【4-1707】

Development of *In Situ* and *Ex Situ* Conservation Procedures for Endangered and Heritage Species in the Amami and Ryukyu Islands

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Key words: endangered species, *ex situ* conservation, *in situ* conservation, metagenomic analysis, OUV, return to the wild, species distribution modeling

Amami-Okinawa is a region that contains one of the highest levels of biodiversity in the world, including many endangered species (endemic species among them). In recent years, the region has attracted attention as a candidate site for World Natural Heritage status. In this project, we focused on the spiny rat (*Tokudaia* spp.), Okinawa rail (*Gallirallus okinawae*), and large tree-hollow-dwelling beetles (*Neolucanus* spp. and *Cheirotonus jambar*), for which urgent conservation measures are required. In parallel with research necessary for the *in situ* conservation of these endangered species, in this study, we collaborated with zoos and insect museums to develop *ex situ* conservation approaches, including breeding techniques, to limit the risk of extinction and contribute to population recovery. Therefore, we first developed techniques according to the conservation status of each target species.

In our *in situ* study of the spiny rat, we aimed to accumulate basic ecological information necessary for its conservation, including distribution data, and used these data to form a conservation plan aimed at expanding their distribution area. We found that the breeding season of the spiny rat is from early summer or early autumn to early spring. In Okinawa, the habitat of the spiny rat is subtropical broadleaf forests and secondary evergreen broadleaf forests at elevations of 200–250 m a.s.l. The Henoko Dam separates these habitats. In Tokunoshima, the habitat of the spiny rat is found at altitudes of 150 m a.s.l. or higher, with suitable habitat on the eastern slope of Mt. Inogawa and the northern part of Mt. Inutabu. The results of dietary analysis revealed that the spiny rat mainly consumes plant materials, but supplements this diet opportunistically with animal matter, suggesting that its diet composition may change seasonally.

In our *ex situ* conservation study of the spiny rat, we aimed to link breeding information obtained in field and laboratory studies and to explore the possibilities of captive breeding and reintroduction. This study represents the world's first successful attempt at captive breeding of the spiny rat. We even produced a second generation from a pair of captive-bred individuals, demonstrating the feasibility of succession management outside its habitat. We also analyzed its gastrointestinal microflora, which are considered to be important for reintroduction to the wild, and found no significant differences between the captive and wild populations.

For the Okinawa rail, our goals were to establish appropriate evaluation criteria for the selection of captive Okinawa rails for release into the wild, and to establish a new population for risk dispersal in cooperation with zoos. In our *ex situ* breeding population, we established breeding techniques that allowed the first successful artificial insemination of the Okinawa rail while maintaining genetic diversity in a small population. We were able to establish an approximate lifespan and an upper limit of suitable breeding ages by analyzing diseases in captivity. Furthermore, the science-based feed production technique based on our non-invasive nutritional physiological analysis is highly versatile and can be applied to other rare species. Acclimatization training for reintroduction to the wild has resulted in a significant increase in the number of days of survival, demonstrating the effectiveness of training for the Okinawa rail.

For return of the Okinawa rail to the wild, we evaluated the adaptation of released individuals to wild environments and accumulated knowledge necessary to improve the efficiency of introducing individuals to the wild. As a result, we clarified that the average area of the action zone of the Okinawa rail is 10.1 ha. We found that wild individuals often use highly natural evergreen broadleaf forests, whereas captive-released individuals use man-made vegetation environments such as field weed communities, green residential areas, and rufous pine communities. Analysis of the survival curve showed that the survival rate of wild birds decreased linearly to a constant level, whereas that of captive-released birds decreased rapidly to 70% within the first 20 days after release and then became constant. There was a difference in the median survival time of about 260 days, from 150 days for captive birds to 411 days for wild birds. In addition, the initial survival rate of captive-released individuals significantly improved after 2018, when training was enhanced, as compared to before 2017, when pre-release acclimation training was inadequate. Captive-bred individuals successfully bred in the wild for the first time in 2019.

In our study of large tree-hollow-dwelling beetles, we aimed to set up a comprehensive conservation management unit for the reintroduction and replenishment of individuals by collecting habitat information and improving their microenvironments, combined with the development of breeding and cultivation techniques for *ex situ* conservation. Through distribution and habitat surveys, we identified new populations of the stag beetle, as well as populations in critical condition that require rehabilitation measures. We also found that the Kume Island population of the Okinawa Stage-beetle is unique in both morphology and genetics, and can be considered a subspecies. The results of our genetic analysis suggested that the populations of each island should be considered evolutionarily significant units (ESUs), such that conservation management units should be set up within each species or subspecies. Techniques for repairing and regenerating tree hollows were established and a manual was prepared. Effective rearing techniques were established and manualized according to the results of rearing experiments and nutritional physiological analysis of food humus and larval gut microflora.