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Genetic structures of *Calophyllum inophyllum* L., a tree employing sea-drift seed dispersal in the northern extreme of its distribution

So Hanaoka · Ching-Te Chien · Shun-Ying Chen · Atsushi Watanabe · Suzuki Setsuko · Kazutaka Kato

Abstract
• *Context* Little is known about the potential of gene flow and resulting genetic structures of trees employing sea-drifting seed dispersal in island populations.
• *Aims* Current genetic structure and the magnitude of historical gene flow were estimated in island populations of *Calophyllum inophyllum* L., a typical plant employing sea-drifting seed dispersal.
• *Methods* Samples were collected from the northern extreme of the species' distribution (Taiwan and the Sakishima, Daito, and Ogasawara Islands, Japan) and genotyped using 15 EST-SSR markers. Genetic differentiation (F_{ST} and AMOVA), genetic structure (STRUCTRE analysis), and historical gene flow (assignment testing) were determined.
• *Results* Frequent gene flow within and rare gene flow among island groups was determined using assignment testing. Clear genetic structures were also detected using the STRUCTURE analysis, which demonstrated differentiation between dominant clusters among geographically constructed island groups.
• *Conclusions* The potential for gene flow via sea-drifting seed dispersal was high, and this was possible even among small islands. However, the extent and frequency of gene flow were not great enough to prevent genetic differentiation in a range of over a few hundred kilometers.

Contribution of the co-authors Ching-Te Chien: Assistance of data analysis and writing paper.
Shun-Ying Chen: Designing and conducting ecological research and sampling.
Atsushi Watanabe: Designing the experiments and supervising the writing paper.
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1 Introduction

Isolation of populations associated with limited gene flow has important implications when determining genetic differentiation and structure, and the effect is generally remarkable in island plant populations. Sea currents are one mode of seed dispersal that allows island littoral flora to overcome spatial obstacles to gene flow (Vatanparast et al. 2011), and plants that are capable of utilizing sea currents as vectors for seed dispersal possess the capacity to transport their seeds over long distances. Therefore, clear genetic differentiation might not be found in such species even in island populations. In fact, previous studies showed that plants that employ sea-drifting seed dispersal exhibited low levels of genetic differentiation throughout certain oceanic regions (Takayama et al. 2006, 2008). While similar results may be expected when examining populations on small islands within a relatively

narrow range, previous studies demonstrated that genetic differentiation is not necessarily low in plant species that employ sea current-based seed dispersal, even at the island chain level (e.g., *Aster miyagii*; Maki 2001). The counterintuitive nature of the results of such studies has not been adequately examined, and further research is required to develop a fuller understanding of gene flow among small islands as well as genetic variations and the structure of plants that employ sea-drifting seed dispersal.

Calophyllum inophyllum. L. is a large, broadleaf, insect-pollinated evergreen tree species distributed within coastal regions ranging from tropical eastern Africa (Kenya, Tanzania, Madagascar etc.) to Southeast Asia (India, Sri Lanka, Indonesia, Malaysia, Philippines etc.) and Polynesia (Palau, Samoa, Fiji, the Cook islands etc.; Lemmens 1994; Whistler 2009). The species is used for windbreak forests in Japan, and its seeds are used in the production of cosmetics, biodiesel fuel, and medicine in other tropical countries. This multitude of practical uses has led to the species' conservation and management having been identified as an important topic of study. *C. inophyllum* was found to be well suited to seawater-based seed dispersal, as its seeds are capable of floating in seawater for longer than 90 days without experiencing a significant decrease in germination rate (Nakanishi 1988). Therefore, it was concluded that *C. inophyllum* possessed a high capacity for increasing its distributional range through sea current-based seed dispersal, a feature that may have contributed to the wide distribution currently associated with the species. Despite its wide range, the distribution of *C. inophyllum* is limited by its inability to tolerate low temperatures, with areas in which the mean minimum temperature of the coldest month was <12 °C having proven inhospitable for growth of the species (Friday and Okano 2006). Taira (1987) also suggested that planting *C. inophyllum* proved difficult in the northern part of Okinawa Island, an area located approximately 250 km to the northeast of the Sakishima Islands where the mean daily minimum air temperature during the coldest months in 1981–2010 was 12.4 °C [28.833 N, 128.268 E] (Japan Meteorological Agency, http://www.jma.go.jp/jma/indexe.html). In addition, observations by the authors of the present study showed that current-year seedlings died within 14 days of exposure to cool temperature conditions (a mean daily minimum air temperature of 8.7±2.7 °C; S. Hanaoka et al. unpublished data). Mean daily minimum air temperatures during the coldest months in 1981–2010 were 16.0, 14.3, and 15.3 °C for Miyako-jima of the Sakishima Islands, Minamidaito-jima of the Daito Islands, and Chichi-jima of the Ogasawara Islands, respectively (Japan Meteorological Agency 2012). Therefore, it was determined that the area ranging from the Sakishima Islands to the Ogasawara Islands in Japan represents the current northern extreme of *C. inophyllum*'s natural distribution (Fig. S1; supplemental data).

The past existence of land bridges may also have affected the current genetic structures of *C. inophyllum*. While land bridges linking China and Okinawa Island formed several times prior to the last glacial maximum (LGM; ca. 25,000–15,000 years BP), the majority of the islands in this region have been independent for the past 20,000 years (Kimura 1996). During the LGM, the distribution of *C. inophyllum* in Japan was thought to have been hampered by the fact that air temperatures within the Sakashima Islands were 5–10 °C colder than they are now (Zheng et al. 2003). The rising air temperature during the postglacial period (ca. 12,000 years BP) allowed the migration of *C. inophyllum* from Southeast Asia and/or southern Pacific islands to the Sakishima, Daito, and Ogasawara Islands. In any case, plants distributed throughout the majority of the islands between Taiwan and the Ogasawara Islands have been isolated from one another for more than 20,000 years, with the existence of continuous populations having been impossible for at least that much time. Therefore, it was expected that evaluation of these areas would allow determination of historical gene flows and genetic structures of *C. inophyllum* that resulted from sea-drifting seed dispersal.

The present study examined (1) the genetic diversity, (2) the degree of historical gene flow, and (3) the genetic structures of island populations of *C. inophyllum* in the northern extreme of its distribution to provide a better understanding of the potential for gene flow in plants with sea-drifting seeds and aid in the conservation management of their genetic resources.

2 Materials and methods

2.1 Sampling sites

Samples were collected from Taiwan and the Sakishima, Daito, and Ogasawara Islands (Fig. 1). The Sakishima Islands were subdivided into the Yaeyama Islands and Miyako Islands, which were classified for the purposes of this study into the Yaeyama and Miyako groups, respectively. Yaeyama group samples were obtained from Yonaguni-jima, Iriomote-jima, Hateruma-jima, Kuro-shima, and Ishigaki-jima, while Miyako group samples were obtained from Tarama-jima and Miyako-jima. The Daito Islands were composed of both Kitadaito-jima and Minamidaito-jima; however, samples were collected only from Minamidaito-jima. Samples were also collected from the Ogasawara Islands, Chichi-jima, and Haha-jima.

Basic information of each sampled island is given in Table 1. Other important features of each island are as noted in the following sections.
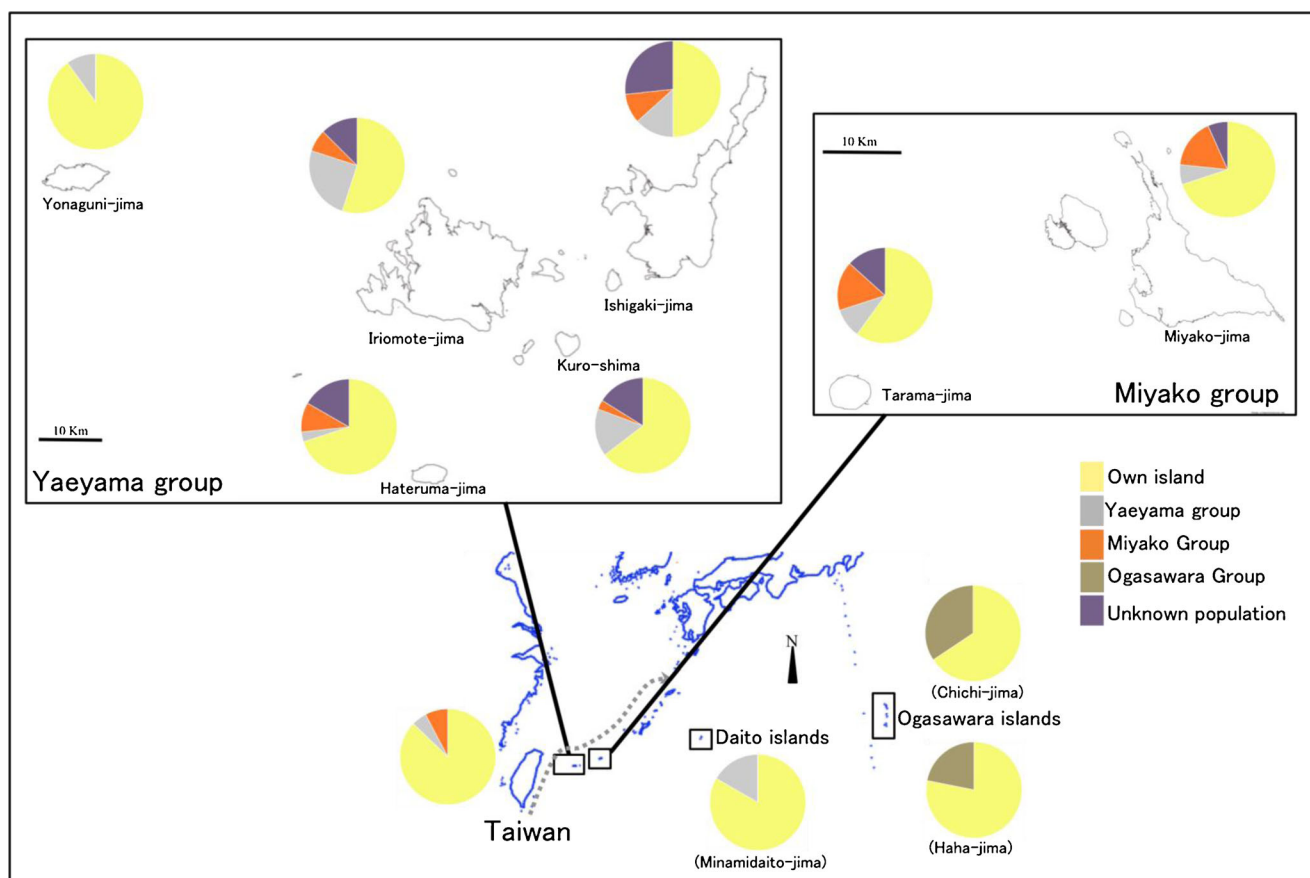
Fig. 1 Maps of islands examined in the present study and results of assignment testing. The *circle graphs* correspond to the summary results of the assignment testing

2.1.1 Taiwan island

Calophyllum inophyllum has not been used commercially in Taiwan; however, a number of trees have been harvested in association with land development. Despite this removal, many naturally occurring trees remain on the southern tip of the island (an area ranging from 21.9 °N–22.3 °N).

2.1.2 Yonaguni-jima and Hateruma-jima

Populations of *C. inophyllum* in Yonaguni-jima and Hateruma-jima were reduced dramatically due to agricultural land development that occurred during 1950s (S. Hanaoka et al., unpublished data); however, some small forests and isolated trees still remain.

Table 1 Summary information regarding each island examined in the present study and the genetic parameters of associated *Calophyllum inophyllum* populations. *N*a Number of alleles, *H*o observed heterozygosity, *H*e expected heterozygosity, *H*eq expected equilibrium gene diversity, *R*s allelic richness, F_{IS} fixation index

* *P*<0.05, ** *P*<0.01 (statistically significant difference between *H*e and *H*eq or from Hardy-Weinberg equilibrium)

Island	Latitude	Longitude	Number of Samples	Island area (km²)	*Na*	*Ho*	*He*	*Heq*	*Rs*	F_{IS}
Taiwan	22.27	120.98	39	35980	4.60	0.44	0.50	0.52	4.38	0.13**
Yonaguni-jima	24.45	122.94	30	29	3.13	0.49	0.48	0.42**	3.12	0.00
Hateruma-Jima	24.06	123.76	30	12	3.20	0.50	0.47	0.43**	3.19	−0.04
Iriomote-jima	24.39	123.75	40	289	3.93	0.47	0.53	0.45**	3.83	0.12**
Kuro-shima	24.24	123.99	31	10	3.73	0.50	0.52	0.48**	3.70	0.05
Ishigaki-jima	24.45	124.08	30	223	3.73	0.48	0.51	0.50*	3.73	0.08*
Tarama-jima	24.65	124.67	30	20	3.33	0.46	0.47	0.43*	3.32	0.04
Miyako-jima	24.75	125.25	30	159	3.40	0.42	0.44	0.43	3.38	0.07
Minamidaito-jima	25.84	131.22	30	31	3.20	0.38	0.39	0.34	3.18	0.04
Chichi-jima	27.08	142.21	32	24	2.80	0.19	0.29	0.35	2.76	0.36**
Haha-jima	26.61	142.18	32	20	2.47	0.27	0.32	0.31	2.44	0.16**

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2.1.3 Iriomote-jima, Ishigaki-jima, and Miyako-jima

Iriomote-jima, Ishigaki-jima, and Miyako-jima islands had areas that were greater than those of the other islands examined in the present study, and each possessed natural *C. inophyllum* populations distributed within coastal areas, although large forests consisting of the species were not present. Based on past changes in sea levels (IPCC 2001) and current sea depths (Minnanokaizu 2012, http://mar-nets.com/contents/), it was concluded that Iriomoete-jima, Kuroshima, and Ishigaki-jima could have been connected prior to ca. 8,000 years BP.

2.1.4 Kuro-shima and Tarama-jima

Kuro-shima and Tarama-jima possessed relatively small areas and had low elevations; however, they were encircled by large natural populations of *C. inophyllum*.

2.2 Sampling of plant material

Mature leaves were collected from 30–40 natural *C. inophyllum* trees selected within a broad range on each island. Trees at least 50 m apart from each other were selected in order to avoid sampling from closely related trees. Collected leaves were desiccated using silica gel and subsequently stored at room temperature prior to DNA extraction.

2.3 DNA extraction and genotyping

DNA was extracted from desiccated leaf samples in accordance with a CTAB method. Genotypes of each DNA sample were scored using 15 pairs of EST-SSR primers that had been developed for *C. inophyllum*: *Ci_0147*, *Ci_0445*, *Ci_0448*, *Ci_0932*, *Ci_1542*, *Ci_1785*, *Ci_1861*, *Ci_2415*, *Ci_2791*, *Ci_3763*, *Ci_4038*, *Ci_4062*, *Ci_4391*, *Ci_5283*, and *Ci_5461* (Setsuko et al. 2012). A polymerase chain reaction (PCR) was performed in a GeneAmp 9700 PCR system (Applied Biosystems, Foster City, CA), with a 6-μL reaction mixture consisting of 1× QIAGEN Multiplex PCR Master mix (Qiagen, Valencia, CA), 0.02 μM of a fluorescent-labeled forward primer, 0.18 μM of a forward primer, and 0.2 μM of a reverse primer. Note that, four sets of primer pairs were constructed: set 1 (*Ci_0147*, *Ci_1785*, *Ci_3763*, and *Ci_4038*), set 2 (*Ci_1861*, *Ci_4062*, *Ci_4391*, and *Ci_5283*), set 3 (*Ci_0445, Ci_0932, Ci_1542*, and *Ci_2415*), and set 4 (*Ci_0448*, *Ci_2791*, and *Ci_5461*), and associated SSR regions were simultaneously amplified. Electrophoresis of PCR products was carried out using an ABI PRISM 3130xl genetic analyzer (Applied Biosystems), and allele sizes and genotypes of all samples were subsequently determined using GeneMapper® ver. 4.1 (Applied Biosystems).

2.4 Data analysis

In order to remove loci that had been subject to natural selection from subsequent analyses, outlier loci were checked using BayeScan ver. 2.1 (Foll and Gaggiotti 2008)—a program based on the multinomial-Dirichlet model. Observed heterozygosity (Ho) and expected heterozygosity (He) were calculated for populations from each island using GenAlex ver. 6.1 (Peakall and Smouse 2006). Allelic richness (Rs) with rarefaction (n=30) and its fixation index (F_{IS}) and deviation from Hardy-Weinberg equilibrium (HWE) were calculated using Fstat 2.9.3.2 (Goudet 1995).

BOTTLENECK ver. 1.2.02 (Cornuet and Luikart 1997) was used to test for recent reductions in the effective population size, since it was believed that genetic diversity on certain islands may have been influenced by recent land development. Significance was determined using a two-phase model (TPM) with a variance of 30 %, a probability of 70 %, and a Wilcoxon test.

Genetic structures among and within islands were evaluated hierarchically through analyses of molecular variance (AMOVAs) using Arlequin 3.11 (Excoffier and Schneider 2005). Samples were divided into those obtained from Taiwan, the Yaeyama group, the Miyako group, Minamidaito-jima, and the Ogasawara group, and genetic variances within islands, among islands within the same group, and among groups were determined. The significance of variance components was tested by calculating their probability based on 1,000 permutations. To detect genetic differentiation among islands, pairwise F_{ST} values were calculated using GenePop 4.0.10 (Raymond and Rousset 1995). In addition, mean numbers of migrants per generation in a given population (Nm) were calculated as $[(1/F_{ST}) - 1] / 4$ (Wright 1969).

In order to evaluate spatial genetic structures, isolation-by-distance was tested by comparing regressed pairwise F_{ST} values $[F_{ST} / (1 - F_{ST})]$ against geographical distances $[\ln(1+\text{distance})]$ between islands. The significance of the resulting regression was determined using a Mantel test with 10^4 permutations.

In order to evaluate the degree of historical gene flow among islands within natural distribution areas, an assignment test using the Bayesian method developed by Paetkau et al. (2004) was performed with GeneClass2 (Piry et al. 2004). Even though all individuals were assigned successfully to the population of one island, if the probability of the most probable population was <0.5, an individual was given the designation "unknown population".

To investigate genetic relationships among islands, a phylogenetic tree analysis based on the Neighbor-joining (NJ) method using Nei's genetic distance (Nei 1972) and a STRUCTURE analysis (Pritchard et al. 2000) were carried out. Branch probabilities within the phylogenetic tree were estimated through 1,000 bootstraps using Population vers. 1.2.30 (Langella 2007). For STRUCTURE analyses, a model with admixture and correlated allelic frequencies without prior

information regarding populations was constructed. Ten independent runs were carried out for each value of K ranging from 1 to 12, each run consisting of 1×10^{6} steps of Markov chain Monte Carlo iterations with a burn-in period of 5×10^{4}. Subsequently, an appropriate number of cluster (K) was determined based on the behavior of LnP(D) values, ΔK (Evanno et al. 2005) and the pattern of clustering when the value of K was changed.

3 Results

3.1 Genetic variation within populations of each island

Candidate loci under natural selection were not detected in any of the 15 EST-SSR markers employed (with a posterior probability of <0.75 and a q-value of <0.1), which indicated that all markers were suitable for analysis of population genetics. Genetic parameters are shown in Table 1. Values of He and Ho were comparatively high in both Taiwan and the Yaeyama group, while these values were extremely low in both Chichi-jima and Haha-jima samples, two of the Ogasawara Islands. However, a significant difference among them was not detected (Tukey's multiple comparison, $P>0.05$). Rs values were greatest in Taiwan, while other comparatively high values were detected in populations on Iriomote-jima, Kuro-shima, and Ishigaki-jima populations. Significant differences in Rs values were detected between Taiwan and two islands belonging to the Ogasawara Islands (Tukey's multiple comparison; $P<0.01$). A significant positive deviation from HWE was detected in F_{IS} values obtained from the populations of Taiwan and Iriomote-jima, Ishigaki-jima, Chichi-jima, and Haha-jima.

3.2 Bottleneck analysis

He values of the populations of Yonaguni-jima, Hateruma-jima, Iriomote-jima, Kuro-shima, Ishigaki-jima, and Tarama-jima were significantly higher than the expected equilibrium gene diversity (Heq) as calculated using BOTTLENECK vers. 1.2.02 (Table 1). These results constituted significant evidence of the existence of a bottleneck in the populations of these islands.

3.3 Genetic differentiation among islands

Pairwise F_{ST} values are shown in Table 2. A significant positive correlation was detected between pairwise F_{ST} values and geographical distance between islands (Mantel test, $P<0.01$; Fig. 2). Numbers of migrants per generation (Nm) are shown in Table 2. The values were comparatively high among islands within same island group. The AMOVA revealed that 85.5 % of the variance was detected within islands, while the variance within and among groups respectively accounted for 3.4 % and 11.1 % of the total variance.

Table 2 Pairwise F_{ST} values (*upper right half*) and Nm values (*lower left half*)

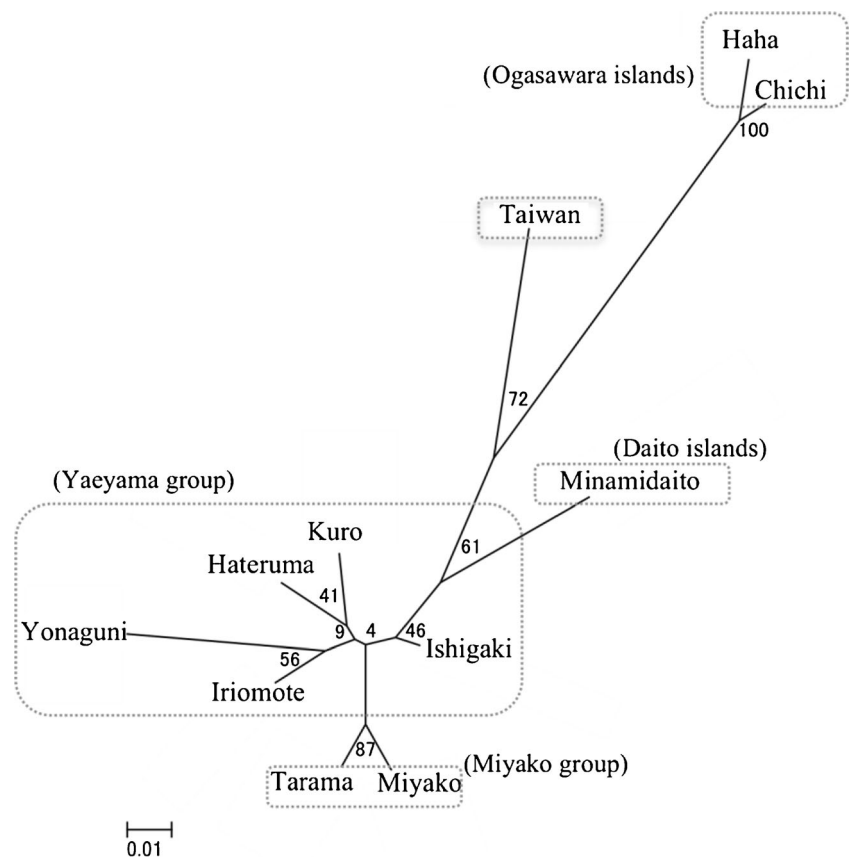|	Taiwan	Yonaguni-jima	Hateruma-jima	Iriomote-jima	Kuro-shima	Ishigaki-jima	Tarama-jima	Miyako-jima	Minamidait-jima	Chichi-jima	Haha-jima
Taiwan	–	0.089	0.075	0.063	0.062	0.047	0.072	0.071	0.080*	0.074	0.078*
Yonaguni-jima	2.568*	–	0.065	0.032	0.058	0.048	0.054	0.050	0.118	0.191*	0.180*
Hateruma-jima	3.087*	0.614*	–	0.024	0.025	0.023	0.027	0.039	0.047	0.137*	0.125*
Iriomote-jima	3.697*	7.562*	10.217*	–	0.016	0.018	0.024	0.033	0.065	0.147*	0.133*
Kuro-shima	3.805*	4.034*	9.599*	15.270*	–	0.017	0.023	0.040	0.050	0.123*	0.116*
Ishigaki-jima	5.067*	4.987*	10.483*	13.998*	14.311*	–	0.018	0.030	0.038	0.105*	0.098*
Tarama-jima	3.210*	4.367*	8.960*	10.123*	10.795*	13.514*	–	0.012	0.051	0.1278	0.114*
Miyako-jima	3.278*	4.789*	6.228*	7.253*	6.063*	0.081*	20.248*	–	0.065	0.1238	0.113*
Minamidait-jima	2.874*	1.877*	5.056*	3.588*	4.764*	6.392*	4.657*	3.616*	–	0.0938	0.092*
Chichi-jima	3.120*	1.061*	1.578*	1.449*	1.785*	2.133*	1.718*	1.779*	2.439*	–	0.015*
Haha-jima	2.953*	1.140*	1.746*	1.625*	1.913*	2.298*	1.951*	1.958*	2.473*	16.709*	–

*Values exhibiting significant differences ($P < 0.05$)
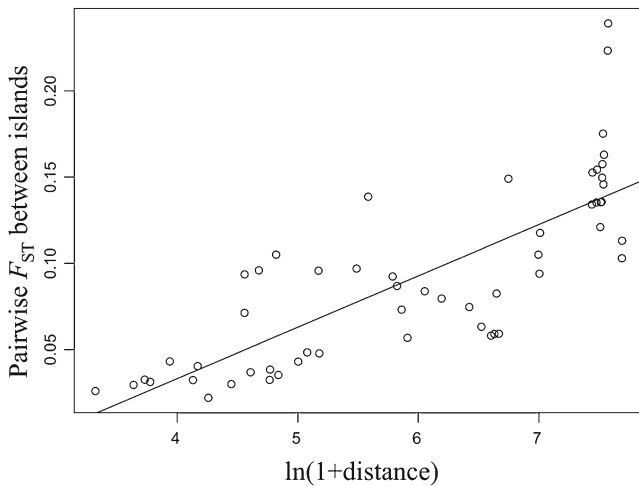
Fig. 2 Comparison of pairwise F_{ST} values $[F_{ST} / (1 - F_{ST})]$ against geographical distances $[\ln(1+distance)]$ among island populations. ($r=0.029$, $P<0.01$)

3.4 Assignment testing

Results of assignment testing are shown in Fig. 1. While the majority of individuals (>87 %) were assigned correctly to their island of origin in samples obtained from Taiwan and Yonaguni-jima, the same was true for only 55 %– 70 % of samples obtained from the other islands examined in the present study. In the Yaeyama group, 65.2 % of samples were

assigned correctly to their island of origin, while 14.3 %, 6.2 %, and 14.3 % of samples were assigned to other islands within the Yaeyama group, islands from the Miyako group, and the unknown population group, respectively. In the Miyako group, 65.0 % of samples were assigned correctly to their island of origin, while 8.3 %, 16.7 %, and 10.0 % of samples were assigned to other islands within the Miyako group, islands from the Yaeyama group, and the unknown population group, respectively. In the Sakishima Islands, 65.1 % of samples were assigned correctly to their island of origin, while 21.8 % and 13.1 % of samples were assigned to other islands and the unknown population group, respectively. Among samples taken from Minamidaito-jima, 83.3 % were assigned correctly to their island of origin, while the remainder were assigned to islands within the Sakishima Islands. Among samples taken from Chichi-jima, 65.6 % were assigned correctly to their island of origin, while the remainder were assigned to Haha-jima. Among samples taken from Haha-jima, 83.3 % were assigned correctly to their island of origin, while the remainder were assigned to Chichi-jima.

3.5 Genetic structures

Results of the phylogenetic tree analyses are shown in Fig. 3. Populations from the Sakishima Islands branched from those of

Fig. 3 Constructed neighbor-joining (NJ) tree based on Nei's genetic distance. Indicated values are the percentage of supported topologies of 1,000 bootstrap replicates. *Dashed squares* indicate the island group

the other islands studied; however, the bootstrap probabilities of branches within this group were not strongly supported.

Results of STRUCTURE analysis showed that LnP(D) values increased as the number of clusters (K) increased, with a peak value reached at around K=10. The value of ΔK was greatest at K=2, while the next-highest ΔK value was observed at K=4 (Fig. S2; supplemental data). Both results were shown in Fig. 4. Because variations of LnP(D) value in each K was very small, and the result of grouping was identical in all runs within same K (data not shown), the result of the run that showed highest LnP(D) value was adopted in this study. Results of the K=2 trial showed that samples taken from the Sakishima Islands as well as those taken from Minamidaito-jima were dominated by cluster 1 (Fst using F model=0.124), while samples taken from the Ogasawara Islands were dominated by cluster 2 (Fst=0.223). Although both clusters were admixed in Taiwan, this pattern was not observed in K=3~6 trials (Fig. 4, S3). Results of the K=4 trial showed that samples taken from Taiwan were dominated by cluster 3 (Fst=0.118), while samples taken from the Ogasawara Islands were dominated by cluster 6 (Fst=0.419). In samples taken from the Sakishima Islands and Minamidaito-jima, cluster 4 (Fst=0.118) and 5 (Fst=0.161) were dominant, and a geographical cline was detected for both clusters. Using K=3, 5 and 6 (ΔK>4), there was clear and robust segregation of clusters among Taiwan, Sakishima Islands and Ogasawara Islands, while only island group within Sakishima islands (Minamidaito-jima, Miyako group and Yaeyama group) were segregated in K=5 and 6 (Fig. S3; supplemental data).

4 Discussion

4.1 Genetic diversity and bottlenecks within the population of each island

It has been shown that genetic variations in island populations are often related to the population size and/or island area; however, correlations between an island's area and genetic parameters (He, Ho, and Rs) were not detected in the present study (single correlation; P=0.61, 0.28, and 0.22 for the mean values of He, Ho, and Rs, respectively). In addition, genetic parameters of populations on both Kuro-shima and Tarama-jima were not necessarily high despite the many naturally occurring trees and forests remaining on these two islands. A decreasing trend in genetic parameters (Rs, He, and Ho) was observed with increasing longitude (Table 1), and detected Rs values differed significantly between Taiwan and two islands belonging to the Ogasawara Islands. A previous phytosociological study demonstrated that certain plant species that employ sea-drifting seed dispersal had migrated from either Southeast Asia or the South Pacific to the Sakishima Islands (Hatsushima 1979). *Calophyllum inophyllum* might also have migrated from those areas, and most of the islands on which it is found were separated more than 20,000 years ago (Kimura 1996). The observed cline of genetic diversity corresponded roughly to the distance from the southern region from which *C. inophyllum* originated, and the effect of distribution range expansion via sea currents and the resultant population bottlenecks may have been conserved. In the Ogasawara Islands,
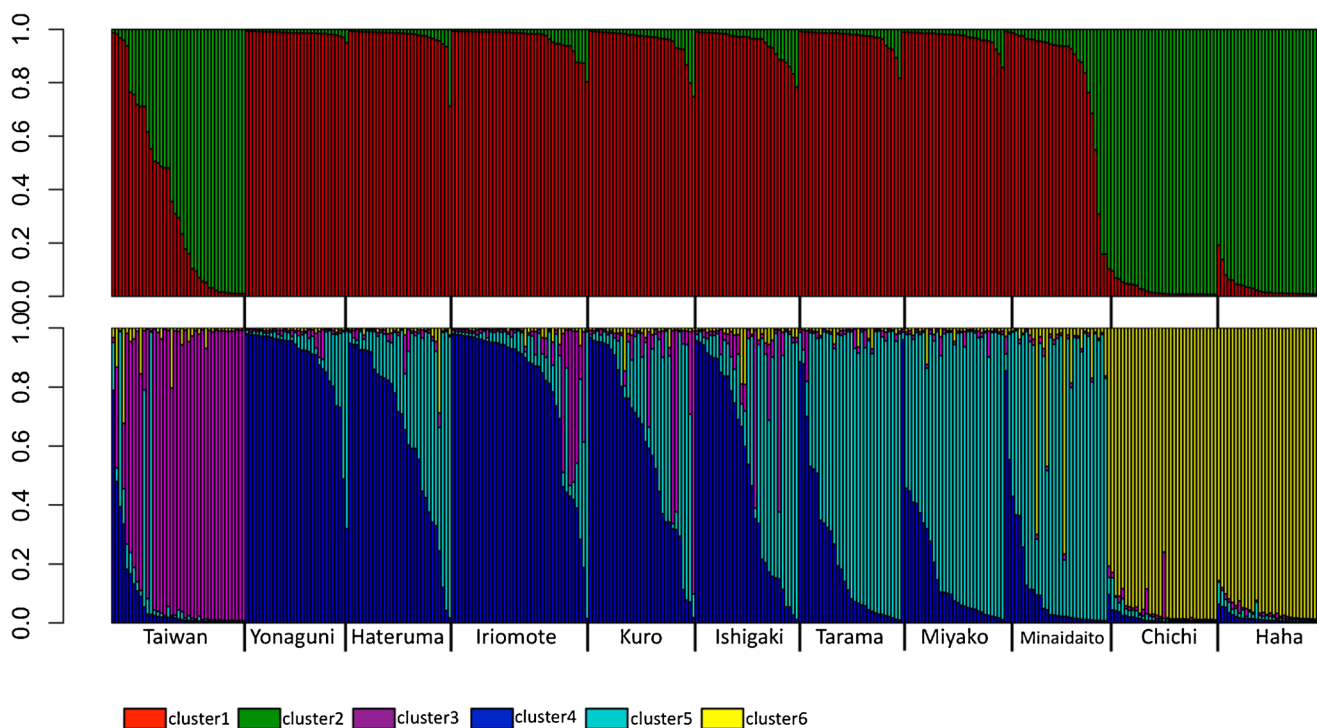

Fig. 4 Results of a STRUCTURE analysis with K=2 (upper diagram), and 4 (lower diagram)

values of all genetic parameters were much lower than they were elsewhere, and significantly high F_{IS} values were also detected (Table 1). These results indicated that early population sizes on the islands were very low and that reproduction among a limited number of individuals had occurred. F_{IS} values obtained from Taiwan, Iriomote-jima, and Ishigaki-jima were also significantly positive; however, these islands have large areas, and samples were collected from a wider area than they were on the other islands examined. Therefore, their significant deviation from HWE may have been the result of the Wahlund effect (Wahlund 1928).

Rs values obtained from populations on Yonaguni-jima and Hateruma-jima were comparatively lower than those obtained from the neighboring Sakishima Islands, although both islands possessed He and Ho values similar to those of their neighbors (Table 1). It was previously demonstrated that the numbers of alleles are more susceptible to a decrease due to recent changes in population size and/or colonization than Ho values (Comps et al. 2001), and the susceptibility to genetic variations of island plant populations due to human activities has been reported many times before (e.g., Nielsen 2004; Wang et al. 2005; Kaneko et al. 2008). Land development resulted in a decrease in the total number of trees on Yonaguni-jima and Hateruma-jima, and was believed to have resulted in a subsequent decrease in genetic diversity. In addition, significant bottlenecks were detected on both islands, and results suggested that a recent decrease in the total number of $C.$ $inophyllum$ individuals had occurred.

The effects of human activity on $C.$ $inophyllum$ populations on Iriomote-jima, Kuro-shima, Ishigaki-jima, and Tarama-jima were believed to have been minor; however, significant bottlenecks were detected in each of these islands. It was established that these islands were hit by a large tsunami in 1771 (Kawana 2000), with a wave greater than 30 m in height recorded on Ishigaki-jima, and two-thirds of the landmasses of both Tarama-jima and Kuro-shima were inundated. A large proportion of $C.$ $inophyllum$ populations located on those islands might have disappeared at that time. No evidence of bottlenecks could be found on islands that experienced only limited damage as a result of the 1771 tsunami (<16 % of samples were collected from the tsunami-affected area). On Iriomote-jima, Kuro-shima, Ishigaki-jima, and Tarama-jima, 45.0 %, 80.6 %, 80.0 %, and 86.7 % of samples were collected from areas hit by waves exceeding 1 m in height, respectively (Okinawa Prefecture in 2012). These proportions were significantly greater than those of samples taken on other islands (test for equality of proportions; $P<0.01$). Therefore, it was concluded that one of the primary causes of the bottlenecks observed in populations from the four islands may have been a rapid decrease in the number of $C.$ $inophyllum$ individuals that occurred as a result of the tsunami 242 years ago (a period of up to 30 generations, as young trees begin flowering approximately 7–8 years after planting) (Friday and Okano 2006).

4.2 Historical gene flow among islands

Genetic differentiation of island species is often determined either by gene flow from a source population or by the age of a given island (Maki 2001; Garcia-Verdugo et al. 2009). Takayama et al. (2006) demonstrated that long-distance sea-drifting seed dispersal alone was sufficient to prevent population differentiation over 2×10^4 km in the distribution range of $Hibiscus$ $tiliaceus$. Takayama et al. (2008) also examined 31 populations of $H.$ $tiliaceus$ in both the Pacific and Indian Ocean regions, with AMOVA results from their study having shown that 68.7 %, 19.1 % and 12.2 % of the total genetic variance were detected within populations, among populations within a given ocean region, and among ocean regions, respectively. F_{ST} values within the Pacific and Indian Oceans were 0.170 and 0.259, respectively.

In the present study, islands distributed within a comparatively short range (<2,200 km) were examined in detail. Results of the AMOVA indicated that variances within and among groups accounted for 3.4 % and 11.1 % of the total variance, respectively, and that a lower degree of differentiation among populations than those reported by Takayama et al. (2006) was present. On Chichi-jima and Haha-jima, which are located approximately 1,600 km from the Sakishima Islands, no individuals were assigned to Taiwan or the Sakishima Islands during assignment testing. Significantly high Fst values were detected between the Ogasawara Islands and the other islands examined, which suggested that gene flow between the two groups has been rare. On the other hand, certain individuals from Minamidaito-jima were assigned to islands within the Yaeyama group (approximately 600 km away), which suggested that gene flow can occur between small islands at distances of over several hundreds of kilometers. The rate of individuals that were assigned to islands other than the one from which they originated was comparatively high within the Sakishima Islands, with rates of at least 21.8 % having been recorded for each island in the group. The average distance between islands within the Sakashima Islands was about 96 km, which suggested that gene flow via sea currents occurred frequently within a range of about 100 km. Although 13.1 % of individuals in the Sakishima Islands were assigned to an unknown population, the majority were initially assigned to their island of origin or an island within the same group. Therefore, those individuals might be the result of hybridization between indigenous individuals and migrants from neighboring islands. In any case, these results suggested that frequent gene flow has occurred among the Sakishima Islands. This suggestion was also supported by the significant isolation-by-distance in examined area (Fig. 2) and Nm values.

None of the individuals sampled from the Japanese islands were assigned to Taiwan during assignment testing, even though the distance between Taiwan and the various Sakishima islands only ranges from 230–340 km. The strong

sea current (Kuroshio Current) that passes from just east of Taiwan to an area approximately 100 km north of the Sakishima Islands (Fig.1) might serve as a natural barrier blocking gene flow between the two areas.

4.3 Genetic structure among islands

Taiwan, the Sakishima Islands, and the Ogasawara Islands were clearly split in the phylogenetic tree constructed in the present study (Fig. 3), with the Sakishima Islands forming a comparatively distant clade. If the stepping stone model had been applicable, a more stepwise tree associated with geographical positions would have been produced (e.g., Garcia-Verdugo et al. 2009); however, the results of the present study were not compatible with that model, which indicated that the current genetic structure of *C. inophyllum* was not necessarily constructed solely by gene flow between neighboring islands.

Results of STRUCTURE analysis showed that LnP(D) values increased as the number of clusters increased; however, the value of ΔK was greatest at K=2, while the next highest ΔK value was observed at K=4. Results of assignment testing suggested that gene flow among archipelagos (i.e., Taiwan, the Sakishima Islands, and the Ogasawara Islands) had been severely limited. Generally, ΔK values tend to be more sensitive to the uppermost level of genetic structure, and their behavior does not necessarily show an optimal number of clusters (K) that reflect population structure (Evanno et al. 2005; in the case of contact zone model). In addition, admixture of clusters in Taiwan were not detected in K=3–6 trials (ΔK>4), and segregation of dominant clusters among Taiwan, the Sakishima Islands and Minamidaito Island, and the Ogasawara Islands was robust (Fig. S3). Based on these reasons, we considered that admixture of clusters in Taiwan at K=2 trial was an artifact caused by insufficient numbers of cluster (K), and the result of the K=4 trial was thought to be a better interpretation of population genetic structure within the studied area. In the result of the K=4 trial, Taiwan, the Sakishima Islands and Minamidaito-jima and the Ogasawara Islands were dominated by a different cluster, indicating that a clear genetic structure has been maintained based on the geographical distribution of the island chain. F_{ST} values of the cluster that dominated in the Ogasawara Islands were very high (>0.4), and these results indicate that individuals from the Ogasawara Islands did not necessarily share recent co-ancestral populations with individuals from the Taiwan, the Sakishima Islands or the Daito Islands; however, Minamidaito-jima, located approximately 600 km from the Sakishima Islands, was dominated by clusters 4 and 5, which were found mainly in the Sakishima Islands. These populations may have shared a common historical background. STRUCTURE analysis indicated that the segregation of clusters between Taiwan and the Sakishima Islands was robust (Fig 4, 7). Some plant species that employ sea-drifting seed dispersal were thought to have migrated from Southeast

Asia to the Sakishima Islands without passing through Taiwan (Hatsushima 1979). *Calophyllum inophyllum*'s distribution in the Sakishima Islands could have potentially followed a similar pattern, and the historical distribution expansion patterns of the species could also have been affected by current patterns of genetic structure. In any case, complex patterns and the magnitude of migration from source regions, limited gene flow between Taiwan and the Sakishima Islands, and frequent gene flow among islands within the same group may have contributed to the formation of the current genetic structure of *C. inophyllum*; however, future research involving sampling from a wider area and more informative markers, such as single-nucleotide polymorphisms of nuclei and chloroplasts, is required in order to reveal the origin and distribution expansion process of the species.

5 Conclusions

In the present study, genetic diversity, degree of gene flow, and genetic structure were determined for island populations of *C. inophyllum*—a plant that employs sea-drift seed dispersal. Results suggested that gene flow among small islands occurred over distances greater than several hundred kilometers; however, a cline of genetic diversity with longitude and a topographical genetic structure were maintained in the area examined. It was determined that, although the potential for gene flow via sea-drift seed dispersal was not necessarily low, the extent and frequency of this gene flow would be insufficient to prevent construction of a genetic structure in island populations. This information will play an important role in the conservation and management of genetic resources of plants that employ sea-drift seed dispersal.

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