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The origin of *Semisulcospira libertina* (Gould, 1859) (Gastropoda; Pleuroceridae) distribution in Take-shima, Northern Ryukyu Islands, Japan as established by a gigantic volcanic explosion approximately 7,300 years ago, based on a DNA analysis.

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Abstract: *Semisulcospira libertina* (Gould, 1859) (Gastropoda; Pleuroceridae) is a common fresh water snail found in Japan. The genetic population structure and invasion pathway of *S.libertina* in the southern Ôsumi peninsula, the southern Satuma peninsula and the Ôsumi islands were studied by analysis of the mtDNA-COI sequences. The samples were collected at seven localities in the southern Ôsumi peninsula, the southern Satuma peninsula and the Ôsumi islands, consisting of five islets (Tanega-shima, Yaku-shima, Kuchierabu-jima, Kuro-shima, and Take-shima). Analysis of the mtDNA-COI gene divides *S.libertina* into two groups: the Kagoshima group and the Yaku・Mishima group. The results of molecular analysis and the geological history of the region under study suggest that *S.libertina* in Take-shima which was formed established by a gigantic volcanis explosion approximately 7,300 years ago, might have been introduced from Yaku-shima or Kuchierabu-jima.

Key words: biogeography, intraspecific variation, DNA analysis, fresh water snail

Introduction

Because fresh water snail species are extremely limited in locomotion, there is little gene interchange among populations Therefore local evolution occurs easily in fresh water snail species, which is remarkable in the islands in particular (Solem, 1976; Tomiyama, 1984). Take-shima, Osumi Islands, Northern Ryukyu Islands, in Japan, is parts of the caldera wall produced after the gigantic volcanis explosion (Kikai-caldera eruption or Akahoya volcanic eruption) approximately 7,300 years ago (Okuno, 2002). It is thought that *Semisulcospira libertina* (Gould, 1859) (Gastropoda; Pleuroceridae) which is a common fresh water snail species in Japan, distributed in Take-shima emigrated from other islands, crossing the sea by unknown means.

The analysis of mtDNA-COI provides useful information in the analysis of intra-specific variation of land or fresh water snail species. Kaneda & Hirano (2016) analyzes a base sequence of mtDNA-COI domain about of the six species belonging to the Bradibaenidae from Ryukyu Islands. To clarify which population of *S. libertina* distributed in Take-shima is closely related to other populations, using mtDNA-COI analysis, we examined the intra-specific variation of this species.

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Materials and Methods

DNA extraction and amplification

In total seven populations (Fig.1: Kuro-shima island, Take-shima island, Kuchierabu-jima island, Yaku-shima island, Tanega-shima island, southern Ôsumi peninsula, and southern Satuma peninsula) were used in this study. *Stenomelania rufescens* (;Thiaridae) from Tanega-shima of the close species, *Crepidula convexa* (Gastropoda, Calyptraeidae), and *Pleurocera pyrenellum* (Gastropoda, Pleuroceridae) were used as outr examination group. Collected samples from each of the populations were stored in a deep freeze at -30°C until the examinations were conducted. Only fully mature specimens were used in the study.

The analysis of DNA was carried out by a method of Wiwegweaw *et al*. (2009). A sample of DNA was extracted from these specimens via a graft of the muscular tissue of the foot The graft (~2 *2mm) which was exfoliated and placed in 1.5 ml of tubes to which was added 500µl of a 2* CTAB solution. Twenty mg/ml of Proteinase K (9µl) was added to the tube, which was then warmed at 50 °C for approximately two hours to dissolve the muscular tissue. The PCI (phenol: chloroform: isoamyl = 25:24:1) of the falling together product (500µl) was added to this solution which was then centrifuged for fifteen minutes under conditions of 25°C and 14,000 rpm. After

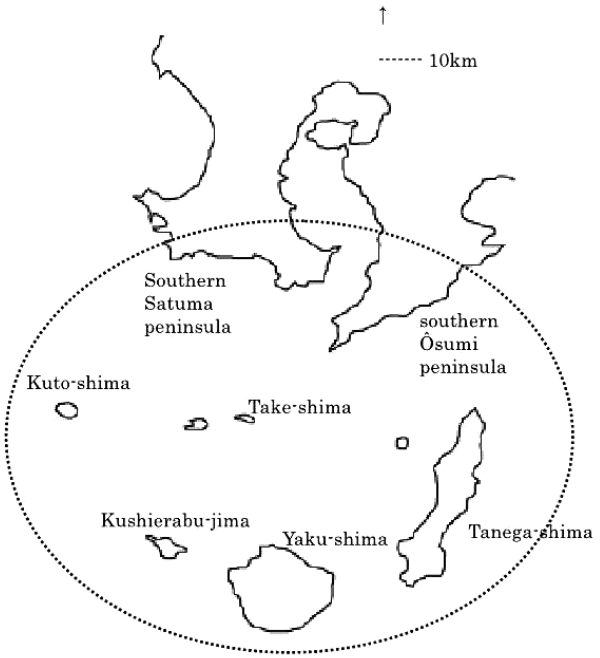

Fig. 1. Map showing the collecting sites of samples in this study.

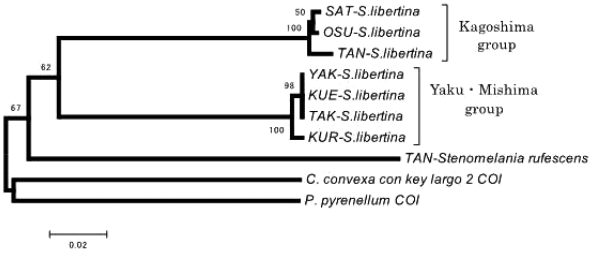

Fig. 2. Phylogenetic trees reconstructed by Neighbor-joining techniques based on COI sequence. Numbers above branches indicate percentage of times in 1000 bootstrap replications that population occured in these clades. Bootstrap values lower than 50% are indicated by (-). OSU: southern Ôsumi peninsula, SAT: southern Satsuma peninsula, TAN: Tanega-shima, YAK: Yaku-shima, TAK: Take-shima, KUE: Kuchierabu-jima, KUA: Kuro-shima,. Out Group Species; TAN- S. rufescens: *Stenomelania rufescens* (;Thiaridae) from Tanega-shima, C. convexa: *Crepidula convexa* (Gastropoda, Calyptraeideae), P. pyrenellum: *Pleurocera pyrenellum* (Gastropoda, Pleuroceridae)

centrifugal separation, only the supernatant liquid in the tube was removed and replaced with 100% ethanol at a ratio of 1 ml to 1.5 ml of the contained new tubes. The deposits of DNA formed as the tube was cooled to -30°C for ten minutes. The tube was centrifuged under a conditions of 4°C and 14,000 rpm for ten minutes. After the ethanol in the tube was removed with a pipette, the DNA pellet was washed with a 70% ethanol solution (500µl). The tube was then centrifuged under conditions of 4°C and 14,000 rpm for five minutes. After the ethanol in the tube was removed, the DNA pellet was air-dried for approximately 10~15 in the share room. The DNA was then dissolved in a TE buffer (50µl, pH 8.0) in a tube.

The full length of the COI gene domain of collected mtDNA was amplified by PCR (polymerase chain reaction). As a primer, COI 017 (5'– GGTCAACAAATCATAAAGATATTGG-3') and COI 018 (5'–TAAACTTCAGGGTGACCAAAAAAT-CA-3') were used. Ti this added 2.5M dTNP mix (0.8µl), 10* ExTaq Polymerase buffer (1.0µl) and 10* ExTaq Polymerase (5µ/5µl). A Mastercycler gradient (eppendorf) was used as a kit, and a PCR reaction was conducted. The PCR amplification was conducted under the following reaction conditions: an initial denaturation for two minutes at 94°C followed by thirty cycles of sixty seconds at 94°C, sixty seconds at 45°C, and sixty seconds at 72°C with a final extension for five minutes at 72°C. As for the provided PCR product, a band of the purpose size (approximately 700bp) was confirmed by agarose gel electrophoresis. A DNA Analyzer Model 4200 (LI-COR) was to sequence the reaction of the PCR product. After the base sequence was automatically determined by e-Seq (LI-COR), the clear error was regulated by manual operation. As for the base sequence of the provided mtDNA-COI domain, multiple alignment was performed using Align IR (LI-COR).

Phylogenetic analyses

MEGA4 (Tamura *et al*., 2007) was used to generate the tree diagram. The tree diagram was made using the Neighbor Joining method (NJ; Saitou & Nei, 1987). The base substitution rate of the NJ method was demanded by Kimara's two variables method of Kimura (Kimura, 1980), and the reliability of the tree diagram was evaluated by the Bootstrap test by 1000 repetitions.

Results

The base sequence of the COI gene of 626bp was decided by seven groups collected in each location. Base substitution was detected in 136 places, and the mean base substitution rate became 5.4±0.5%. The dendrogram constructed by the Maximum Parsimony method (MP method) generated the same result as the Neighbor Joining method (NJ method).

As a result, 7 populations were grouped by three main systems:

Group I: Kagsoshima Group: Tanega-shima, southern Ôsumi peninsula, and southern Satuma peninsula

Group II: Yaku-Mishima Group: Kuro-shima, Take-shima, Kuchierabu-jima,and Yaku-shima

The Haplotype of CO1 from Take-shima population was the most closely related to the populations from Kuchierabu-jima or Yaku-shima.

Discussion

The analysis of the base sequence of the COI gene showed, the population of Take-shima to have an identical haplotype. Furthermore, this island and the heredity distance between Kuchierabu-jima or Yaku-shima were small when compared to with other populations. Take-shima is comprised of parts of the caldera wall produced after the gigantic explosion (Kikai-caldera eruption or Akahoya eruption) of the volcano which they produced approximately 7,300 years ago (Okuno, 2002). This eruption is said to have been the most intense volcanic activity on the earth in the past 10,000 years (Machida & Arai, 1978). Take-shima is thought to be the result of caldera walls formed at the time of explosion. In other words, this island was temporarily comprised of inanimate objects. Because the last glacial epoch is over, and it is estimated that both islands were in isolation following the eruption, it is thought that the creatures who now inhabit this island arrived there by some kind of means of crossing the sea. In light of the facts mentioned above, when we consider both the geographical distance and the heredity distance among Take-shima, Kuchierau-jima and Yaku-shima, most biota inhabiting in Take-shima might have been introduced from Kuchierau-jima or Yaku-shima. The the results of this study lend support to the hypothesis that *S. libertina* distributed in Take-shima were introduced from neighboring either Kuchierabu-jima or Yaku-shima.

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