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Optimum Conservation of Species Designated by the Endangered Species Preservation Act Using Information Obtained from Sequencing Breakthrough

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Biodiversity, the variety of natural organisms living on Earth at the genetic, specific and ecological levels, is essential for our life. Despite our recognition of the importance of biological diversity, its loss has progressed: many species around the world have been threatened and/or have already disappeared due to recent environmental changes. Minimizing the loss of biodiversity is important from the standpoint of continuing to receive services from biodiversity.

Many plant species are threatened with extinction, so the Act on Conservation of Endangered Species of Wild Fauna and Flora in Japan was revised in 2013 to implement better conservation practices for endangered plant species. The revised Act set a target of hundreds of new designations of rare domestic species of wild fauna and flora in Japan. There are still concerns, however, about increasing extinction risk from overharvesting, insufficient information such as regarding deleterious genes, adaptability and genetic pollution, and a paucity of knowledge for recognizing appropriate management units for optimal conservation strategies. To support valid implementation of the Act, we constructed effective, rational conservation guidelines for critically endangered species, taking the advantage of recent DNA sequencing breakthroughs. This project achieved four objectives: (1) to conduct transcriptome and genome sequencing for species sets consisting of the endangered species designated in the Act, and phylogenetically related non-endangered species, (2) to evaluate the vulnerability and sustainability of species by analyzing transcriptome information, (3) to estimate historical demographic changes in endangered species by analyzing genomic data and (4) to develop and improve a cost-effective method of genome sequencing using small or degraded DNA samples.

We found that some endangered species had accumulated larger amounts of deleterious variations in their genomes and fewer gene duplications as compared with non-threatened congeners (Hamabata et al., 2019). These genomic traits in endangered species can result in vulnerability and less adaptability to diverse environments and/or environmental changes. Conservational conditions of endangered species have been conventionally evaluated on the basis of the number and reduction rate of wild individuals. The present finding is epoch-making for biodiversity conservation in that some genomic traits, including the amount of accumulated deleterious genetic variations and gene duplications, can be used to evaluate the feasibility and effect of conservation activities, and the sustainability of endangered species (Fig. 1).

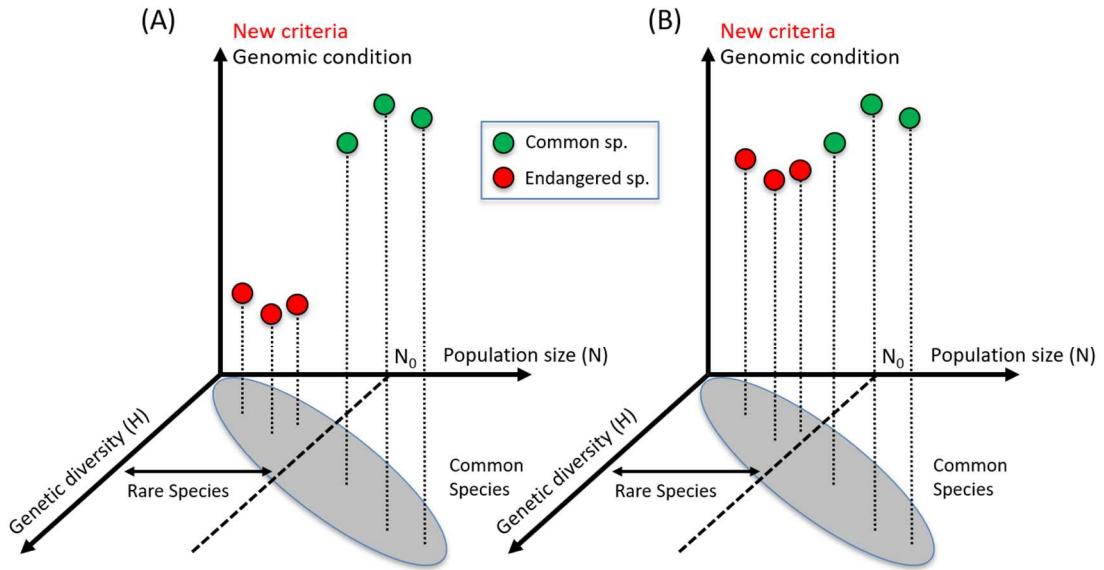


Figure 1 New categorization of endangered species taking into account genomic conditions. Endangered species can be differentiated between those with deteriorated (A) and sound (B) genomic conditions

Demographic changes in critically endangered species were successfully inferred and compared with those of less threatened congeners. Most endangered species exhibited smaller effective population numbers and more frequent population decline. For example, the results for an endangered species, *Primula kisoana* var. *kisoana*, suggest that its genetic diversity is lower than that of its congener (*Primula kisoana* var. *shikokiana*), and the former population size has decreased twice, at 200 and 1,000 generations prior, while the latter has decreased only once at 1,000 generations prior. In the case of a non-endemic species of Ericaceae, *Vaccinium amamianum*, the endangered Japanese population was genetically divergent from the Taiwanese wild populations. Demographic analyses suggest that the domestic population has maintained a small number of individuals since the divergence at approximately 5,500 generations prior from larger Taiwanese population. On the other hand, there are other cases such as an endangered species of Orchidaceae, *Dendrobium okinawense*. No genetic differentiation can be detected among the samples from all of its known geographic ranges. The demography of this species has fluctuated in the past, but the effective population size has stayed large (more than 200,000) for the last 60,000 generations. These outcomes indicate the importance of evaluating previous demography for understanding the conservation status of each species, irrespective of its current situation.

Sufficient amounts and quality of samples of endangered species for DNA extraction are not always available. MIG-seq (Multiplexed ISSR Genotyping by sequencing) analysis (Suyama & Matsuki, 2015), a simple and cost-effective contracted genome sequencing method, is highly useful in these cases and the method was greatly improved during this project. Now, the method produces larger outputs (several times larger) and more accurate sequencing information. Genetic analyses of spatial genetic structure, phylogeny, detection of genetic pollution and construction of appropriate management units for many critically endangered species, such as *Piper postelsianum*, *Cypripedium*

acranthos var. *rebunense*, and *Gentiana yakushimensis*, were successfully performed using small amounts of degraded DNA samples. As a result, for endangered species listed in the Act, species-specific genetic information can be expected to be used in the construction of future conservation plans.

Finally, the findings for each objective were integrated into a new scheme for biological conservation of rare domestic species of wild fauna and flora in Japan using large amounts of genetic information obtained from DNA sequencing breakthroughs. In addition to the conventional criteria (e.g., number of individuals, rate of population decline and degree of genetic diversity) of the guidelines for critically endangered species, information on vulnerability, sustainability, phylogenetic uniqueness and demography should also be included to construct more effective and more rational conservation plans. The strategy developed in this project will provide new criteria for assessing extinction risk and sustainability of endangered species, including National Endangered Species of Wild Fauna and Flora, directly. The outcome of this project will aid in achieving Aichi Biodiversity Targets 12 and 19.

Journal articles:

- Hamabata, T., Kinoshita, G., Kurita, K., Cao, P.-L., Ito, M., Murata, J., Komaki, Y., Isagi, Y. and Makino, T. (2019) Endangered island endemic plants have vulnerable genomes. *Communications Biology*, 2: 244.
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