Chapter 5
Expanded Home Range of Pollinator Birds Facilitates Greater Pollen Flow of *Camellia japonica* in a Forest Heavily Damaged by Volcanic Activity

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5.1 Introduction

Ecological observations during and after volcanic activity provide valuable opportunities to study how organisms respond to environmental devastation. Previous studies of volcanic islands have mainly addressed the colonization processes of pioneer plants to examine the primary succession of plant communities (e.g., Kitayama et al. 1995; Tsuyuzaki and del Moral 1995; Thornton et al. 1996; Shanahan et al. 2001; Kamijo et al. 2002; Kamijo and Hashiba 2003). However, no study has focused on the recovery processes of late climax forest communities by secondary succession according to various symbiotic species interactions. Because secondary succession dominates the recovery process, a better understanding of the detailed interactions between organisms during recovery is important.

In an effort to study these community recovery processes, we examined the effects of volcanic activity on a plant–animal system comprising a common broad-leaved evergreen tree species, *Camellia japonica*, from climax forests, and a pollinating bird, the Japanese white-eye, *Zosterops japonica*. We examined the hypothesis that *Camellia* trees are sufficiently robust to serve as a core species in natural forest recovery, even in severely damaged areas. We previously reported the effects of volcanic activity on the maternal reproductive success of *C. japonica* (Abe and Hasegawa 2008). We found that the net fruit production of *C. japonica* in a heavily damaged area was nearly the same as that in a less damaged area; this was true because the heavily damaged area had better pollination and seed-set rates compared to the less damaged area, which compensated for the suppressed blossoming and fruit growth in the heavily damaged area where poisonous volcanic gas exposure was intense. Despite the similar maternal reproductive success in terms of the final fruit-set rates in heavily damaged and less damaged sites, the genetic diversity of subsequent generations (seeds) may be different among sites. Knowledge about the genetic diversity of pollen transported by pollinators will deepen our understanding of population maintenance mechanisms and reproductive strategies in *C. japonica*. These types of studies are currently quite limited for bird-pollinated species (Ward et al. 2005).

In this study, we show how pollinator behavior affects the pollen dispersal of *C. japonica* at sites having variable flower densities. We do this using a single-pollen genotyping method and radio tracking of pollinator birds. Our previous study (Abe and Hasegawa 2008) suggested that because pollinator birds transport *C. japonica* pollen grains collected from various areas with low flower density, genetic diversity should increase in *C. japonica* progeny as a consequence of long-distance pollen dispersal vectors. Recently, direct genotyping of single pollen grains was developed (Matsuki et al. 2007, 2008). In the present study, we reveal characteristics of pollen movement of *C. japonica* pollinated by *Z. japonica* by comparing the genetic diversity of pollen grains on the birds between sites having different flower densities. In addition, we also compare the results from radio tracking of pollinator birds with pollen haplotyping. We examined the stability of plant–pollinator systems with regard to volcanic disturbance by combining the single-pollen genotyping method (Matsuki et al. 2007, 2008) with radio tracking,

which demonstrated that genetic diversity in pollen grains found on the birds was more diverse in the heavily damaged area that had low flower density. The consequences of these results for plant–pollinator system stability following volcanic disturbances are discussed.

5.2 Materials and Methods

5.2.1 Study Areas

The Izu Islands are a group of volcanic islands located on the western rim of the Pacific Ocean. They are characterized by a humid warm temperate climatic zone with an annual rainfall of more than 2,000 mm and an average air temperature ~17°C. The volcanically active island Miyake-jima (Fig. 5.1), located about 180 km south of Tokyo, was selected as the study island because the volcano explosively erupted during the summer of 2000. The main vegetation is evergreen broad-leaved dominated by species such as *Castanopsis sieboldii*, *Machilus thunbergii*, and *Camellia japonica* before the 2000 volcanic eruption (Kamijo et al. 2002; Kamijo and Hashiba 2003).

In the winter of 2006, we established three study sites on Miyake-jima [Tubota (TU), Igaya (IG), and Nanto-road 2 (N2); Figs. 5.1 and 5.2] to examine the genetic aspects of reproductive success under various flowering conditions related to volcanic activity.

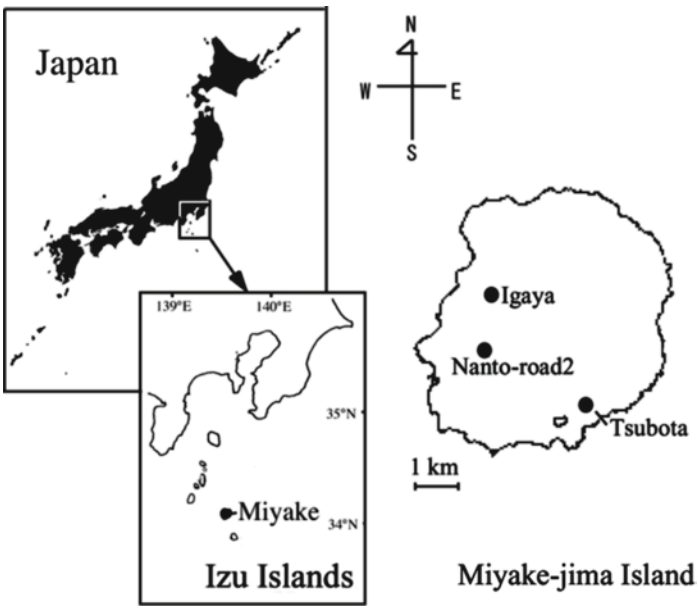

Fig. 5.1 Three study sites on Miyake-jima Island, Japan

Fig. 5.2 *Camellia japonica* blossoming at the area (Nanto-road 2, N2) heavily damaged by volcanic gases

5.2.2 Study Species

Camellia japonica was selected as the study species. In eastern Asia, *C. japonica* is a common broad-leaved evergreen tree species in climax forests (Wendel and Parks 1985; Katsuta 1999; Nagamasu 2006). This species is monoecious in its reproductive system. It produces conspicuous red flowers with a large quantity of nectar that attracts birds (*Z. japonica* and *Hypsipetes amaurotis*). It flowers during November to March with a peak during January to February on Yakushima Island (Yumoto 1988) and the Izu islands (Abe et al., unpublished). *C. japonica* is also self-incompatible (Shibata and Ieyumi 1991), and flowers isolated from pollinating birds do not bear fruit (Kunitake et al. 2004). Kunitake et al. (2004) experimentally demonstrated that *Z. japonica* (Fig. 5.3) is the most effective pollinator of *C. japonica* on Niijima Island adjacent to Miyake-jima. Although *H. amaurotis*, *Cettia diphone*, and *Parus major* are also potential pollinators at Miyake-jima, *Z. japonica* was always dominant and represented 78–96% of the flower-visiting birds (Abe and Hasegawa 2008). *Z. japonica* migrates to warmer places in winter, and some birds fly from mainland Honshu to the Izu islands. Resident birds on the Izu islands are classified as subspecies *Z. japonica* var. *stejnegeri*. Kikkawa and Kakizawa (1981) determined that about 9% of *Z. japonica* caught in winter on Miyake-jima were mainland subspecies. Abe et al. (2006) reported secondary seed dispersal by the Japanese wood mouse (*Apodemus speciosus*) on Niijima.

Fig. 5.3 *Zosterops japonica* visiting *Camellia* flower. (Photograph by Akio Ogura)

5.2.3 Flowering Conditions of C. japonica and Home Ranges of Z. japonica

During winter 2006, the number of flowering trees and flowers per flowering tree were counted, and the flower density (per hectare, ha) was estimated at each study site to analyze the relationship among flowering conditions, pollen genetic diversity, and the home range size of the pollinator bird *Z. japonica*. Home range sizes were estimated using radiotelemetry and analyzed for birds tracked for more than 1 week.

In the radiotelemetry tracking, birds were captured with 12- to 18-m mist nets (mesh size, 36 mm) that were placed at a central location between the radio-tracking sites (~100 ha). The capture of birds using mist nets was permitted by the Ministry of the Environment, Government of Japan (permit no. 051118001). Transmitters (model LB-2; Holohil Systems, Canada; http://www.holohil.com) weighing less than 0.5 g were glued to the backs of adult birds heavier than 11 g in body mass, based on the methods of Raim (1978). Any two birds captured at the same time were determined to be a pair. Therefore, we only attached a transmitter to one of them. The life of a transmitter was 12 days (lifespan range, 8–15 days), and its dimensions were $13 \times 6.5 \times 3.5$ mm (L × W × H). We radio-tracked birds with transmitters between 5 January and 5 February 2006 at high- and low-flower-density sites at TU, IG, and N2 on Miyake-jima (see Fig. 5.1). The birds were tracked more than three different times per day, between sunrise and sunset (normally at 0700, 1200, and 1600) for active birds and once during the night for inactive birds. The home range of the birds that were continually tracked for more than 1 week was depicted using minimum convex polygons. Radio-tracking equipment comprised a receiver (FT-817; Vertex Standard) that had three handheld Yagi aerial-antenna elements.

5.2.4 Pollen Grain Sampling and Haplotyping

For all birds captured using the methods just described, we first checked for the presence or absence of pollen grains on the beak and then wiped any visible pollen grains off the beak using a cotton-tipped swab (Fig. 5.4). Pollen grains were dropped on a glass slide from the swab, and a single pollen grain was picked up using a needle under a stereomicroscope.

DNA extraction and microsatellite haplotyping of a single pollen grain were conducted according to the method described by Matsuki et al. (2007). Haplotypes were determined using eight pairs of microsatellite polymerase chain reaction (PCR) primers: *TMSE-27B03T*, *TMSE-9E07S*, *TMSE-25E07T*, *TMSE-11D02T*, *TMSE-4B07S*, *TMSE-10C05T* (Taniguchi et al., personal communication), *MSCjaH38* (Ueno et al. 1999), and *MSCjaR2* (Abe et al. 2006). Microsatellite amplification was then performed using a Multiplex PCR Mix according to the manufacturer's protocol (Takara Bio). Reactions were incubated in a TaKaRa PCR Thermal Cycler Dice Gradient TP600 (Takara Bio). After an initial denaturation phase of 15 min at 95°C, 30 thermal cycles were performed under the following conditions: denaturation for 30 s at 94°C, annealing for 90 s at 58°C, and extension for 90 s at 72°C, with final extension for 10 min at 72°C. The PCR amplification products were detected using a genetic analyzer (ABI Prism 3100; Applied Biosystems). Individual genotypes were determined using Genotyper software (Applied Biosystems).

5.2.5 Mature Tree Sampling and Genotyping

Because the distribution of *C. japonica* at the study sites tended to be clumped, we selected a dense area of *C. japonica* trees as the study plot within each site. From late July to early August 2005, we collected leaves from all mature trees that had

Fig. 5.4 Wiping pollen from the beak of *Cettia diphone* using a cotton swab

floral buds in each 50×60 m area (0.3 ha) at the three study sites. The number of mature trees within each population was 48, 24, and 17 for TU, IG, and the N2 population, respectively. Genomic DNA was extracted from dried or frozen leaves using a modified CTAB method (Murray and Thompson 1980). Genotypes were determined using the same method as in the pollen grain analysis already described.

5.2.6 *Analysis of Genetic Diversity*

We calculated three genetic parameters: number of alleles (*Na*), gene diversity (*H*), and allelic richness (A_r). *Na* and *H* were estimated using the FSTAT 2.9.3 software (Goudet 2000). The number of gene copies [*g*] was standardized to 20 for the calculation of A_r, according to El Mousadik and Petit (1996). To compare genetic diversity among the study sites, the partitioning of allelic richness among pollen grains ($A_{st}[g]$) was calculated as $A_{st}[g]=1-(A_s[g]-1)/(A_t[g]-1)$ (El Mousadik and Petit 1996; Petit et al. 1998; Comps et al. 2001), where $A_s[g]$ and $A_t[g]$ signify the mean allelic richness within each pollen pool adhering to a bird and the total allelic richness in the pollen pools adhering to birds within each study site, respectively. Values of A_{st} depend on the distribution of alleles among populations. If rare alleles are clustered in some populations (pollen pools, here), high values of A_{st} are expected. On the other hand, even distribution of alleles among pollen pools will give low A_{st} values. From a conservation point of view, more emphasis should be placed on rare alleles, and therefore A_{st} is a more suitable measure than F_{st} (El Mousadik and Petit 1996). Comparisons of genetic diversity among sites were carried out using $A_{st}[g]$ values. Differences in the mean values of the analyzed genetic parameters between bird species and among study sites were analyzed using Mann–Whitney *U* tests and Kruskal–Wallis tests after a test of equal variances.

5.3 Results

5.3.1 *Flowering Conditions and Home Range of Zosterops japonica*

The flower and flowering tree densities were highest at TU and lowest at N2 (Table 5.1). For the three sites described above, a total of 18 *Zosterops* birds were captured (Table 5.2). One bird at TU was determined, by the length of its beak, to

Table 5.1 Flowering conditions of *C. japonica* in the Miyake-jima Island study sites

Study site	Census area (ha)	Flower density (/ha)	Flowering tree density (/ha)	Number of flowers per flowering tree	(SD)
TU (Tubota)	0.3	10,106.7	193.3	207.6	(156.1)
IG (Igaya)	0.5	206.0	36.0	16.2	(18.7)
N2 (Nanto-road 2)	1.0	53.0	12.0	16.6	(8.1)

Table 5.2 Number of birds captured and those from which pollen grains on their beak were analyzed

	Zosterops japonica		*Hypsipetes amaurotis*		*Cettia diphone*		*Parus major*	
Study site	Captured	Analyzed	Captured	Analyzed	Captured	Analyzed	Captured	Analyzed
TU (Tubota)	13 (3)	16	0	0	6	0	1	0
IG (Igaya)	3 (1)	4	1	1	7	3	9	1
N2 (Nanto-road 2)	2 (0)	2	0	0	2	1	3	3
Total	18 (4)	22	1	1	15	4	13	4

Number of recaptured birds is in parentheses

be a *Z. japonica* that had migrated from the Honshu mainland. The others were identified as a nonmigratory resident subspecies from the Izu Islands, *Z. japonica* var. *stejnegeri*.

In total, ten birds were tracked for 7–20 days ($n=7$, 16.6 days at TU; $n=3$, 7.5 days at IG). The home range sizes were estimated using the minimum convex polygon method (Table 5.3). The mean home range size at the high-flower-density site (TU) was smaller than at the low-flower-density site (IG), although no significant differences were found (Welch's T test, $P=0.13$; Table 5.3 and Fig. 5.5).

The mean number of flowers estimated to be within all home ranges was greater at TU than at IG (Table 5.3). In contrast, the mean number of flowering trees estimated to be within all home ranges at TU tended to be smaller than at IG (Table 5.3). However, these differences were not significant (Mann–Whitney U test: $Z=1.03$, $P=0.31$).

Table 5.3 Mean home range sizes of *Zosterops japonica* and estimated mean number of flowers and flowering trees at two study sites based on the home range sizes and flowering density in Table 5.1

		Mean home range size		Estimated number within the home range			
Study site	Number of birds	Hectares (ha)	(SD)	Flowers	(SD)	Flowering trees	(SD)
TU (Tubota)	7	0.26	0.24	2,599	2,466	49.7	47.2
IG (Igaya)	3	1.97	1.20	405	247	74.7	45.7

No birds were analyzed at site N2 (Nanto-road 2)

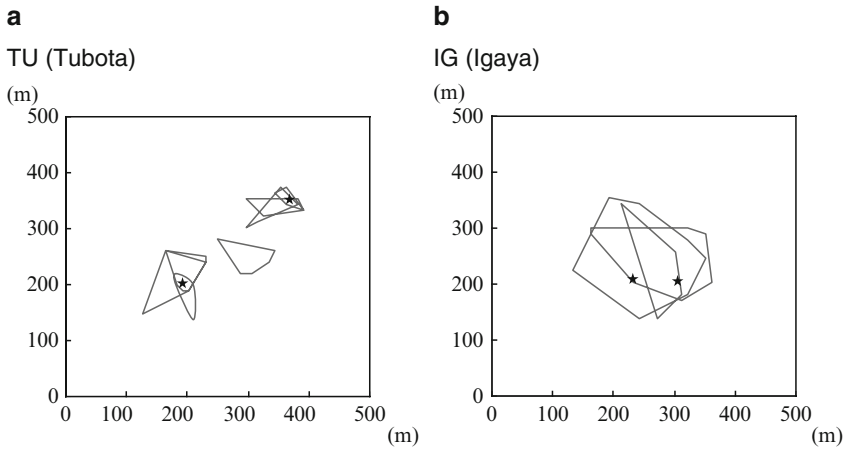

Fig. 5.5 Home ranges of *Zosterops japonica* individually tracked using radiotelemetry in winter 2006 on Miyake-jima. Outermost points of locations tracked for more than 1 week were connected by *lines* to form minimum convex polygons of home ranges. (**a**) High-flower-density area of Tubota (*TU*). (**b**) Low-flower-density area of Igaya (*IG*). *Stars* represent points where birds were captured

5.3.2 Microsatellite Haplotyping from Single Pollen Grains

All *Zosterops* captured using nets ($n=22$) had pollen grains adhered to their entire beak surfaces. Four *Cetta diphone* ($n=15$; 27%) and four *Parus major* ($n=13$; 31%) individuals had small amounts of pollen on their beaks. Only one *Hypsipetes amaurotis* individual was captured with a small quantity of pollen on its beak (see Table 5.2).

The haplotypes of pollen grains found on the 18 captured *Z. japonica* and 4 recaptured birds were determined (Table 5.2). Additionally, we determined the haplotypes of pollen grains on 1 *H. amaurotis*, 4 *C. diphone*, and 4 *P. major* captured in the low-flower-density areas of IG and N2 (Table 5.2).

In total, 878 pollen grain samples were haplotyped for more than five microsatellite loci and used in the genetic analyses ($n=599$, mean 27.2, and range 16–48 for *Z. japonica*; $n=279$, mean 31.0, and range 22–92 for other birds). All microsatellite loci were amplified in 83% of the pollen grains.

5.3.3 Genetic Diversity

The genotypes of all 89 mature trees were determined at the three study plots (each 0.3 ha: $n=48$ at TU; $n=24$ at IG; $n=17$ at N2). The genetic parameters of the pollen grains found on *Z. japonica* are summarized in Table 5.4. We summed the data at IG and N2 in the low-flower-density area because of the small number of birds analyzed for pollen. A comparison of the diversity parameters (*Na*, *H*, and *Ar*) for adult trees in the high-flower-density site (TU) to those in the low-flower-density sites (IG and N2) revealed that diversity only slightly differed among different densities and that this difference was not significant (Table 5.4a). On the other hand, the three genetic parameters for the pollen were significantly higher in the low-flower-density sites (IG and N2) than in the high-flower-density site (TU) (Table 5.4b). In addition, A_{st} were significantly lower in the low-flower-density sites (Table 5.4b and Fig. 5.6). Higher genetic diversity parameters in the low-flower-density sites were probably caused by the larger numbers of pollen donors sampled by *Z. japonica* (see Table 5.3), whereas the lower values of A_{st} in the low-flower-density sites were considered to be the result of more homogeneous distribution of alleles among pollen pools on beaks of *Z. japonica* in the low-flower-density sites.

The genetic parameters for the pollen found on *H. amaurotis*, *C. diphone*, and *P. major* are also summarized in Table 5.4. A comparison of the genetic diversity of the pollen found on *Z. japonica* and the other birds in the low-flower-density sites (IG and N2) showed a significant difference in three parameters (Table 5.4c). However, the mean A_{st} of *Z. japonica* did not differ from that of the other birds (Table 5.4c).

Table 5.4 A comparison of genetic diversity between study sites (**a** and **b**) and among bird species (**c**), based on eight microsatellite markers

a Mature trees

	Study sites		
Gene diversity	TU	IG and N2 (IG | N2)	Significance[a]
Na	7.3	7.2 (8.0 | 6.3)	NS
H_e	0.68	0.70 (0.72 | 0.67)	NS
A_r	5.71	5.26 (5.42 | 5.10)	NS

b Pollen pools on *Z. japonica* beaks

	Study sites		
Gene diversity	TU	IG and N2 (IG | N2)	Significance[a]
Na	3.6	4.7 (4.2 | 5.2)	**
H	0.48	0.54 (0.53 | 0.56)	*
A_s	3.28	3.95 (3.75 | 4.15)	***
A_t	4.48	4.37 (4.40 | 4.33)	NS
A_{st}	0.35	0.15 (0.19 | 0.06)	***

c Pollen pools on beaks of *Z. japonica* and other bird species

	Bird species		
Gene diversity	*Z. japonica*	Other birds	Significance[a]
Na	4.5	3.5	*
H	0.54	0.43	**
A_s	3.95	2.84	**
A_t	4.37	3.71	–
A_{st}	0.15	0.23	NS

For comparison between study sites, the high-flower-density site (TU) and low-flower-density sites (IG and N2) were considered. Values for IG and N2 are also listed separately

Each genetic diversity parameter indicates *Na*, no. of alleles; H_e, expected heterozygosity; *H*, gene diversity; A_r, allelic richness; A_s, mean allelic richness within each pollen pool adhering to a bird; A_t, total allelic richness in the pollen pools adhering to birds within each study site; A_{st}, partitioning of allelic richness among pollen grains

Gene copies of allelic richness were standardized at 20

[a]Significance: NS, nonsignificant; $*P<0.05$, $**P<0.01$, $***P<0.005$ (Mann–Whitney *U* test)

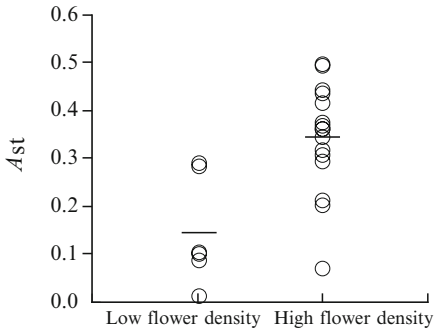

Fig. 5.6 Relationship between flower density and the partitioning of allelic richness among pollen grains on each *Zosterops* individual (A_{st}). Site TU had a high flower density, whereas sites IG and N2 had low flower densities. *Circles* and *bars*, each sampled bird and the mean value for each site, respectively

5.4 Discussion

5.4.1 *Resilience of the Camellia japonica–Zosterops japonica System Against Environmental Perturbation*

Cascading negative impacts of reduced flower density on pollination, fruit-set, and seed-set rates have been observed in many plant species (Ågren 1996; Mustajärvi et al. 2001; Leimu and Syrjänen 2002). Low fruit-set rates have generally been attributed to pollen or resource limitation (Stephenson 1981; Bawa and Webb 1984; Zimmerman and Pyke 1988; Sperens 1996). Even with efficient seed production that produces a sufficient quantity of pollen, the genetic variation of progeny might decrease because of a decrease in pollen donors. Although long-distance pollen dispersal by insects occurs when there is low tree density as a consequence of sparse distribution or fragmented landscapes (Stacy et al. 1996; Konuma et al. 2000; Dick et al. 2003), the low population numbers or low flowering density increases selfing and inbreeding depression (Aldrich and Hamrick 1998; Obayashi et al. 2002; Latouche-Hallé et al. 2004; Naito et al. 2008). For *Shorea leprosula*, it was demonstrated that flowering tree density was negatively correlated with the rare allele frequency in the seedling population as well as with pollen dispersal distance, without reference to the biological systems that caused these results (Fukue et al. 2007). In contrast to the conclusions of numerous previous studies, reduced flower density in *C. japonica* engendered markedly high genetic diversity in pollen grains associated with *Zosterops* (see Table 5.4) as well as an expansion in the pollen dispersal area (see Fig. 5.5). Moreover, the F_{st} values, or the partitioning of heterozygosity among pollen grains, were lower in the low-flower-density areas (0.267 at IG; 0.169 at N2) compared to the high-flower-density area (0.301 at TU). We propose that specialized interactions between *C. japonica* and *Z. japonica* along with the foraging behavior of *Z. japonica* account for the resilience within the *C. japonica–Z. japonica* system against environmental perturbation, such as volcanic eruption and anthropogenic habitat destruction, which is discussed next.

Because *Z. japonica* strongly depends on *Camellia* flower nectar as a food resource in winter, it is considered a seasonal specialist (Kunitake et al. 2004). During the winter season, invertebrates, fruits, and other flowering plants are scarce, whereas *C. japonica* flowers that secrete large amounts of nectar are abundant. This phenological concordance between the pollinator's resource shortage and *C. japonica* flowering might have stimulated seasonal specialization by omnivorous birds that produced effective pollination services. This specialization may never stop *Z. japonica* from visiting *Camellia* flowers, even if volcanic disturbance severely limits the flowering activities of *C. japonica*; that is, birds without access to high-flower-density areas were forced to seek *Camellia* flowers in low-flower-density areas. Consequently, the shortage of flower resources increases the home range size of the pollinator (Table 5.3 and Fig. 5.5) and the number of visited flowering trees (Table 5.3) under low flower densities. The corresponding increase in pollen donors not only enhanced

genetic diversity of the pollen grains on *Zosterops* individuals (Table 5.4), but also increased the proportion of flowers visited by pollinators, thereby ensuring higher pollination rates in low-flower-density areas (Abe and Hasegawa 2008).

At the high-flower-density site (TU), 70% of tracked *Zosterops* moved within the study area, while at the low-flower-density sites (IG and N2) 100% of tracked birds disappeared from the study plot for 9 days or fewer (data not shown). The home ranges in the low-flower-density sites may be more flexible than those in high-flower-density sites. The existence of pollinator birds with a large home range in low-flower-density habitats (Fig. 5.5) must be one of the factors preventing genetic differentiation among pollen grains, because the number of trees visited by *Z. japonica* in low-flower-density areas was larger than in the high-flower-density area, which would increase the number of pollen donors for each mother tree. High genetic diversity of pollen grains in low-density sites is therefore likely a result of the resilient relationship between *C. japonica* and *Z. japonica*. The enlarged area is generated in response to environmental perturbation, leading to an increase in potential pollen donors for recipient *Camellia* trees in low-flower-density areas.

5.4.2 Conclusions and Future Research

This study examined how reduced flower density affected the pollen movement of *Camellia japonica* through a combination of single-pollen haplotyping and radio tracking of the specialized pollinator bird, *Zosterops japonica*. Greater foraging movements of the pollinator in areas with low flower density promote higher genetic diversity of pollen grains transported by *Zosterops*. These results corresponded to an enhanced efficiency in maternal reproductive success (pollination rate and seed-set rate) of *C. japonica* in low-flower-density areas (Abe and Hasegawa 2008). A greater dependence on nectar, which results in an increase in the transporting rates of *C. japonica* pollen grains, makes *Z. japonica* the most effective pollinator of *C. japonica* among the potential avian pollinators. The greater genetic diversity within the transported pollen (see Table 5.4) and higher visitation rates by pollinator birds (Kunitake et al. 2004; Abe and Hasegawa 2008) together provide solid evidence that the *C. japonica–Z. japonica* system, with its innate mechanisms, is robust against environmental perturbation such as volcanic eruption and anthropogenic habitat destruction.

Finally, there are two other issues that should be addressed in future studies. One concern is to determine whether the high genetic diversity found within the pollen adhering to pollinator birds is successfully delivered to the trees by examining the genetic diversity of pollen donors within a fruit or seedling, which would determine the genetic diversity of the next generation in a more direct way. The second issue is to examine how spatial differences in flowering phenology (i.e., flowering time and the number of flowers) among individual trees affect both maternal and paternal reproductive success via pollen flow. Variations in flowering phenology

might induce spatial and temporal variation in flower density and affect the degree of spatial restriction of pollen flow. In fact, previous studies have reported that flower density can strongly affect pollen flow (Levin and Kerster 1969). For example, at the TU site with high flower density, the number of flowers per flowering tree varied greatly (see Table 5.1). There may be large variations in genetic diversity among pollen pools that individual trees have received. If *Z. japonica* were only attracted to *Camellia* trees that had large amounts of flowers, the number of pollen donors received per tree would decrease, which would lead to genetic differentiation. Therefore, the differences in genetic diversity of pollen pools between the low- and high-flower-density sites might be caused by variations in flowering phenology within each population. To better understand the *C. japonica–Z. japonica* system, we must determine the consequences of variable pollen flow on genetic differentiation within the populations considering the interactions among flowering time, the number of flowers, and visitation rates of pollinators.

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