

## LABORATORY OF BIOLOGICAL DIVERSITY

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While genomic structures as well as their genetic information appear to stably transmit into daughter cells during cell division, and also into the next generation, they can actually vary genetically and/or epigenetically. Such variability has had a large impact on gene expression and evolution. To understand such genome dynamisms in eukaryotes, especially in plants, we are characterizing the flower pigmentation of morning glories.

### I. Flower pigmentation patterns of the morning glories

*Ipomoea nil* (Japanese morning glory), *I. purpurea* (the common morning glory), and *I. tricolor* have been domesticated well as floricultural plants, and their various spontaneous mutations have been isolated. The wild type morning glories produce flowers with uniformly pigmented corolla, whereas a number of mutants displaying particular pigmentation patterns have been collected (Figure 1). Because flower pigmentation patterns are easily observed, the molecular mechanisms underlying these phenomena provide fine model systems for investigating genome variability.

*Margined*, *Rayed* and *Blizzard* of *I. nil* are dominant mutations. While these mutants show distinct flower pigmentation patterns, the same pigmentation gene is repressed by non-coding small RNA in the whitish parts of the corolla. It is suggested that distinct regulation of small RNA cause the difference in pigmentation patterns. The recessive mutations, *dusky* of *I. nil* and *pearly-v* of *I. tricolor*, confer variegated flowers, and epigenetic mechanisms are thought to regulate flower pigmentation. We are currently characterizing detailed molecular mechanisms of these mutations.



Figure 1. Flower phenotypes of the morning glories.

### II. *de novo* sequencing of Japanese morning glory genome

Although morning glories are studied worldwide, especially in plant physiology and genetics, no whole nuclear genome sequences of any *Ipomoea* species are available. To facilitate the studies of our group as well as all morning glory researchers, we are conducting *de novo* genome sequencing of *I. nil*, having a genome of about 800 Mbp. We chose the Tokyo-kokei standard line for genome sequencing, and employed not only shotgun sequencing using high-throughput DNA sequencers but also BAC end sequencing. We are collaborating with several laboratories in Japan.

### III. BioResource of morning glories

NIBB is the sub-center for National BioResource Project (NBRP) for morning glory. In this project, we are collecting, maintaining and distributing standard lines, mutant lines for flower pigmentation, and DNA clones from EST and BAC libraries of *I. nil* and its related species. *I. nil* has been one of the most popular floricultural plants since the late Edo era in Japan. It has extensive history of genetic studies and also has many advantages as a model plant; simple genome, large number of mutant lines, and efficient self-pollination. Our collection includes 220 lines and 147,000 DNA clones.

### IV. Flower color and vacuolar pH

Flower color is not only determined by pigments. It is dependent on several factors, such as colorless pigments, metal ions, and pH in the vacuole where flower pigments are accumulated. Petunia blooms red or violet flowers, and mutations in any one of the seven loci, named *PH1-PH7*, result in a bluish flower color (Figure 2). We successfully isolated *PH1* that encodes P3<sub>B</sub>-ATPase, hitherto known as Mg<sup>2+</sup> transporters in bacteria. Although *PH1* itself is not a proton transporter, it can boost *PH5* (P3<sub>A</sub>-ATPase) proton transport activity that has been known to be essential for vacuolar hyperacidification. *PH1* and *PH5* physically interact with each other, and co-localize in the vacuolar membrane. The heteromeric P-ATPase pump of *PH1* and *PH5* is sufficient to hyperacidify vacuoles creating red pigmentation of petunia flowers.



Figure 2. The petunia unstable *ph1* mutant. The bluish pigmentation is due to a failure to hyperacidify vacuoles.