Abstract

[Research Title]

Study on Cryopreservation of Butterfly Ovary for Future Utilization of the Cryopreserved Tissues of *Celastrina ogasawaraensis*

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The Ogasawara Holly Blue, Celastrina ogasawaraensis, has been considered to have undergone species-level extinction because no wild or reared individuals have been observed since 2020. However, a small number of larval ovaries, eggs, and spermatozoa were cryopreserved from individuals of the breeding population before its extinction. In Lepidoptera, a cryopreservation technique for larval ovaries has been developed in the silkworm, Bombyx mori. The technique consists of several steps: removal of larval ovaries from the donors, slow freezing, storage in liquid nitrogen, thawing, transplantation of the ovaries into the recipients, and post-surgery rearing. Then, the cryopreserved ovaries develop mature eggs in the recipients. Therefore, new individuals of C. ogasawaraensis might be obtained from the cryopreserved ovaries and spermatozoa if the xenotransplantation technique of cryopreserved ovaries is available for butterfly species. Because few studies have focused on butterfly species in terms of the cryopreservation of ovaries, the present study applied the transplantation of cryopreserved ovaries between individuals in a pierid butterfly Eurema mandarina as the first step to our final purpose. Then, xenotransplantation experiments of cryopreserved ovaries were conducted between E. mandarina and several other pierid species. The effects of the degree of inbreeding or starvation of the donors on the success of transplantation of cryopreserved ovaries were also investigated. The modified cryopreservation method allowed the recipient females to develop mature eggs in the donor ovaries in E. mandarina. Mature eggs were also observed in the xenotransplanted ovaries under specific conditions. The degree of inbreeding and starvation of the donors did not

remarkably affect the development of the donor ovaries in the recipient females. The present study confirms the cryopreservation technique developed in *B. mori* is useful for the ex-situ conservation of butterflies. Future directions of the cryopreservation technique and the conservation of butterflies were discussed.

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