

Jun Yokoyama

Cospeciation of figs and fig-wasps: a case study of endemic species pairs in the Ogasawara Islands

Received: 8 October 2003 / Accepted: 10 November 2003 / Published online: 5 December 2003
© The Society of Population Ecology and Springer-Verlag Tokyo 2003

Abstract The interactions between figs (*Ficus*, Moraceae) and fig-wasps (Agaonidae, Chalcidoidea, Hymenoptera) are special plant-pollinator relationships that are highly species-specific, in that each fig species is pollinated by a single fig-wasp species that can breed only in that particular fig species. This study examined the degree of pre-mating isolation and genetic differentiation for three fig/fig-wasp pairs endemic to the Ogasawara Islands. Simple Y-tube tests revealed that fig-wasps from *Ficus nishimurae* and “Higashidaira type” chose their own host figs significantly more often, while fig-wasps from *F. boninsimae* did not. Based on RAPD markers, the genetic differentiation among the fig species was low, but *F. boninsimae* formed a cluster within the *F. nishimurae* cluster. The Higashidaira type has been derived from a subpopulation of *F. nishimurae*. Five mitochondrial DNA haplotypes were found in the fig-wasps and each haplotype correlated well with the fig species on their island of origin. These results led to a three-step hypothesis on the cospeciation process: (1) spatial separation of fig/fig-wasp populations arises; (2) gene flow is restricted and character differentiation of fig-wasps occurs; (3) there is further restriction of gene flow and genetic and character differentiation of figs.

Keywords Host recognition · Mitochondrial DNA · Oceanic islands · RAPD · Speciation

Introduction

In terrestrial ecosystems, insects and land plants have the greatest species diversity, with insects comprising

over half of the recorded macroscopic organisms and land plants accounting for half of the remainder. There are close ecological relationships between the two groups of organisms, such as between insect herbivores and their food plants or between entomophilous plants and their insect pollinators. Therefore, insects and land plants have probably each had great effects on the evolution of the other, and coevolution is considered one of the most important processes contributing to the current diversity of both groups of organisms (Ehrlich and Raven 1964; Bock 1972; Mitter et al. 1988; Thompson 1989; Farrell et al. 1991, 1992; Mopper and Strauss 1998). To reveal how this diversity has arisen in the course of insect-plant coevolution, accurate information on phylogenies in both groups of organisms is indispensable. In addition, detailed knowledge of the morphological, biochemical, or behavioral traits related to insect-plant interactions and their genetic background is also necessary for studying coevolution and evolutionary changes of traits that sometimes lead to speciation (Thompson 1986, 1994).

An interaction between figs (*Ficus* spp., Moraceae) and fig-wasps (Agaonidae, Chalcidoidea, Hymenoptera) is a special plant-pollinator relationship in which fig-wasps are both seed parasites and pollinators of figs. These interactions are highly species-specific in that each species of fig is pollinated only by a single species of fig-wasp, and each fig-wasp species can raise their larvae only in the flowers of that particular species of fig. No other organisms can replace the partnership between these two organisms; therefore, this interaction is considered an extreme case of obligate mutualism (Ramirez 1974; Wiebes 1979; Berg 1990; Yokoyama 1995; Herre et al. 1996; Anstett et al. 1997; Machado 2001; Weiblen 2001; Weiblen and Bush 2002; but see Molbo et al. 2003).

The marked species specificity of this inseparable interaction indicates that diversification of both figs and fig-wasps proceeds simultaneously, and that cospeciation occurs during the diversification process. The process of cospeciation in this interaction, however, is

J. Yokoyama
Graduate School of Life Sciences, Tohoku University,
Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan
e-mail: jyokoyam@mail.tains.tohoku.ac.jp
Tel.: +81-2-2176689
Fax: +81-2-2176689

uncertain. Speciation mediated by ecological interactions is considered one of the most important processes of diversification, especially in plant/insect interactions, in which close ecological relationships may promote extensive diversification of both organisms. However, this process of interaction-mediated speciation has not been well documented. The fig/fig-wasp interaction is an excellent system for studying this process. Therefore, I examined the process of cospeciation in the fig/fig-wasp interaction as a model case of speciation mediated by plant/insect coevolution.

To study the process of speciation, it is useful to study closely related species pairs derived from a common ancestor. For this purpose, I studied the fig species endemic to the Ogasawara Islands, which are typical oceanic islands about 1,000 km south of mainland Japan (Fig. 1). There are three endemic fig species on these islands; *Ficus iidaiana* Rehder et Wilson, *F. boninsimae* Koidz. and *F. nishimurae* Koidz. *Ficus iidaiana* is a tree species that grows to 10 m and is easily distinguished from other species morphologically (Yamazaki 1983; Yokoyama 2003). The species is reproductively isolated from the others since it is the only tetraploid species in Japan, while the others are all diploid (Ono 1990). By contrast, *F. boninsimae* and *F. nishimurae* are closely related to each other, but usually occupy different habitats: *F. boninsimae* occurs in open sites and *F. nishimurae* in the forest understory. They differ only in a few morphological characters (Toyoda 1981; Yokoyama 2003), and are sometimes considered the same species (e.g., Yamazaki 1983). There are also few qualitative differences in the morphological characters of the pollinating fig-wasps that use these two fig species. Therefore, the two diploid species, *F. boninsimae* and

F. nishimurae, are considered to have a very close common ancestor and to be in the process of speciation, making them suitable for studying cospeciation in fig/fig-wasp systems.

This study focused on the following two points to reveal the cospeciation process of diploid fig species in the Ogasawara Islands: (1) the mechanisms of pre-mating isolation of the two diploid fig species and the degree of difference in the mechanism, and (2) the degree of genetic differentiation in the figs and fig-wasps as an estimate of the level of gene flow in both figs and fig-wasps.

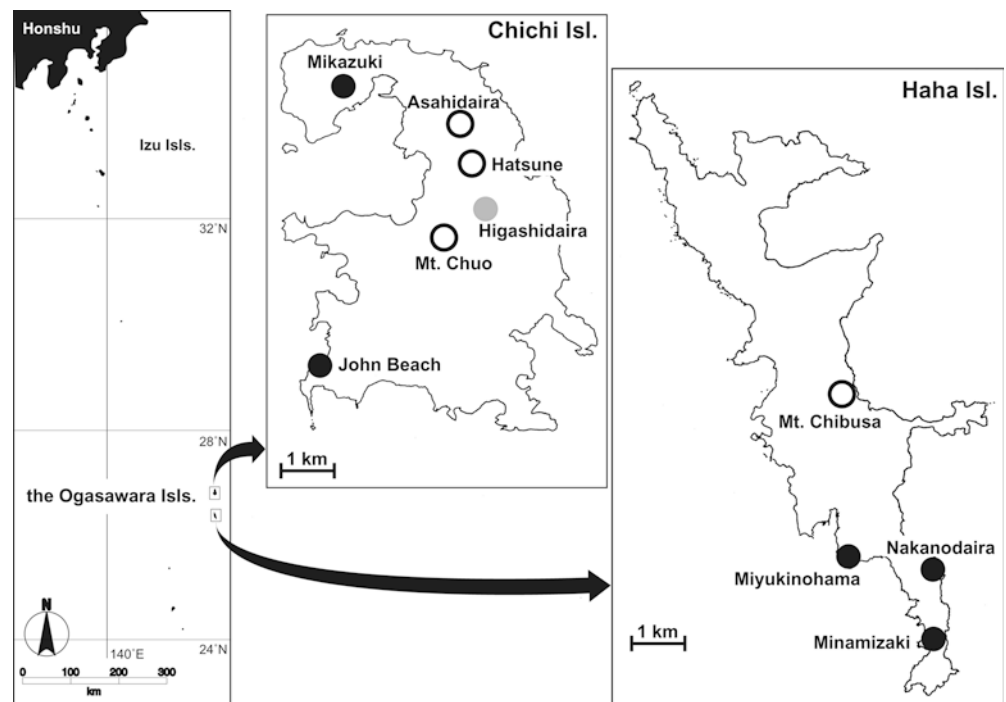
Materials and methods

Study organisms

Ficus boninsimae and *F. nishimurae* are two of the three species of figs endemic to the Ogasawara Islands (Toyoda 1981; Yokoyama 2003). Both species are dioecious (morphologically gynodioecious), diploid, and are shrubs or small trees up to 2–4 m tall. *Ficus boninsimae* grows in open sites as a shrub, while *F. nishimurae* occurs in the forest understory, especially along the coast. In central Chichi Island, I found an additional form of diploid fig that is similar to *F. nishimurae*, but has a unique set of morphological characters. Hereafter, I will refer to this fig as the “Higashidaira type” and analyzed it separately.

The fig-wasps of *F. boninsimae* and *F. nishimurae* belong to the genus *Blastophaga*. The wasp associated with each fig species is considered an undescribed species that is closely related to *B. nipponica*, the pollinator of *F. erecta* Thunberg (Yokoyama and Iwatsuki 1998; J. Yokoyama, unpublished data). Although the fig-wasp of *F. nishimurae* is slightly larger than that of *F. boninsimae*, the two wasps are morphologically similar and are difficult to distinguish by size. This study treated wasp samples based on the origin of their particular fig population.

Fig. 1 Location of the Ogasawara Islands and the sampling sites in this study



Testing host plant recognition by fig-wasps

In both figs and fig-wasps, host plant recognition by fig-wasps acts as a strict pre-mating isolation mechanism, because each species of fig is pollinated by only a single species of fig-wasp. The fig-wasps mate only inside the syconia of the host fig species. Fig-wasps recognize specific combinations of volatile chemicals released from the inside of receptive syconia of figs (Barker 1985; Ware et al. 1993; Hossaert-McKey et al. 1994; Ware and Compton 1994). I conducted simple Y-tube tests to reveal the host recognition of wasps from the Ogasawara Islands. The samples used in the test are listed in Table 1. A Y-shaped test tube (a straight 9-cm entrance part, and two 8.5-cm branches, with an inside diameter of 1.5 cm) was used for the selection experiments. Volatile chemicals were extracted from the figs by washing the inner surface of receptive syconia with ethyl acetate. Ethyl acetate was injected into receptive syconia via a hole opened at the top of the syconia with a glass capillary and collected using a mechanical pipette. About 50 receptive syconia were used to obtain 5 ml ethyl acetate containing volatile chemicals from *F. boninsimae*. To wash approximately the same area of inner surface of the syconia of *F. nishimurae* and Higashidaira type with ethyl acetate, 10–15 receptive syconia were used to obtain equivalent chemical samples, because the receptive syconia of these two figs are twice as long as those of *F. boninsimae*. A 1-cm² piece of filter paper infiltrated with 50–100 µl the extract was placed in each branch of the Y-shaped tube. Then a single fig-wasp was released at the entrance of the tube to see whether it would select the volatile sample from its specific host. The chi-square test was used to compare the results.

RAPD analysis of figs

Preliminary investigations of the genetic differentiation of the fig species endemic to the Ogasawara Islands based on RFLP analyses of chloroplast DNA showed no interspecific differences at more than 600 restriction sites (J. Yokoyama et al., unpublished data). Therefore, the degree of genetic differentiation among species or types of fig from the Ogasawara Islands was expected to be quite low. This study surveyed the genetic diversity of figs from the Ogasawara Islands using randomly amplified polymorphic DNA (RAPD) analysis. Sample sites and sizes are listed in Table 1. Total DNA was isolated from 200–300 mg fresh leaves using the modified 2× CTAB method (Hasebe and Iwatsuki 1990). The DNA samples were then resuspended in an appropriate volume of TE buffer and kept at –20°C until used.

The following twelve 10-mer primers from the Operon kit A (Operon Technology) were used for the analysis: OPA-3, 5, 6, 7, 8, 9, 10, 14, 16, 18, 19, and 20. The PCR reaction mix contained 100–200 ng total DNA, 1 µM each primer, 200 mM each deoxynucleotide, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, and 1.25 units of *Taq* polymerase. Double-stranded DNA was amplified by first incubating it at 94°C for 3 min, followed by 45 cycles of 94°C for 1 min, 37°C for 2 min, and 72°C for 3 min, and a final extension at 72°C for 15 min. The PCR products were

electrophoresed in 2.0% agarose gels. Profiles of the amplified products were recorded photographically. Each PCR was repeated at least twice for each primer to check the reproducibility of the profiles.

The genetic distance between individuals was calculated using the Nei and Li method (1979) with RESTDIST in PHYLIP 3.6a (Felsenstein 2000). A dendrogram was obtained using the UPGMA method with NEIGHBOR in the same program package. A single individual each of *F. erecta* from Ishigaki, Okinawa, and *F. iidaiana* from Haha Island were used as outgroups for the dendrogram.

Analysis of fig-wasp mitochondrial DNA

To examine haplotype diversification in the wasps, the nucleotides in the intergenic region between the mtDNA cytochrome oxidase subunit I and II genes (called *coxI* and *coxII* hereafter) were sequenced. Sampling sites and year of collection are listed in Table 1. From 1992 to 1994, 15–30 wasps from a single syconium were used for DNA isolation, because usually a single wasp enters each syconia, which is less than 1 cm in diameter (J. Yokoyama, personal observations). Five syconia from each sample site were used for DNA isolation. In the course of this study, it was found that the syconia of figs from the Ogasawara Islands sometimes contained a few wasps. Consequently, ten syconia were used to collect fig-wasps from each sample site and DNA was isolated from three wasps per syconium for the samples collected in 1995 and 1996 to avoid haplotype contamination. At Nakanodaira, only five syconia were sampled due to the small fig population. For the samples collected from 1992 to 1994, three independent amplifications were performed to see whether different fig-wasp haplotypes occurred within the same syconia.

Total DNA was isolated from each entire insect body using the method of Latorre et al. (1986) with the following slight modifications. The insects were ground with a pre-cooled disposable pestle in a 1.5-ml microcentrifuge tube containing 320 µl 10 mM Tris/60 mM NaCl/5% (wt/vol) sucrose/10 mM EDTA-2Na, pH 8.0. Then, 400 µl 1.25% SDS/300 mM Tris/5% (wt/vol) sucrose/10 mM EDTA-2Na, pH 9.0, were added and the mixture was incubated at 60°C for 30 min. The procedures following incubation were the same as in the original protocol. The DNA samples were resuspended in an appropriate volume of TE buffer and kept at –20°C until use.

DNA amplification using the PCR method (Saiki et al. 1988) was carried out in 50-µl reactions containing about 50–100 ng total DNA, 10 mM Tris-HCl buffer (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM each dNTP, 1.25 units *Taq* DNA polymerase (Takara), and 0.5 µM each primer (COint-F: 5'-CCACGACGTTATTCAGACTATC CAGAT-3', COint-R: 5'-TGGATATTCATATGATCAATAACATTG-3'). The primers used in this study were designed by comparing the *coxI* and *coxII* sequences of *Drosophila yakuba* (Clary and Wolstenholme 1985) and *Apis mellifera ligustica* (Crozier and Crozier 1993). The following thermal cycle profile was used for amplification: 1 min at 94°C, 2 min at 45°C, and 3 min at 60°C for 30 cycles, followed

Table 1 Samples of figs and fig-wasps used in this study

| Fig species | Islands | Locality | Y-tube test (figs) ^a | Y-tube test (fig-wasps) ^a | DNA (figs) ^b | DNA (fig-wasps) ^c | |
|-------------------------|-------------|--------------|---------------------------------|--------------------------------------|-------------------------|------------------------------|------------|
| <i>Ficus boninsimae</i> | Chichi Isl. | Asahidaira | + | + | 3 | 92–96 | |
| | | Hatsune | + | + | 1 | 92–96 | |
| | | Mt. Chuo | | | 1 | 93–96 | |
| <i>F. nishimurae</i> | Haha Isl. | Mt. Chibusa | + | + | 9 | 92, 93, 95, 96 | |
| | | Chichi Isl. | John Beach | + | + | 6 | 92–96 |
| | Chichi Isl. | Mikazuki | + | + | 1 | | |
| | | Haha Isl. | Minamizaki | + | + | 6 | 93, 95, 96 |
| | | Miyukinohama | | | 1 | 95, 96 | |
| 'Higashidaira type' | Chichi Isl. | Nakanodaira | | | | 96 | |
| | | Higashidaira | + | + | 2 | 93, 95, 96 | |

^a Localities of samples used for the test were indicated as +

^b Numbers indicate the individuals used in the analysis

^c Numbers indicate the year in which collections were made

by a 15-min extension at 72°C. The PCR products were electrophoresed in 1.0% agarose gels and purified using a GENECLEAN II kit (BIO 101) following the manufacturer's instructions. Cycle sequencing reactions were performed using approximately 200 ng purified PCR product and an AutoCycle sequencing kit (Pharmacia) or using approximately 80–100 ng purified PCR products for the PRISM Ready Reaction DyeDeoxy Terminator Cycle sequencing kit (Applied Biosystems) following the manufacturer's instructions. The DNA was sequenced using an ALF Autosequencer (Pharmacia) and a 373A DNA Sequencer (Applied Biosystems). Haplotypes were determined from the sequences. The ratio of each haplotype was calculated for each population in each collection year.

Phylogenetic analysis was carried out using the maximum parsimony method using PAUP* 4.0b (Swofford 1998). The nucleotide sequence of *Blastophaga nipponica* Grandi was used as the outgroup. All characters were weighted equally. The analysis was conducted using a branch-and-bound search with the TBR branch-swapping option.

Results

Tests of host-plant recognition by fig-wasps

In control experiments (fig volatile samples vs ethyl acetate), each fig-wasp selected the volatile sample from its own host (data not shown). In all combinations of trials, fig-wasps from *F. nishimurae* and the Higashidaira type selected their own host fig odors significantly more often, while wasps of *F. boninsimae* did not show specific preference for a particular fig odor (Table 2). These results indicate that host recognition by fig-wasps acts as an effective pre-mating isolation mechanism for *F. nishimurae* and the Higashidaira type, but not between *F. boninsimae* and the other types.

RAPD analysis of figs

Ninety-seven bands were detected using 12 random primers; 5 appeared in all samples including the outgroup, and 10 were specific to *F. erecta*. There were no species-specific bands for either *F. boninsimae* or

F. nishimurae, reflecting the low genetic differentiation between these species. The ratio of polymorphic bands was higher in *F. nishimurae* (64%) than in *F. boninsimae* (31%). The bands for the Higashidaira type were more similar to those of *F. nishimurae* than those of *F. boninsimae*.

Figure 2 shows the UPGMA dendrogram based on genetic distances calculated using the Nei and Li method (1979). *Ficus boninsimae* formed a single cluster within the *F. nishimurae* cluster. The Higashidaira type was relatively close to some *F. nishimurae*. In both *F. boninsimae* and *F. nishimurae*, no obvious clusters appeared in the dendrogram related to their geographic origins (e.g., for different islands).

Analysis of fig-wasp mitochondrial DNA

For the fig-wasps from the Ogasawara Islands, 692 bp of the *coxI-coxII* mtDNA region were sequenced. A comparison of the fig-wasp mtDNA nucleotide sequences distinguished five haplotypes. For Chichi Island, there were three haplotypes, which were completely correlated with the host fig species. Hereafter, these haplotypes for fig-wasps from *F. boninsimae*, *F. nishimurae*, and Higashidaira type are referred as CB, CN, and CH, respectively. A similar correlation was also observed for the two Haha Island haplotypes: HB and HN for fig-wasps from *F. boninsimae* and *F. nishimurae*, respectively. Except for CN, all the haplotypes have one to three apomorphic substitution sites. CB and HB share two synapomorphic substitutions and the latter has an additional substitution. CH and HN each have a single autoapomorphic substitution. The sequences determined in this study have been submitted to the DDBJ/EMBL/GenBank nucleotide sequence databases (accession nos. AB121669–AB121673).

In the eight populations for which data were obtained for two or more years, no haplotype polymorphisms were observed (Fig. 3). Each fig-wasp population consisted of a single haplotype, in contrast to the recent

Table 2 Results of the Y-tube selection experiments for the volatile chemical compounds extracted with ethyl acetate

| Fig-wasp (origin) ^a | Host fig (origin) ^a | Non-host fig (origin) ^a | <i>N</i> | No. selected host | No. selected non-host | Departure from random selection ^b |
|--------------------------------|--------------------------------|------------------------------------|----------|-------------------|-----------------------|--|
| B (Asahidaira) | B (Asahidaira) | N (John) | 91 | 44 | 47 | NS |
| B (Asahidaira) | B (Asahidaira) | N (Mikazuki) | 34 | 18 | 16 | NS |
| B (Hatsune) | B (Asahidaira) | N (John) | 31 | 16 | 14 | NS |
| B (Hatsune) | B (Asahidaira) | H (Higashidaira) | 80 | 38 | 42 | NS |
| B (Chibusa) | B (Chibusa) | N (Minamizaki) | 19 | 11 | 8 | NS |
| N (John) | N (John) | B (Asahidaira) | 51 | 35 | 16 | <i>P</i> < 0.01 |
| N (John) | N (John) | B (Hatsune) | 32 | 23 | 9 | <i>P</i> < 0.05 |
| N (John) | N (Mikazuki) | B (Hatsune) | 37 | 25 | 12 | <i>P</i> < 0.05 |
| N (John) | N (John) | H (Higashidaira) | 34 | 24 | 10 | <i>P</i> < 0.05 |
| N (Minamizaki) | N (Minamizaki) | B (Chibusa) | 35 | 26 | 9 | <i>P</i> < 0.01 |
| H (Higashidaira) | H (Higashidaira) | B (Asahidaira) | 46 | 32 | 14 | <i>P</i> < 0.01 |
| H (Higashidaira) | H (Higashidaira) | B (Hatsune) | 48 | 35 | 13 | <i>P</i> < 0.01 |
| H (Higashidaira) | H (Higashidaira) | N (John) | 38 | 27 | 11 | <i>P</i> < 0.01 |

^a B *Ficus boninsimae*, H Higashidaira type, N *F. nishimurae*

^b Statistical analyses were conducted using the chi-square test (NS not significant; *P* > 0.05)

Fig. 2 A dendrogram of individual figs based on the genetic distance data from the RAPD analysis. The abbreviations are as follows: *CB* *Ficus boninsimae* from Chichi Island, *CH* the Higashidaira type, *CN* *F. nishimurae* from Chichi Island, *HB* *F. boninsimae* from Haha Island, *HN* *F. nishimurae* from Haha Island

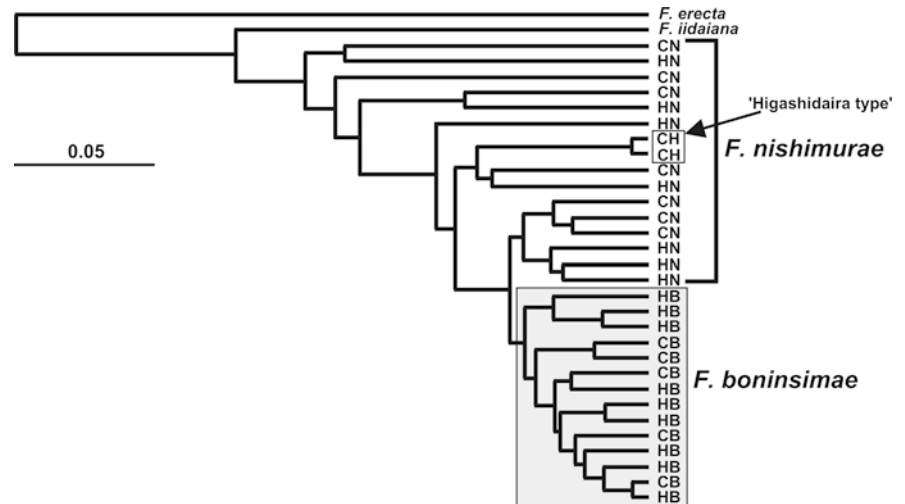
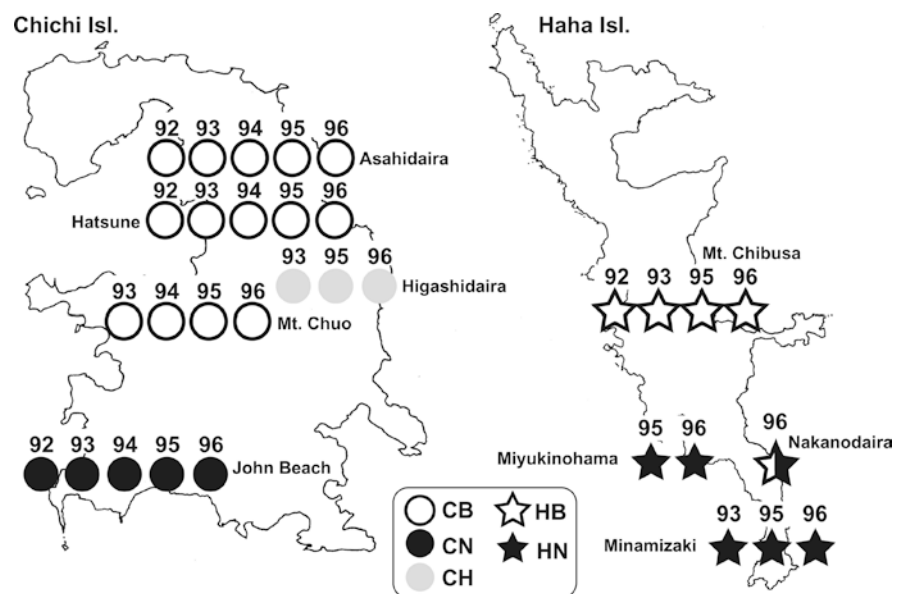


Fig. 3 Distribution of the mtDNA haplotypes of fig-wasps in the Ogasawara Islands. The numbers on the symbols indicate the year of sampling. Both the HB and HN haplotypes were found in Nakanodaira, Haha Island



study of neotropical monoecious figs (Molbo et al. 2003). Two haplotypes were observed simultaneously in a single population from Haha Island (Nakanodaira; most of the fig-wasps were haplotype HN, but fig-wasps from one syconium were haplotype HB). These results indicate that gene flow in fig-wasps, especially between populations of different haplotypes, is quite rare.

The MP analysis produces a single most parsimonious tree with 41 steps and a CI of 1.00 (Fig. 4). The relationships among CN, CH, and HN were not resolved. Fig-wasps of *F. boninsimae* from both Chichi and Haha islands (CB and HB) formed a monophyletic group. The results indicate a single origin for the *F. boninsimae* fig-wasp.

Discussion

As far as I know, this is the first study on the cospeciation of closely related species pairs of figs and fig-wasps

based on an extensive survey of pre-mating isolation mechanisms and genetic differentiation in both organisms. Host fig recognition by fig-wasps was significant in all cases, except for the *F. boninsimae* wasps. This level of specificity of host recognition reflects the pre-mating isolation mechanism of both figs and fig-wasps. The genetic differentiation observed in both figs and fig-wasps may have resulted in part from specific host recognition by fig-wasps. Direct analysis of the volatile chemicals indicated that *F. boninsimae* and *F. nishimurae* have different volatile profiles (Yokoyama 1995). This chemical difference may lead to the exact host recognition by the *F. nishimurae* fig-wasp. However, it is not clear why the fig-wasps from *F. boninsimae* showed no specific preference for their host fig. Based on the RAPD analysis, *F. boninsimae* is considered to have evolved from a population of *F. nishimurae*. Therefore, *F. boninsimae* is a derivative species with newly gained morphological features, such as persistent stipules, small leaves, globose syconia, and syconia with short

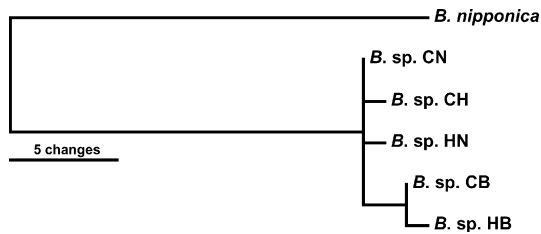


Fig. 4 A most parsimonious tree for the fig-wasps based on the nucleotide sequences of the *coxI-coxII* intergenic region of mtDNA

peduncles (Yokoyama 2003). Consequently, *F. boninsimae* has likely lost its ancestral pre-mating isolation. In the courtship behavior of Hawaiian *Drosophila*, both the gain and loss of pre-mating isolation mechanisms by derivative species have been reported (Kaneshiro 1983). If the case of *F. boninsimae* is similar to the latter type of Hawaiian *Drosophila*, the newly evolved fig-wasps may continue to recognize plesiomorphic composition of host volatile chemicals (i.e., *F. nishimurae* in this case); however, fig-wasps that remain with the previous host cannot recognize the newly evolved fig as the host due to the apomorphic composition of volatile chemicals. More detailed analyses of the behavior and responses of wasps to each volatile chemical or combinations of compounds are needed to confirm this. By contrast, the Higashidaira type is also thought to have been derived from *F. nishimurae*, but these fig-wasps have developed specific host recognition. This also needs to be confirmed by further analyses of the volatile chemicals.

Another important factor leading to reproductive isolation is the distance between populations. Preliminary mark-recapture experiments indicated that fig-wasps on the Ogasawara Islands usually migrate less than 100 m (J. Yokoyama, unpublished). Female adult fig-wasps on the Ogasawara Islands survive only a few hours after emergence from the syconia and this may be one of the major factors restricting the range of gene flow (J. Yokoyama, personal observations). A similar result was reported for the common fig, *F. carica* L. (Kjellberg et al. 1988). By contrast, the fig-wasps of monoecious figs are thought capable of dispersal exceeding 10 km (Nason et al. 1998). Observations of the recolonization of fig-wasps in Borneo indicated that most species of fig-wasps from dioecious figs could not recolonize after local extinction due to a severe drought, while those of monoecious figs recolonized successfully (Harrison 2000). Therefore, an inability to migrate long distances seems to be common to fig-wasps of dioecious fig species. This directly affects the range of gene flow of figs via pollen. In addition, gene flow of figs is facilitated by seed dispersal, which is independent of the movement of fig-wasps. Most species of figs with small syconia have bright colors when mature and are bird-dispersed. Seed (female) syconia of *F. erecta*, a probable sister species of the figs in the Ogasawara Islands, also turn purple when mature. By contrast, the mature seed (female) syconia of all diploid fig species on the Ogasawara Islands are yellow-green and less frequently eaten by birds

(Yokoyama 2003; J. Yokoyama, personal observations). Even after seed dispersal, the three types of fig examined in this study occupied largely different habitats, which might inhibit the co-occurrence of different types. This habitat differentiation might also act as a barrier to gene flow via seeds. Therefore, the range of gene flow in both figs and fig-wasps might be restricted to individuals in a single population or to close populations in similar habitats. Moreover, the production of syconia and release of fig-wasps are usually continuous in the male (wasp-producing) trees in dioecious figs belonging to the subgenus *Ficus* (Okamoto and Tashiro 1981; Kjellberg et al. 1987; Harrison et al. 2000). The Ogasawara Islands' figs are no exception, and most male trees produce new syconia and wasps year-round, except during January and February (J. Yokoyama et al., unpublished data). If the number of figs in a given fig population is saturated by continuously emerging fig-wasps year-round, there is little room for immigrants from other populations. This might explain why the pre-mating isolation mechanism associated with volatile chemicals promotes the genetic differentiation among species and populations that was observed in this study.

Considering these points, I developed the following hypothesis for cospeciation in the fig/fig-wasp system of the Ogasawara Islands. First, a population of a diploid fig species pollinated by a fig-wasp species was established on the Ogasawara Islands. Then, this population became subdivided with expansion of its range. When the distance among subpopulations exceeded the flying ability of fig-wasps, gene flow was restricted within the subpopulations. This led to genetic differentiation over time. Fig-wasp differentiation precedes fig differentiation due to the short generation time of fig-wasps. Restricted fig gene flow via pollen leads to the further genetic differentiation of the figs; consequently, a new species pair might evolve. Further diversification will occur with repetition of this process. Therefore, cospeciation of the fig/fig-wasp system on the Ogasawara Islands might proceed via the following steps: (1) spatial separation of fig/fig-wasp populations, (2) restriction of the range of gene flow and genetic and character differentiation of fig-wasps, (3) further restriction of gene flow and genetic and character differentiation of figs.

Some cases in which geographically separated populations of a conspecific fig species were pollinated by different species of fig-wasp have been reported. Such cases are more frequent than cases in which different fig species are pollinated by the same fig-wasp species (Rasplus 1996). These situations can be understood in terms of the above hypothesis, but all of these cases were observed on a much larger scale than the cases studied here. For example, *F. sur* Forsskal (Rasplus 1996) is distributed from central to southern Africa, which is a much larger range than the area of the Ogasawara Islands, which amounts to less than 50 km² including both Chichi and Haha Islands. Therefore, the case presented here is an extreme instance of local differentiation of fig/fig-wasp species pairs. As with the diversification

of other organisms on oceanic islands, founder effects and the process of adaptive radiation might enforce local differentiation and rapid diversification (Gavrilets and Hastings 1996; Barton 1998; Grant 1998; Thorpe and Malhotra 1998; Schluter 2000). The most critical problem with the above hypothesis is that it is not known whether there has been sufficient time in isolation for differentiation. However, there are examples in which the morphological or genetic differentiation of animals on small islands has occurred in a relatively short time (e.g., Malhotra and Thorpe 1991; Losos et al. 1997; Britton-Davidian et al. 2000). Therefore, rapid diversification should be possible in the fig/fig-wasp species pairs in the Ogasawara Islands. Further studies on ecological differentiation and fine-scale genetic analyses using microsatellite loci should add insight to the cospeciation process of fig/fig-wasp systems in the Ogasawara Islands.

Acknowledgments I wish to express my gratitude to Prof. M. Kato for valuable advice and encouragement throughout my work. I wish to thank Drs. R. Yamaoka and M. Odaki for their help and suggestion on bioassays for chemical compounds. Thanks are also due to Drs. M. Hasebe, M. Ito, N. Murakami, N. Minaka, and T. Yahara for their helpful comments during this study, Dr. M. Ono and Mr. T. Yasui for their help with field work, and Dr. T. Fukuda, Ms. M. Sato, and Ms. A. Yokoyama for their help with laboratory work. This study was partly supported by Grants in Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

- Anstett MC, Hossaert-McKey M, Kjellberg F (1997) Figs and fig pollinators: evolutionary conflicts in a coevolved mutualism. *Trend Ecol Evol* 12:94–98
- Barker NP (1985) Evidence of volatile attractant in *Ficus ingens* (Moraceae). *Bothalia* 15:607–611
- Barton NH (1998) Natural selection and random genetic drift as causes of evolution on islands. In: Grant PR (ed) *Evolution on islands*. Oxford University Press, Oxford, pp 102–123
- Berg CC (1990) Reproduction and evolution in *Ficus* (Moraceae): traits connected with the adequate rearing of pollinators. *Mem N Y Bot Gard* 55:169–185
- Bock WJ (1972) Species interactions and macroevolution. *Evol Biol* 5:1–24
- Britton-Davidian J, Catalan J, Ramalhinho MG, Ganem G, Auffray J-C, Capela R, Biscoito M, Searle J, Mathias ML (2000) Rapid chromosomal evolution in island mice. *Nature* 403:158
- Clary DO, Wolstenholme DR (1985) The mitochondrial molecule of *Drosophila yakuba*: nucleotide sequence, genome organization, and genetic code. *J Mol Evol* 22:252–271
- Croizier RH, Croizier YC (1993) The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. *Genetics* 133:97–117
- Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. *Evolution* 18:586–608
- Farrell B, Dussourd DE, Mitter C (1991) Escalation of plant defense: do latex and resin canals spur plant diversification? *Am Nat* 138:881–900
- Farrell BD, Mitter C, Futuyma DJ (1992) Diversification at the insect-plant interface. *BioScience* 42:34–42
- Felsenstein J (2000) PHYLIP: phylogeny inference package, version 3.6(alpha). University of Washington, Seattle
- Gavrilets S, Hastings A (1996) Founder effect speciation: a theoretical reassessment. *Am Nat* 147:466–491
- Grant PR (1998) Speciation. In: Grant PR (ed) *Evolution on islands*. Oxford University Press, Oxford, pp 83–101
- Harrison R (2000) Repercussion of El Niño: drought causes extinction and the breakdown of mutualism in Borneo. *Proc R Soc Lond B* 267:911–915
- Harrison R, Yamamura N, Inoue T (2000) Phenology of a common roadside fig in Sarawak. *Ecol Res* 15:47–61
- Hasebe M, Iwatsuki K (1990) *Adiantum capillus-veneris* chloroplast DNA clone bank: as useful heterologous probes in the systematics of the leptosporangiate ferns. *Am Fern J* 80:20–25
- Herre EA, Machado CA, Bermingham E, Nason JD, Windsor DM, McCafferty SS, van Houten W, Bachmann K (1996) Molecular phylogenies of figs and their pollinator wasps. *J Biogeog* 23:521–530
- Hossaert-McKey M, Gibernau M, Frey JE (1994) Chemosensory attraction of fig wasps to substances produced by receptive figs. *Entomol Exp Appl* 70:185–191
- Kaneshiro KY (1983) Sexual selection, and direction of evolution in the biosystematics of Hawaiian Drosophilidae. *Ann Rev Entomol* 28:161–178
- Kjellberg F, Gouyon P-H, Ibrahim M, Raymond M, Valdeyron G (1987) The stability of the symbiosis between dioecious figs and their pollinators: a study of *Ficus carica* L. and *Blastophaga psenes* L. *Evolution* 41:693–704
- Kjellberg F, Doumesche B, Bronstein JL (1988) Longevity of a fig wasp (*Blastophaga psenes*). *Proc K Ned Akad Wet C* 91:117–122
- Latorre A, Moya A, Ayala FJ (1986) Evolution of mitochondrial DNA in *Drosophila subobscura*. *Proc Natl Acad Sci USA* 83:8649–8653
- Losos JB, Warheit KI, Schoener TW (1997) Adaptive differentiation following experimental island colonization in *Anolis* lizard. *Nature* 387:70–73
- Machado CA, Emmanuelle J, Kjellberg F, Compton SG, Herre EA (2001) Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. *Proc R Soc Lond B* 268: 685–694
- Malhotra A, Thorpe RS (1991) Experimental detection of rapid evolutionary response in natural lizard populations. *Nature* 353:347–348
- Mitter C, Farrell BD, Wiegmann B (1988) The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? *Am Nat* 132:107–128
- Molbo D, Machado CA, Sevenster JG, Keller L, Herre EA (2003) Cryptic species of fig-pollinating wasps: Implications for the evolution of the fig-wasp mutualism, sex allocation, and precision of adaptation. *Proc Natl Acad Sci USA* 100:5867–5872
- Mopper S, Strauss SY (1998) Genetic structure and local adaptation in natural insect populations. Chapman & Hall, New York
- Nason JD, Herre EA, Hamrick JL (1998) The breeding structure of a tropical keystone plant resource. *Nature* 391:685–687
- Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci USA* 76:5269–5273
- Okamoto M, Tashiro M (1981) Preliminary study on phenology of syconial production of *Ficus erecta* Thunberg (in Japanese). *Bull Osaka Mus Nat Hist* 35:43–53
- Ono M (1990) Chromosome number of some endemic plant species of the Bonin Islands, III. *Ogasawara Res* 16:1–27
- Ramirez B W (1974) Coevolution of *Ficus* and Agaonidae. *Ann Missouri Bot Gard* 61:770–780
- Rasplus JY (1996) The one-to-one species specificity of the *Ficus*-Agaoninae mutualism: how casual? In: van der Maesen LJG (ed) *The biodiversity of African plants*. Kluwer, Dordrecht, pp 639–649
- Saiki RK, Gelfland DH, Stoffel S, Scharf S, Higuchi R, Horn R, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487–491
- Schluter D (2000) *The ecology of adaptive radiation*. Oxford University Press, Oxford

- Swofford DL (1998) PAUP*. Phylogenetic analysis using parsimony (*and other methods), vers 4. Sinauer, Sunderland, Mass.
- Thompson JN (1986) Patterns in coevolution. In: Stone AR, Hawksworth DL (eds) *Coevolution and systematics*. Oxford University Press, Oxford, pp 119–143
- Thompson JN (1989) Concepts of coevolution. *Trend Ecol Evol* 4:179–183
- Thompson JN (1994) *The coevolutionary process*. University of Chicago Press, Chicago, Ill.
- Thorpe RS, Malhotra A (1998) Molecular and morphological evolution within small islands. In: Grant PR (ed) *Evolution on islands*. Oxford University Press, Oxford, pp 66–82
- Toyoda T (1981) *Flora of Bonin Is.* (in Japanese). Aboc-sha, Kamakura
- Ware AB, Compton SG (1994) Dispersal of adult female fig wasps: arrivals and departures. *Entomol Exp Appl* 73:221–229
- Ware AB, Kaye PT, Compton SG, Van Noort, S (1993) Fig volatiles: their role in attracting pollinators and maintaining pollinator specificity. *Plant Syst Evol* 186:147–156
- Weiblen GD (2001) Phylogenetic relationships of fig wasps pollinating functionally dioecious figs based on mitochondrial DNA sequences and morphology. *Syst Biol* 50:243–267
- Weiblen GD, Bush GL (2002) Speciation in fig pollinators and parasites. *Mol Ecol* 11:1573–1578
- Wiebes JT (1979) coevolution of figs and their insect pollinators. *Ann Rev Ecol Syst* 10:1–12
- Yamazaki T (1983) Taxonomic review of the Moraceae from Japan, Korea, Taiwan and adjacent areas (2) (in Japanese). *J Phytoecog Taxon* 31:1–15
- Yokoyama J (1995) Insect-plant coevolution and speciation. In: Arai R, Kato K, Doi Y (eds) *Biodiversity and evolution*. The National Science Museum Foundation, Tokyo, pp 115–130
- Yokoyama J (2003) *Ficus*. In: Iwatsuki K, Yamazaki T, Boufford DE, Ohoba H (eds) *Flora of Japan*. Iia, Kodansha, Tokyo (in press)
- Yokoyama J, Iwatsuki K (1998) A faunal survey of fig-wasps (Chalcidoidea, Hymenoptera) distributed in Japan and their association with figs (*Ficus*, Moraceae). *Entomol Sci* 1:37–46