

# **The current hybridization between the endemic *Morus boninensis* Koidz. and the invasive *Morus australis* Poir. in the Ogasawara Islands**

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## **Abstract:**

Impacts of invasive alien plant species on its closely related endemic ones in oceanic islands are often problematic because hybridization may occur between them. This study focused on endemic *Morus boninensis* (tetraploid of  $2n=56$ ) and its related invasive species *M. australis* (diploid of  $2n=28$ ) in the Ogasawara Islands. Aim of this study is to estimate proportion of the hybrids between the two species in wild seedling of native habitats of *M. boninensis* and to evaluate the current genetic diversity of *M. boninensis*. Observations and collections of plant samples were conducted in two wild seedling populations, two mature tree populations of *M. boninensis* and 12 populations of *M. australis* in the Ogasawara Islands. The 14 microsatellite markers were used for population genetic analyses. Based on the results of UPGMA analysis, the Principal Coordinate Analysis (PCoA), and the Bayesian Clustering, clear genetic differentiation was detected between the two species in the Ogasawara Islands. Gene flows between them unlikely or rarely occur maybe due to the difference in ploidy level. Hybrids were not found in the seedling populations, either. Limited effects of *M. australis* to the endangered *M. boninensis* through hybridization were detected in this study. It was also shown that *M. boninensis* still maintains high genetic diversity ( $H_E = 0.53$ ;  $A_R = 3.27$ ) despite that it is endangered species. It may be able to survive if the number of the individuals of the endangered species could be increased.

## Key words

Conservation, Genetic diversity, Microsatellite markers

## Introduction

Oceanic islands often have an exceptional rank in global biodiversity conservation due to their high degree of endemism (Hobohm, 2000; Whittaker & Fernández-Palacios, 2007). Oceanic islands are volcanic islands in the ocean such as the Hawaiian, Galapagos and Canary Islands, which have never been connected to large landmasses. They usually have unique land biota. For example, 524 endemic vascular plant species were found in the Canary Islands (Fernández-Palacios & Whittaker, 2008). Consequently, oceanic islands contribute to a disproportionately high degree to global biodiversity despite their relatively low percentage of surface area and limited number of species growing in the islands (Cowie & Holland, 2006).

The Ogasawara Islands in Japan are one of the oceanic islands and have many endemic plant species, which should be carefully conserved. The islands locate about 1,000 km south of the main Island of Japan, and consist of several island groups, Chichijima, Hahajima and others with total terrestrial area of 63.58 km<sup>2</sup> (Government of Japan, 2010). The Ogasawara Islands have been noted for their distinctive flora. Kobayashi & Ono (1987) reported 112 endemic land plant taxa in the islands, and among which 40% of vascular plant species, including 70% of the tree species, are endemic (Toyoda, 2003). In a recent report, the number of endemic plant taxa reached 161 of a total number of 441 vascular plant species and 32% (144 taxa) of them were recognized as endangered (Government of Japan, 2010). As oceanic islands, ecosystem of the Ogasawara Islands has already suffered from various negative effects by human impacts, biological invasions and others. To control the effects, several studies for establishing relevant management plans have been conducted in the Ogasawara Islands (Hata *et al.*, 2006; Osawa, 2019), but more information are still needed to conserve the endemic species in the islands.

*Morus boninensis* is one of the endemic plant species in the Ogasawara Islands that need careful conservation. It is dioecious tree species whose pollen and seeds are dispersed by wind and birds, respectively and tetraploid (Tani *et al.*, 2003). Its population size has been reduced by over-exploitation before World War II

(Government of Japan, 2010) since the Japanese government permitted free logging to encourage immigration to the islands during the last quarter of the 19th century and early 20th century (Tani *et al.*, 2003). Due to this, it is currently categorized as endangered species (Environment Agency of Japan, 2000; Government of Japan, 2010; Tani *et al.*, 2006). Its distribution range is now limited to several small areas of Chichijima, Hahajima and Otoutojima (Toyoda, 2003, 2014). Therefore, the current conservation status of *M. boninensis* needs to be investigated for establishing better conservation strategies. Especially, the main concerns about the conservation of *M. boninensis* at this moment are i) effects of *M. australis*, an Invasive Alien Species (IAS) closely related to *M. boninensis* and ii) an expected decline of genetic diversity due to the reduction of its population size.

IAS closely related to native species such as *M. australis* are expected to have strong impacts on the native species by hybridization (Mooney & Cleland, 2001). Hybridization between IAS and native species not only helps IAS to locally adapt to their new environments by incorporating beneficial alleles from the native species (Prentis *et al.*, 2008) but also make species boundary between them obscure and replace pure strains of native species with admixed ones (Levin *et al.*, 1996). *Morus australis*, the IAS closely related to endemic *M. boninensis*, was introduced to the Ogasawara Islands for sericulture of silkworm *Bombyx mori* (Awasthi *et al.*, 2004; National Institute for Environmental Studies, Japan, 2017; Tokyo Prefecture Ogasawara Island Branch Office, 1938). Because it recently expanded even into conserved areas in the islands where *M. boninensis* is distributed, even after the cultivation of *M. australis* has been stopped. It is the reason why *M. australis* has been recognized as IAS (National Institute for Environmental Studies, Japan, 2017).

The hybridization between *M. boninensis* and *M. australis* in the Ogasawara Islands have been reported (Tani *et al.*, 2003, 2006). They suggested that the hybridization between the two *Morus* species potentially leads to loss of purity of endemic *M. boninensis* and the hybrids should be removed from the seedling pools. It is because the previous study focused only on artificially germinated seeds collected from a single female tree of *M. boninensis* and did not check any naturally growing seedlings or mature trees in wild conditions (Tani *et al.*, 2003, 2006). Thus, it is very urgent to confirm hybrids in wild seedlings and mature trees in the Ogasawara Islands to clarify

the present conservation status of *M. boninensis*.

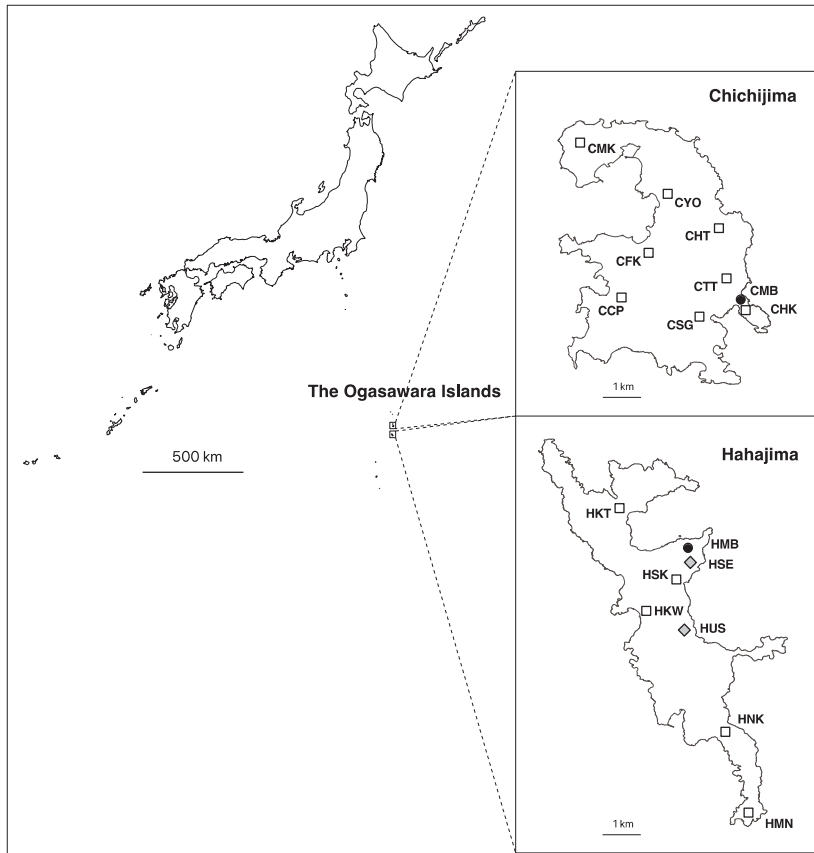
In addition, evaluation of levels of genetic diversity in endemic *M. boninensis* is necessary. Genetic diversity reduction leads to maladaptation to changing environmental conditions and are susceptible to extinct even after small alterations in the environments (Dostálek *et al.*, 2010; Isagi *et al.*, 2020). Endemic species in islands are usually illustrated by small and isolated populations (Dostálek *et al.*, 2010), and oceanic insular species often possess lower genetic diversity than their continental relatives (Frankham, 1997). Moreover, population size reduction is expected to cause decreasing of the genetic diversity. Given that oceanic endemic species, *M. boninensis* have experienced rapid declines in their individual number and population size, the species is expected to currently exhibit very low level of genetic diversity. Therefore, investigation of the level of genetic diversity in *M. boninensis* is urgently necessary from conservation aspects. In general, a proper evaluation of genetic diversity in target species is challenging due to a lack of appropriate closely related taxon to compare. In the previous study, Tani *et al.* (2006) analyzed *M. boninensis* populations using genetic markers without any congeners. However, in our previous study (Yulianti *et al.*, 2021), it was revealed that *M. australis* in Ogasawara possesses similar amount of genetic diversity to the populations in its native range. This result enables us to describe and evaluate the levels of genetic diversity in *M. boninensis* by comparing with those of *M. australis* in the Ogasawara Islands and other native populations of the invasive species.

In this study, for better understanding of conservation status of endemic *Morus*, population genetic analysis was conducted for both of endemic *M. boninensis* and invasive *M. australis* growing in the Ogasawara Islands. Specifically, the following questions were addressed here: 1) What actual impact does hybridization with invasive *M. australis* have on native *M. boninensis*? 2) What level of the current genetic diversity is observed in *M. boninensis* in the Ogasawara Islands?

## Materials and methods

Plant materials of two populations of *M. boninensis* (CMB, HMB), one of *M. australis* (CHK) and two wild seedling populations (HSE, HUS) were collected during 2019-2020, and they were analyzed together with materials of *M. australis* from 12

populations which were used in previous study (Yulianti *et al.*, 2021) (Table 1 and Fig. 1). All samples except in seedling populations were sampled from mature trees. Both population of *M. boninensis* on Chichijima (CMB) and on Hahajima (HMB) were found neighboring to *M. australis* (CHK) and to seedling (HSE) population, respectively. Individuals of the seedling populations containing some juvenile trees were collected without identifying the taxa. Fresh-young leaves in the apical stems were collected from each individual in order to obtain total DNAs with the best quality. The collected leaves were stored in plastic bags with silica gel to keep them dry until DNA extraction. Voucher specimens were also collected during the sample collection and deposited in the Makino Herbarium of Tokyo Metropolitan University (MAK) (Table 1).



**Fig.1** Map of sampling localities for *M. boninensis* (black circle), *M. australis* (open square) and wild seedling populations (gray diamond) in the Ogasawara Islands. Population IDs are shown in Table 1.

Extraction of total DNAs and amplification of microsatellite markers were conducted using the same methods as reported by Yulianti *et al.* (2021). Total of 14 microsatellite primers developed for *M. boninensis* (Tani *et al.*, 2005) and for *M. indica* (Aggarwal *et al.*, 2004; Krishnan *et al.*, 2014) were used for population genetic analyses. The amplified DNA was examined by using the ABI 3130xl genetic analyzer. The obtained data were processed by using the GeneMapper software version 4.0 supplied by ABI to determine the genotype of each sample. Since *M. boninensis* is a tetraploid species, the genotype determination for the species was based on the dosage of each allele that implied partial heterozygotes, following Tani *et al.* (2005) and Tsuda *et al.* (2017). For example, when only one allele is found then written as *AAAA*, when four alleles are found then written as *ABCD*, when two alleles are found then written as *AABB*, and when three alleles are found then the one allele with the highest peak written double, such as *AABC*.

To show the genetic similarity among populations of *M. boninensis*, *M. australis* and seedling, UPGMA dendrogram based on Nei's genetic distances,  $D_A$  (Nei *et al.*, 1983) was drawn by using Populations 1.2.30 (Langella, 1999) and FigTree v.1.4.4 (Rambaut, 2018). The Principal Coordinate Analysis (PCoA) based on pairwise  $F_{ST}$  values was conducted by using GenAlEx 6.503 (Peakall & Smouse, 2012).

The Bayesian clustering analysis was conducted to evaluate genetic structure and to detect hybrids by using the software STRUCTURE version 2.3.4 (Falush *et al.*, 2003, 2007; Pritchard *et al.*, 2000). All investigated population were tested together by using the setting number of burn-in 100000 and MCMC 100000 which  $K = 1-19$  by applied 10 runs for each  $K$  value. The  $\Delta K$  value was adopted through Evanno's method (Evanno *et al.*, 2005) by using STRUCTURE HARVESTER (Earl & vonHoldt, 2012). Results were visualized with software CLUMPP 1.1.2 (Jakobsson & Rosenberg, 2007) and Distruct 1.1 (Rosenberg, 2004).

The allele frequencies of *M. boninensis*, *M. australis* and seedlings were obtained by SPAGeDi software (Hardy & Vekemans, 2002). Prior to this analysis, the putative admixed individuals in each population were excluded based on the result of STRUCTURE analysis mentioned above. Number of alleles per locus ( $N_A$ ), expected heterozygosity ( $H_E$ ), allelic richness ( $A_R$ ), and fixation index ( $F_{IS}$ ) were also calculated as genetic parameters by SPAGeDi software.

**Table 1** Sampling localities and genetic parameters of *M. boninensis*, *M. australis* and seedling in the Ogasawara Islands.

Island	Location	Pop ID	Lat. (°N)	Long. (°E)	Voucher ID	<i>N</i>	<i>N<sub>A</sub></i>	<i>H<sub>E</sub></i>	<i>A<sub>R</sub></i>	<i>F<sub>IS</sub></i>
<i>M. boninensis</i>										
Chichijima	Higashi-Kaigan	CMB	27.0602	142.2273	MAK467151	17	4.43	0.53	3.32	0.06
Hahajima	Sekimon	HMB	26.6841	142.1614	MAK467152	8	3.71	0.52	3.21	-0.03
					Mean	12.50	4.07	0.53	3.27	0.02
<i>M. australis</i>										
Chichijima	Mikazuki	CMK	27.0986	142.1869	MAK467119	21	5.86	0.66	4.55	0.08
	Yoakeyama	CYO	27.0856	142.2172	MAK467124	12	5.64	0.68	4.89	0.14
	Hatsuneura	CHT	27.0770	142.2177	MAK467125	11	4.71	0.59	4.30	0.21
	Fukiagedani	CFK	27.0755	142.2087	MAK467120	23	5.64	0.61	4.28	0.04
	Tatsumidoro	CTT	27.0640	142.2199	MAK467123	15	5.07	0.60	4.24	0.02
	Higashi-Kaigan	CHK	27.0649	142.2276	MAK467153	15	4.21	0.58	3.69	0.14
	Kitafukurozawa	CCP	27.0602	142.2031	MAK467122	12	4.86	0.60	4.39	0.11
	Siguredamu	CSG	27.0599	142.2068	MAK467121	16	4.57	0.59	3.93	0.06
Hahajima	Kitako	HKT	26.6963	142.1436	MAK467128	25	5.93	0.63	4.26	0.08
	Sekimon	HSK	26.6744	142.1562	MAK467129	33	6.21	0.63	4.40	0.14
	Kuwanokiyama	HKW	26.6638	142.1520	MAK467130	13	5.93	0.65	4.98	0.11
	Nakanotaira	HNK	26.6321	142.1736	MAK467127	14	5.14	0.61	4.33	0.06
	Minamizaki	HMN	26.6114	142.1762	MAK467126	22	5.36	0.58	4.07	0.11
					Mean	17.85	5.32	0.62	4.33	0.10
Seedling										
Hahajima	Sekimon	HSE	26.6846	142.1614	MAK467154	52	7.21	0.65	4.56	0.04
	Uchusawa	HUS	26.6649	142.1566	MAK467155	8	4.00	0.60	4.00	-0.05
					Mean	30.00	5.61	0.63	4.28	-0.01

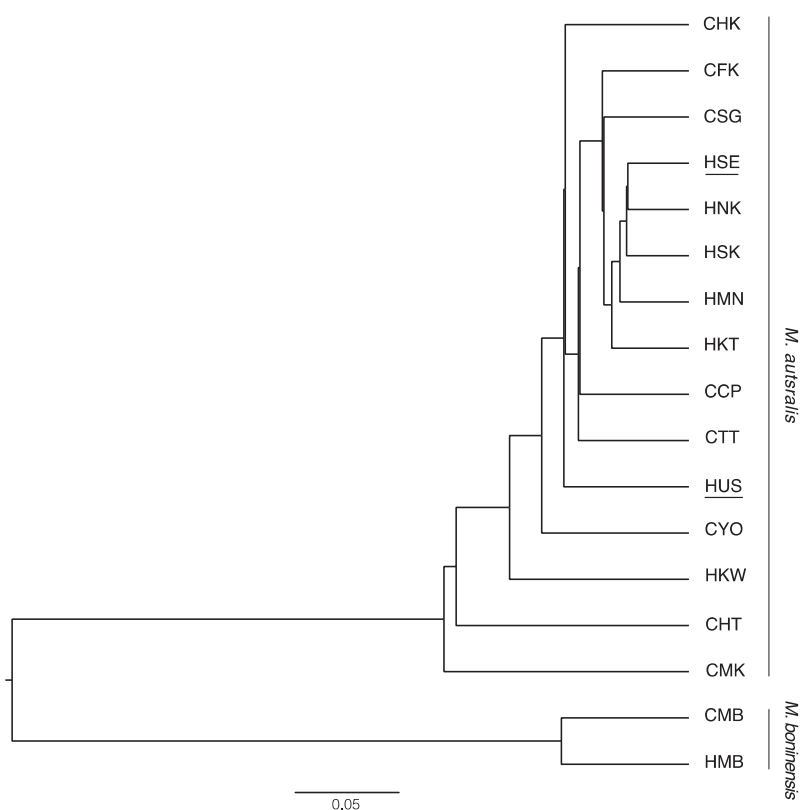
*N*, number of individuals; *N<sub>A</sub>*, number of alleles; *H<sub>E</sub>*, expected heterozygosity; *A<sub>R</sub>*, allelic richness; *F<sub>IS</sub>*, fixation index.

## Results

All the used microsatellite loci showed polymorphisms for both the species. A total of 200 alleles were obtained from the 14 loci. The highest number of alleles was obtained from Mos0340-2 and MulSSR 258 (22 alleles), followed by MESTSSR 31 and Mos0031 (19 alleles), MulSSR 338 (18 alleles), MulSSR 313 (16 alleles), MulSSR 147 and MulSTR 2 (15 alleles), Mos0157-2 (13 alleles), MESTSSR 48, MulSTR 4 (11 alleles), MESTSSR 126 (eight alleles), and MUL3SSR6 (six alleles). The fewest number of alleles was detected by MESTSSR 73, which showed only five alleles.

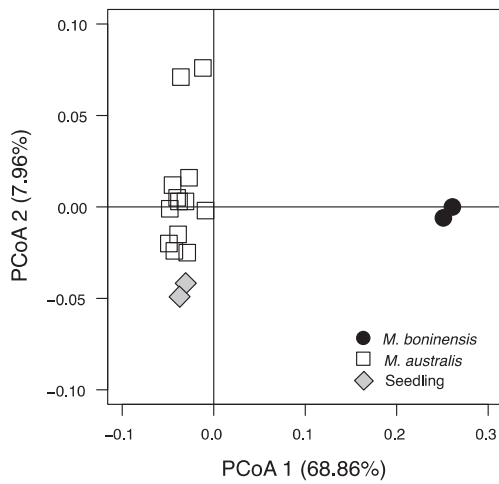
The genetic similarity among all the investigated population was revealed in the UPGMA dendrogram based on Nei's genetic distances, *D<sub>A</sub>* (Fig. 2). Two clusters were revealed in the dendrogram. The first cluster comprises of all the population of *M. boninensis*. The second cluster comprises of all the population of *M. australis* and seedling/juvenile populations. The result of the Principal Coordinate Analysis (PCoA) is

shown in Fig. 3. The first axis showed 68.86 % of the total variation. This axis clearly distinguished the two species, *M. boninensis* and *M. australis*. The seedling population formed a group together with *M. australis* (Fig. 3). The second axis showed only 7.96 % of the total variation. There was no obvious grouping found along this axis either between the two species or between populations in the island groups of the Ogasawara Islands.



**Fig.2** The UPGMA dendrogram among all populations of *M. boninensis*, *M. australis* and seedling in the Ogasawara Islands based on Nei's  $D_A$  genetic distance (Nei *et al.*, 1983). Underline represents seedling populations.

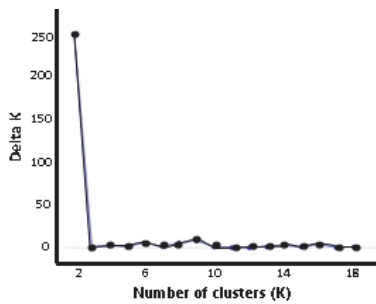




**Fig.3**

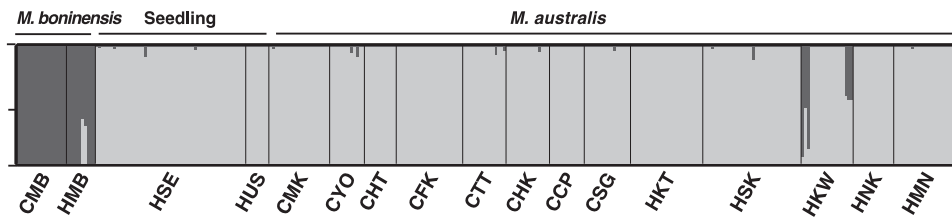
Result of the PCoA among all populations of *M. boninensis*, *M. australis* and seedling in the Ogasawara Islands based on  $F_{ST}$  values.

a)



b)

$K = 2$



**Fig. 4** Result of Bayesian clustering (STRUCTURE) analysis of *M. boninensis*, *M. australis* and seedling. a) delta  $K$  as number of clusters, b) Bar plot at  $K = 2$ . Each bar represents an individual of *Morus* in this study, and the heights of the bars of each color are proportional to the posterior means of the estimated admixture proportions. Locations of population are shown in Fig. 1 and Table 1.

The result of the STRUCTURE analysis for *M. boninensis* and *M. australis* is shown in Fig. 4. The  $\Delta K$  value was maximal at  $K = 2$ . Based on this maximal  $K$  value, two clusters corresponding to the two species, *M. boninensis* and *M. australis* were observed. No hybrid individual was detected in seedling populations. However, eight individuals admixed between two clusters were found in the populations of *M. boninensis* (HMB) and *M. australis* (HKW). Two individuals found in HMB exhibited the genetic admixture in close to 2:1 ratio (0.62:0.38 and 0.68:0.32) between the *M. boninensis* (dark gray) and the *M. australis* (light gray) clusters.

A relatively high level of genetic diversity was observed for all the population of *M. boninensis* and *M. australis* even after excluding all the putative hybrids. The mean genetic diversity for *M. boninensis* ( $H_E = 0.53$ ,  $A_R = 3.27$ ) was almost the same as those of *M. australis* ( $H_E = 0.62$ ,  $A_R = 4.33$ ) and those of seedling populations ( $H_E = 0.63$ ,  $A_R = 4.28$ ). The values of heterozygosity ( $H_E$ ) for *M. boninensis* were 0.52 and 0.53, while in *M. australis*, it ranges from 0.58 to 0.68, and for seedling populations, 0.60 and 0.65, respectively. The allelic richness ( $A_R$ ) for *M. boninensis* were 3.21 and 3.32, it ranged from 3.69 to 4.98 in *M. australis*, and 4.00 and 4.56 in seedling populations (Table 1).

## Discussion

Genetic research on hybridization between native species and IAS are necessary specially to conserve endangered native species. As mentioned in the introduction part, IAS is hypothesized to have the strong impacts on native closely related species by hybridization (Mooney & Cleland, 2001). Such interspecific hybridization with native species not only enables IAS to assimilate the invaded area by introduction of the genotype adapted to the area (Prentis *et al.*, 2008) but also replace pure strains of the native species with admixed ones, namely ‘genetic contamination’.

In this study, extent of hybridization between an endangered species, *M. boninensis* and its closely related IAS, *M. australis* in natural condition of the Ogasawara Islands was investigated. Tani *et al.* (2003) investigated the extent of the hybridization between the two species by examining a proportion of hybrids in embryos of seeds collected from a mother tree of *M. boninensis*. They detected hybrids in some

of the artificially germinated embryos and concluded that the hybridization with *M. australis* has a strong negative impact on the regeneration of native *M. boninensis*. In their study, however, seedlings and mature trees were not investigated in the natural condition and the seeds used were collected only from a single female tree. Thus, the impact of the hybridization in wild still remains unclear. In this paper, naturally growing *Morus* individuals were collected by especially focusing on seedlings and juveniles to examine the impact of hybridization in natural conditions.

Of the 60 seedlings and juveniles from two populations, all individuals were identified as *M. australis* and no hybrid was suggested even though seedling population at Sekimon (HSE) was adjacent to *M. boninensis* population (HMB) (Figs.1 & 4). Considering high proportion (13%) of hybrids in the artificially germinating seeds of *M. boninensis* was reported (Tani *et al.* 2003), the hybrid seedlings might not be able to survive and/or grow in wild conditions. Although there is no direct evidence, natural selections against seedlings/juveniles of hybrids may act in the *Morus* species.

On the other hand, several individuals genetically admixed between the two species, possibly F<sub>1</sub> hybrids, were found in the populations of the mature trees (Fig. 4). Two individuals found in HMB exhibited the proportions of genetic admixture between the *M. boninensis* cluster (dark gray) and the *M. australis* cluster (light gray) as close to 2:1 ratio. Given that *M. australis* and *M. boninensis* are diploid and tetraploid species, respectively, the proportion of genetic admixture in F<sub>1</sub> hybrids is expected to be 1/3 of their genetic constitution from diploid *M. australis* and 2/3 from tetraploid *M. boninensis*. Thus, these two individuals can be interpreted as triploid F<sub>1</sub> hybrids between them. In addition, the admixed individuals found in HKW population of *M. australis* exhibited various patterns of genetic admixture differentiated from that in typical F<sub>1</sub> hybrid mentioned above: Two showing similar proportions genetically close to pure strain of *M. boninensis* and the other four showing more admixed from *M. australis* than the expected ratio for the F<sub>1</sub> hybrids.

These possible hybrid individuals other than F<sub>1</sub> can be explained by following two hypotheses. Firstly, the two hybrids genetically close to the pure strain of *M. boninensis* could be artifacts resulting from the possession of shared alleles between the two species. They could be interpreted as pure strain of *M. boninensis*. If this is true, the other four hybrids can be also considered as F<sub>1</sub> hybrids with more shared alleles

between *M. australis* and *M. boninensis*. Thus, the presence of the shared alleles might have caused the pattern observed in HKW population. Secondly, the hybrids could be those backcrossing with parental species. A few studies reported interploidal gene flow in angiosperms (e.g., *Betula* by Tsuda *et al.*, 2017 & Wang *et al.*, 2014; *Arabidopsis* by Arnold *et al.*, 2015). In general, however, triploid rarely produces viable gametes due to failure in homologous chromosome pairing during meiosis. Thus, this hypothesis might be unlikely. In future study, these hypotheses may need to be tested by incorporating population genetic analysis with cytological analysis such as those using flow cytometry.

In conclusion, our study showed no hybrid seedlings in wild condition and no evidence of the expected genetic contamination in *M. boninensis* populations despite of the presence of some F<sub>1</sub> hybrids. Thus, the degree of hybridization between native *M. boninensis* and alien *M. australis* in the Ogasawara Islands is relatively more limited than previously expected.

The level of genetic diversity within *M. boninensis*, an endangered endemic plant species in the Ogasawara Islands, was observed almost same as that within its widely distributed relative, *M. australis*. Reductions of genetic diversity in insular endemics compared to continental counterparts (*ex. related taxa in the mainland*) have been discussed in several previous studies (Frankham, 1997; García-Verdugo *et al.*, 2015; Stuessy *et al.*, 2014). The island endemics are assumed to display low levels of genetic diversity (Stuessy *et al.*, 2014). However, the mean level of heterozygosity and allelic richness of *M. boninensis* ( $H_E = 0.53$ ;  $A_R = 3.27$ ) was only slightly lower than those of *M. australis* in the Ogasawara Islands ( $H_E = 0.62$ ;  $A_R = 4.33$ ) in this study (Table 1). *Morus australis* is widely distributed from the Himalayas to Japanese archipelagos including the Ryukyu and Izu Islands of Japan. Genetic diversities of *M. australis* in other areas of Japan ranged from 0.51 to 0.73 ( $H_E$ ) and from 3.70 to 5.80 ( $A_R$ ) (Yulianti *et al.*, 2021). Frankham (1997) investigated genetic diversity reductions by comparing heterozygosities in populations of island endemic plants ( $H_{IS}$ ) to those of mainland related species ( $H_M$ ) based on allozyme data, and the ratio ( $H_{IS}/H_M$ ) was 0.54. In this study, the ratio between endemic *M. boninensis* and wide-spread *M. australis* was 0.85. It suggests that *M. boninensis* maintains high levels of genetic diversity and it does not seem to follow tendency of genetic diversity reducing in insular endemics.

No reduction of genetic diversity in *M. boninensis* may be attributed to biological characteristics of this species. Stuessy *et al.* (2014) suggested biological characteristics influencing levels of genetic variation in insular endemics. For example, the generation time (Stuessy *et al.*, 2014) regulates the development of genetic variation within populations by influencing the rates of recombination. As another possible reason, outcrossing of dioecious plant might be suitable for maintenance of higher levels of genetic diversity (Government of Japan, 2010; Paschoa *et al.*, 2018). On the other hand, small population size was thought as negatively affecting to maintain genetic diversity. *Morus boninensis* is dioecious and produces many seeds which are dispersed by birds, suggesting this species is predominantly outbreeder. Additionally, the generation time of *M. boninensis* seems very long. A stump over 2000 years inferred from annual rings was reported in the Ogasawara Islands (Toyoda, 2003). These characteristics are suitable to accumulate and maintain genetic diversity within the species. However, the population size of *M. boninensis* rapidly declined because the trees had been heavily logged during the last quarter of the 19th century and the start of 20th century with encouragement by Japanese government (Tani *et al.*, 2003). This reduction in population size is likely to lead to the loss of genetic diversity within *M. boninensis* in near future.

Tani *et al.* (2006) also reported that there was no observation of seedling recruitment since 1995. In this study any seedlings or juveniles of *M. boninensis* were not detected even in adjacent site to mother (mature) population on Hahajima (Fig. 4). This suggests that *M. boninensis* failed any regenerations in recent 20 years, despite intensive efforts to the conservation of the species. In current condition, although *M. boninensis* was maintained relatively high amount of genetic diversity compatible to *M. australis*, *M. boninensis* will decline if no seedling recruitment will occur in future.

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## References

- Aggarwal RK, Udaykumar D, Hendre PS, Sarkar A & Singh LI (2004) Isolation and characterization of six novel microsatellite markers for mulberry (*Morus indica*). *Molecular Ecology Notes* 4: 477-479. doi: 10.1111/j.1471-8286.2004.00718.x
- Arnold B, Kim S-T, & Bomblies K (2015) Single geographic origin of a widespread autotetraploid *Arabidopsis arenosa* lineage followed by interploidy admixture. *Molecular Biology and Evolution* 32: 1382-1395. doi: 10.1093/molbev/msv089
- Awasthi AK, Nagaraja GM, Naik GV, Kanginakudru S, Thangavelu K & Nagaraju J (2004) Genetic diversity and relationships in mulberry (genus *Morus*) as revealed by RAPD and ISSR marker assays. *BMC Genetics* 5: 1. doi: 10.1186/1471-2156-5-1
- Cowie RH & Holland BS (2006) Dispersal is fundamental to biogeography and the evolution of biodiversity on oceanic islands. *Journal of Biogeography* 33: 193-198. doi: 10.1111/j.1365-2699.2005.01383.x
- Dostálek T, Münzbergová Z & Plačková I (2010) Genetic diversity and its effect on fitness in an endangered plant species, *Dracocephalum austriacum* L. *Conservation Genetics* 11: 773-783. doi: 10.1007/s10592-009-9879-z
- Earl DA & vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359-361. doi: 10.1007/s12686-011-9548-7
- Environment Agency of Japan (Ed.). (2000) *Threatened Wildlife of Japan. Red Data Book 2nd ed: Vol. 8 Vascular Plants*. Japan Wildlife Research Center. 660p. (in Japanese)
- Evanno G, Regnaut S & Goudet J (2005) Detecting the number of clusters of

- individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611-2620. doi: 10.1111/j.1365-294X.2005.02553.x
- Falush D, Stephens M & Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164: 1567-1587. doi: 10.1093/genetics/164.4.1567
- Falush D, Stephens M & Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes* 7: 574-578. doi: 10.1111/j.1471-8286.2007.01758.x
- Fernández-Palacios JM & Whittaker RJ (2008) The Canaries: An important biogeographical meeting place. *Journal of Biogeography* 35: 379-387.
- Frankham R (1997) Do island populations have less genetic variation than mainland populations? *Heredity* 78: 311-327. doi: 10.1038/hdy.1997.46
- García-Verdugo C, Sajeve M, La Mantia T, Harrouni C, Msanda F & Caujapé-Castells J (2015) Do island plant populations really have lower genetic variation than mainland populations? Effects of selection and distribution range on genetic diversity estimates. *Molecular Ecology* 24: 726-741. doi: 10.1111/mec.13060
- Government of Japan (2010) *Nomination of the Ogasawara Islands for inscription on the World Heritage List*. Ministry of the Environment, Government of Japan. [http://ogasawara-info.jp/pdf/isan/recommendation\\_en.pdf](http://ogasawara-info.jp/pdf/isan/recommendation_en.pdf) Accessed 24th December 2021.
- Hardy OJ & Vekemans X (2002) SPAGeDi: A versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2: 618-620. doi: 10.1046/j.1471-8286.2002.00305.x
- Hata K, Suzuki J-I, Kachi N & Yamamura Y (2006) A 19-year study of the dynamics of an invasive alien tree, *Bischofia javanica*, on a subtropical oceanic island. *Pacific Science* 60: 455-470. doi: 10.1353/psc.2006.0029
- Hobohm C (2000) Plant species diversity and endemism on islands and archipelagos, with special reference to the Macaronesian Islands. *Flora* 195: 9-24. doi: 10.1016/S0367-2530(17)30943-X
- Isagi Y, Makino T, Hamabata T, Cao P, Narita S, Komaki Y, Kurita K, Naiki A, Kameyama Y, Kondo T & Shibabayashi M (2020) Significant loss of genetic diversity and accumulation of deleterious genetic variation in a critically

- endangered azalea species, *Rhododendron boninense*, growing on the Bonin Islands. *Plant Species Biology* 35: 166–174. doi: 10.1111/1442-1984.12270
- Jakobsson M & Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23: 1801-1806. doi: 10.1093/bioinformatics/btm233
- Kobayashi S & Ono M (1987) A revised list of vascular plants indigenous and introduced to the Bonin (Ogasawara) and Volcano (Kazan) Islands. *Ogasawara Research* 13: 1-55.
- Krishnan RR, Naik VG, Ramesh SR & Qadri SMH (2014) Microsatellite marker analysis reveals the events of the introduction and spread of cultivated mulberry in the Indian subcontinent. *Plant Genetic Resources: Characterization and Utilization* 12: 129-139. doi: 10.1017/S1479262113000415
- Langella O (1999) *Populations*, 1.2.30. [https://bioinformatics.org/populations/#ancre\\_bibliographie](https://bioinformatics.org/populations/#ancre_bibliographie), Accessed 24th December 2021.
- Levin DA, Francisco-Ortega J & Jansen RK (1996) Hybridization and the extinction of rare plant species. *Conservation Biology* 10: 10-16. doi: 10.1046/j.1523-1739.1996.10010010.x
- Mooney HA & Cleland EE (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the United States of America* 98: 5446-5451. doi: 10.1073/pnas.091093398
- National Institute for Environmental Studies, Japan (2017) *Morus australis*. Invasive Species of Japan. <https://www.nies.go.jp/biodiversity/invasive/DB/detail/80900e.html>, Accessed 24th December 2021.
- Nei M, Tajima F & Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *Journal of Molecular Evolution* 19: 153-170. doi: 10.1007/bf02300753
- Osawa T (2019) Establishing a strategic management plan for alien invasive plants in the Ogasawara Islands. *Global Environmental Research* 23: 21-28.
- Paschoa RP da, Christ JA, Valente CS, Ferreira MF da S, Miranda FD de, Garbin ML & Carrijo TT (2018) Genetic diversity of populations of the dioecious *Myrsine coriacea* (Primulaceae) in the Atlantic Forest. *Acta Botanica Brasilica* 32:



- 376-385. doi: 10.1590/0102-33062017abb0355
- Peakall R & Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* 28: 2537-2539. doi: 10.1093/bioinformatics/bts460
- Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM & Lowe AJ (2008) Adaptive evolution in invasive species. *Trends in Plant Science* 13: 288-294. doi: 10.1016/j.tplants.2008.03.004
- Pritchard JK, Stephens M & Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.
- Rambaut A (2018) *FigTree v1.4.4*. <http://tree.bio.ed.ac.uk/software/figtree/>, Accessed 24th December 2021.
- Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes* 4: 137-138. doi: 10.1046/j.1471-8286.2003.00566.x
- Stuessy TF, Takayama K, López-Sepúlveda P & Crawford DJ (2014) Interpretation of patterns of genetic variation in endemic plant species of oceanic islands. *Botanical Journal of the Linnean Society* 174: 276-288. doi: 10.1111/boj.12088
- Tani N, Kawahara T, Yoshimaru H & Hoshi Y (2003) Development of SCAR markers distinguishing pure seedlings of the endangered species *Morus boninensis* from *M. boninensis* × *M. acidosa* hybrids for conservation in Bonin (Ogasawara) Islands. *Conservation Genetics* 4: 605-612. doi: 10.1023/A:1025655331429
- Tani N, Kawahara T & Yoshimaru H (2005) Development and diversity of microsatellite markers for endangered species, *Morus boninensis* Koidz., to establish conservation program. *Molecular Ecology Notes* 5: 398-400. doi: 10.1111/j.1471-8286.2005.00940.x
- Tani N, Yoshimaru H, Kawahara T, Hoshi Y, Nobushima F & Yasui T (2006) Determination of the genetic structure of remnant *Morus boninensis* Koidz. trees to establish a conservation program on the Bonin Islands, Japan. *BMC Ecology* 6: 14. doi: 10.1186/1472-6785-6-14
- Tokyo Prefecture Ogasawara Island Branch Office (1938) *Survey of Plants of the Ogasawara Islands*. Chu-gai Print Press. 75p. (in Japanese)
- Toyoda T (2003) *Flora of Bonin Islands (2nd ed, enlarged & revised)*. Aboc & Co., Ltd.

522p. (in Japanese)

- Toyoda T (2014) *The Endemic Plants of the Bonin Islands*. Woods Press. 623p. (in Japanese)
- Tsuda Y, Semerikov V, Sebastiani F, Vendramin GG & Lascoux M (2017) Multispecies genetic structure and hybridization in the *Betula* genus across Eurasia. *Molecular Ecology* 26: 589-605. doi: 10.1111/mec.13885
- Wang N, Borrell JS, Bodles WJA, Kuttapitiya A, Nichols RA & Buggs RJA (2014) Molecular footprints of the Holocene retreat of dwarf birch in Britain. *Molecular Ecology*, 23, 2771-2782. doi: 10.1111/mec.12768
- Whittaker RJ & Fernández-Palacios JM (2007) *Island Biogeography: Ecology, Evolution, and Conservation* (2nd ed.). Oxford University Press. 401p.
- Yulianti W, Katoh S, Sugita N, Kokubugata G, Kato H & Murakami N (2021) Microsatellite markers reveal genetic differentiation of an invasive mulberry species *Morus australis* Poir. among the island groups in Japan and its introduction event into the Ogasawara Islands. *Acta Phytotaxomica et Geobotanica*: in press.

## ABSTRACT IN JAPANESE

小笠原諸島における固有種オガサワラグワと侵略的外来種シマグワとの交雑の現状

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侵略的外来種が近縁な自生種に与える悪影響は、それらの間での交雑が起きる可能性があるため、特に固有種が多い海洋島で深刻である。本研究では、小笠原諸島の固有種であるオガサワラグワ (*Morus boninensis*, 4 倍体) と、それに近縁な侵略的外来種のシマグワ (*M. australis*, 2 倍体) に焦点を当てた。この研究の目的は、オガサワラグワの自生地の実生集団におけるシマグワとの雑種の割合を推定すること、ならびにオガサワラグワの種内の遺伝的多様性を評価することである。本研究では、小笠原諸島におけるクワ属植物の 2 つの野生実生集団、オガサワラグワの 2 つの成熟個体集団、および 12 のシマグワの成熟個体集団で観察と植物サンプルの採集を行い、14 個のマイクロサテライト・マーカーを用いて集団遺伝学的解析を行った。UPGMA 解析、主座標分析 (PCoA)、およびベイズ・クラスタリング解析の結果、小笠原諸島産のこれら 2 種の間で明確な遺伝的分化が認められた。そして、おそらく倍数性レベルの違いによって、2 種間の遺伝子流動はほとんど起きてはいなかった。雑種は、実生集団でも全くみられなかった。シマグワの交雑を通じての固有種オガサワラグワへの悪影響は、このように限定的であることが分かった。さらにオガサワラグワは、絶滅危惧種であるにもかかわらず、種内に高い遺伝的多様性 ( $H_e=0.53$ ;  $A_r=3.27$ ) を維持していることも明らかになった。オガサワラグワの個体数を増やすことができれば、この種を絶滅から救える可能性がある。

## キーワード

遺伝的多様性、保全、マイクロサテライト・マーカー