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Harue Abe · Rikyu Matsuki · Saneyoshi Ueno
Makoto Nashimoto · Masami Hasegawa

Dispersal of *Camellia japonica* seeds by *Apodemus speciosus* revealed by maternity analysis of plants and behavioral observation of animal vectors

Abstract Seed dispersal determines a plant's reproductive success, range expansion, and population genetic structures. *Camellia japonica*, a common evergreen tree in Japan, has been the subject of recent genetic studies of population structure, but its mode of seed dispersal has been assumed, without detailed study, to be barochory. The morphological and physiological features of *C. japonica* seeds, which are large and nutritious, suggest zoochorous dispersal, however. We compared actual distances between mother trees and seedlings with distances attributable to gravity dispersion only, to test the zoochory hypothesis of *C. japonica*. The animals that transport the seeds for caching were identified experimentally. We also examined the extent to which seed dispersal is affected by the behavior of animal vectors. Seed dispersal by *Apodemus speciosus* was confirmed by taking photographs of animals that were consuming seeds experimentally deposited on the ground. *Camellia* seeds hoarded by the rodents under the litter or soil were protected from drying. On the basis of microsatellite analysis of maternal tissue from the seed coat, the mother trees of 28 seedlings were identified. Maternity analysis revealed the average seed-dispersal distance from mother trees was 5.8 m ± 6.0 SD, a distance greater than initial dispersal by gravity alone. These results indicate that *C. japonica* is a zoochorous species dispersed by *A. speciosus*. Fifty percent of the seed dispersal occurred from mature evergreen forests to dwarf bamboo thickets. This directional seed dispersal would contribute to range expansion of *C. japonica*. Home range sizes of *A. speciosus* were 0.85 ha at most and covered with different types of vegetation, from evergreen forests to grassland. This low specificity of their microhabitat use might enhance seed dispersal to different types of vegetation.

H. Abe (✉) · M. Hasegawa
Laboratory of Geographical Ecology, Toho University,
2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan
E-mail: aag62170@pop02.odn.ne.jp
Tel.: +81-47-4721162
Fax: +81-47-4721162

R. Matsuki · M. Nashimoto
Environmental Science Research Laboratory,
Central Research Institute of Electric Power Industry,
Chiba 270-1194, Japan

S. Ueno
Tree Genetics Laboratory, Department of Forest Genetics,
Forestry and Forest Products Research Institute,
Ibaraki 305-8687, Japan

Introduction

Seeds are important in the life history of rooted plants. They establish new generations in locations where the seedlings can grow successfully (e.g. Willson and Traveset 2000; Wang and Smith 2002). Plants have evolved many adaptations that promote movement of seeds away from the parent. For example, some plants dispersed by animals bear fruit with high nutrient value as a reward to animals which, consequently, transport them (e.g. Harper et al. 1970; Van Der Pijl 1982; Stiles 2000; Wenny 2001). On the other hand, seeds categorized as barochory (dispersal by gravity) are extremely large and nutritious. For those reasons, they might provide a high-quality food resource for animals. Van Der Pijl (1982) suggests that the seeds of most plants assumed to be barochory are frequently dispersed by scatter hoarding animals. It is important to reveal whether secondary dispersal by animals substantially affects the locations at which seeds germinate and the distance the seeds are dispersed from the mother trees. Those characteristics of seed dispersal might ultimately determine the plants' reproductive success, speed of range expansion, and population genetic structures (e.g. Hamrick et al. 1993;

Stiles 2000). They not only identify animal species precisely as secondary dispersal agents, therefore, but the place, distance, and direction of seed dispersal can also be estimated accurately.

Camellia japonica, a common evergreen tree in Japan, bears large and oily seeds (Xu et al. 1995; Katsuta 1999). This species has been the subject of genetic studies of population structure in recent years (Wendel and Parks 1982, 1985; Ming and Zhang 1996; Oh et al. 1995, 1996; Chung and Kang 1996; Yeeh et al. 1996; Chung and Chung 2000; Ueno et al. 2000, 2002; Chung et al. 2003) but its mode of seed dispersal has been simply assumed to be barochory without further detailed study (Chung and Chung 2000; Ueno et al. 2000, 2002).

The morphological and physiological features of seeds dispersed by scatter hoarding, which are large and calorie-rich, etc., have been summarized in many studies (Van Der Pijl 1982; Gautier Hion et al. 1985; Yumoto 1992; Vander Wall 2001; Forget and Wenny 2005), however, and these characteristics are applicable to *Camellia* seeds. Thus *C. japonica* seems to be classifiable as a zoochorous species.

The initial objective of some studies of large-seed dispersal by scatter hoarding was to identify which animal species or groups are seed dispersers. Such studies have also been intended to measure independently the distance between the places seeds were artificially deposited and those cached in the forest floor by animals (e.g. Miyaki and Kikuzawa 1988; Sone and Kohno 1996; Ouden et al. 2005). Recently, actual seed dispersal has also been studied by molecular methods. Dispersal distances of seeds are measurable by identification of mother (source) trees and their offspring (seedlings) by comparing particular sequences of source plant DNA with those of DNA extracted from the seed endocarps, which is maternal tissue (Godoy and Jordano 2001; Suyama 2004; Grivet et al. 2005). Notwithstanding, few studies simultaneously identify animal dispersers, measure seed transportation distance by animals, and estimate seed-dispersal distance with molecular identification of mother trees and seedlings.

The primary purpose of this study was to test the zoochory hypothesis for *C. japonica* by comparing the distances between mother trees and seedlings identified by molecular techniques with the distances of seeds dispersed solely by gravity. The second objective was experimental identification of the animals that transport the seeds for caching. Finally, we discuss the extent to which seed dispersal is affected by behavior of animal vectors and the extent that these vectors increase dispersal distance beyond barochory.

Materials and methods

Natural history of the study system

Camellia japonica is a tree species common in the broad-leaved evergreen forests of northeast Asia, distributed from Ryukyu to Aomori prefecture of Japanese main islands and the southern coastal region of the Korean peninsula (Wendel and Parks 1985; Katsuta 1999). This species is widely exported to Europe and America as a garden ornamental tree. The flowering season is very long on Niijima Island—from late October to early April. *Camellia* trees produce large apple-shaped fruits called capsules from which three to seven mature seeds are dropped on to the ground in late summer (Saito 1992). Weights of individual seeds are 0.8–1.5 g (Katsuta 1999), and these large seeds are highly nutritious because their succulent and oily hypogeal cotyledons contain 11.6–52.6% w/w kernel oil (Xu et al. 1995), protected by a hard seed coat. The presence of two seed predators, the Japanese wood pigeon (*Columba janthina*) and the large Japanese wood mouse (*Apodemus speciosus*), has been confirmed on Niijima Island (Yamashina 1936; Iwasaki 1998; Abe 2004). Of these seed-disperser candidates, *A. speciosus*, for which caching behavior has been observed, is a possible dispersal agent (Miyaki and Kikuzawa 1988; Iida 1996; Sone and Kohno 1996; Hoshizaki et al. 1999; Sato 2000; Seiwa et al. 2002a). The wood pigeon can be excluded, however, because it does not cache seeds and because the seeds, once consumed, cannot be passed intact through the pigeon's powerful gizzard (Yamashina 1936; Iwasaki 1998).

Study site

Field study was conducted on Niijima Island (34°22′N, 139°16′E), located 150 km south of Tokyo, Japan (Fig. 1). The annual mean air temperature is 17.3°C;
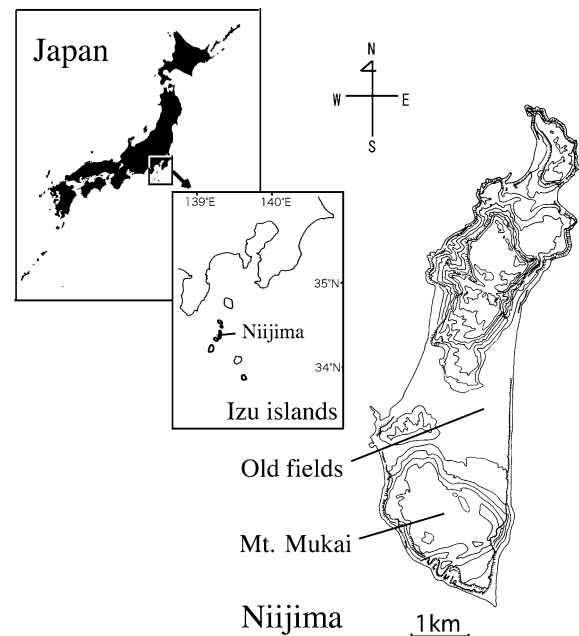

Fig. 1 Map showing two study plots (old fields in the central plain and dwarf evergreen forest on Mt. Mukai) on Niijima Island, the Izu Islands, Japan

rainfall is 2356 mm annually. The dominant tree species in the undisturbed forest is the evergreen oak *Castanopsis cuspidata*, but *C. japonica* is distributed throughout the island (Ohyama 2001).

Two study sites for seed dispersal were established on Niijima Island. One was located in old fields with secondary forests, with a variety of succession stages, at 30 m altitude located in the central plain between the two volcanic mountains. The other was in the dwarf evergreen forest on Mt. Mukai, at an altitude of ca. 280 m (Fig. 1). The central plain of Niijima Island was cultivated extensively as vegetable fields in the late 1940s. Most of the fields have been abandoned, however, and the timing of abandonment and the location of fallow fields and secondary forests was determined by examining aerial photographs taken in 1947, 1968, 1978, 1990 and 2000 (Geographical Survey Institute). Since abandonment, vegetation of the old fields has changed gradually to grassland, dense thickets of dwarf bamboo (*Pseudosasa japonica*), and deciduous broad-leaved forests of *Alnus sieboldiana*. They eventually became broadleaved evergreen forests—this region's climax vegetation.

The first study site on Niijima Island consists of the old field plots abandoned 30 years ago, and adjacent evergreen broad-leaved forest. Vegetation of the old fields is mainly bamboo bush; some saplings and small mature trees of *C. japonica* also occur there. Close examination of aerial photographs proved that the adjacent evergreen broad-leaved forest has existed since 1948 at least. Many mature trees of *C. japonica* are more than 50 years old.

The second site was on Mt. Mukai, where a volcanic eruption occurred in the late ninth century. The forest of Mt. Mukai consists of evergreen broad-leaved trees that resemble those of the central plain. *C. cuspidata*, *Machilus thunbergii*, *Elaeocarpus sylvestris*, *Clethra barbinervis*, and *Pinus thunbergii* are present in the canopy layer, and *Daphniphyllum teijsmannii*, *Ilex integra*, *Eurya japonica*, *C. japonica*, *Myrsine seguinii* in the sub-tree layer and shrub layers (Ohyama 2001). In this region *C. japonica* is a canopy tree, however, because the forest canopy height is sufficiently low (less than 10 m). *Descoreba simplex* moth larvae reproduced explosively during 2000–2003 and extensively defoliated *C. japonica*, exclusively, on Mt. Mukai.

Material trees in old fields

This study designated mature trees as those that set fruit in late summer or that blossom during the winter and early spring. First, we plotted the location of mature trees on the map in old fields (approx. 700 m²); a leaf was sampled from each mature tree for DNA identification in March 2003. In addition to leaf sampling, leaves of mature trees in adjacent evergreen broad-leaved forests (approx. 300 m²) were also sampled for additional DNA analysis in September 2003 (Fig. 2). Minimum ages of mature trees were estimated by close
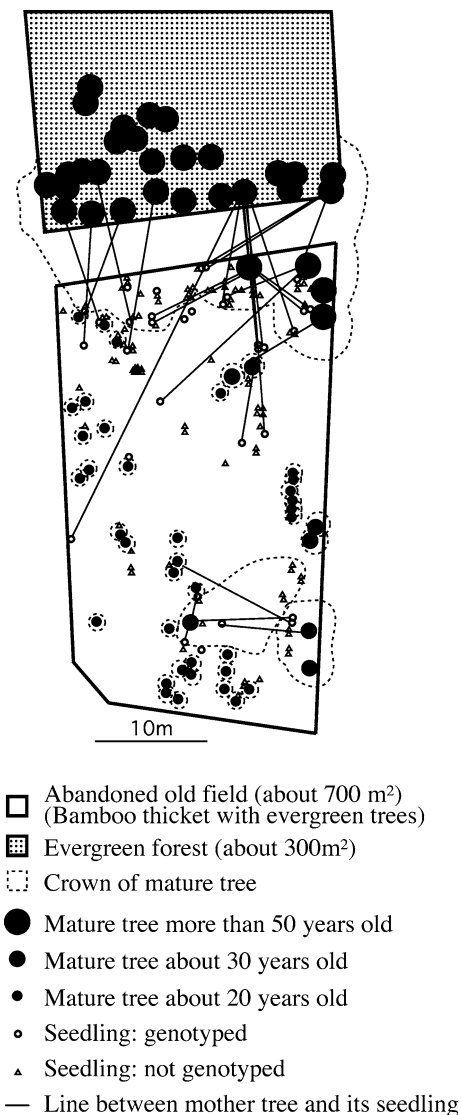

Fig. 2 Map showing the spatial relationships, dispersal events, and distances between mother trees and their seedlings, determined by use of microsatellite DNA in old fields

inspection of aerial photographs from 1947, 1968, 1978, 1990, 1999 and 2000 (Geographical Survey Institute). If the tree crowns were clearly visible in the aerial photographs, we defined the ages of these trees as equal to the years the photographs were taken. Results revealed 46 mature trees in the old field plot and 27 mature trees from the adjoining evergreen forest. Inspection of aerial photographs revealed that four mature trees estimated to be more than 50 years old were present in the study plot before abandonment of the vegetable field. Of 73 mature trees at the study site, seven were estimated to have been established 30 years before, and 35 trees were established 20 years after abandonment (Fig. 2).

Dispersion patterns, locations, and genetic characteristics were studied for the seedlings present in the study plot. The location of all seedlings less than 3 years old was plotted on the map (Fig. 2). Their seed coats were sampled in March and June 2003 to extract DNA.

Because the seed coat is maternally derived from fruit tissue, matching of multilocus genotypes of seed coat and mother tree leaves can be used to identify parent–offspring relationships with high reliability. Ages of seedlings were determined by examining the number of bud-scale scars. Seedling ages were designated as the first, second, or third-year since germination.

Initial seed dispersal by gravity

Initial seed dispersal by gravity was studied for seeds falling from the ten mature trees of *C. japonica* in the week of 14–25 September, 2002, in terms of the distance between the fallen seeds and the canopy edges of tentative mother trees. The mean height of the sampled trees was 420 cm $\pm$ 150 SD (range 250–600 cm); the maximum diameter of the crowns averaged 230 cm $\pm$ 162 SD (range 100–600 cm). Seeds that had fallen to ground with little understory vegetation and were located within the tree canopy were counted separately from those outside the canopy edge. Data were obtained during days when a typhoon with wind velocity 11 m s^{-1} hit Niijima Island during the survey. For that reason, dispersal distance by gravity might be somewhat overestimated.

Use of microsatellite DNA for identification of mother trees and their seedlings

Plant tissue (seed coat and leaf) was crushed with a Mixer Mill (MM300; Retsch). DNA was extracted both from the leaves and from seed coats using a DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. DNA from seed coats was also purified by use of a QIAquick PCR Purification Kit (Qiagen).

Six microsatellite markers developed for *C. japonica* (MSCjaF25, MSCjaF37, MSCjaH38; Ueno et al. 1999; MSCjaQ11, MSCjaR2 and MSCjaT25; DNA Data Bank of Japan, accession numbers AB211361–AB211363) were used for parent–offspring identification. PCR analysis was performed with a PCR Thermal cycler (TP600; Takara Bio). Amplification products were analyzed by use of an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Individual genotypes were determined by using GeneScan Analysis software ver. 3.1 (Applied Biosystems). We identified the seed and mature tree as parent and offspring when the genotypes of seed coats were identical with those of the mature tree. Only samples for which the same multilocus genotypes were observed at least twice were used for parent–offspring identification to avoid genotyping errors at this stage of analysis.

Identification of seed disperser and measurement of the transported distance

Photographic shots of potential seed dispersers were taken by sensor cameras placed in front of *Camellia* seed bait deposited on the forest floor. This procedure was called the Bait Photo shot method, abbreviated BP method. In addition to the BP method, the spool and line method (Yasuda et al. 1991) was used to assess seed dispersal. Polyester thread 15 m long and a spool coated with vinyl tape were prepared for these purposes. Straight-line distances of the threads were measured 1–7 days after setting up the seeds; the conditions of seeds were evaluated and classified into five categories: eaten, scatter hoarding, larder hoarding, carried-only seed, and intact (not carried). Scatter hoarding is defined as the caching of separate seeds; larder hoarding is caching of more than two seeds at the same point.

On Mt. Mukai, we performed the BP method on 17 nights from September 2002 to April 2003. On each sampling date 20–50 seeds were placed as bait; a total of 600 seeds was used. The spool and line method was applied four times during the period; a total 280 seeds with spools was placed.

In the old field study plot of the central plain, the BP method was used to identify seed dispersers on three nights—1 April and 29 August 2002, and 8 September 2003. A total of one-hundred and twenty seeds was used. To measure carriage distances of seed dispersal, 20 seeds attached with thread to a spool were placed on 8 September.

Home ranges of seed disperser

Radiotelemetry studies were conducted to examine patterns of space used by *A. speciosus*, a candidate seed disperser of *C. japonica*. We radio-tracked *A. speciosus* for 2 months of the fruiting season from 15 August to 15 October 2002 in the old field study site at which relationships between mother trees and seedling were investigated (Fig. 2). Mice for the radio-tracking study (1.5 ha) were captured by use of 60 aluminum live traps placed at 10 m intervals in the area (0.5 ha) inside the site. Live trapping was conducted for 21 nights (three nights of 14–17 August, 5 nights of 20–25 August, 6 nights of 31 August–6 September, and 7 nights of 9–16 September). Five types of vegetation cover occur there—evergreen forest, deciduous forest, bamboo thicket with evergreen trees, grassland, and others (road and farming facilities) (Fig. 3).

Transmitters (Sirtrack) emitting different radio frequencies between 144.0 and 144.4 MHz, mounted on collars weighing less than 2 g, were attached to adult mice of more than 30 g body mass. The tagged mice were released at the site of capture and were tracked at three different times from sunset to midnight (19:00, 21:00 and 24:00) for active mice and once during daytime for inactive mice. The home ranges of mice that were tracked continuously for about 1 month were analyzed using minimum convex polygons. Equipment for radio tracking included a receiver (FT-817; Vertex Standard) with three hand-held Yagi aerial-antenna elements.
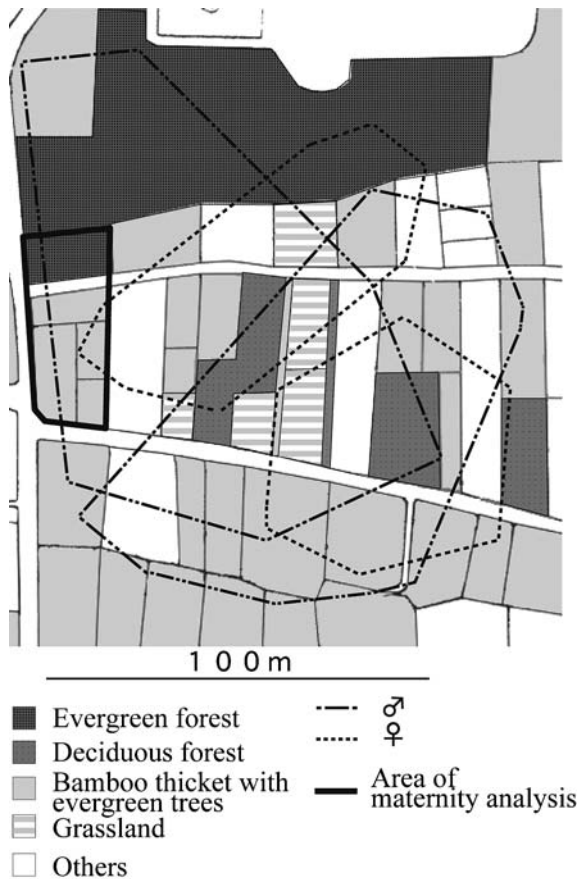
Fig. 3 Home ranges of *Apodemus speciosus* followed with radiotelemetry in the late summer of 2002 at the old field study plot of Niijima Island, the Izu Islands

Results

Initial seed dispersal by barochory

During the fruiting season of 2002, 5,649 seeds were counted as fallen from ten mature trees. Of those, 81.6% were located under the canopy of their presumed mother trees; only 0.2% were dispersed more than 2 m from the edge of the tree canopy (Fig. 4a).

Seed-dispersal distance revealed by identification of mother trees and their seedlings

Genotypes were determined for 73 mature trees using six microsatellite loci as genetic markers. All 73 trees were identified individually using this combination of microsatellite markers. In all, 126 seedlings of the first, second, and third years of germination were found in the study plot by late May of 2003: 84 seed coats were selected from those seedlings. Genotypes were identified for 34 of 84 seed coats with six microsatellite loci; 34 seedlings were identified individually. Of those 34 seedlings, 28 were matched to genotypes determined for the mature

trees in the study plot or the area adjacent to the plot. These 28 pairs were recognized as mother trees and their seedlings (Fig. 2). Six seedlings did not match the mature trees at the study site.

Identification of mother trees and their seedlings enabled us to directly measure seed-dispersal distance. The average dispersal distance from the canopy edge was 5.79 m ± 5.96 SD (range 0–29 m) (Fig. 4c). Of 28 seedlings, 22 were dispersed from mature trees that were older than 50 years; 50% of seedlings in the old field plot were dispersed from the mother trees located in the nearby evergreen broad-leaved forest (Fig. 2). Of 28 seedlings, 23 that had the same multilocus genotypes as mature trees were located at distances of more than 2 m from the respective canopy edges of the mother trees (Figs. 2, 4c).

Identification of the seed disperser by BP method

On Mt. Mukai, all 600 seeds placed for 17 nights were removed. Three-hundred and five images were obtained with a sensor-activated camera; of these, 274 showed images of animals. *A. speciosus* mice appeared in 271 images (98.9%), and 44 of the 271 images showed the mice carrying seeds with their jaws. Other animals photographed were a cat (twice, 0.8%) and a Japanese white-toothed shrew, *Crocidura dsinezumi* (once, 0.4%).

In the old field study site, 12 of 120 seed sets were carried away during three nights. Only 16 images were obtained using the sensor-activated camera. Three animal species were shown in eight of the sixteen photographs. Of these, four shots were of lizards and two each were of *A. speciosus* and *C. dsinezumi*.

Transported distance and destination as determined by the spool and line method

All 280 seeds attached by thread to a spool were transported on Mt. Mukai. Of 33 seeds later discovered, nine had already been consumed, 22 were stocked as larder hoarding at three different places, and two were stocked separately as scatter hoarding.

The average distance carried for larder hoarding was 1.5 m ± 0.6 SD (range 1–3 m) (Fig. 4b). All seeds were hoarded under rocks. In the case of scatter hoarding, each seed was discovered under leaves. The distances carried were 4 and 6 m (Fig. 4b). Six months later these seeds had disappeared from the original hoarding location. The remaining 247 seeds were not tracked to their destinations; 24 seeds were lost with their spool thread and 223 were carried away after the spool thread had fallen off.

At the old field site, 18 of 20 seeds with attached spool thread were transported. The places of hoarding of four of those 18 seeds were later discovered. One had been consumed and three seeds were hoarded separately at three different places. Larder hoarding was not
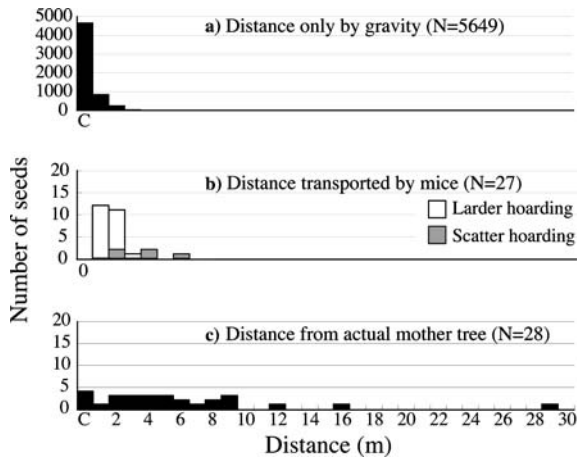
Fig. 4 Seed-dispersal distance by four methods. **a** Distance transported by *A. speciosus*, by use of the spool and line method, **b** distance between seedling and nearest mother tree, and **c** distance from the actual mother tree as determined by DNA analysis. *C* indicates locations under the crown of the mother tree

Fig. 5 Tracked points of *Apodemus speciosus* ($N = 4$) by use of radiotelemetry in five vegetation types: evergreen forest 0.38 ha (evergreen), deciduous forest 0.13 ha (deciduous), bamboo thicket with evergreen trees 0.60 ha (bamboo), grassland 0.08 ha, and areas with road and farming facilities 0.29 ha (others). The numbers of tracked points for each mouse were $a = 47$, $b = 70$, $c = 58$, and $d = 60$. The values are corrected for the sizes of each type of vegetation

observed at the old field site. Distances carried for scatter hoarding were 2, 2 and 4 m (Fig. 4b). All were buried in soil at depths of 2 cm. A month later, one of the three buried seeds was observed to have germinated.

Home range of *A. speciosus* as determined by radiotelemetry

In the study plot, 19 *A. speciosus* (♂10, ♀9) were captured. Transmitters were attached to 17 adult mice weighing more than 30 g (♂9, ♀8) to track their movement and spatial location. Of these tracked mice, two died within 2 days and eleven were tracked in the study area for less than 1 week.

Four mice (♂2, ♀2) were tracked for approximately 1 month (26–33 days); the frequency of space use was determined for each type of vegetation (Fig. 5). The mice used different vegetation types from evergreen forest to grassland. The minimum convex polygons method showed the home ranges of the mice were 7,906 m² (diameter 100 m) and 8,500 m² (diameter 104 m) for males, and 3,062 m² (diameter 62 m) and 3,175 m² (diameter 64 m) for females (Fig. 3).

Discussion

Generality of seed dispersal by *A. speciosus*

This study was primarily intended to test the zoochory hypothesis of *C. japonica*. It was shown that:

1. Results from the spool and line method confirmed evidence in photographic images (BP method) of *A. speciosus* handling and hoarding seeds; and
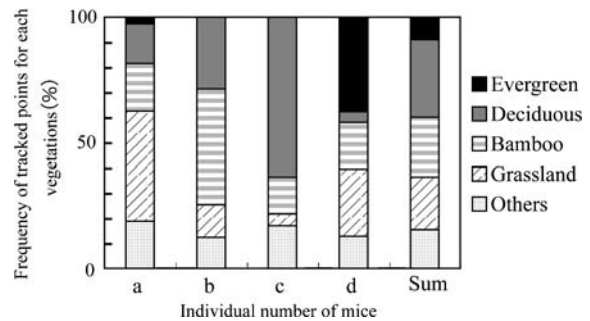
2. Dispersal distances estimated by the spool and line method and the molecular method were greater than initial dispersal solely by gravity.

These results indicate that *C. japonica* is actually a zoochorous species. It is also doubtful this close biological seed-dispersal interaction between *C. japonica* and *A. speciosus* is a special case in an insular environment on Niijima Island. The seed dispersal interaction between *C. japonica* and *Apodemus* mouse is prevalent and general, as argued below.

Apodemus mice cache large seeds, for example nuts (Miyaki and Kikuzawa 1988; Iida 1996; Sone and Kohno 1996; Hoshizaki et al. 1999; Abe et al. 2000; Sato 2000; Seiwa et al. 2002a). Their distribution in Japan, South Korea, and China overlap greatly with *C. japonica* (Xia 1985). In recent years, evidence that *Camellia* seeds are dispersed by *Apodemus* mice has rapidly accumulated from field studies. By use of a video camera, Tamura et al. (2005) recorded the process of animals removing seeds in Hachioji city, Western Tokyo, and she showed that *C. japonica* seeds were transported by Japanese field mice only. We have also confirmed *Camellia* seed dispersal by *Apodemus* mice using a sensor camera system on Miyake-jima, one of the Izu Islands, and on Tsushima Island, located between Kyushu and Korea (Abe et al. unpublished).

Second, the morphological and ecological features of seeds dispersed by scatter hoarding have been summarized in many studies (Van Der Pijl 1982; Gautier Hion et al. 1985; Yumoto 1992; Vander Wall 2001; Forget and Wenny 2005). These characteristics are applicable to *Camellia* seeds. According to Yumoto's review, seeds that are hoarded are large and have high nutritive value. Typically, the outer coat of the seed fruit is hard. Plants produce many seeds and mast seeding is frequent. The fruits fall when they have ripened, seeds can germinate even if a substantial part of the seed has been eaten, and few seeds are protected by potent chemicals. In addition, Saito (1992) suggested that the hypogeal cotyledon is

characteristic of scatter hoarding. *C. japonica* produces large and highly nutritional seeds that are protected with a hard seed coat; the nutritional part of the seed is the cotyledon (Katsuta 1999). The seeds leave the mother trees in fruits that have ripened (Katsuta 1999). Although *Camellia* seeds have some defensive chemical—a triterpene saponin (Xu et al. 1995; Katsuta 1999; Yoshikawa et al. 2001)—this sort of defensive chemical is not sufficiently potent to prevent *A. speciosus* from eating *Camellia* seeds. *Apodemus* mice are resistant to some plant secondary compounds because other plant secondary compounds (saponin and tannin) from the seeds of *Quercus serrata*, *Quercus crispula*, and *Aesculus turbinata* are not deadly for *A. speciosus* (Shimada 2001). Consequently, almost all seed characteristics are valid for *Camellia*, and *C. japonica* seeds would evolve to accommodate the hoarding habits of *Apodemus* mice.

In other geographic areas, however, *C. japonica* might be dispersed by other animals. For example, Cheng et al. (2005) reported that seed dispersal of *Camellia oleifera* was mediated by mice in China. Kondo et al. (1982) suggested that the seeds of *Camellia* species including *C. japonica* are often eaten by *Microscelis amaurotis amaurotis* in captivity, and usually passed through the digestive tract without being killed (Kondo et al. 1982). Higuchi (1975) observed that *Parus varius owstoni* in Miyake Island hoarded the seeds of *C. japonica*, whereas the subspecies *Parus varius varius* in the mainland did not. These reports suggest that some rodents and birds also contribute to the seed dispersal for *Camellia* species by ingestion-defecation or seed-hoarding. Thus it is necessary to identify the dispersal agents in other areas and clarify the qualitative and quantitative contribution to the range expansion or metapopulation dynamics of *C. japonica*.

Effects of seed dispersal by *A. speciosus*

Apodemus speciosus used a variety of vegetation types from evergreen forest to grassland (Fig. 5). Low specificity of macrohabitat use by *A. speciosus* might enhance seed dispersal to a variety of successional stages and range expansion of *C. japonica*. Actually, in the preliminary survey, *Camellia* seedlings were found in a variety of types of vegetation. For some, there were few source mature trees nearby. Moreover, half of seed dispersal occurred from the late to early succession stages (Fig. 2). Seed dispersal from the source forest to early succession stages, for example gaps and grasslands has been attributed to rodents (e.g. Abe et al. 2000; Seiwa et al. 2002a; Li and Zhang 2003; Gorchov et al. 2004).

Second, *A. speciosus* buried *Camellia* seeds under litter or soil in scatter hoarding; one seed was observed to germinate. Buried seeds are more likely to establish new plants than unburied seeds (Seiwa et al. 2002b). The germination rates of *C. japonica* decrease in dry conditions (Kayumi and Shibata 1989). Therefore, burial by *A. speciosus* served to prevent the seeds from dying. In

comparison with dispersal by gravity alone, secondary seed dispersal and subsequent burial by *A. speciosus* might help *Camellia* seeds survive and, subsequently, promote their germination. However, *C. japonica* is not a pioneer species. Early succession stages, for example bamboo thickets and grassland, might reduce the seedling recruitment probability by increasing competition for water or nutrition. Future studies will clarify if seed dispersal to different types of vegetation facilitates survival of *C. japonica* seedlings.

Effectiveness of maternity analysis by microsatellite analysis using maternal tissue

It is important to estimate actual realized seed-dispersal distances in natural communities because only 11% of the nearest mature trees of the seedlings matched their actual mother trees (Fig. 2). Even if the seedlings occur within the crown of a mature tree, they might not be a mother and offspring pair.

Genetic analysis has been used to estimate seed dispersal (e.g. Schnabel et al. 1998; Godoy and Jordano 2001; Jordano and Godoy 2002; Richardson et al. 2002; Nathan et al. 2003; Grivet et al. 2005), but different problems arise in such studies. Ouborg et al. (1999) reviewed the use of molecular markers in population genetics approaches to study seed dispersal. According to their review, cytoplasmic markers could be used to exclude maternity, but they usually lack sufficient variation for individual maternity assignment. In contrast, microsatellite markers are suitable for parental analysis, because they are polymorphic genetic markers. It is, however, difficult to distinguish the father and mother in cosexual species when both parents are unknown (Godoy and Jordano 2001; Jordano and Godoy 2002; Suyama 2004; Grivet et al. 2005). In this study the paternity-exclusion probability for the first parent was 0.8588 ($N = 73$). The probability of correctly assigning all mothers for 28 seedlings was therefore $0.8588^{28} = 0.0141$. When we used seedling tissue to estimate the candidate parents by the maximum likelihood method, using Cervus ver. 2.0 (Marshall et al. 1998), 14 out of 28 mother trees were not selected as the two most likely parents for each seedling. That method is, therefore, insufficient for individual maternity assignments in this study. Microsatellite analysis using maternal tissue is, in contrast, a superior technique for determination of actual mother and seedling relationships, even when the paternity exclusion probability is low. It depends, however, on maternal tissue still being attached to the seedling (Ziegenhagen et al. 2003).

Sampling area for estimating the seed-dispersal distance

In this study, six of 34 genotyped seedlings might have been dispersed from the area outside of the study site because their genotypes did not match those of mature trees at the site. Genotypes of mother trees inhabiting a

much larger area should be examined to provide satisfactory datasets of dispersal distance. It is useful to determine the sampling area from the standpoint of animal vector behavior.

Studies of the home range and movement pattern of animals can provide indirect estimates of the distances to which the seeds are dispersed. Theoretically, the seed-dispersal distance should be proportional to home range area of animals, but few studies have actually compared home range size and seed-dispersal distance. In this study, the home range size of dispersal agents was 8,500 m² with a diameter of 104 m at most. Maximum distances of *C. oleifera* to search for seeds marked by tagging are 32.5 m in primary hoarding and 38.0 m in secondary (Xiao et al. 2004). According to information related to large-seed dispersal by *Apodemus* mice in Japan, the maximum value is 23.5 m for *Pasania edulis* marked by transmitters (Sone and Kohno 1996), 38.5 m for *Q. serrata* by magnets (Iida 1996) and 114.5 m for *A. turbinata* by ink (Hoshizaki et al. 1999). These values are shorter or similar to the home range diameters estimated in this study. The home range size therefore seems to be good index for determining the search area for a seed-dispersal study. Home ranges of *Apodemus* mice overlapped between sexes and among males, however (Fig. 3); also, *Apodemus* mice are known to scatter hoarded seeds repetitively (Seiwa et al. 2002a; Vander Wall 2003; Xiao et al. 2004). There is, therefore, a possibility of secondary caching by other mice individuals. Considering the home range size and extent of home range overlap, more than 1.5 ha surrounding the intensive study plot where seedlings were mapped should be surveyed to determine the exact locations and genotypes of mother trees in this study.

Appendix

Table 1

Table 1 DNA analysis of mature trees ($N = 73$) for six microsatellite loci

Locus	Number of alleles	H_e[a]	H_o[b]
MSCjaF25	5	0.557	0.534
MSCjaF37	8	0.706	0.630
MSCjaH38	8	0.717	0.685
MSCjaT25	6	0.715	0.630
MSCjaQ11	9	0.756	0.397
MSCjaR2	6	0.684	0.589

[a]Effective no. of alleles
[b]Observed heterozygosity

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