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## Plant distribution patterns in the Ryukyu Archipelago, Japan in the light of seed dispersal abilities

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The Ryukyu Archipelago is an assemblage of continental islands that lie between Taiwan and mainland Japan for approximately 1300 km. The climate is subtropical and moderate throughout the year and the islands are covered by well developed broad-leaved evergreen forests. On the basis of its submarine topography, the Ryukyu Archipelago is subdivided into three areas (the northern, central, and southern Ryukyus) at the Tokara tectonic strait (the Tokara Gap) and the Kerama Gap, where the sea bottom is more than 1000 m deep. These two gaps are the oldest channels in the Ryukyu Archipelago, which first segmented the land bridge connecting the Asian continent, via Taiwan and the Ryukyu Archipelago, with mainland Japan in the Pliocene or early Pleistocene. In consideration of this geographical characteristics, preceding floristic geographic studies in the Ryukyu Archipelago placed demarcation lines between the northern and central Ryukyus, and/or between the central and southern Ryukyus, based on distribution records of a few particular elements of the flora, such as endemics, mangrove plants and sea grasses (e.g., Hara, 1959; Good, 1974; Mackawa, 1974; Takhtajan, 1986; Kitamura *et al.*, 1994). Also, we conducted more quantitative analysis on the floristic demarcations, collecting distribution records of all the seed plants (approximately 1800 species) on major islands from several literature sources into a data matrix, and revealed that the flora of the Ryukyu Archipelago is hierarchically structured as (northern Ryukyus, (central Ryukyus, southern Ryukyus)).

This time, we use subsets of the floristic data in the light of different seed dispersal abilities and further investigate the influence of these deep oceanic channel divisions on the floristic differentiation in this archipelago, taking account of the influence of isolation by mere geographic distances among islands. We examined local patterns of the correlation between pairwise floristic dissimilarity and geographic distance among the islands; if floristic dissimilarity distance is not correlated with geographic distance locally, barriers (not isolation by geographic distance) are likely causing the differentiation of floras. Analysis on the influence of the deep oceanic channel divisions on the floristic differentiation is expected to advance our understanding of floristic plant geography in such island chains.

Oral -21

### Population genetics of the Joey palms, *Johannesteijsmannia* H.E.Moore (Palmae)

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*Johannesteijsmannia* is a palm genus of four tropical rain forest understorey species. Only *Jt. altifrons* is widespread, ranging from southern Thailand, Peninsular Malaysia, Sumatra to western Borneo while *Jt. lanceolata*, *Jt. magnifica* and *Jt. perakensis* are endemic to Peninsular Malaysia. Their increasing commercial exploitation as ornamental plants makes conservation a priority. To conserve effectively, more understanding is needed about their genetics variation within and between populations of each species.

AFLP fingerprinting was generated using six primer combinations on DNA samples from 222 individuals collected from 27 populations throughout the distribution of *Johannesteijsmannia*. *Jt. altifrons* exhibited the highest genetic diversity of all. Populations of each species exhibited moderate genetic diversity (Nei's genetic diversity values ranged from 0.0861–0.1968 and the Shannon information index, from 0.0677–0.2355). Results of the Analysis of Molecular Variance (AMOVA) showed that all congeners partition higher genetic diversity within population (63%–88%) than between populations

(12%–37%). The population differentiation measure, the  $F_{st}$  value, was highly significant ( $P < 0.001$ ) and ranged from 0.1235 (*Jt. magnifica*) to 0.3277 (*Jt. altifrons*) indicating that there is gene flow between populations of each species. AMOVA results were corroborated by Bayesian analysis with significant  $F_{st}$  values for all species. The best model selected was  $f = 0$  ( $f$  being the inbreeding coefficient), suggesting all congeners are outcrossing species. Results from Mantel test demonstrated there is a significant correlation between geographical and genetic distance in *Jt. altifrons* but insignificant for the others.

Oral -22

**Cytological diversity of the genus *Hydrangea* (Saxifragaceae)  
in the Ryukyu Archipelago of Japan  
- On the origin of tetraploid *Hydrangea liukiuensis* endemic to Okinawajima  
Island –**

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Despite corresponding to roughly 1 % of the area of Japan, the Ryukyu Archipelago support more than 1600 seed plant species. This marvelous plant diversity in the current Ryukyu Archipelago must have been established due to the influence of the paleogeographical dynamics of this area. In addition, such speciation processes as interspecific hybridization and polyploidization appear to play an important role in creating plant diversity in the Ryukyus. Some examples of the intraspecific polyploidy have been reported from the *Hydrangea chinensis*-complex that comprises five species distributed in the Ryukyu - Taiwan region. Of the five species, *H. chinensis*, *H. grosseserrata* and *H. kawagoeana* are diploid ( $2n=36$ ), while *H. liukiuensis* has diploid ( $2n=36$ ) and tetraploid ( $2n=72$ ) cytotypes, and *H. yayeyamensis* has octoploid ( $2n=144$ ) and decaploid ( $2n=180$ ) cytotypes. In this workshop, I will focus on the origin of tetraploid *H. liukiuensis* inferred from chloroplast and nuclear DNA sequences.

*Hydrangea liukiuensis* is a small shrub endemic to Okinawa-jima Island of the central Ryukyus. In a preliminary cytogeographical survey of this species, only diploids were found in 15 of 18 populations investigated, whereas tetraploids occurred at remaining three populations (Mt. Nekumachiji, Taiho Riv., Mt. Yae). Intra-population variation in the ploidy level was observed in Taiho Riv., in which diploids were dominant and only four tetraploids grew next to each other. Ten diploids and six tetraploids of *H. liukiuensis*, together with *H. chinensis* (Taiwan), *H. grosseserrata* (Yaku-shima Isl.) and *H. yayeyamensis* (Ishigaki-jima and Iriomote-jima Isls.) were used for molecular analysis. *Cardiandra alternifolia* (Hyogo Pref.) was also used as an out-group. The *trnS/trnG* intergenic spacer region (*trnS*-*G* region) of the chloroplast DNA and the internal transcribed spacer region (ITS) of nuclear ribosomal DNA were PCR amplified and sequenced. Most parsimonious (MP) and maximum likelihood (ML) trees were then constructed based on the nucleotide sequence of each region. On the MP and ML trees of *trnS*-*G* region, *H. liukiuensis* was monophyletic with high bootstrap values (97% and 95%, respectively) and this clade was sister to one composed of remaining three *Hydrangea* species. On the other hand, tetraploids of *H. liukiuensis* were nested with *H. chinensis*, *H. grosseserrata* and *H. yayeyamensis* to construct a monophyletic clade on the MP and ML trees of ITS region (bootstrap values of 60% and 55%, respectively). This clade was sister to a monophyletic clade composed of all diploids of *H. liukiuensis*. This incongruence between chloroplast and nuclear DNA phylogenies of *H. liukiuensis* seem to suggest an allopolyploid origin of tetraploid *H. liukiuensis*. However, no other *Hydrangea* species except *H. liukiuensis* are distributed on Okinawa-jima Island. Past interspecific hybridization event(s) between diploid *H. liukiuensis* and an extinct parent may have caused the origin of tetraploid *H. liukiuensis*.