Genetic Diversity of the Natural Monument *Nypa fruticans* (Palmae) at Funaura, Iriomote Island

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Abstract. Since the population of *Nypa fruticans* at Funaura on the Iriomote Island was declared as a natural monument in 1959, it has been rapidly reduced in size. Its genetic diversity examined by the Random Amplified Polymorphic DNA (RAPD) method showed that all 28 "individuals" examined were genetically identical and had no diversity. They are thus considered clones derived from a single individual by vegetative propagation. Because flowers of a few "individuals" failed to set fertile seeds in 1998, the species is likely to be self-incompatible. A totality of data available indicates that the population at Funaura is at a crisis of extinction.

Key words: conservation, genetic diversity, natural monument, *Nypa fruticans*, Palmae, RAPD

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*Nypa fruticans* (Thunb.) Wurmb. (Palmae) is a major element of mangrove plants in Southeast Asia. It is widely distributed from Ceylon, the Ganges Delta, Burma, and the Malay Peninsula through Indonesia to New Guinea and the Solomon Islands, and northward to the Ryukyu Islands and southward to north Queensland (Tomlinson, 1986). In the Ryukyu Islands, the species is restricted to Funaura of the Iriomote Island (Funaura population) and to the Uchibanare Island (Uchibanare population; Figs. 1, 2). The Funaura population represents the northern limit of distribution of the species.

In 1959 the Ryuku (Okinawa) Government designated the Funaura population as a natural monument and reported the presence of ca. 150 individuals (see Ryuku Government, 1960). However, the population size progressively decreases for the last few decades: 38 individuals were found in 1981 (Niijima et al., 1983), and 25 in 1993 (Nakazato et al., 1996). The population seems to have minutely recovered its size because 28 individuals were counted in 1998. Fruiting has not been recorded during the past five years. Although 11 individuals produced flowers in 1998, none of them set fertile fruits or seeds. Conservation programs are thus needed to sustain the population.

In this paper we present genetic variation in the Funaura population of *Nypa fruticans* to understand a capability of seed production. The data presented is expected to contribute to getting idea for the conservation of *Nypa fruticans* in this and other regions.
Materials and Methods

RAPD analysis

The Random Amplified Polymorphic DNA (RAPD) method has been successful in analyzing genetic diversity in plant populations (e.g., Brauner et al., 1992; Vazquez et al., 1999), and we apply this method to the Funaura population of Nypa fruticans.

Leaf pieces were collected from all 28 individuals in the Funaura population and were dried using silica gel and preserved with it. They were frozen using liquid nitrogen and pulverized into fine powder. Prior to DNA extraction, the powder was suspended in HEPES buffer (pH 8.0) and centrifuged at 10,000 rpm for 5 min at 20 °C to remove polysaccharides (Setoguchi and Ohba, 1995). Total DNA was isolated from collected pellets using the CTAB (Cetyltrimmmonium Bromide) method (Hasebe and Iwatsuki, 1990).

Amplifications were performed in 20 μl reaction volumes containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl₂, 200 μM each of dATP, dCTP, dGTP, and dTTP, 15 ng of primer, 20 ng of template DNA, and 0.8 units of Takara Taq DNA polymerase (Takara Shuzo, Tokyo). The primer kit (A Kit, A1 - A20: Operon Technologies, CA, U.S.A.) consisted of 20 random 10-mer sequences, and each amplification was performed using a single primer. Amplifications were performed in a Takara Thermal Cycler 480 (Takara Shuzo, Tokyo) programmed for 30 cycles of symmetric polymerase chain reaction (PCR). Conditions of PCR

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Fig. 1. Location of the Iriomote Island.
in the first cycle were 2 min at 94 °C for denaturation, 2 min at 40 °C for primer annealing, and 1 min at 72 °C for primer extension. Denaturation time at 94 °C was reduced to 1 min during the next 28 cycles. An extension time at 72 °C was increased to 5 min in the last cycle. The PCR products were separated by electrophoresis in 0.5% agarose gel (SeaKem LE Agarose: FMC Bio Products, CA, USA) using 1 x TBE buffer. The gels were stained in ethidium bromide and photographed on a UV transilluminator. We used λDNA digested with Hind III and included on the gels as a size reference.

**Terminology**

In this paper we often use a term "individual." This needs some explanation. *Nypa fruticans* is rhizomatous, and massive rhizomes buried in estuarine mud undergo repeated dichotomous branching (Tomlinson, 1971, 1986). When an old part of rhizomes dies, daughter shoots or rhizomes develop as independent individuals. It is often difficult to distinguish truly independent individuals (whose mother rhizome has already decayed and thus are not alive) from apparently independent individuals (whose mother rhizome is connected and alive) due to the muddy and crowded habitat. The term "individual" thus refers to a terminal shoot regardless the status of its mother rhizome.

**Results and Discussion**

The 20 RAPD primers produced ca. 136 visible bands per individual. The appearance of those bands was consistent in 27 of the 28 individuals, but one small individual growing somewhat separately from the others showed a difference in one band when the Primer A-11 was used (see individual with number 1 in lane number 1 in Fig. 3A). The band of ca. 1160 kb was always observed in that small
Fig. 3. Typical agarose gels showing RAPD bands obtained on different gels using primers A-11 (A) and A-17 (B) from the A Kit. The gel obtained with primer A-11 contained a RAPD band of ca. 1160 kb (indicated by arrowhead) which represents a diversity in the Nypa fruticans population at Funaura. The first lane is λDNA digested with Hind III as a size reference; the bands from the top down represent: 23,130 bp; 9,416 bp; 6,557 bp; 4,361 bp; 2,322 bp; 2,027 bp; and 564 bp, respectively.

individual but never found in the 27 remaining individuals.

The fact that the 27 of the 28 individuals in the Funaura population have no genetic variation suggests that the individuals are mostly clones derived from a single original individual and that the population has been alive largely by vegetative propagation, in other words, by branching of rhizomes. As discussed in the introductory remark, 11 individuals produced flowers in 1998 although none of them set fertile seeds. They are all included in the 27 individuals with no genetic diversity, and their flowers are thus probably what were produced by a single individual rather than by genetically different individuals. The reason why they did not produce fertile seeds may lie in their self-incompatibility. On the other hand, the one remaining individual with a different genotype appears to be too young to bear flowers. Thus we cannot expect that the population will produce fertile seeds by outcrossing in near future.

In conclusion, the population of Nypa fruticans at Funaura is likely to have been maintained by vegetative propagation, or branching of rhizomes. The species might be self-incompatible. These conditions, along with the fact that the size of population has been rapidly decreasing, indicate that the population is now at a crisis of extinction.

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References


要　旨

瀬戸口浩彰¹・渡邉かよ¹・高相德志郎²・仲里長浩³・戸部博⁴：天然記念物西表島船浦のニッパヤシの遺伝的多様性

南西諸島西表島の船浦にあるニッパヤシ群集は1959年に天然記念物に指定されて以来、縮小の一途をたどっている。この群集の全ての個体（28個体）の遺伝的多様性をRAPDで解析した結果、27個体は全く同じRAPDバンドをもち、小型の1個体だけが催かなる多様性を示した。従って、この27個体は遺伝的に同一なるクローンである可能性がある。これは群集内で開花しても種子が全く形成されない事実にも関連していると思われる。ニッパヤシは根茎が水平方向に伸長して2分岐し、その各々の先端にシュートを形成しながら栄養繁殖をする性質があり、船浦においてもこの栄養繁殖によってのみ群集が維持されていると考えられる。集団サイズの急激な縮小、群集が栄養繁殖によるクローンであること、結実しないことなどを考えると、船浦のニッパヤシは絶滅の途をたどっていると言える。

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