests that some

18 processes of long-distance pollen dispersal are at work, in addition to local

1   pollination due to the optimal foraging behavior of birds. The dispersal

2   kernels estimated by KINDIST in our study also indicate a high potential for

3   long-distance pollen dispersal. The shape parameters (*b*) estimated here were

4   similar to those in previous studies on other forest tree species that used

5   KINDIST and assumed exponential power functions, all of which suggested

6   long-distance mean (δ) of pollen dispersal (Lander *et al*., 2010; Breed *et al*.,

7   2015b; Kassa *et al*., 2018) (Supplementary Table S3). The low shape

8   parameters estimated here also indicate a fat-tailed dispersal distribution that

9   may reflect the high mobility of bird pollinators.

10   However, the actual mean distance of pollen dispersal is likely

11   shorter than the values (1487.4 and 1476.9 m in 2006 and 2008, respectively)

12   estimated by KINDIST, which are based on the assumption of a population

13   with an infinite area, even though the area of our study site was only

14   approximately 100 ha, relatively small for estimating long pollen dispersal.

15   Therefore, although the dispersal kernel may be valid at our scale because the

16   dispersal kernels estimated for the two different years were very similar, the

17   long mean distances found here may reflect an extrapolation of the observed

18   data beyond the limited spatial scale of analysis.

1   *Zosterops japonica* (Japanese white-eye) is the main pollinator of

2   *Camellia japonica* and is a territorial bird, and the agonistic interactions

3   among the conspecific individuals of *Z. japonica* has been reported (Kikkawa

4   and Kakizawa, 1981). The extent of dispersal by territorial birds should

5   depend on the magnitude of aggressive interactions (Krauss *et al.*, 2017). The

6   combination of agonistic interactions, high mobility, and limited grooming

7   has been thought to promote pollen dispersal and consequently induce

8   deviation from optimal foraging behavior (Krauss *et al.*, 2017). Although

9   such characteristics of *Z. japonica* might induce the long-distance pollen

10   dispersal, the bird pollinator of *C. japonica* would also conduct optimal

11   foraging behavior, as reflected by the dependency of the correlated paternity

12   of pollen pool on the distance between seed-parent pair at a local scale and

13   the low N_{ep} as previously noted.

14   Although the seed dispersal of *Camellia japonica*, which is mediated

15   by gravity and mouse, is spatially limited [mean distance for 28 of 34

16   seedlings estimated by maternity analysis was 5.8 m ± 6.0 SD, Abe *et al.*

17   (2006)], the fine-scale spatial genetic structure (FSGS) of *C. japonica* in this

18   study site is weak (Ueno *et al.*, 2000). Ueno *et al.* (2000) considered

31

1   overlapping seed shadow and extensive pollen dispersal as factors that

2   weaken the FSGS. Therefore, the long-distance pollen dispersal found here

3   would contribute to the weakening of the FSGS of the *C. japonica* population.

4   A strong FSGS induced by limited seed dispersal should result in a small

5   effective number of individuals in a neighborhood (N_e), thus inducing random

6   local genetic differentiation within a population (Crawford, 1984). However,

7   extensive pollen dispersal might mitigate the effect of restricted seed dispersal

8   on FSGS (Grivet *et al*., 2009). Furthermore, bird-mediated pollen dispersal

9   may also promote gene flow from outside the population more frequently than

10   seed dispersal via gravity and mouse. Therefore, bird pollination may largely

11   contribute to the maintenance of genetic diversity in *C. japonica* populations.

12

13   *Genetic effects of spatial variation in bird pollination on pollen pools*

14   *accepted by Camellia japonica individuals*

15

16   We found that the genetic diversity of accepted pollen pools tended to be

17   negatively related to the density of flowering individuals around seed parents

18   during winter. Abe *et al*. (2011) found that the area covered by *Zosterops*

japonica was larger and the genetic diversity of pollen attached to *Z. japonica*

individuals was higher in *Camellia japonica* populations with lower

flowering density. This characteristic of bird pollination may increase the

genetic diversity of offspring in *C. japonica* populations with decreased

flowering density (Abe *et al*., 2013). While these studies would indicate

negative density-dependence of bird-pollination distance and the consequent

mitigating effects over the entire population, we demonstrate in the current

study the mitigating effects at local sites within a continuous population at the

landscape level. The density of flowering individuals tended to be lower at

higher altitude sites during winter when *C. japonica* pollination may be

mediated mostly by *Z. japonica*. Furthermore, the average number of flowers

per individual was lowest at the highest altitude site (s5). Therefore, low

flowering density at sites such as s5 during winter may broaden the foraging

area of *Z. japonica* and consequently increase the genetic diversity of pollen

pools accepted by *C. japonica* individuals. The effect of low flowering

density rather than the agnostic interaction of birds, as noted previously, could

explain the long-distance pollen dispersal of *C. japonica*. Furthermore, the

optimal foraging behavior of birds, as noted above, at sites with high

1   flowering densities (i.e., effect of high flowering density) could explain the

2   limited pollen dispersal of *C. japonica*. Thus, responses in foraging behavior

3   to variations in local flowering density may induce spatial variations in bird

4   pollination within the population and in the genetic diversity of pollen pools.

5   Again, in the model, $N_{ep} = 8\delta^2 d$ (Austerlitz and Smouse, 2001), where N_{ep}, δ,

6   and d are the effective number of pollen parents, mean distance of pollen

7   dispersal, and density of reproductive individuals, respectively, N_{ep}

8   theoretically decrease with decreasing d if δ is assumed to be constant.

9   However, due to negative density-dependence of bird-pollination distance, δ

10   would increase with decreasing d, and the effect of increasing δ on N_{ep} and

11   the consequent genetic diversity of pollen pools accepted by seed parents

12   would exceed the effects of decreasing d on N_{ep}. Therefore, the spatial

13   variations in bird pollination associated with variations in flowering density

14   may induce mitigating effects.

15   Similar mitigating effects of low population density have also been

16   reported in other bird-pollinated forest tree species in fragmented forests

17   (Breed *et al*., 2015a, b). In this study, we detected an extreme mitigating effect

18   not only in maintaining but also increasing the genetic diversity of pollen

1   pools within an intact and continuous forest. Therefore, such mitigating effect

2   of bird pollination may be innate characteristics of the bird (*Z. japonica*) and

3   plant (*C. japonica*) species, and may reflect the strong interaction between

4   the two species, in which *C. japonica* strongly depends on *Zosterops japonica*

5   for pollination, and the bird strongly depends on *C. japonica* for food

6   resources (Kunitake *et al.*, 2004).

7

8   *Stability of bird pollination across different reproductive years*

9

10 The Two-Gener analysis based on model 2 showed the small but significant

11 genetic differentiation of pollen pools between the two reproductive years

12 within each seed parent. However, the analysis based on model 3 showed no

13 genetic differentiation in the global pollen pool (averaged over all seed

14 parents sampled across the entire population) between the two years. This

15 may reflect small differences in combinations of mating pairs between years,

16 although the relative contributions of each pollen parent to overall seed

17 production in the population did not differ between years. The interannual

18 variation of pollen pools within a seed parent in this study ($\Phi_{YF} = 0.029$) was

1   lower than wind-pollinated forest tree species (0.039; Nakanishi *et al*., 2005)

2   and insect-pollinated forest tree species (0.084; Irwin *et al*., 2003). Therefore,

3   the interannual differences in the patterns of bird pollination in *Camellia*

4   *japonica* may be smaller than those for wind or insect pollination.

5   Furthermore, patterns of correlated paternity of pollen pools and pollen

6   dispersal kernels showed similar tendencies across the two years, and there

7   was a significant positive correlation between years in the correlated paternity

8   of pollen pools for each seed-parent pair. These results may indicate the

9   stability of the pattern of bird pollination over time. Additionally, the

10   significantly positive correlation of the gene diversity values (H_E) of pollen

11   pools accepted by each seed parent between years suggests a stable effect of

12   bird pollination on the genetic diversity of pollen pools across years.

13   Therefore, the overall patterns of bird pollination and their genetic effects,

14   including the mitigation effect, may be stable over time.

15

16   *Conclusions*

17

18   Genetic diversity of pollen pools in *Camellia japonica* tended to be negatively

related to the local density of flowering individuals, which may be due to the

negative density-dependence of bird pollination. This dependency of bird

pollination in *C. japonica* may affect the spatial variation of pollination within

populations, which should affect the overall pattern of pollen dispersal, and

the consequent genetic effect could contribute to the maintenance of genetic

diversity within populations by increasing genetic diversity of offspring from

seed parents at local sites with low flowering density. Such process may be

innate characteristics of the bird (*Zosterops japonica*) and plant (*C. japonica*)

species and may reflect the strong interaction between the two species.

However, it may be further necessary for elucidating the overall pattern of

bird pollination in more detail to examine patterns of pollen dispersal taking

account of such dependency and effects of flowering density, e.g., estimation

of a pollen dispersal kernel having parameters determined by the flowering

density. Furthermore, because the dependence itself may be an important

characteristic of bird pollination, it could be useful for characterizing the

pattern of pollen dispersal for the bird-pollinated tree species to examine the

parameters concerned with negative density-dependence of pollination, e.g.,

the slope for regression of genetic diversity of pollen pool on density of

1 flowering individuals, as examined in this study.

2 　　　Most of evergreen broad-leaved forests, where *Camellia japonica*

3 grows, in Japan are small, at most a few hectares in size, and scattered due to

4 strong and frequent anthropogenic disturbances for a long time (Manabe *et*

5 *al.*, 2000; Miura *et al.*, 2001). With respect to *C. japonica*, the mitigating

6 effects of bird pollination might contribute to the maintenance of genetic

7 diversity within populations with decreased flowering density due to the

8 disturbances. However, the disturbances could affect population dynamics of

9 the bird pollinator (*Zosterops japonica*) by destroying habitats of the bird

10 species, and consequently affect the mitigating effects. In order to evaluate

11 such impacts of anthropogenic forest disturbances on the mitigating effects,

12 future studies should examine the mitigating effects in the anthropogenically

13 disturbed and fragmented forests and compared their results to those in the

14 intact and continuous forest indicated by this study.

15

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17

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

DATA ARCHIVING

Sampling locations, microsatellite genotypes, and flowering data are available from the Dryad Digital Repository: https://datadryad.org/resource/doi:10.5061/dryad.17q550q

Supplementary information is available at *Heredity*'s website.…

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6

1   **Titles and legends to figures**

2

3   **Figure 1.** Locations of the 19 seed parents (circles with ID numbers) of

4   *Camellia japonica*.

5   **Figure 2.** Relationship between the reproductive years 2006 and 2008 of

6   correlated paternity values of pollen pools accepted by each seed-parent pair.

7   **Figure 3.** Distributions of the correlated paternity between pollen pools as

8   functions of the spatial distances between seed-parent pairs in 2006 (filled

9   circles) and 2008 (open circles). The quadratic functions of the distance

10   were fitted to the data. Solid and dashed curves indicate the functions for the

11   reproductive years 2006 and 2008, respectively.

12   **Figure 4.** Pollen dispersal kernels estimated by KINDIST model assuming

13   the exponential power functions for reproductive years 2006 and 2008. The

14   vertical axis indicates the probability density $P(r)$ that a pollen lands at a seed

15   parent r m away from the source individual (a value of e^{-x} in the scale

16   indicates 10^{-x}, in the display style of R program). The abscissa axis indicates

17   the distance r. Solid and dashed curves indicate the kernels for the

18   reproductive years 2006 and 2008, respectively.

1   **Figure 5.** Distribution of gene diversity (H_E) values of pollen pools accepted

2   by seed parents in 2008 versus the densities of flowering individuals within

3   10 m radii from the respective seed parents during winter (from December

4   20–21, 2007, to February 22–23, 2008). Filled circles, open circles, filled

5   squares, crosses, and filled triangles indicate the values for seed parents at the

6   s1, s2, s3, s4, and s5 sites, respectively. Line shows a linear function fitted to

7   the data.

8

1   **Table 1.** Numbers of analyzed seeds from 19 seed parents in the two

2   reproductive years.

3

Site	Seed parent	N_{2006}	N_{2008}
s1	s1-1	26	58
	s1-2	35	78
	s1-3	28	57
s2	s2-1	17	53
	s2-2	20	40
	s2-3	29	59
s3	s3-1	28	59
	s3-2	25	66
	s3-3	19	37
s4	s4-1	31	54
	s4-2	29	70
	s4-3	28	57
s5	s5-1	21	
	s5-2	33	40
	s5-3	35	
	s5-4	25	
	s5-5		51
	s5-6		67
	s5-7		48
Total		429	894

4

5   N_{2006} and N_{2008} indicate the numbers of analyzed seeds in the reproductive

6   years of 2006 and 2008, respectively.

1   **Table 2.** Mean altitude and mean density of *Camellia japonica* individuals

2   flowering during winter at the five sites.

3

Site	Mean altitude (m)	Mean density during winter (ha^{-1})
s1	162	202
s2	241	64
s3	248	85
s4	266	170
s5	541	48

4

5

1 **Table 3.** Mating system parameters in the two reproductive years.

2

Mating system parameter	Reproductive year	
	2006	2008
t_s	0.894 (0.024)	0.921 (0.017)
t_m	0.966 (0.017)	0.985 (0.008)
t_m - t_s	0.072 (0.025)	0.063 (0.015)
r_p	0.193 (0.039)	0.108 (0.023)
N_{ep}	5.2	9.3

3

4 t_s, single-locus outcrossing rate; t_m, multilocus outcrossing rate; $t_m - t_s$, level

5 of biparental inbreeding; r_p, multilocus correlation of paternity; N_{ep}, effective

6 number of pollen parents ($1/r_p$).

7 Standard deviations estimated by 1000 times bootstrap resampling are in

8 parentheses.

1 **Table 4.** Parameters estimated by quadratic models to examine the

2 regressions of the correlated paternity of pollen pools between seed-parent

3 pairs against the spatial distances between the seed-parent pairs in the two

4 reproductive years.

5

Parameters	Estimate	Stndard error	t statistic	
Reproductive year 2006				
a	$5.917\,\mathrm{E}^{-2}$	$9.805\,\mathrm{E}^{-3}$	6.035	***
b	$-3.150\,\mathrm{E}^{-4}$	$4.217\,\mathrm{E}^{-5}$	-7.469	***
c	$2.581\,\mathrm{E}^{-7}$	$4.181\,\mathrm{E}^{-8}$	6.174	***
Reproductive year 2008				
a	$4.162\,\mathrm{E}^{-2}$	$6.322\,\mathrm{E}^{-3}$	6.584	***
b	$-1.988\,\mathrm{E}^{-4}$	$2.698\,\mathrm{E}^{-5}$	-7.370	***
c	$1.458\,\mathrm{E}^{-7}$	$2.643\,\mathrm{E}^{-8}$	5.514	***

6
7

8 Equation of the model: $C_p = a + bD + cD^2$, where C_p is correlated paternity

9 and D is spatial distance between seed-parent pairs.
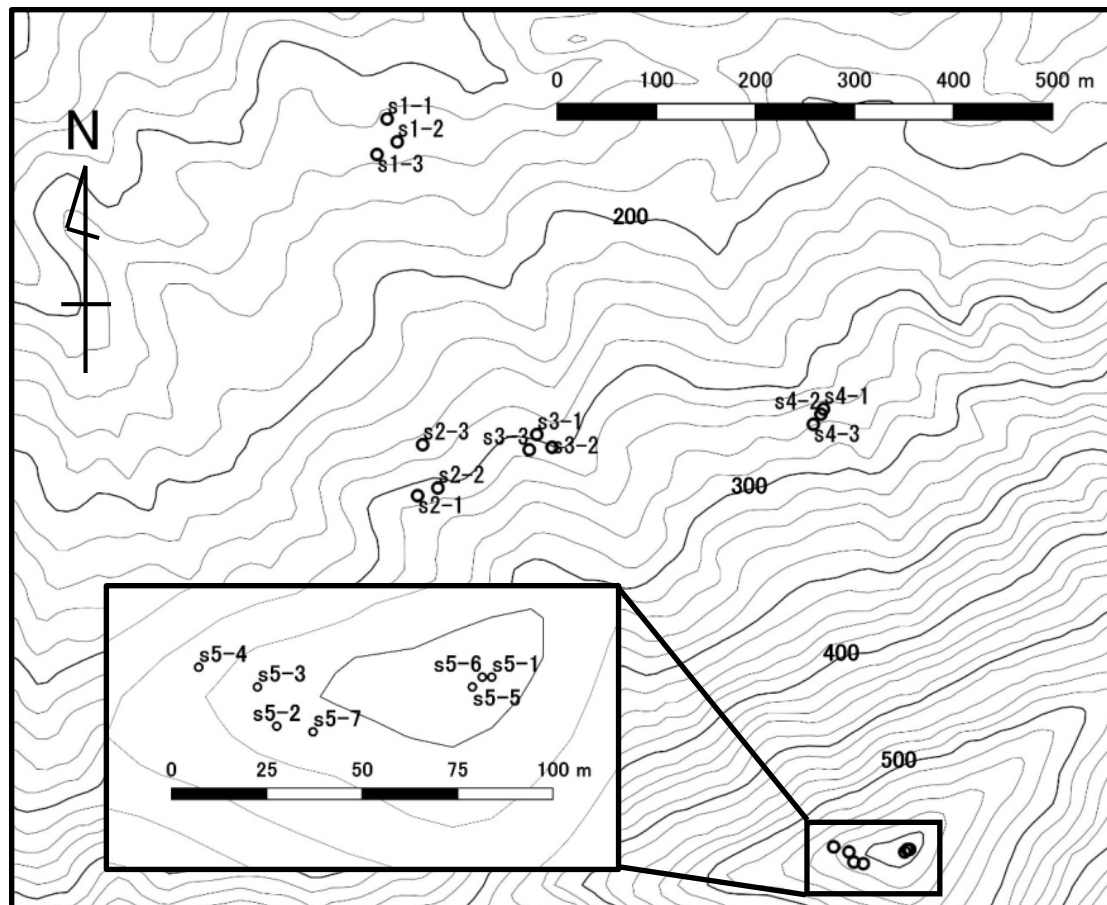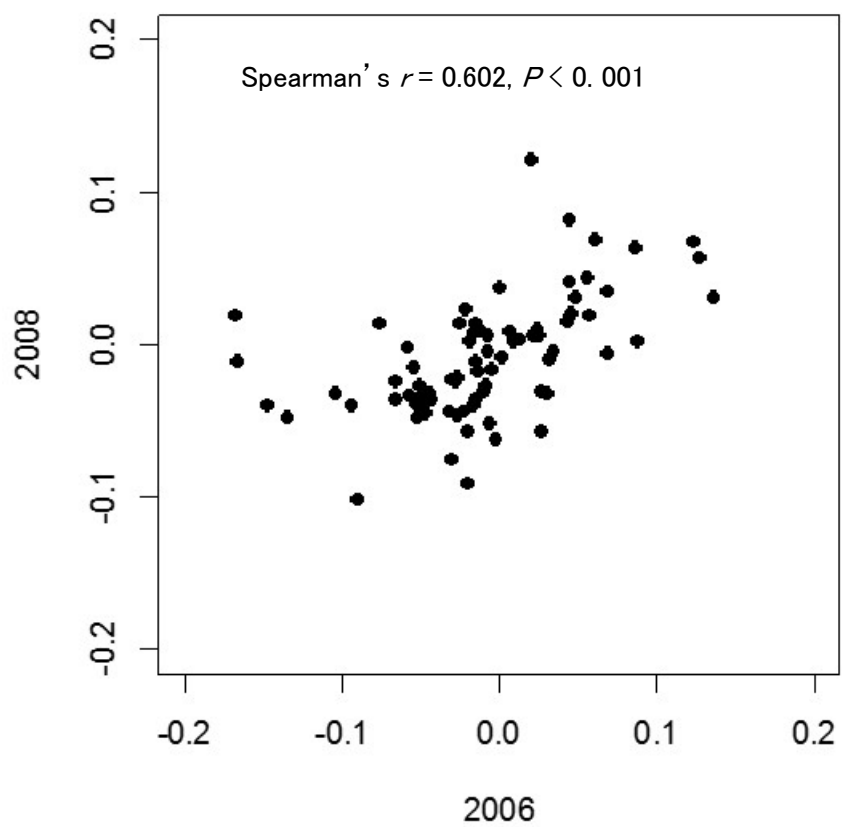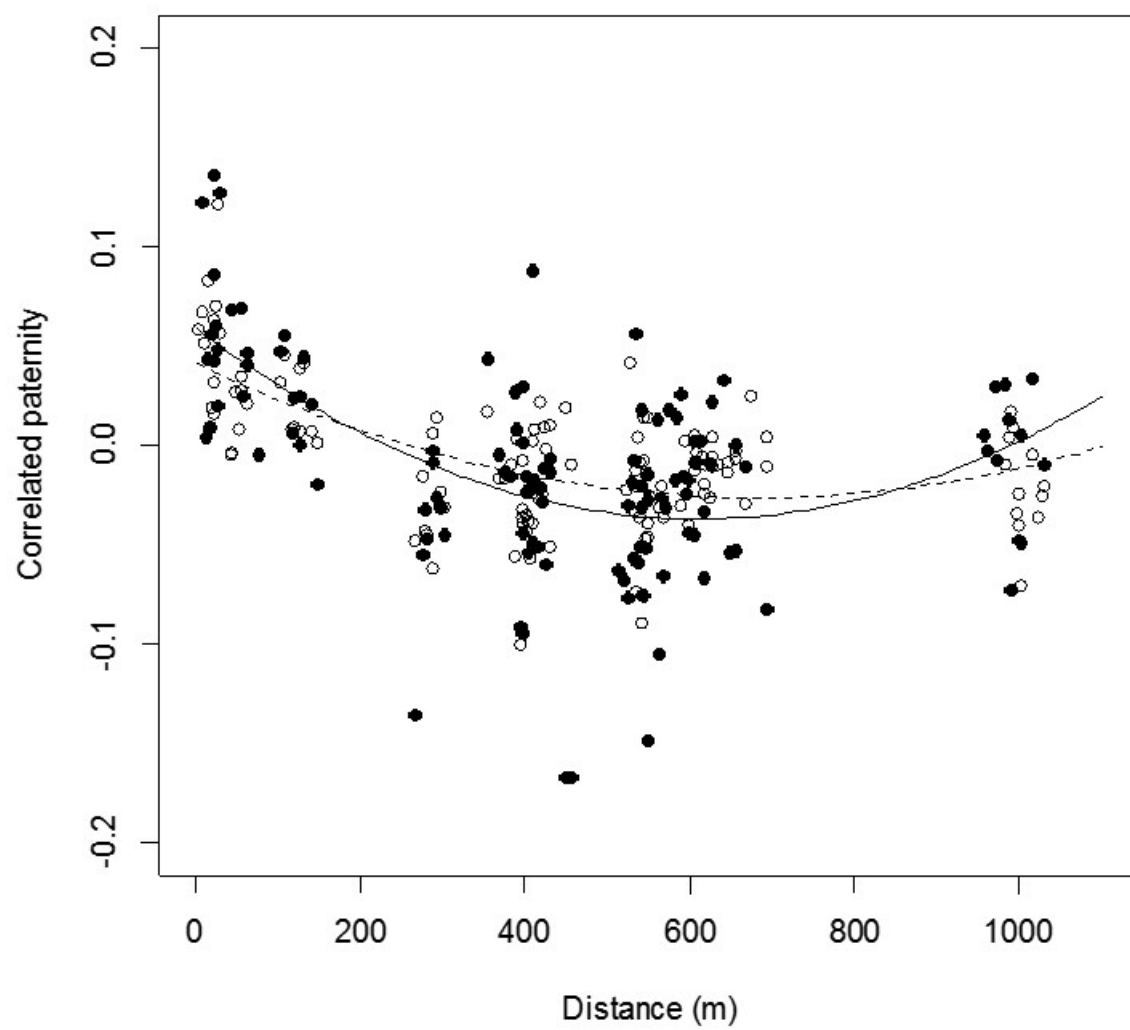
10 E^x means 10^x. ***$P < 0.001$.

11

Figure 1

Figure 2

Spearman's *r* = 0.602, *P* < 0. 001

Figure 3

Figure 4

$P(r) = \{b \, / \, 2\,\pi\,a^2\,\Gamma\,(2/b)\}\,\exp\,\{-(r/a)^b\}$

$P(r)$: probability density that a pollen lands at a seed parent r m away from the source individual

——— $a = 0.000036$, $b = 0.156$ for 2006

- - - - - $a = 0.000190$, $b = 0.168$ for 2008

Probability density
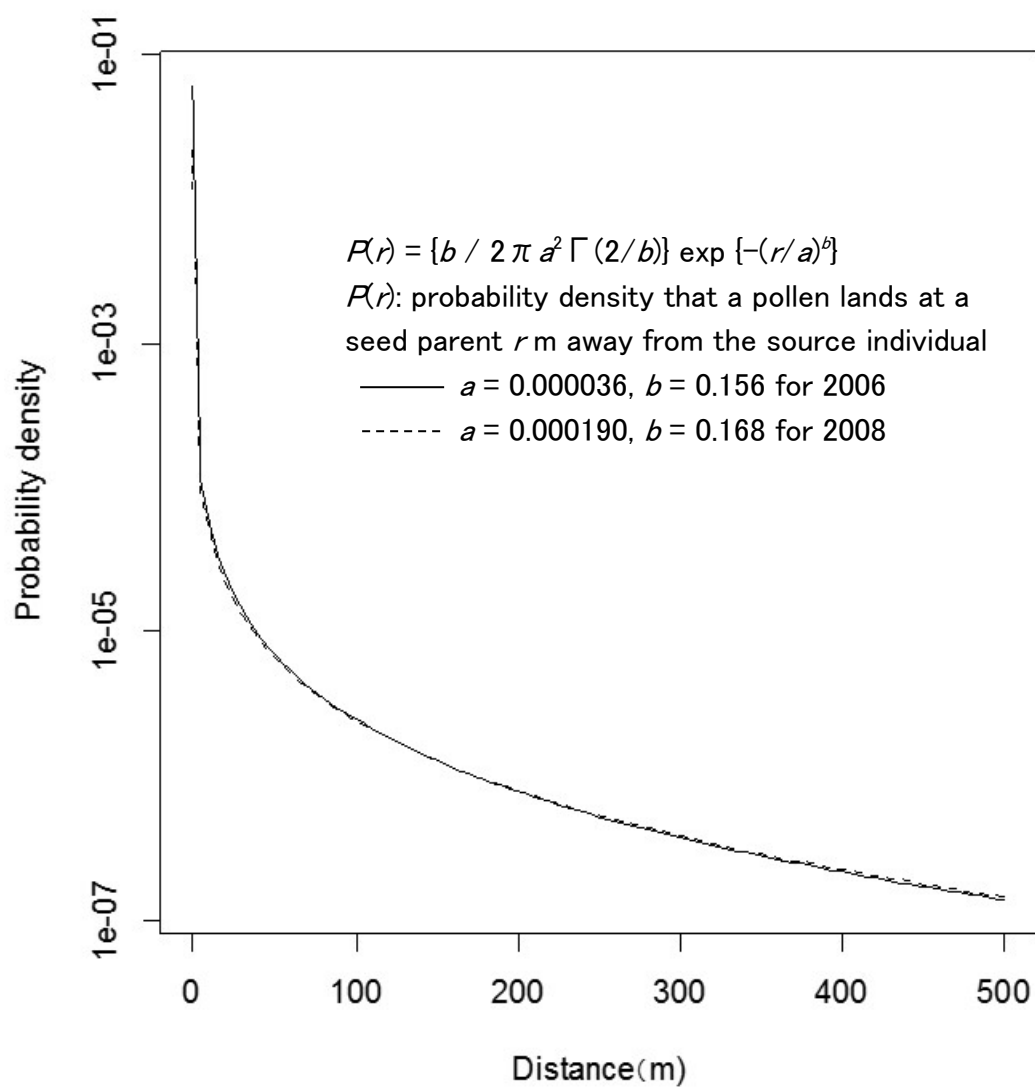
Distance(m)

Figure 5
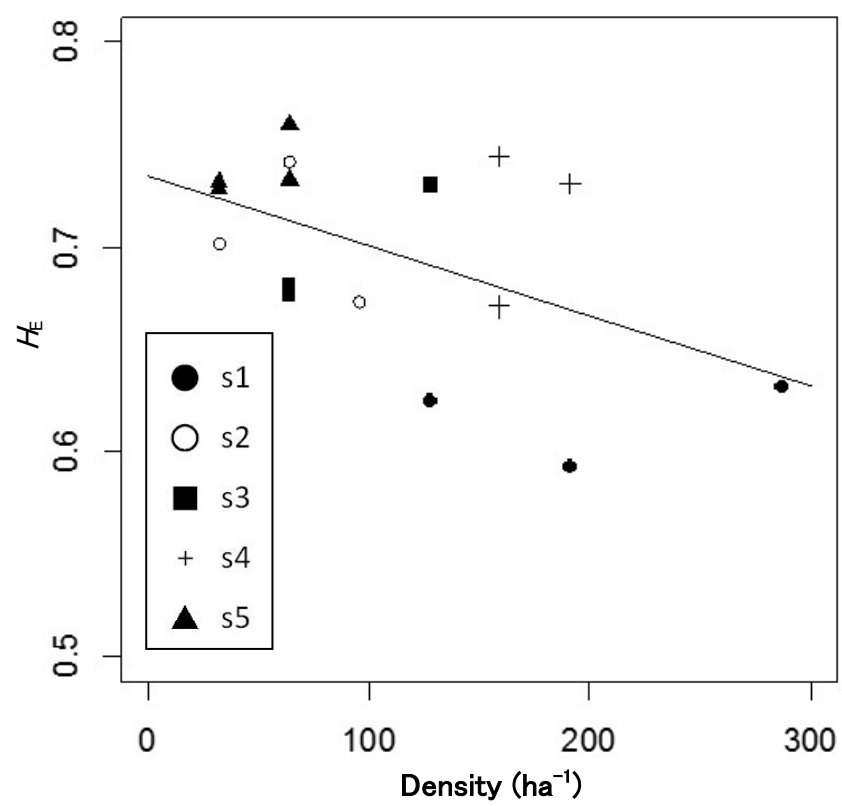
Supplementary Materials

Method S1. Estimation of the pollen dispersal kernels by KINDIST model implemented in the POLDISP software package

KINDIST model estimates the distribution of pollen dispersal distances from the spatial coordinates and genotypes of a sample of seed-plants and their respective maternal progenies (Robledo-Arnuncio *et al*., 2006) as follows.

KINDIST model examines the expected decay with the spatial distance of a normalized measure of correlated paternity between female pairs $\Psi(z)$, which is defined as the ratio of the correlated paternity between maternal sibship pairs with distance z to the average correlation of paternity within maternal sibships (Robledo-Arnuncio *et al*., 2006). Assuming a given pollen dispersal kernel with parameter set θ, the model yields θ estimates by nonlinear least-squares regression of observed $\Psi(z)$ on the expected values of $\Psi(z, \theta)$, which are formulated as a function of the dispersal distribution (Robledo-Arnuncio *et al*., 2007). Given that $\Psi(z, \theta)$ is independent of the effective density of the pollen donors, the approach yields accurate estimates for dispersal parameters (θ) under a wide range of sampling and flowering scenarios (Robledo-Arnuncio *et al*., 2006).

We examined Spearman's rank correlation coefficient between the correlated paternity and distance between the seed-parent pair for each of the two years because a negative coefficient < −0.1 will yield acceptable dispersal estimates (Robledo-Arnuncio *et a*l., 2007). We then set the threshold distance D_u (the approximate observed value beyond which the correlated paternity between maternal sibships typically stabilize at a slightly negative value) to 300 m and applied the

following exponential power function (characterized by the θ consisting of scale (*a*) and shape (*b*) parameters) (Clark, 1998) for the pollen dispersal kernel:

$$P(r) = [b/\{2\pi a^2 \Gamma(2/b)\}] \exp\{-(r/a)^b\},$$

where *P*(*r*) is the probability density that the pollen lands at a seed parent *r* away from the pollen parent, Γ is the gamma function, and shape parameter *b* affects the "fatness" of the tail of the dispersal distribution (Austerlitz *et al.*, 2004). When *b* < 1, the dispersal kernel is fat tailed (Clark, 1998), i.e., the long-range decay of probability is slower than that for the exponential distribution. By using this method, we calculated the scale (*a*) and shape (*b*) parameters and mean distances of pollen dispersal for each of the two reproductive years.

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Robledo-Arnuncio JJ, Austerlitz F, Smouse PE (2006). A New Method of Estimating the Pollen Dispersal Curve Independently of Effective Density. *Genetics* **173**: 1033–1045.

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Method S2. Two-Gener analyses based on the three models

First, to obtain the estimates of the genetic variation of pollen pools among seed parents (Φ_{FT}), we applied a standard Two-Gener analysis by using the model presented by Smouse *et al.*, (2001):

$$G_{ij} = \mu + f_i + \omega_{ij}, \text{ (model 1)}$$

where G_{ij} is a vector representing a haplotype of the *j*th pollen gamete from the *i*th seed parent, μ is a mean vector over all pollen gametes, f_i is the average effect of the *i*th seed parent, and ω_{ij} is the replication error associated with the *j*th pollen gamete from the *i*th seed parent. On the basis of the estimated among- and within-seed-parent variance components, the Φ_{FT} values among all seed parents were estimated separately for 2006 and 2008 by the analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992). The analysis was conducted for all 16 seed parents in each year.

Second, we used the three-level nested model of Irwin *et al.*, (2003) to examine the genetic variation of pollen pools between reproductive years within seed parents (Φ_{YF} over all seed parents) and to obtain an exact estimate of Φ_{FT} that takes into account the temporal genetic variation of pollen pools:

$$G_{ijk} = \mu + f_i + y_{j(i)} + \omega_{ijk}, \text{ (model 2)}$$

where G_{ijk} is a vector representing a haplotype of the *k*th pollen gamete from the *i*th seed parent in the *j*th year, μ is a mean vector over all pollen gametes, f_i is the average effect of the *i*th seed parent, $y_{j(i)}$ is the average effect of the *j*th year, nested within the *i*th seed parent, and ω_{ijk} is the replication error associated with the *k*th pollen gamete from the *i*th seed parent in the *j*th year. On the basis of the variance components estimated among seed parents, between years nested within seed parents,

and within seed parents in single years, the Φ_{FT} and Φ_{YF} of all seed parents were estimated by AMOVA. All 13 seed parents sampled in both years were included in this analysis.

Third, we used the following three-level nested model of Irwin *et al.*, (2003) to examine the genetic variation of the global pollen pools averaged for all seed parents between reproductive years (Φ_{YG}) and the genetic variation of pollen pools among seed parents nested within the years (Φ_{FT}):

$$G_{ijk} = \mu + y_i + f_{j(i)} + \omega_{ijk}, \text{(model 3)}$$

where G_{ijk} is a vector representing a haplotype of the kth pollen gamete from the jth seed parent in the ith year, μ is a mean vector of all pollen gametes, y_i is the average effect of the ith year, $f_{j(i)}$ is the average effect of the jth seed parent nested within the ith year, and ω_{ijk} is the replication error associated with the kth pollen gamete from the jth seed parent in the ith year. On the basis of the variance components estimated between years, among seed parents within years, and within seed parents in single years, the Φ_{YG} and Φ_{FT} of all seed parents were estimated by AMOVA. All 13 seed parents sampled in both years were included in this analysis.

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Method S3. Identification of paternal alleles within seeds

The paternal allele of each seed was identified by subtracting the maternal allele from the seed genotype. In ambiguous cases in which both the seed and its seed parent were heterozygotes with the same alleles, the frequency of each candidate paternal allele within the seed was estimated from allele frequencies in a global pollen pool estimated by unambiguous pollen haplotypes. For example, when a seed and its seed parent have the same heterozygous genotype AB at a loci and when the frequencies of alleles A and B in a global pollen pool are q_A and q_B (estimated by unambiguous pollen haplotypes), respectively, the pollen is defined to have alleles A and B with frequencies of q_A / $(q_A + q_B)$ and q_B / $(q_A + q_B)$, respectively.

Table S1. Parameters of polymorphism at the eight microsatellite loci

Locus	N_O	N_E	H_O	H_E
MSE0030	9	7.5	0.895	0.890
MSE0045	9	5.9	0.789	0.853
MSE0049	3	2.0	0.368	0.511
MSE0051	16	11.1	0.789	0.935
MSE0062	9	3.5	0.895	0.737
MSE0078	6	4.2	0.895	0.785
MSE0079	8	4.5	0.789	0.798
MSE0087	4	2.4	0.579	0.596
Mean	8.0	5.1	0.750	0.763
SE	1.3	1.0	0.062	0.048

N_O, observed number of alleles; N_E, effective number of alleles; H_O, observed heterozygosity; H_E, gene diversity; SE, standard error.

Table S2. Mating system parameters of forest tree species pollinated by birds or mobile animals in this study and other studies

Reference	Pollinator type	Density (ha^{-1})	1 - t_m	t_m - t_s	r_p
This study	Bird	127-684	0.02-0.03	0.06-0.07	0.11-0.19
Bezemer *et al*. (2016)	Bird	Unknown	0.17	0.06	0.14
Breed *et al.* (2015a)	Bird	2-51	0.04-0.07	0.08-0.18	0.08-0.18
Breed *et al.* (2015b)	Bird	2-13	0.06	0.16-0.19	0.16-0.18
Breed *et al*. (2015a)	Mobile animal	Unknown	0.00-0.46	0.02-0.21	0.10-0.74

t_s, single-locus outcrossing rate; t_m, multilocus outcrossing rate (thus, 1 - t_m indicates multilocus selfing rate); t_m - t_s, level of biparental inbreeding; r_p, multilocus correlation of paternity.

References

Bezemer N, Krauss SL, Phillips RD, Roberts DG, Hopper SD (2016). Paternity analysis reveals wide pollen dispersal and high multiple paternity in a small isolated population of the bird-pollinated Eucalyptus caesia (Myrtaceae). *Heredity* **117**: 460–471.

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Breed MF, Ottewell KM, Gardner MG, Marklund MHK, Stead MG, Harris JBC, *et al.* (2015b). Mating system and early viability resistance to habitat fragmentation in a bird-pollinated eucalypt. *Heredity* **115**: 100–107.

Table S3. Pollen dispersal kernel parameters estimated by the KINDIST model with assumption of an exponential power function as the dispersal kernel in this study and other studies

Reference	Pollination vector	b	δ (m)
This study	Bird	0.16-0.17	1477-1487
Lander *et al*. (2010)	Insect	0.17	1189
Breed *et al*. (2015b)	Bird	0.15-0.16	870-1134
Kassa *et al*. (2018)	Wind	0.18	417

b, shape parameter; δ, mean distance of pollen dispersal.

References

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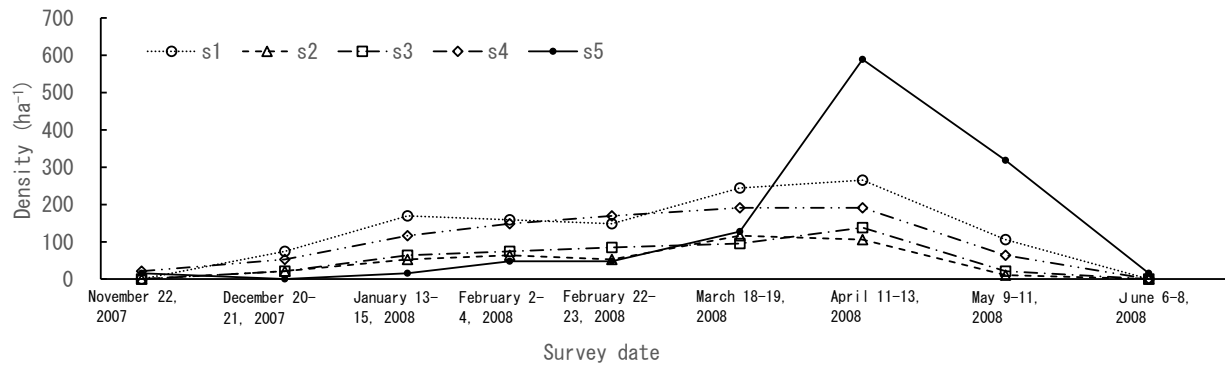
Figure S1. Change in the local density of individuals with open flowers at the five sites in the

2007/2008 flowering season. The local density was estimated by averaging the densities within
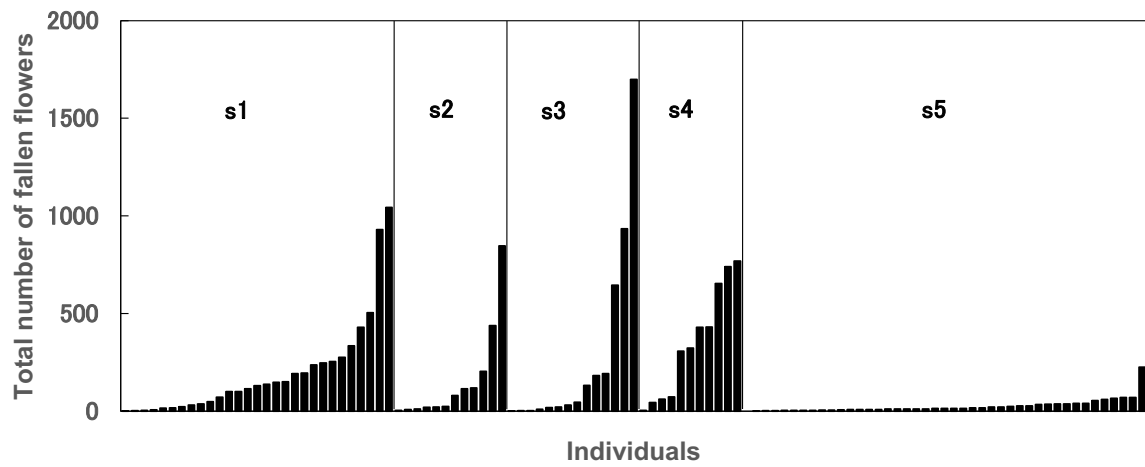
10-m radii from the seed parents at each site.

Figure S2. Cumulative numbers of flowers that had fallen from each individual at the five sites recorded in surveys of 14 seed parents and all individuals within 10-m radii from the respective seed parents. Bars indicating the numbers of fallen flowers for respective individuals were sorted in ascending order along the abscissa axis.
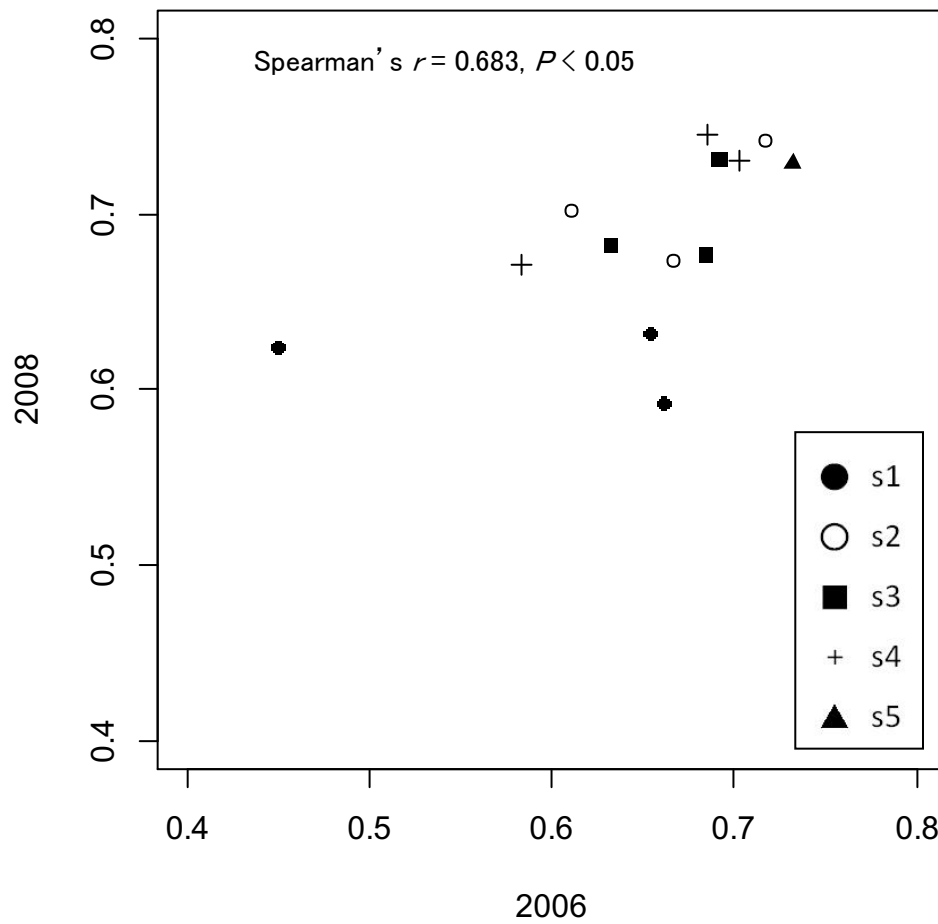
Figure S3. Relationship between the reproductive years 2006 and 2008 of the gene diversity (H_E) values of pollen pools accepted by each seed parent. Filled circles, open circles, filled squares, crosses, and filled triangles indicate the values for seed parents at the s1, s2, s3, s4, and s5 sites, respectively.