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Origin and diversification of *Hibiscus glaber*, species endemic to the oceanic Bonin Islands, revealed by chloroplast DNA polymorphism

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Abstract

Two woody *Hibiscus* species co-occur in the Bonin Islands of the northwestern Pacific Ocean: *Hibiscus glaber* Matsum. is endemic to the islands, and its putative ancestral species, *Hibiscus tiliaceus* L., is widely distributed in coastal areas of the tropics and subtropics. To infer isolating mechanisms that led to speciation of *H. glaber* and the processes that resulted in co-occurrence of the two closely related species on the Bonin Islands, we conducted molecular phylogenetic analyses on chloroplast DNA (cpDNA) sequences. Materials collected from a wide area of the Pacific and Indian Oceans were used, and two closely related species, *Hibiscus hamabo* Siebold Zucc. and *Hibiscus macrophyllus* Roxb., were also included in the analyses. The constructed tree suggested that *H. glaber* has been derived from *H. tiliaceus*, and that most of the modern Bonin populations of *H. tiliaceus* did not share most recent ancestry with *H. glaber*. Geographic isolation appears to be the most important mechanism in the speciation of *H. glaber*. The co-occurrence of the two species can be attributed to multiple migrations of different lineages into the islands. While a wide and overlapping geographical distribution of haplotypes was found in *H. tiliaceus*, localized geographical distribution of haplotypes was detected in *H. glaber*. It is hypothesized that a shift to inland habitats may have affected the mode of seed dispersal from ocean currents to gravity and hence resulted in geographical structuring of *H. glaber* haplotypes.

Keywords: Bonin Islands, chloroplast DNA, *Hibiscus*, oceanic islands, phylogeography, seed dispersal

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Introduction

Oceanic islands that have never been connected to any continental landmass offer opportunities for the study of speciation (Darwin 1859; Carlquist 1974). By studying combinations of species endemic to islands and their continental ancestors, it is possible to infer mechanisms of speciation. The ancestors of plant species of oceanic islands are typically capable of long-distance seed dispersal via birds, wind, or ocean currents. However, the loss of seed dispersal ability has been reported from a number of derived taxa on oceanic islands. It has been hypothesized that these species have lost the mechanisms for long-distance seed dispersal during local adaptation to inland island environments (Carlquist 1974; Cody & Overton 1996).

Adaptive radiation has often been recognized in oceanic islands in spite of their small size (Carlquist 1974; Wagner & Funk 1995). Ecological and morphological diversification observed in oceanic islands may be attributable to a combination of genetic structure resulting from reduced seed dispersal and ecological release provided by a series of unoccupied habitats.

The Bonin (Ogasawara) Islands are extinct volcanic islands in the northwestern Pacific Ocean, approximately 1000 km south of the Japanese archipelago. The islands consists of 24 small islands (> 0.6 km^2) and many islets scattered throughout a rectangular area bordered by 26°30′N, 27°40′N, 142°00′E, and 142°15′E. The three island groups, Mukojima, Chichijima and Hahajima, are aligned from north to south (Fig. 1A). The Bonin Islands were

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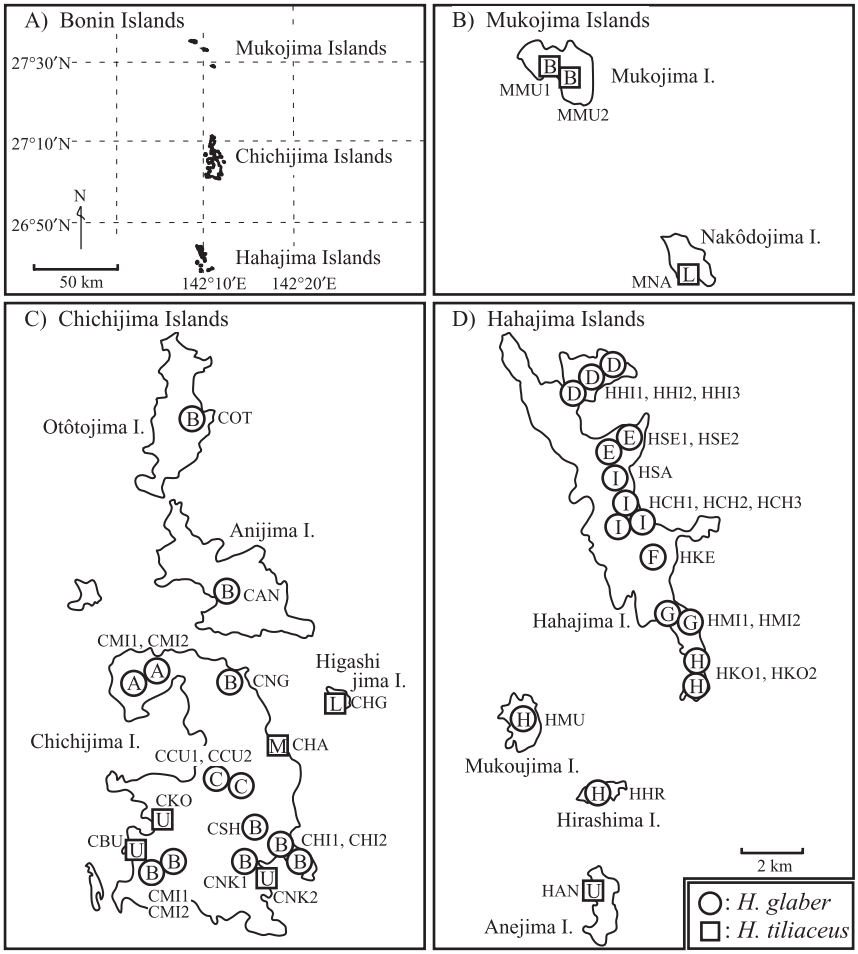

Fig. 1 The geographical location (A) and distribution of cpDNA haplotypes in *Hibiscus glaber* and *Hibiscus tiliaceus* (B–D) in the Bonin Islands. Circles and squares represent *H. glaber* and *H. tiliaceus*, respectively. For individual acronyms refer to Table 1.

formed during the Palaeocene, but they appeared above sea level before the middle Pleistocene (Kaizuka 1977; Imaizumi & Tamura 1984). It has been estimated that the islands have supported a biota for the past 1.8–3.2 million years (Myr) (Ito 1998; Chiba 1999). The Bonin Islands are older than other islands in the northwestern Pacific Ocean, such as the Izu Islands, the Volcano Islands, and the Northern Marianas (Asami 1970). The advanced age of the islands has resulted in high endemism of vascular plants (40% of 369 indigenous species, Kobayashi 1978). Most endemic species do not have their closest allies in the Bonin Islands, but rather in surrounding continental regions such as Southeast Asia, Taiwan, and the Japanese archipelago (Yamazaki 1981; Shimizu 1991). The dominant mode of speciation in these systems may well be geographical isolation rather than ecological and reproductive isolations.

One exception to this pattern in the Bonin Islands is the genus *Hibiscus* (Malvaceae). Two woody species, the endemic, *Hibiscus glaber* Matsum., and a widely distributed relative, *Hibiscus tiliaceus* L., coexist on the islands (Kobayashi 1978; Kudoh *et al*. 1998; Ohba 1999; Takayama & Kato 2001). Among the taxa ascribed to section *Azanza* in *Hibiscus*, *H. glaber* and *H. tiliaceus* are most closely related to each other and show considerable morphological similarity (Ono & Kobayashi 1985; Shimizu 1989; Kudoh & Kachi 1997; Ohba 1999). Therefore, it is plausible to assume that *H. glaber* is derived from *H. tiliaceus* (Shimizu 1989; Kudoh & Kachi 1997; Takayama & Kato 2001; Takayama *et al*. 2002). The two species are distinct from each other by characters of the fruits; i.e. capsules of *H. tiliaceus* have 10 loculi divided by five true and five false septa, but those of *H. glaber* have five loculi divided by five true septa (Takayama *et al*. 2002). The leaf surface of *H. tiliaceus* is densely covered by long stellate hairs, whereas in *H. glaber*, the leaf surface is covered by short stellate hairs, and their distribution varies from nearly absent to dense (Takayama & Kato 2001). *Hibiscus tiliaceus* is restricted to coastal habitats in the Bonin Islands (Kudoh & Kachi 1997) as is the case elsewhere in its wider tropical and subtropical range (Waalkes 1966; Tomlinson 1986; Fryxell 2001). *Hibiscus glaber* occurs on most inland areas of the islands, from the coast to the highest ridges around 400 m above sea level (a.s.l.) (Shimizu 1984). However, the two species do sometimes co-occur in coastal areas.

DNA sequence data are often used to reconstruct phylogenetic relationships among species, and they have been used in many recent studies on insular speciation (Sang *et al*. 1994; Baldwin *et al*. 1998; Kim *et al*. 1998; Francisco-Ortega *et al*. 2001; Richardson *et al*. 2001; Emerson 2002). In most angiosperms, the chloroplast genome is transmitted through maternal lineages (Mogensen 1996), and geographical variation of the chloroplast DNA (cpDNA) is expected to reflect patterns of historical seed movements. The spatial distribution of cpDNA polymorphisms therefore is useful to infer colonization where long-distance seed dispersal is suspected to have been important (McCauley 1994, 1996). In this study, we conducted a phylogenetic analysis using nucleotide sequences of cpDNA to reveal the processes that have resulted in the co-occurrence of the widespread *H. tiliaceus* and its derived endemic *H. glaber* in the Bonin Islands. We analysed multiple *H. glaber* and *H. tiliaceus* populations from different localities covering the wide range of geographical area of the species. We also included another member of section *Azanza*, *Hibiscus hamabo* Siebold & Zucc., IPNI (International Plant Name Index) which is distributed along the Pacific coasts of the Japanese archipelago (Nakanishi 1979; Ohba 1999; Fryxell 2001).

Two major questions were addressed. First, what type of isolation has been responsible for speciation in *H. glaber*. In this regard, we evaluated two alternative hypotheses concerning which different types of isolating mechanisms play critical roles during speciation. The first hypothesis is that *H. glaber* has been derived from sympatric populations of *H. tiliaceus*. Reproductive isolating mechanisms working on a local level should have been important at least for the initial stages of speciation (Mayr 1963; Schluter 2001). If this hypothesis is correct, we would expect to see monophyletic pairings of co-occurring *H. glaber* and *H. tiliaceus* populations in the Bonin Islands. The second hypothesis assumes that geographical isolation promoted speciation, and requires at least two independent colonization of the Bonin Islands by *H. tiliaceus*. In this hypothesis, we considered that the first *Hibiscus* lineage to colonize the islands adapted to inland habitats and was transformed into *H. glaber*. After this speciation event, ancestors of the current *H. tiliaceus* populations migrated to the Bonin Islands. In this case, we expect that *H. glaber* and *H. tiliaceus* populations on the Bonin Islands would not share most recent common ancestors. We also expect that the *H. tiliaceus* populations that are most closely related to *H. glaber* would be found from areas outside the Bonin Islands. We have included *H. hamabo* in the analyses therefore as the possibility exists that *H. hamabo* and *H. glaber* share a most recent ancestor. Seeds of *H. hamabo* are buoyant and are dispersed by ocean currents, but the present distribution of the species is restricted to the warm-temperate area of the northwestern Pacific Ocean (Nakanishi 1979; Ohba 1999).

The second question concerned how a habitat-shift toward inland areas of *H. glaber* could have affected the geographical distribution of cpDNA haplotypes in the species. Direct consequences of the habitat-shift would be changes in mode of seed dispersal. Seeds of *H. glaber* are dispersed primarily by gravity. Distances for seed dispersal in *H. glaber* are expected to be much shorter than those in hydrochorous *H. tiliaceus*. We hypothesized therefore that, in *H. glaber*, geographical structures have been formed in haplotype distributions within the Bonin Islands. Such geographical structuring may also explain the morphological diversity among *H. glaber* populations. We previously reported morphological diversification between populations in Chichijima and Hahajima, the two major islands in the Bonin Islands (Takayama & Kato 2001). In contrast to what is predicted for *H. glaber*, we might expect that in *H. tiliaceus*, haplotypes would be distributed across a wide geographical area with extensive overlap because of seed dispersal by ocean currents.

Materials and methods

Plant materials

Four species of section *Azanza* in the genus *Hibiscus* i.e. *Hibiscus glaber*, *Hibiscus tiliaceus*, *Hibiscus hamabo* and *Hibiscus macrophyllus* Roxb., were included in the present study. We included the last species as an outgroup in the phylogenetic analyses. Section *Azanza* is characterized by large, more or less oblong stipules, broadly ovate or elliptic leaves, and distinctive fruits. It has sometimes been segregated from *Hibiscus* as the genus *Taliparitі* (Fryxell 2001). Leaf samples for DNA analyses were collected from 29 individuals of *H. glaber* from the Bonin Islands, 36 individuals of *H. tiliaceus* from Southeast Asia and Sri Lanka, three individuals of *H. hamabo* from the Japanese archipelago, and one individual of *H. macrophyllus* from the Malay Peninsula (Table 1). Voucher specimens were deposited in the Makino Herbarium, Tokyo Metropolitan University (MAK) and the Herbarium of the University of Tokyo (TI).

DNA isolation, PCR amplification, and sequencing

Total DNA was extracted either from fresh or silica-dried leaves. Leaf tissue was pulverized to a fine powder under liquid nitrogen. Because extracts from *Hibiscus* leaves are sticky, prior to DNA extraction the leaf powder was washed using SEB for fresh materials (Sorbitol Extraction Buffer; Tai & Tanksley 1990) and HEPES {2-[4-(2-Hydroxyethyl)-1-piperazinyle] ethanesulphonic acid} for dried materials (Setoguchi & Ohba 1995). Total DNA was isolated from a washed leaf pellet using the CTAB (hexadecyltrimethyl ammononium bromide) extraction method (Doyle & Doyle 1987) and purified using CIA (chloroform–

Table 1 Localities of samples for *Hibiscus glaber*, *Hibiscus tiliaceus*, *Hibiscus hamabo* and *Hibiscus macrophyllus* used in this study

Taxon	Locality	Voucher	Acronym	Haplotype
H. glaber Matsum.	Bonin Islands; Chichijima Islands			
	Otôtojima I.	Kato 00-1	COT	Type B
	Takinoura, Anijima I.	Kudoh 285	CAN	Type B
	Mount Mikazukiyama, Chichijima I.	Kudoh 00-024	CMI1	Type A
	Mount Mikazukiyama, Chichijima I.	Takayama 298	CMI2	Type A
	Nagasaki, Chichijima I.	Takayama 190	CNG	Type B
	Mount Chuozan, Chichijima I.	Kudoh 99-639	CCU1	Type C
	Mount Chuozan, Chichijima I.	Takayama 85	CCU2	Type C
	Mount Shigureyama, Chichijima I.	Takayama 151	CSH	Type B
	Higashikaigan, Chichijima I.	Kudoh 99-032	CHI1	Type B
	Higashikaigan, Chichijima I.	Takayama 176	CHI2	Type B
	Nakakaigan, Chichijima I.	Kudoh 99-038	CNK1	Type B
	Minamifukurozawa, Chichijima I.	Takayama 137	CMI1	Type B
	Minamifukurozawa, Chichijima I.	Takayama 146	CMI2	Type B
	Bonin Islands; Hahajima Islands			
	Mount Higashiyama, Hahajima I.	Fujita Hy001	HHI1	Type D
	Mount Higashiyama, Hahajima I.	Takayama 240	HHI2	Type D
	Mount Higashiyama, Hahajima I.	Takayama 241	HHI3	Type D
	Sekimon, Hahajima I.	Kato 980065	HSE1	Type E
	Sekimon, Hahajima I.	Takayama 234	HSE2	Type E
	Mount Chibusayama, Hahajima I.	Kato 980074	HCH1	Type I
	Mount Chibusayama, Hahajima I.	Takayama 215	HCH2	Type I
	Mount Chibusayama, Hahajima I.	Takayama 226	HCH3	Type I
	Mount Sakaigatake, Hahajima I.	Miyazaki 00-204	HAS	Type I
	Mount Kensakiyama, Hahajima I.	Takayama 1	HKE	Type F
	Minamizaki, Hahajima I.	Kudoh 99-638	HMI1	Type G
	Minamizaki, Hahajima I.	Takayama 257	HMI2	Type G
	Mount Kofuji, Hahajima I.	Kudoh 99-631	HKO1	Type H
	Mount Kofuji, Hahajima I.	Takayama 278	HKO2	Type H
	Mukoujima I.	Kudoh 99-634	HMU	Type H
	Hirashima I.	Kudoh 99-632	HHR	Type H
H. tiliaceus L.	Bonin Islands; Chichijima Islands			
	Hatsuneura, Chichijima I.	Kudoh s.n.	CHA	Type M
	Kominatokaigan, Chichijima I.	Kato 980089	CKO	Type U
	Butakaigan, Chichijima I.	Takayama 173	CBU	Type U
	Nakakaigan, Chichijima I.	Kudoh 99-037	CNK2	Type U
	Higashijima I.	Takayama 591	CHG	Type L
	Bonin Islands; Hahajima Islands			
	Anejima I.	Kudoh 99-637	HAN	Type U
	Bonin Islands; Mukojima Islands			
	Mukojima I.	Kato 010383	MMU1	Type B
	Mukojima I.	Kato 010391	MMU2	Type B
	Nakôdojima I.	Takayama 590	MNA	Type L
	Kazan Islands			
	Kita-Ioujima I.	Fujita KI-001	KKI	Type L
	Okinawa Pref.; Ryukyu Islands			
	Tamashiro Vill., Okinawahonto I.	Tateishi 52045	ROT	Type U
	Ada, Okinawahonto I.	Takayama 790	ROA	Type L
	Ikeijima I.	Takayama 835	RIK	Type L
	Akajima I.	Takayama 785	RAK	Type L
	Okinawa Pref.; Miyako Islands			
	Shigira beach, Miyakojima I.	Takayama 647	MMS	Type N
	Sawadanohama, Irabujima I.	Takayama 623	MIS	Type K
	Shimojijima I.	Takayama 637	MSH	Type U

Table 1 *Continued*

Taxon	Locality	Voucher	Acronym	Haplotype
	Okinawa Pref.; Yaeyama Islands			
	Ishigakijima I.	Kudoh 00-34	YIS	Type L
	Ohta, Ishigakijima I.	Takayama 686	YIO	Type K
	Taketomijima I.	Takayama 776	YTA	Type K
	Haimida, Iriomotejima I.	Kudoh 00-037	YIHI	Type L
	Haimida, Iriomotejima I.	Takayama 753	YIH2	Type L
	Haterumajima I.	Takayama 780	YHA	Type L
	Kagoshima Pref.			
	Kasari Town, Amamiohshima I.	Kudoh 99-647	KAK	Type L
	Southeast Asia			
	Labrador Park, Singapore	Boo-199-01	SSL	Type T
	Noordin, Ubin, Singapore	Ohi HTS102	SSU	Type K
	Pelabuhanratu, Java, Indonesia	Ohi HTI004	SIJ	Type S
	Hua Thanon, Samui, Thailand	Ohi HTT002	STS	Type K
	Phuket, near Laem Sai, Thailand	Ohi HTT012	STP	Type J
	Noordin, Ubin, Singapore	Ohi HTS102	SSU	Type K
	Dodanduwa, Sri Lanka	Ohi S002	SSD	Type U
	Taiwan	Senni 3	STA	Type L
	Pacific Islands			
	Saipan I.	Kato SP6	PSA1	Type O
	Saipan I.	Kato SP11	PSA2	Type P
	Hawaii I., Hawaiian Is.	Fujita HW0010	PHH	Type Q
	Kauai I., Hawaiian Is.	Fujita HW0041	PHK	Type K
	Faleolupo, Savaii I., Samoa	Fujita Ht0004	PSS	Type R
H. hamabo Siebold and Zucc.	Kagoshima Pref.			
	Sumiyou Town, Amamiohshima I.	Kudoh 98-733	KAS	Type V
	Mie Pref.			
	Meiwa Town	Kudoh 1	MME	Type V
	Aichi Pref.			
	Atsumi Town	Kato 000001	AAT	Type V
H. macrophyllus Roxb.	Phuket, near Karon, Thailand	Ohi HTT009		Type W

The Bonin and Kazan islands are western North Pacific oceanic islands. Okinawa, Kagoshima, Mie and Aichi prefectures are in the Japanese archipelago. Voucher information, acronyms, and cpDNA haplotypes are also shown.

isoamylalcohol; 24:1) and PCI (phenol–chloroform–isoamylalcohol; 25:24:1). For difficult materials, DNA was isolated using the DNeasy Plant Mini Kit (QIAGEN).

Six cpDNA regions, *atpB-rbcL* intergenic spacer (IGS), *accD-psaI* IGS, *rpl16* intron, *ndhF* gene, *trnK-psbA* IGS (including *matK* gene), and *trnH-psbA* IGS (Table 2), were amplified by polymerase chain reaction (PCR) and were sequenced for all samples. Reaction mixtures (30 μL/reaction) contained *c*. 10–30 ng of template DNA, 0.12 μL (0.6 unit) of *ExTaq* DNA polymerase (TaKaRa Bio), 3 μL of *ExTaq* PCR buffer [10 mmol/L Tris-HCL (pH 8.3), 50 mmol/L KCL, 1.5 mmol/L $MgCl_2$], 2.4 μL of 0.2 mmol/L dNTP solution, 2.4 μL of 2.0 mM $MgCl_2$, 0.8 μL of 10 pmol/L for each of two primers. Programmed PCR temperatures were 94 °C for 3 min for initial denaturation, followed by 35 annealing–extension cycles. The temperatures for primer annealing (1 min/cycle) were 55 °C, 53 °C and 51 °C for the 1st–5th, 6th–10th, and 11th–35th cycles, respectively. The extension periods were at 72 °C for 2 min for all cycles. The extension periods for the first five cycles were followed by 1-min denaturation at 94 °C. The extension period for the last cycle was 10 min at 72 °C. For the *atpB-rbcL* IGS, the annealing temperatures were 53 °C, 50 °C and 47 °C for the 1st–5th, 6th–10th, and 11th–35th cycles, respectively. Electrophoreses of PCR products were made on 1.0% agarose gels. The products were excised from the gels and purified using a GENECLEAN III Kit (BIO 101) to remove the nonincorporated primers and nucleotides. Sequencing reactions were carried out using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). The sequencing reaction products were purified, concentrated by ethanol precipitation with sodium acetate, and their sequences were determined by a DNA sequencer (ABI PRISM 377, Applied Biosystems) with Long Ranger Gel (FMC Bio Products). Nucleotide sequences obtained were deposited in the DNA Data Bank of Japan (DDBJ) under the accession

Table 2 Primer sequences used in the analyses of chloroplast DNA

Analyzed region	Primer	5′ to 3′ sequence	Reference
atpB-rbcL IGS	***atpB*-F**	AAG TAG TAG GAT TGG TTC TCA T	Terachi 1993
	***rbcL*-R**	TAG TTT CTG TTT GTG GTG ACA T	Terachi 1993
accD-psaI IGS	***accD*-796F**	GGA AGT TTG AGC TTT ATG CAA ATG G	Small *et al*. 1998
	***psaI*-75R**	AGA AGC CAT TGC AAT TGC CGG AAA	Small *et al*. 1998
rpll6 intron	***rpl16*-F71**	GCT ATG CTT AGT GTG TGA CTC GTT G	Jordan *et al*. 1996
	***rpl16*-R1516**	CCC TTC ATT CTT CCT CTA TGT TG	Kelchner & Clark 1997
psbA-trnH IGS	***psbA***	CGA AGC TCC ATC TAC AAA TGG	Hamilton 1998
	trnH	ACT GCC TTG ATC CAC TTG GC	Hamilton 1998
trnK-psbA IGS	***trnK*-3914F**	TGG GTT GCT AAC TCA ATG G	Johnson & Soltis 1994
(including *matK*)	*matK*-10	ATG ATT CTG TTG ATA CAT TC	Kato *et al*. 1998
	matK AF(*Hibiscus)*	CTA TAC CCA CTT ATT TTT CGG GAG T	this study
	matK-987F(*Hibiscus*)	CTC GAC TTT CTG GGC TAT CTT TCA A	this study
	matK-1094R(*Hibiscus*)	TCC AAC GTC TTC ATA GCA TTA T	this study
	***psbA*-R**	CGC GTC TCT CTA AAA TTG CAG TCA T	Johnson & Soltis 1994
ndhF	***ndhF* F1**	GAA TAT GCA TGG ATC ATA CC	Seelanan *et al*. 1997
	ndhF R972	CAT CAT ATA ACC CAG TTG GGA C	Olmstead & Sweere 1994
	***ndhF* R1318**	CGA AAC ATA TAA AAT GCG GTT AAT CC	Olmstead & Sweere 1994

PCR amplification primers are shown in bold.

nos: *atpB-rbcL* IGS AB180981–AB181004; *accD–psaI* IGS AB181005–AB181028; *rpl16 intron* AB181029–AB181052; *ndhF* AB181053–AB181076; *trnK-psbA* IGS (including *matK*) AB181077–AB181100; *trnH-psbA* IGS AB181101–AB181124.

Phylogenetic analysis

Sequence alignment was performed manually using the DNASIS-MAC program (Hitachi Software Engineering). The alignment and mutational interpretation criteria were made with reference to Golenberg *et al*. (1993) and Kelchner (2000). Based on nucleotide substitutions, indels, and inversions in the analysed sequences, the phylogenetic analyses were performed using Wagner maximum parsimony (MP) with PAUP* version 4.0 beta10 (Swofford 2002). Indels and inversion were used as a fifth character and were scored as binary states, i.e. 0 or 1. Length polymorphisms in mononucleotide repeat units (poly A or poly T) were excluded from the analyses. All characters (nucleotide substitutions, indels, and inversions) were unordered and equally weighed. MP method was conducted using the heuristic search option with 100 random addition replicates. The tree bisection reconnection (TBR) branch-swapping algorithm was used with the 'MulTrees' option. In the algorithm, if the maximum branch length was equal to zero, tree branches were collapsed to create polytomies. Branch support was assessed by bootstrap analysis (Felsenstein 1985) with 1000 replicates using the full heuristic search option.

Molecular phylogenetic studies of the genus *Hibiscus* have been already completed (Pfeil *et al*. 2002), including two species of section *Azanza*, *H. tiliaceus* and *H. macrophyllus* from the Malay Peninsula. To test the monophyly of section *Azanza* and to reveal phylogenetic positions of the species analysed in this study, we reconstructed phylogenetic trees for the genus *Hibiscus* based on combined sequence data of the *ndhF* gene and *rpl16* intron of Pfeil *et al*. (2002), plus new sequences of *H. glaber*, *H. tiliaceus*, *H. hamabo* and *H. macrophyllus* from the present study. The strict consensus tree was constructed with ACCTRAN character-optimization.

Phylogenetic analyses among haplotypes of *H. glaber*, *H. tiliaceus* and *H. hamabo* were also conducted using *H. macrophyllus* as an outgroup. In the six cpDNA regions analysed, the *trnH-psbA* IGS region showed no variation and was henceforth excluded from further analysis.

Results

Phylogenetic positions of Hibiscus glaber *and* Hibiscus tiliaceus *in the genus* Hibiscus

The strict consensus tree of the genus *Hibiscus* was constructed from 699 most parsimonious trees. The consensus tree strongly supports monophyly of the four species of section *Azanza* with 100% bootstrap probability (BP) (Fig. 2). In the clade of section *Azanza*, *Hibiscus glaber*, *Hibiscus tiliaceus*, and *Hibiscus hamabo* formed a subclade (BP 94%, Fig. 2). Furthermore, we found three haplotypes among four accessions of *H. glaber* and *H. tiliaceus*. Two *H. glaber* and one of two *H. tiliaceus* samples formed a monophyletic group (BP 65%), indicating a close relationship between the two species (Fig. 2). *Hibiscus macrophyllus* is revealed to be sister to the subclade of *H. glaber*, *H. tiliaceus* and *H. hamabo* (Fig. 2), and therefore it was used as outgroup in further analyses.

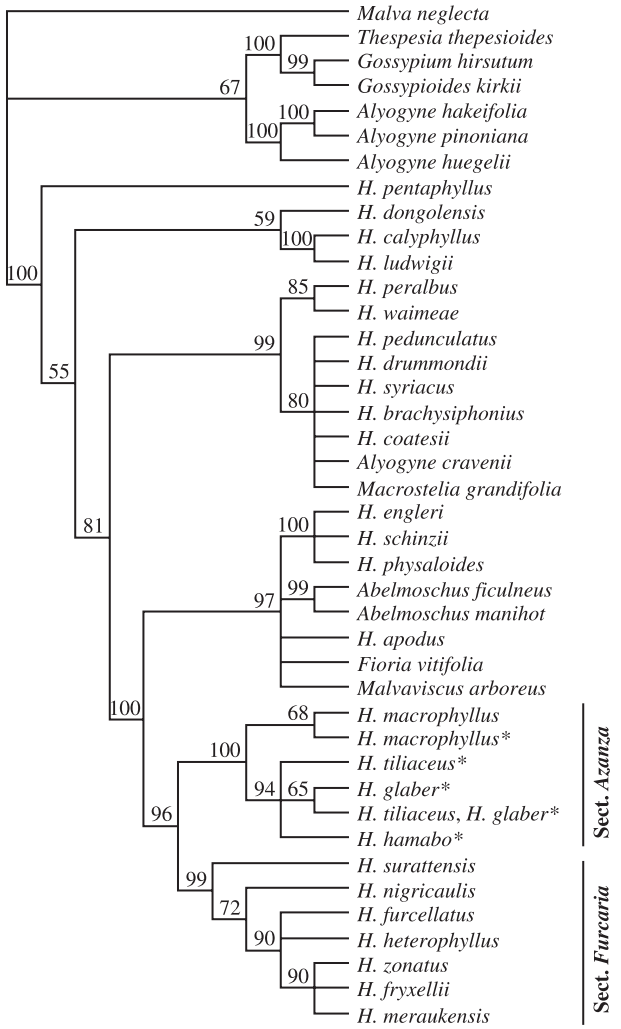

Fig. 2 Strict consensus cladogram of 699 most parsimonious trees based on *ndhF* and *rpl16* intron sequences (length = 447 steps, CI = 0.852, RI = 0.902 and RC = 0.768) from genus *Hibiscus* (sequence data from this study and Pfeil *et al*. 2002). Bootstrap probabilities greater than 50% are shown above the branches. Asterisk indicates sequences newly determined in this study.

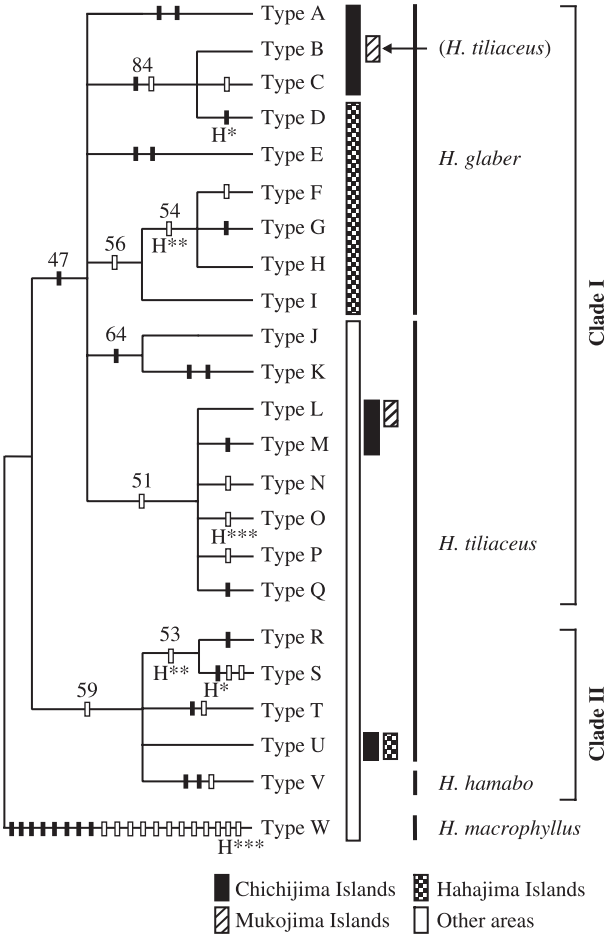

Fig. 3 Single most parsimonious tree based on the sequences of five cpDNA regions, *atpB-rbcL* IGS, *accD-psaI* IGS, *rpl16* intron, *ndhF*, *trnK-psbA* IGS (including *matK*) (length = 53 steps, CI = 0.943, RI = 0.893 and RC = 0.842) among haplotypes of *Hibiscus glaber*, *Hibiscus tiliaceus* and *Hibiscus hamabo*. Numbers above branches are bootstrap probabilities. Solid and open bars on branches show substitutions and indels (or an inversion), respectively. H with asterisk indicates homoplasies. Bars with different patterns next to the haplotype names indicate different geographical areas.

Phylogenetic relationships among cpDNA haplotypes of H. glaber, H. tiliaceus *and* H. hamabo

In the combined sequences of five polymorphic cpDNA regions, 17 substitutions, 13 indels and one inversion were found among all samples of *H. glaber*, *H. tiliaceus* and *H. hamabo* (Table 3). Intraspecific variations were found within *H. glaber* (seven substitutions and five indels) and *H. tiliaceus* (10 substitutions, nine indels, and one inversion) (Table 3). There was no variation among the three samples of *H. hamabo* (Type V in Table 3). Based on these polymorphisms, a total of 22 cpDNA haplotypes was distinguished (Tables 1 and 3). Nine (denoted as A–I), 13 (B, J–U) and one (V) haplotypes were detected for *H. glaber*, *H. tiliaceus* and *H. hamabo*, respectively (Tables 1 and 3). The B haplotype was shared between *H. glaber* from the Chichijima Islands and *H. tiliaceus* from the Mukojima Islands.

From the phylogenetic analysis of 22 haplotypes, we obtained a single most parsimonious tree of 53 steps with a consistency index (CI) of 0.943, a retention index (RI) of 0.893, and a rescaled consistency index (RC) of 0.842 (Fig. 3). In this tree, two major clades, I and II, were resolved, although both were weakly supported (BP 47% and 59% for the clades I and II, respectively, Fig. 3). Clade I was comprised of all haplotypes of *H. glaber* and haplotypes B and J–Q of *H. tiliaceus*, and the clade II was comprised of haplotypes R–U of *H. tiliaceus* and the single haplotype of *H. hamabo* (Fig. 3).

Table 3 Variable sites of the aligned sequences of the five regions cpDNA in *Hibiscus glaber*, *Hibiscus tiliaceus* and *Hibiscus hamabo* haplotypes

			atpB-rbcL IGS																			*accD-psaI* IGS				*rpl16* intron	*ndhF*		*trnK-psbA* IGS (5′*trnK-matK* IGS)				(*matK*)		(*matK3′-psvA* IGS)	
																				1					1									1	1	
			1	1	2	4	6	6	7	7	7	7	8	8	8	8	8	9	9	0	6	7	9	0	8		7		5	6	1	4	1	
			4	5	3	4	2	4	0	1	5	9	0	1	6	8	9	3	4	5	7	5	5	7	4	9	8	1	8	5	0	3	7	
Haplotype	*N*	Taxon	4	8	1	6	3	9	3	4	3	9	8	4	4	7	9	9	7	2	9	5	5	7	7	7	8	7	5	5	0	0	0	
Type A	2	*H. glaber*	0	G	T	C	A	1	1	1	0	0	1	0	A	A	0	0	0	G	T	0	1	A	G		C	T	T	C	C	G	T	2^{n}
Type B	9/2	*H. glaber*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	0^{m}	.	.		G	.	.	T	.	.	.	.	
		/*H. tiliaceus*																							.							.	.	.	
Type C	2	*H. glaber*	1^{a}	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	0^{m}	.	.		G	.	.	T	.	.	.	.	
Type D	3	*H. glaber*	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	0^{m}	.	.		G	.	.	T	.	.	.	.	
Type E	2	*H. glaber*	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	C	.	.	.	.	.		G	.	.	.	.	.	.	.	
Type F	1	*H. glaber*	.	.	.	.	.	.	0^{c}	.	.	.	1^{h}	.	.	1^{i}	.	.	.	C	.	.	.	.	.		G	.	.	.	.	.	.	.	
Type G	2	*H. glaber*	.	.	.	.	.	.	0^{c}	.	.	.	1^{h}	.	.	.	.	.	.	C	.	.	.	T	.		G	.	.	.	.	.	.	.	
Type H	4	*H. glaber*	.	.	.	.	.	.	0^{c}	.	.	.	1^{h}	.	.	.	.	.	.	C	.	.	.	.	.		G	.	.	.	.	.	.	.	
Type I	4	*H. glaber*	.	.	.	.	.	.	.	.	.	.	1^{h}	.	.	.	.	.	.	C	.	.	.	.	.		G	.	.	.	.	.		.	
Type J	1	*H. tiliaceus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.		G	.	.	.	.	A	.	.	
Type K	6	*H. tiliaceus*	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.		G	.	.	.	T	A	.	.	
Type L	12	*H. tiliaceus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.		G	.	.	.	.	.	.	2^{o}	
Type M	1	*H. tiliaceus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	G	.	.	.	.		G	.	.	.	.	.	.	2^{o}	
Type N	1	*H. tiliaceus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	1^{l}	.	.	.		G	.	.	.	.	.	.	2^{o}	
Type O	1	*H. tiliaceus*	.	.	.	.	.	0^{b}	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.		G	.	.	.	.	.	.	2^{o}	
Type P	1	*H. tiliaceus*	.	.	.	.	.	.	.	.	1^{e}	.	.	.	.	.	.	.	.	C	.	.	.	.	.		G	.	.	.	.	.	.	2^{o}	
Type Q	1	*H. tiliaceus*	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.		G	.	.	.	.	.	.	2^{o}	
Type R	1	*H. tiliaceus*	.	.	.	.	.	.	0^{c}	.	.	.	.	.	.	.	.	.	1^{k}	C	.	.	.	.	A		G	.	C	.	.	.	.	.	
Type S	1	*H. tiliaceus*	.	C	.	.	.	.	0^{c}	0^{d}	.	.	.	.	.	.	1^{j}	1^{k}	C	.	.	.	.	A		G	.	.	.	.	.	.	.	
Type T	1	*H. tiliaceus*	.	.	.	.	.	.	.	.	.	1^{f}	.	.	.	.	.	.	1^{k}	C	.	.	.	.	A		G	.	.	.	.	.	C	.	
Type U	7	*H. tiliaceus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1^{k}	C	.	.	.	.	A		G	.	.	.	.	.	.	.	
Type V	3	*H. hamabo*	.	.	.	.	C	.	.	.	.	.	0^{g}	.	.	.	.	.	1^{k}	C	.	.	.	.	A		G	C	.	.	.	.	.	.	

a, CAATTGGATT; b, TTCTAA; c, ATTT; d, ATTATATATTTTATTT; e, ATAATATAATTTTATTTATAA; f, ATATTT; g, AATATTT; h, ATATTAATATTTAATATTTATTTAATTT; I, AATATTTAATATTTATTTAATTT; j, ATTATTATTT; k, ATTATTTATTATTATTT; L, AAAAATTGAAATTAGAAATTCAAA; m, AGTCC; n, TTCCC; o, GGGAA.

Sequences are numbered from the 5′ to the 3′ ends in each region. Dots (.) indicate that the character states are the same as for *H. glaber* haplotype A. Numbers (0, 1 and 2) in the sequences indicate length polymorphisms; 0, 1, and 2 represent absence, presence, and inversion of sequences cited by uppercase letters, respectively.

Geographic distribution of cpDNA haplotypes

The geographical distribution of the nine haplotypes of *H. glaber* was structured between the two main island groups in the Bonin Islands (Fig. 1). Haplotypes A–C and haplotypes D-I were unique to the Chichijima and Hahajima islands, respectively, and most of them were restricted to limited areas (Fig. 1). Of particular note was the clear geographical structure from north to south within the Hahajima Islands (Fig. 1). The northern haplotype in the Hahajima Islands (type D) showed close relatedness (84% BP) with two haplotypes (types B and C) in the Chichijima Islands (Fig. 3). The central and southern four haplotypes in the Hahajima Islands (types F, G, H and I) showed relatively close relations within *H. glaber* lineages (Fig. 3). In *Hibiscus tiliaceus*, three major haplotypes (K, L and U) were widely distributed (Fig. 4). For example, the K haplotype is distributed across the Pacific Ocean, from the Malay Peninsula, the Ryukyu Islands of the Japanese archipelago, and Hawaii (Fig. 4). Other nine haplotypes were found from single accessions (Fig. 4). In the Bonin Islands, four haplotypes of *H. tiliaceus* were found, two of which were widely distributed (types L and U), and the other two (B and M) were unique to the Bonin Islands (Figs 1 and 4). The V haplotype from *H. hamabo* was distributed in the Pacific coast areas of the Japanese archipelago (Fig. 4).

Discussion

Phylogenetic relationships among Hibiscus glaber, Hibiscus tiliaceus, *and* Hibiscus hamabo

Phylogenetic relationships in the genus *Hibiscus*, based on the sequences of the *ndhF* gene and *rpl16* intron, indicated

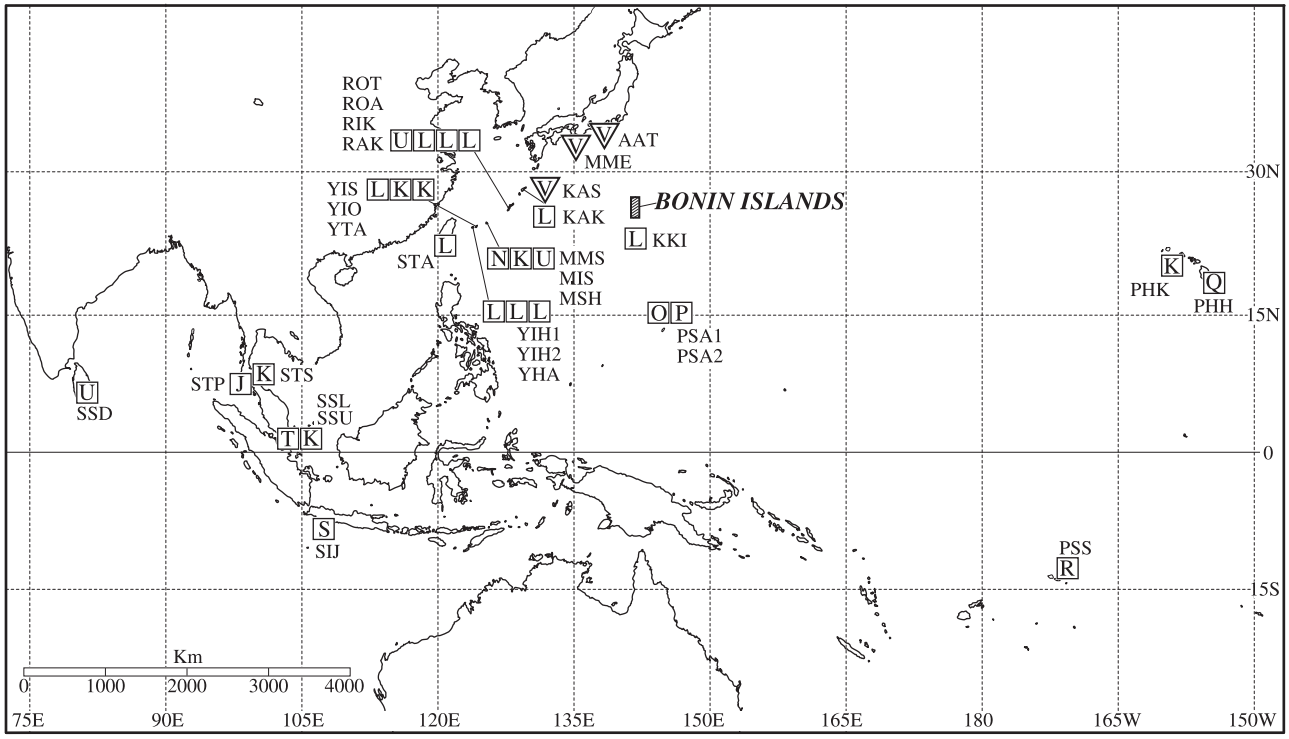

Fig. 4 The geographical distribution of cpDNA haplotypes found in *Hibiscus tiliaceus* and *Hibiscus hamabo*. Squares and triangles show *H. tiliaceus* and *H. hamabo*, respectively. For individual acronyms refer to Table 1.

that section *Azanza* is monophyletic. *Hibiscus glaber*, *Hibiscus tiliaceus*, and *Hibiscus hamabo* formed a subclade (Fig. 2). These three species are also morphologically similar to each other within section *Azanza*. *Hibiscus macrophyllus* is distinct from these three species in several morphological traits, such as prominent yellowish shaggy hairs on leaves and stems, and large leaves which grow up to 40 cm long and 50 cm wide (Waalkes 1966; Fryxell 2001).

Although the phylogenetic relationships among haplotypes of *H. glaber*, *H. tiliaceus* and *H. hamabo* resolve only to a polytomy with low bootstrap support, the data suggest that at least two lineages exist (clades I and II in Fig. 3). Haplotypes of *H. tiliaceus* were found within both clades, and *H. glaber* and *H. hamabo* were nested in separate clades (clades I and II, respectively). The results suggest that *H. glaber* and *H. hamabo* have been derived from independent lineages of ancestral *H. tiliaceus*.

The origin and speciation of H. glaber

At the beginning of this study, we offered two different hypotheses for the speciation of *H. glaber.* The first hypothesis assumes that *H. glaber* and the modern Bonin populations of *H. tiliaceus* have differentiated sympatrically within the Bonin Islands. The second hypothesis assumes that *H. glaber* differentiated under geographical isolation from other *H. tiliaceus* populations, and modern Bonin populations of the latter have immigrated more recently. With the former hypothesis, we would expect to find a monophyletic relationship between co-occurring *H. glaber* and *H. tiliaceus* populations on the Bonin Islands. In the latter hypothesis, we would expect that co-occurring *H. glaber* and *H. tiliaceus* populations on the Bonin Islands would not share most recent ancestral populations.

Of four haplotypes of *H. tiliaceus* from the Bonin Islands (B, L, M, and U in Fig. 3), the U haplotype was in clade II and did not obviously share a most recent ancestor with *H. glaber* (Fig. 3). The results indicate multiple migration of this ancestral group to the Bonin Islands (hypothesis 2). In the Bonin Islands, haplotype U was detected from the three largest populations in the area (CKO, CBU and CNK2 in the Chichijima Island, Fig. 1; Kudoh & Kachi 1997) and from the population in Anejima Island (in the Hahajima Islands, Fig. 1). Sites CKO and CNK2 were the areas where we observed co-occurrence of individuals of *H. tiliaceus* and *H. glaber*. The U haplotype was also found in *H. tiliaceus* plants in the Ryukyu Islands (ROT) and Miyakojima Islands (MSH), southwestern islands of the Japanese archipelago (Fig. 4).

Although the L and M haplotypes were nested in clade I with the haplotypes of *H. glaber*, the geographical distributions of the L and M haplotypes were more supportive of independent migration of these lineages to the Bonin Islands from ancestral populations of *H. glaber* (hypothesis

2). In the Bonin Islands haplotype L was found in a small islet (CHG in the Chichijima Islands, Fig. 1) or on an island (MNA in the Nakôdojima Island, Fig. 1) where *H. glaber* is absent. The M haplotype was found in a small isolated population of *H. tiliaceus* on the eastern shore of Chichijima Island (CHA, Fig. 1). Outside the Bonin Islands, the L haplotype was found from the Kazan Islands (KKI), the Ryukyu Islands (ROA, RIK and RAK), the Yaeyama Islands (YIS, YIH1, YIH2, and YHA), and Amamioshima Island (KAK) and Taiwan (STA). Furthermore, the L and M haplotypes form a subclade (51% bootstrap value, Fig. 3) with the other haplotypes of *H. tiliaceus* from wide-ranging areas, such as the Miyakojima Islands (MMS), Saipan Island (PSA1 and PSA2), and the Hawaiian Islands (PHH).

The existence of one haplotype of *H. tiliaceus*, the B haplotype, from the Bonin Islands may be supportive of the first hypothesis. The B haplotype was shared between nine individuals of *H. glaber* in the Chichijima Islands and two individuals of *H. tiliaceus* in the Mukojima Island (Figs 1 and 3). The B haplotype was included in the subclade with haplotypes C and D of *H. glaber* with an 84% BP. It should be noted that the Mukojima Islands are located 50 km north of the Chichijima Islands, and we did not find *H. glaber* in the Mukojima Islands during this study nor in herbarium surveys. The results suggested that the *H. tiliaceus* population of Mukojima Island is a geographically isolated lineage originating from ancestral populations that have differentiated into B, C and D haplotypes of *H. glaber* in the Chichijima Islands and the Hahajima Island (Figs 1 and 3). We consider that the ancestors of *H. glaber* were similar to *H. tiliaceus* when they first arrived in the Bonin Islands because *H. tiliaceus* has characteristics that are important for long-distance colonization, such as ocean-current dispersal of buoyant seeds and adaptation to coastal habitats. It is plausible to imagine that the Mukojima Island lineages have maintained ancestral characters because adaptation to an inland habitat has not been significant on this small island; the Mukojima population is therefore morphologically referable to as *H. tiliaceus*. An alternative explanation might be that past hybridization events have introduced the cpDNA haplotype of *H. glaber* into *H. tiliaceus*. Further studies on variation in nuclear DNA are required to reveal the origin of haplotype B on Mukojima Island.

Overall, the data emphasize the importance of geographical isolation during speciation of *H. glaber*. The co-occurrence of *H. glaber* and *H. tiliaceus* on the Bonin Islands are attributable to multiple migration events of different lineages into the islands. We detected at least three independent migration events during the formation of current distributions of *H. tiliaceus* and *H. glaber* on the Bonin Islands. The importance of multiple migration events during plant speciation on oceanic islands has been reported from other systems: *Lavatera* (Malvaceae) in the Canary Islands (Ray 1995), and *Asteriscus* (Asteraceae) and *Sonchus* (Asteraceae) alliances in Macaronesia (Kim *et al*. 1996a, b; Francisco-Ortega *et al*. 1999).

All nine haplotypes of *H. glaber* were in clade I (Fig. 3), suggesting a close relationship among them. However, the results neither support nor reject monophyly of *H. glaber* because the basal relationships of the haplotypes still remain unresolved. Lack of phylogenetic resolution of the basal relationships in island-plant groups may reflect either rapid diversification of lineages, unstable rates of molecular evolution, or effects of homoplasy (Baldwin *et al*. 1998). The additional comparison of gene trees based on unlinked molecular data sets is required before any firm conclusion can be reached.

The diversification within H. glaber *in the Bonin Islands*

Despite its narrow geographical range, nine haplotypes of *H. glaber* showed a clear geographical structure in their distributions (Fig. 1). Except for the B haplotype in the Chichijima Islands, all haplotypes showed localized distributions even within the islands. In contrast, corresponding with the wide range of species distribution, some haplotypes of *H. tiliaceus* showed remarkably wide distributions across the Pacific Ocean (Fig. 4). The haplotypes K, L and U were widely distributed throughout the northwestern Pacific and even to the Indian Ocean (haplotype U) or to the Hawaiian Islands (haplotype K). The distributions of these haplotypes overlap with one another. The wide and overlapping distribution of haplotypes in *H. tiliaceus* is attributable to the historical and current migration events among distant areas by ocean-current seed dispersal, as seeds of *H. tiliaceus* are known to be buoyant and able to float in saltwater for more than 3 months, and with high level of germination (Nakanishi 1985, 1988, 1991).

Geographic structuring of haplotypes in *H. glaber* may be one of the consequences of adaptation to inland habitats during speciation. Habitat-shift into inland areas is likely to result in changes of seed-dispersal to more local gravity modes. Many generations would have been needed for the earlier ancestors of *H. glaber* to become distributed throughout the Bonin Islands. Historical and current short-distance seed dispersals would have formed restricted geographical distributions of cpDNA haplotypes. This idea is supported by the tendency to have proximate distributions genetically related haplotypes (e.g. haplotypes F, G, H and I in the Hahajima Islands, Figs 1 and 3). As discussed previously, our results do not rule out the possibility of multiple origins of *H. glaber*, which could also explain geographical structuring between northern (B, C, and D) and southern haplotypes (F, G, H, and I, Figs 1 and 3).

No haplotypes were commonly found in both the Chichijima and Hahajima islands that are separated from each other by *c*. 50 km (Fig. 1). The D haplotype, detected in the northern part of the Hahajima Islands, however, formed a

clade with two haplotypes of the Chichijima Islands (Figs 1 and 3), suggesting that migrations of *H. glaber* have occurred between the Chichijima and Hahajima islands.

Loss of dispersal ability is a common feature of evolution on oceanic islands (reviewed in Whittaker 1998). Carlquist (1974) reported that the loss of seed dispersal mediated by habitat-shift has occurred in several plant families. It is also thought that easily dispersed propagules would be more likely lost from the gene pool in island environments (Roff 1990; Cody & Overton 1996). Cody & Overton (1996) monitored populations of *Hypochaeris radicata* Hook. and *Lactuca muralis* E. May, both wind-dispersed members of Asteraceae, for 10 years. They showed striking examples of short-term evolution of reduced seed dispersal ability in small and isolated island populations.

Short-distance seed dispersal might result in genetic and morphological diversification among local populations of *H. glaber*. We previously reported (Takayama & Kato 2001) variation in leaf morphology of *H. glaber*, and seven out of 10 measured traits were significantly different between the Chichijima and Hahajima islands. Combined analyses showed that the geographical structuring of the haplotypes of *H. glaber* corresponded well to geographical patterns in morphological diversification (data not shown). The strong genetic structuring and moderate morphological diversifications in *H. glaber* may represent on-going processes toward accelerated diversification or adaptive radiation common among island plants. In the Bonin Islands, adaptive radiation has been reported in the genera *Crepidiastrum* (Asteraceae, three species), *Callicarpa* (Verbenaceae, three species), *Pittosporum* (Pittosporaceae, four species,) and *Symplocos* (Symplocaceae, three species) (Shimizu 1984; Kawakubo 1986). Furthermore, the extensive morphological diversifications within species, corresponding to a variety of habitats, are also observed in *Syzygium cleyeraefolium* Makino (Fujita *et al*. 2002). The low allozyme genetic diversity within these groups, except *Callicarpa* and *Syzygium* (Ito & Ono 1990; Soejima *et al*. 1994; Ito *et al*. 1997), suggests that the diversification has occurred at accelerated rates.

Ricklefs & Cox (1972, 1978) applied a taxon cycle model to the biogeography of the avifauna of the West Indies. In their model, they recognized four stages of island species evolution (reviewed in Whittaker 1998). Stage I is the invasion of an island by a mainland species. In stage II, the colonist expands its niche, but has patchy distributions because selection against mobility reduces gene flow. At stage III, the species become highly differentiated endemic. In stage IV, a highly differentiated endemic species persists as a relict on a few islands and becomes extinct, to be replaced by new colonists from the mainland or from neighbouring islands. Recent molecular phylogeographical approaches in the West Indian avifauna (see review in Emerson 2002) allow the time dimension of the taxon cycle to be measured quantitatively. Although the taxon cycle model does not necessarily apply to all cases (Pregill & Olson 1981), if we apply this model to *H. glaber*, our data suggest that *H. glaber* may be in a transition from stage II to stage III, and that *H. glaber* is still at an early stage of adaptive radiation. Further studies, particularly focusing on the relationship between genetic diversification and local adaptations, are required to gain a fuller understanding of the evolution of *H. glaber* in the Bonin Islands.

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Reference

Asami S (1970) Geological features. In: *Ogasawara No Shizen* (eds Tuyama T, Asami S), pp. 91–108. Hirokawa-shoten, Tokyo, Japan (in Japanese).

Baldwin BG, Crawford DJ, Francisco-Ortega J *et al.* (1998) Molecular phylogenetic insights on the origin and evolution of oceanic islands plants. In: *Molecular Systematics of Plants II: DNA Sequencing* (eds Soltis DE, Soltis PS, Doyle JJ), pp. 410–441. Kluwer Academic Publishers, Norwell, Massachusetts.

Carlquist S (1974) Loss of dispersibility in island plants. In: *Island Biology*, pp. 429–486. Columbia University Press, New York.

Chiba S (1999) Accelerated evolution of land snails *Mandarina* in the oceanic Bonin Islands: evidence from mitochondrial DNA sequences. *Evolution*, **53**, 460–471.

Cody ML, Overton JM (1996) Short-term evolution of reduced dispersal in island plant populations. *Journal of Ecology*, **84**, 53–61.

Darwin C (1859) *The Origin of Species*. John Murray, London, UK.

Doyle JJ, Doyle JS (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, **9**, 11–15.

Emerson BC (2002) Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. *Molecular Ecology*, **11**, 951–966.

Felsenstein J (1985) Confidence limits on phylogenies — an approach using the bootstrap. *Evolution*, **39**, 783–791.

Francisco-Ortega J, Goertzen LR, Santos-Guerra A, Benabid A, Jansen RK (1999) Molecular systematics of the *Asteriscus* alliance (Asteraceae: Inuleae) I: evidence from the internal transcribed spacers of nuclear ribosomal DNA. *Systematic Botany*, **24**, 249–266.

Francisco-Ortega J, Barber JC, Santos-Guerra A, Febles-Hernández R, Jansen RK (2001) Origin and evolution of the endemic genera of Gonosperminae (Asteraceae: Anthemideae) from the Canary Islands: evidence form nucleotide sequences of the internal transcribed spacers of nuclear ribosomal DNA. *American Journal of Botany*, **88**, 161–169.

Fryxell PA (2001) *Taliparitti* (Malvaceae), a segregate from *Hibiscus*. *Contribution of the University of Michigan Herbarium*, **23**, 225–270.

Fujita T, Kato H, Wakabayashi M (2002) Morphological variation and environmental condition of *Syzygium* (Myrtaceae) in the Bonin Islands. *Acta Phytotaxonomica et Geobotanica*, **53**, 181–199.

Golenberg EM, Clegg MT, Durbin ML, Doebley J, Ma DP (1993) Evolution of a noncoding region of the chloroplast genome. *Molecular Phylogenetics and Evolution*, **2**, 52–64.

Hamilton MB (1998) Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology*, **8**, 521–523.

Imaizumi T, Tamura T (1984) Geomorphology of the Chichijima and Hahajima islands. *Ogasawara-Kenkyu-Nenpo*, **7**, 3–11 (in Japanese).

Ito M (1998) Origin and evolution of endemic plants of the Bonin (Ogasawara) Islands. *Researches on Population Ecology*, **40**, 205–212.

Ito M, Ono M (1990) Allozyme diversity and the evolution of *Crepidiastrum* (Compositae) on the Bonin (Ogasawara) Islands. *Botanical Magazine, Tokyo*, **103**, 449–459.

Ito M, Soejima A, Ono M (1997) Allozyme diversity of *Pittosporum* (Pittosporaceae) on the Bonin (Ogasawara) Islands. *Journal of Plant Research*, **110**, 455–462.

Johnson LA, Soltis DE (1994) *MatK* DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. *Systematic Botany*, **19**, 143–156.

Jordan WC, Courtney MW, Neigel JE (1996) Low levels of intraspecific genetic variation at a rapidly evolving chloroplast DNA locus in north American duckweeds (Lemnaceae). *American Journal of Botany*, **83**, 430–439.

Kaizuka S (1977) Geology and geomorphology of the Bonin Islands. *Ogasawara-Kenkyu-Nenpo*, **1**, 29–34 (in Japanese).

Kato H, Oginuma K, Gu Z, Hammel B, Tobe H (1998) Phylogenetic relationships of Betulaceae based on *matK* sequences with particular reference to the position of *Ostryopsis*. *Acta Phytotaxonomica et Geobotanica*, **49**, 89–97.

Kawakubo N (1986) Morphological variation of three endemic species of *Callicarpa* (Verbenaceae) in the Bonin (Ogasawara) Islands. *Plant Species Biology*, **1**, 59–68.

Kelchner SA (2000) The evolution of noncoding chloroplast DNA and its application in plant systematics. *Annals of the Missouri Botanical Garden*, **87**, 482–498.

Kelchner SA, Clark LG (1997) Molecular evolution and phylogenetic utility of the chloroplast *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). *Molecular Phylogenetics and Evolution*, **8**, 385–397.

Kim S-C, Crawford DJ, Jansen RK (1996a) Phylogenetic relationships among the genera of the subtribe Sonchinae (Asteraceae): evidence from ITS sequences. *Systematic Botany*, **21**, 417–432.

Kim S-C, Crawford DJ, Francisco-Ortega J, Santos-Guerra A (1996b) A common origin for woody *Sonchus* and five related genera in the Macaronesian Islands: molecular evidence for extensive radiation. *Proceedings of the National Academy of Sciences, U.S.A.*, **93**, 7743–7748.

Kim H-G, Keeley S-C, Vroom PS, Jansen RK (1998) Molecular evidence for an African origin of the Hawaiian endemic *Hesperomannia* (Asteraceae). *Proceedings of the National Academy of Sciences, U.S.A.*, **95**, 15440–15445.

Kobayashi S (1978) A list of the vascular plants occurring in the Ogasawara (Bonin) Islands. *Ogasawara Research*, **1**, 1–33.

Kudoh H, Kachi N (1997) Distribution of *Hibiscus tiliaceus* L. in the Chichijima Island, the Bonin Islands. *Ogasawara-Kenkyu-Nenpo*, **21**, 56–60 (in Japanese).

Kudoh H, Uchiyama M, Kachi N (1998) Flower size variation in *Hibiscus glaber* and *H. tiliaceus* in Chichijima Island, the Bonin (Ogasawara) Islands. *Ogasawara Research*, **24**, 25–34.

Mayr E (1963) *Animal Species and Evolution*. Belknap Press of Harvard University Press, Cambridge, Massachusetts.

McCauley DE (1994) Contrasting the distribution of chloroplast DNA and allozyme polymorphism among local populations of *Silene alba*: implications for studies of gene flow in plants. *Proceedings of the National Academy of Sciences, U.S.A.*, **91**, 8127–8131.

McCauley DE (1996) The spatial distribution of chloroplast DNA and allozyme polymorphisms within a population of *Silene alba* (Caryophyllaceae). *American Journal of Botany*, **83**, 727–731.

Mogensen HL (1996) The hows and whys of cytoplasmic inheritance in seed plants. *American Journal of Botany*, **83**, 383–404.

Nakanishi H (1979) Distribution and ecology of a semi-mangrove plant, *Hibiscus hamabo* Sieb. et Zucc. and its community. *Acta Phytotaxonomica et Geobotanica*, **30**, 169–179.

Nakanishi H (1985) Geobotanical and ecological studies on three semi-mangrove plants in Japan. *Japanese Journal of Ecology*, **35**, 85–92.

Nakanishi H (1988) Dispersal ecology of the maritime plants in the Ryukyu Islands, Japan. *Ecological Research*, **3**, 163–173.

Nakanishi H (1991) Oceanic dispersal and the formation of oceanic islands flora. *Shuseibutsugaku Kenkyu*, **15**, 1–14 (in Japanese).

Ohba H (1999) Malvaceae. In: *Flora of Japan, Volume IIc* (eds Iwatsuki K, Boufford DE, Ohba H), pp. 134–142. Kodansha, Tokyo, Japan.

Olmstead RG, Sweere JA (1994) Combining data in phylogenetic systematic: an empirical approach using three molecular data sets in the Solanaceae. *Systematic Biology*, **43**, 467–481.

Ono M, Kobayashi S (1985) Synopsis of flowering plants endemic to the Bonin Islands. In: *Endemic Species and Vegetation of the Bonin Islands*, pp. 1–96, Aboc-Sha, Kamakura, Japan (in Japanese with English Summary).

Pfeil BE, Brubaker CL, Craven LA, Crisp MD (2002) Phylogeny of *Hibiscus* and the tribe Hibisceae (Malvaceae) using chloroplast DNA sequences of *ndhF* and the *rpl16* intron. *Systematic Botany*, **27**, 333–350.

Pregill GK, Olson SL (1981) Zoogeography of West Indian vertebrates in relation to Pleistocene climatic cycles. *Annual Review of Ecology and Systematics*, **12**, 75–98.

Ray ML (1995) Systematics of *Lavatera* and *Malva* (Malvaceae, Malvae) — a new perspective. *Plant Systematics and Evolution*, **198**, 29–53.

Richardson JE, Weitz FM, Fay MF *et al*. (2001) Phylogenetic analysis of *Phylica* L. (Rhamnaceae) with an emphasis on island species: evidence from plastid *trnL-F* and nuclear internal transcribed spacer (ribosomal) DNA sequences. *Taxon*, **50**, 405–427.

Ricklefs RE, Cox GW (1972) Taxon cycles in West Indian avifauna. *American Naturalist*, **106**, 195–219.

Ricklefs RE, Cox GW (1978) Stage of taxon cycle, habitat distribution, and population density in the avifauna of West Indies. *American Naturalist*, **112**, 875–895.

Roff DA (1990) The evolution of flightlessness in insects. *Ecological Monographs*, **60**, 389–421.

Sang T, Crawford DJ, Kim S-C, Stuessy TF (1994) Radiation of the endemic genus *Dendroseris* (Asteraceae) on the Juan Fernandez Islands: evidence from sequences of the ITS regions of nuclear ribosomal DNA. *American Journal of Botany*, **81**, 1494–1501.

Schluter D (2001) Ecology and the origin of species. *Trends in Ecology & Evolution*, **16**, 372–380.

Seelanan T, Schnabel A, Wendel JF (1997) Congruence and consensus in the cotton tribe (Malvaceae). *Systematic Botany*, **22**, 259–290.

Setoguchi H, Ohba H (1995) Phylogenetic relationships in *Crossostylis* (Rhizophoraceae) inferred from restriction site variation of chloroplast DNA. *Journal of Plant Research*, **108**, 87–92.

Shimizu Y (1984) Comparison of the woody species between the Bonin (oceanic) and the Ryukyu (continental) Islands concerning the ecological release of plants in islands. *Ogasawara Research*, **11**, 25–49.

Shimizu Y (1989) Ökologische eigenschaften der Waldvegetation of auf den ozeanischen Inseln, Ogasawara. In: *Vegetation of Japan Okinawa and Ogasawara* (ed. Miyawaki A), pp. 159–203. Shibundo, Tokyo, Japan (in Japanese).

Shimizu Y (1991) Forest structures, composition, and distribution on a Pacific island, with reference to ecological release and speciation. *Pacific Science*, **45**, 28–49.

Small RL, Ryburn JA, Cronn RC, Seelanan T, Wendel JF (1998) The tortoise and the hare: choosing between noncoding plastome and nuclear *ADH* sequences for phylogeny reconstruction in a recently diverged plant group. *American Journal of Botany*, **85**, 1301–1315.

Soejima A, Nagamasu H, Ito M, Ono M (1994) Allozyme diversity and the evolution of *Symplocos* (Symplocaceae) on the Bonin (Ogasawara) Islands. *Journal of Plant Research*, **107**, 221–227.

Swofford DL (2002) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4*. Sinauer, Sunderland, Massachusetts.

Tai TH, Tanksley SD (1990) A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. *Plant Molecular Biology Reporter*, **8**, 297–303.

Takayama K, Kato H (2001) Morphological variation of leaf characters in *Hibiscus* in the Bonin (Ogasawara) Islands. *Ogasawara Research*, **26**, 1–13.

Takayama K, Ohi T, Kato H, Kudoh H, Wakabayashi M (2002) Capsule morphology and geographic distribution of *Hibiscus glaber* and *H. tiliaceus*. *Ogasawara Research*, **27**, 31–55.

Terachi T (1993) Structural alterations of chloroplast genome and their significance to the higher plant evolution. *Bulletin of the Institute for National Land Utilization Development, Kyoto Sangyo University*, **14**, 138–148 (in Japanese).

Tomlinson PB (1986) *The Botany of Mangroves*. Cambridge University Press, Cambridge.

Waalkes JVB (1966) Malesian Malvaceae revised. *Blumea*, **14**, 23–57.

Wagner WL, Funk VA eds (1995) *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago*. Smithsonian Institution Press, Washington, D.C.

Whittaker RJ (1998) *Island Biogeography: Ecology, Evolution and Conservation*. Oxford University Press, Oxford.

Yamazaki T (1981) The floristic position of the Bonin Islands plants. In: *Illustrated Book of the Bonin Islands Plants* (ed. Toyoda T), pp. 303–308. Aboc-sha, Kamakura, Japan (in Japanese).

This work forms part of Koji Takayama's Masters' thesis at Tokyo Metropolitan University. It was one of several cooperative research projects on the endemic plants of the Bonin Islands between the plant ecology and the plant systematics & taxonomy laboratories of the University. Koji Takayama's current research is focused on the population genetics of taxa with pan-tropical seed dispersal plants. Tetsuo Ohi-Toma is interested in systematics and phylogeography of plants in the Sino-Japanese region. Hiroshi Kudoh is working on the genetics of environmental adaptations in plants. Hidetoshi Kato, the project leader, is interested in the systematics and conservation of island plants.
