

An Investigation into the Distribution
Pattern of *Limonium wrightii*

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Bio 330
Feb 12, 2014

“Origin of the disjunct distribution of flower colour polymorphism within *Limonium wrightii* (Plumbaginaceae) in the Ryukyu Archipelago”

- Shun’Ichi Matsumura, Jun Yokoyama, Tatsuya Fukuda, & Masayuki Maki

Introduction

- Islands provide a natural way to observe evolution in action. Organisms are confined to a small area defined by its oceanic boundaries. This “reduces gene flow between islands”, and creates interesting speciation events.
- Several islands were connected to the mainland during the Pleistocene epoch. For some reason there has been little research into plant groups on the land-bridge islands, despite the fact they are excellent demonstrations for species divergence. Multiple factors play into the evolution of these communities, however, fauna/flora interactions and various pollination systems are the key components.

Location of Study

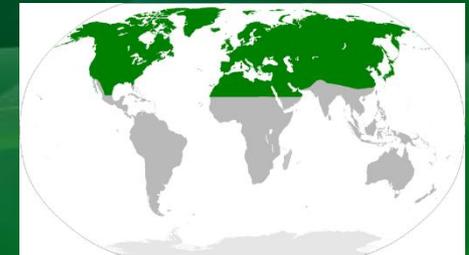
Ryukyu Archipelago



Mix of temperate and tropical communities

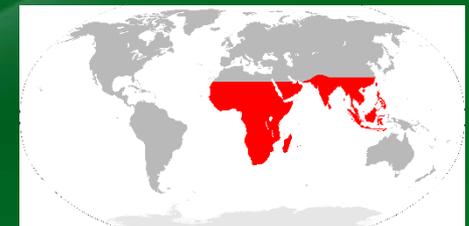
Part of the Holarctic Floral Kingdom

- flora relative of evergreen forests of Sino-Japanese Region
- some tropical plant origins



Zoogeographically considered Palaeotropical

- contains fauna similar to Taiwan, south-east Asia, and continental China



Limonium wrightii

Order Caryophyllales

Belongs to Plumbaginaceae (“leadwort”) Family

- consists mainly of shrubs and herbaceous plants
- basal or alternate leaves
- flowers consist of five sepals and stamens
- five petals fused at the base to form a tubular structure
- found mainly along coastal limestone rocks

Six flower color morphs have been observed

- pink, yellow, yellow corolla with white calyx flowers, white, orange and ivory
- pink and yellow morphs are the most frequent

Distribution

- Daito and Izu-Ogasawara Islands of Japan
- Taiwan (offshore eastern region)
- Ryukyu Archipelago



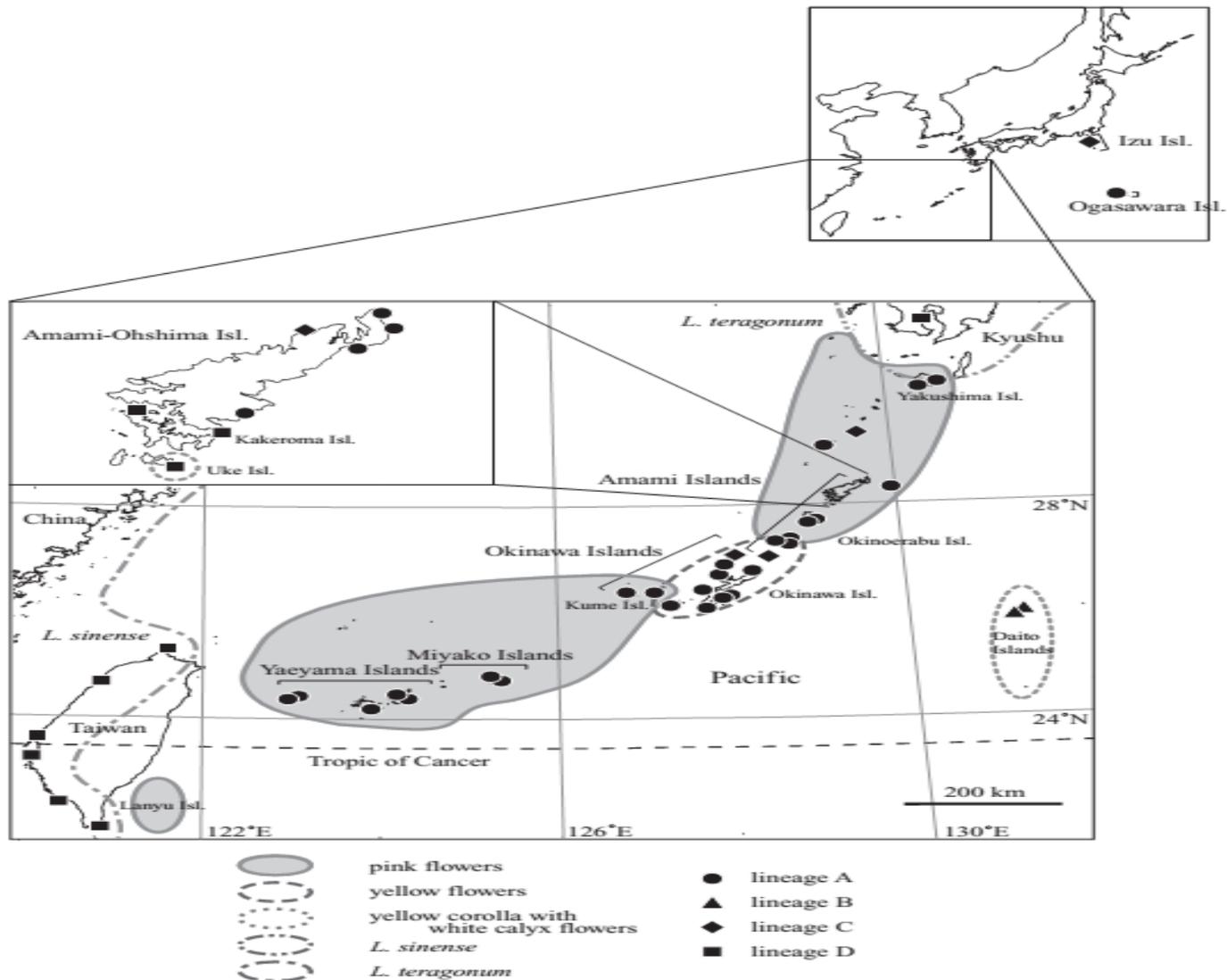


Figure 1. Geographical pattern of the distribution of flower colour variations within *Limonium wrightii* and its closely-related species in the vicinity of the Ryukyu Archipelago. The distribution of each flower colour variation within *L. wrightii* and the related species *Limonium sinense* and *Limonium tetragonum* is indicated by line pattern. The locations of the haplotypes of lineages A, B, C, and D in Fig. 2 are indicated by a circle, triangle, diamond, and square, respectively.

Distribution Pattern

Leapfrog Distribution: “two populations very similar in appearance are geographically separated from each other by a different, intervening population of the same species”

2 possible hypotheses:

I. Vicariance and Dispersal

- a. populations on one side of an island must have come from the other
- b. far distance dispersal results in communities with a similar phenotype

These hypotheses were discarded due to a lack of connection between morphs in the Ryukyu Archipelago. It could be possible that the northward running Kuroshio Current dispersed some southern pink morphs with the northern yellow. However, this would not be a major factor in creating a Leapfrog pattern.

- II. Phenotypic Change: “assumes unequal rates of phenotypic changes among populations” as a result of
- a. stochastic (random) processes - separate communities undergo convergent evolution or central populations experience quick transformation of phenotypic traits
 - b. natural selection

If this was the case, neutral markers should not show genetic and phenotypic similarities.

Materials and Methods

Collection of Plants

- 58 samples were collected from the 28 islands composing the entire distribution of *L. wrightii*
- 3 *Limonium tetragonum*, 7 *Limonium sinense*, and 1 sample of out-group *Limonium bicolor* and *Limonium australe*
- orange, ivory, and white morphs were not collected as they are assumed to be a hybridization of the pink/yellow morphs; Matsumura et al. determined that these exclusions were unlikely to affect the data
- “The collected samples were quickly dried with silica gel and stored as herbarium samples”

Preparation of Chloroplast DNA (cpDNA) for Analysis

- 2x CTAB procedure used to extract and isolate 200-300 µg of genomic DNA from samples
- placement of DNA in 100µl TE to prevent degradation
- PCR amplification of intergenic spacers *psbA-trnH*, *trnG (GCC)-trnfM (CAU)* and *trnV (UAC)-trnM (CAU)*

Making a Cladogram

- compilation of PCR product sequences into one data set
- alignment of cpDNA sequences by Se-Al ver. 2.0a11
- 3 regions of cpDNA were considered in the creation of a cladogram

Table 1. List of analysed samples and the localities in *Limonium wrightii* and its closely-related species in the north-western Pacific for chloroplast DNA sequences

Island	Number of localities	Flower colour	Haplotype (N)
Izu-Ogasawara Islands			
Shikine	2	Pink	8 (2)
Chichi-jima	1	PinK	1 (1)
Subtotal	3		
Ryukyu Archipelago			
Yaku-shima	2	Pink	2 (2)
Akuseki	1	Unknown*	9 (1)
Takara	1	Pink	1 (1)
Kikai	1	Pink	1 (1)
Amami-ohshima	6	Pink	1 (3), 2 (1), 8 (1), 11 (1)
Kakeroma	1	Pink	11 (1)
Uke	1	Yellow (white calyx)	11 (1)
Tokunoshima	2	Pink	1 (2)
Okinoerabu	2	Pink	1 (2)
Okinoerabu	2	Yellow	1 (2)
Okinoerabu	1	Orange†	1 (1)
Yoron	3	Yellow	1 (1), 7(2)
Iheya	3	Yellow	3 (1), 7 (2)
Izena	1	Yellow	3 (1)
Islet. Yanoshita	1	Yellow	1 (1)
Ie-jima	1	Yellow	1 (1)
Okinawa	5	Yellow	1 (2), 2 (2), 4 (1)
Islet. Oh-u	1	Yellow	4 (1)
Ikei	1	Yellow	2 (1)
Yabuchi	1	Yellow	4 (1)
Aka-jima	1	Yellow	1 (1)
Aguni	1	Pink	2 (1)
Kume	1	Pink	2 (1)
Kita-Daito	4	Yellow (white calyx)	6 (3)
Minami-Daito	4	Yellow (white calyx)	6 (3)
Miyako	2	Pink	5 (2)
Ishigaki	2	Pink	1 (1), 2 (1)
Kuroshima	1	Pink	2 (1)
Yonaguni	4	Pink	1 (4)
Subtotal	57		
<i>Limonium sinense</i>			
Taiwan			
Chunan	1	Yellow (white calyx)	11 (1)
Peimen	1	Yellow (white calyx)	13 (1)
Longkeng	1	Yellow (white calyx)	10 (1)
Yuwengtao	1	Yellow (white calyx)	11 (1)
Tongtunyu	1	Yellow (white calyx)	11 (1)
Shiao-liuchiu	1	Yellow (white calyx)	10 (1)
Subtotal	6		
<i>Limonium tetragonum</i>			
Japan			
Isl. Tashiro, Miyagi	1	Yellow (white calyx)	12 (1)
Akoh, Hyogo	1	Yellow (white calyx)	11 (1)
Kiire, Kagoshima	1	Yellow (white calyx)	11 (1)
Subtotal	3		
<i>Limonium bicolor</i>‡			
China			
Mt. Dachinshan, Nei-Mongole	1	Y.-C. Ma 2-87 in TUS	10 (1)
<i>Limonium australe</i>‡			
Australia			
New South Wales	1	Yonekura K., <i>et al.</i> 99334 in TUS	11 (1)
Total	71		

*This is most likely the pink type because the calyx was white, although it was old.

†Putative hybrid origin of the pink and yellow morphs.

‡Herbarium specimens.

§General traits of flower colour in the species.

TUS, Tohoku University.

Results

- 14 Haplotypes and 4 lineages (A,B,C,D) derived from data
 - *L. wrightii* contains 10 haplotypes within A, B, and C
 - A = 5 haplotypes, numbered 1-5 with 1 being the most frequent
 - B = 1 haplotype (9)
 - C = 3 haplotypes from one island
 - Haplotype 11
 - The remaining 3 haplotypes belong to the other 3 closely related species composing lineage D + haplotype 11

The sharing of haplotype 11 indicates possible gene flow among species, “although we have no other evidence at present”.

Distribution of Lineages

- A: Izu-Ogasawara Islands + most areas of distribution
- B: Daito Islands
- C: Okinawa + Amami Islands
- D: Amami-Ohshima Island + offshore islands

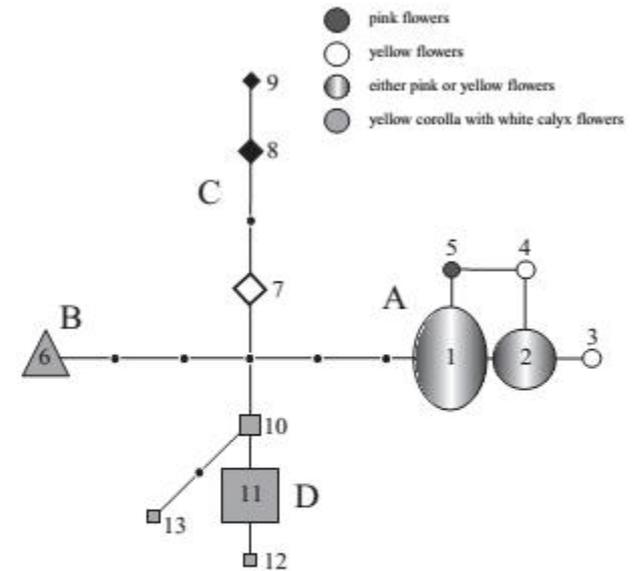


Figure 2. Statistical parsimony network of chloroplast DNA haplotypes in *Limonium wrightii* and its closely-related species. A line and small black dots between haplotypes indicate mutational steps between them. The relative frequency of each haplotype is represented by the size of the symbol. The state of flower colour is indicated by shading pattern.

Conclusion

- There is no connection between cpDNA sequences and the variation in pink and yellow flowers. Common haplotypes were not restricted to a certain flower color. Pink flower morphs in the north and south lacked any kind of haplotype relationship. The evidence leans toward stochastic processes or natural selection as the reason for leapfrog distribution.
- However, it is hard to prove which one is responsible. “Stochastic processes may also affect the frequencies of flower colour morphs, even if natural selection has mediated the evolution of flower colour polymorphisms”.
- Pollinators could have an effect on flower color morphs. A certain small bee (*Ceratina* spp.) visits both yellow and pink flowers. However, the pink morphs receive several different kinds of hymenoptera. Perhaps the different colors are a response generated by the “divergent selective pressures generated by pollinators, even if pollinator visitations differ only quantitatively”. Further research is required to verify this idea.
- Matsumura et al. were unsure as to how natural selection affects the dispersal of flower color, but claim stochastic processes are not the only cause. Previous studies showed a single color (pink or yellow) between 30 populations in 3 major regions. Natural selection is assumed to be the major contributor to color variation.

● Reference:

- Shun'Ichi Matsumura, Jun Yokoyama, Tatsuya Fukuda, & Masayuki Maki. "Origin of the disjunct distribution of flower colour polymorphism within *Limonium wrightii* (Plumbaginaceae) in the Ryukyu Archipelago" *Biological Journal of the Linnean Society* 2009, 97, 709-717