Researchers develop cryopreservation method for ladybird beetle ovaries

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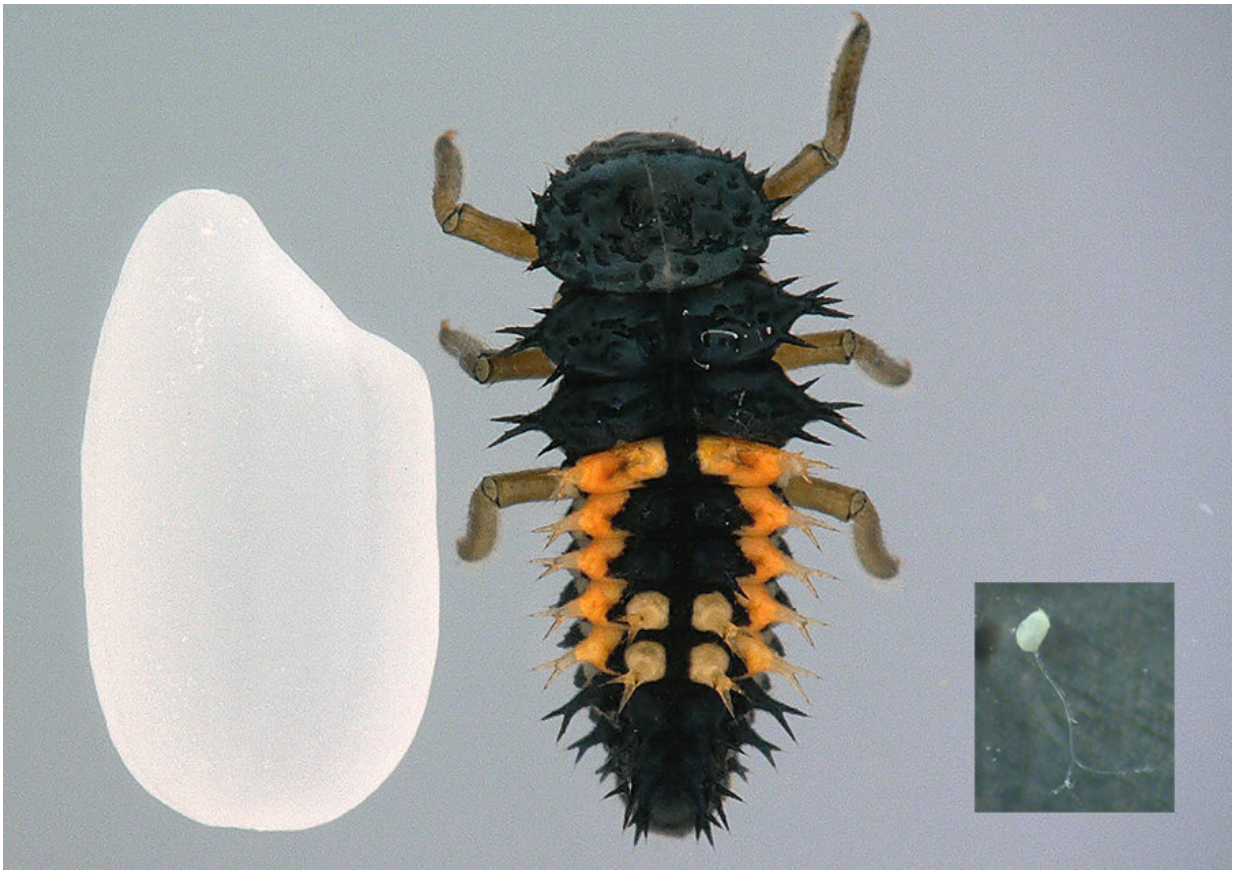

A 4th instar larvae (center) and an ovary (right) of the multicolored Asian ladybird beetle, *Harmonia axyridis*. The size of a larva is comparable to that of a grain of rice (left). (Scale = 1mm.) Credit: NIBB

A new study has found an effective way to cryopreserve and

subsequently transplant ovaries of the multicolored Asian ladybird beetle, Harmonia axyridis. In mammals (including humans), long-term cryopreservation of fertilized eggs, sperm and ovaries is possible. However, in insects, cryopreservation of fertilized eggs has not been successful, and cryopreservation of sperm and ovaries has been put to practical use only in silkworms.

The study, published in the *Journal of Insect Biotechnology and Sericology* in July 2018 is the first to report a successful ovary cryopreservation method in a small non-model insect, or an insect that has not previously been widely studied in this manner. It shows that female ladybird beetles that received transplants of previously frozen ovaries gave viable offspring. The investigators highlight that transplantation of cryopreserved ovaries is a viable alternative to all the complications associated with maintenance of insect colonies in the laboratory.

The team, led by professor Teruyuki Niimi at the National Institute for Basic Biology (NIBB) in Japan, found that the average rate of viable females that were transplanted with originally cryopreserved ovaries was 26 percent, with a peak rate of 40 percent, which the researchers deem sufficient for the technique to be of practical use for the maintenance of genetically diverse H. axyridis lines.

Niimi, the corresponding author of the study, points out that the Asian ladybird beetle is known for having more than 200 color patterns in its forewing. This striking feature makes the tiny insect an important model for scientists studying how genetic and genomic changes can lead to the different physical traits in plants or animals.

Niimi adds that his laboratory currently maintains more than 1,000 ladybirds, but that it "is very laborious task. In addition, long-term rearing of animals increases risk factors such as disease contamination

and human errors that can wipe out valuable genetic lines, genetic mutations interfere with specific desirable genotypes and inbreeding depression." He emphasizes that cryopreservation is the best alternative to avoid these, which is why they have focused on developing an effective procedure of H. axyridis ovary preservation.

The methods were adapted from previously reported methods of ovary cryopreservation in the silkworm Bombyx mori. The team successfully preserved ladybird beetle ovaries by first slowly cooling them down to -80 degrees C in a freezing container at a rate of 1 degree C per minute. The frozen ovaries were then placed into liquid nitrogen, where they were stored until further use. Immediate thawing at 37 degrees C was done at the time of transplant, and thawed donor ovaries were then placed in the recipient larva's body.

Despite several difficulties, including the small body size of H. axyridis, the challenge of visualizing the insects, rapid clotting, and the tiny size of the organs, the researchers developed fine manipulation tools as well as dissection techniques with which they successfully obtained fertile females that received originally cryopreserved ovaries.

While the researchers indicate that the rate of fertile females from frozen ovaries is sufficient for the maintenance of an H. axyridis genetic line, they are confident that this number will be improved by further enhancing surgical techniques as well as transplanting ovaries into younger insects.

The researchers also believe that the tools and techniques that they have developed in this study will benefit research in other insect species, including small insects. Haruka Kawaguchi, one of the authors of the paper, says, "We believe that this achievement will be useful not only for experimental insects, but also for endangered insect species that are difficult to maintain in a laboratory."

They state that their next steps are geared toward developing methods for the preservation of genetic resources derived from males, and will focus on the development of cryopreservation of testis and sperm.

More information: A method for cryopreservation of ovaries of the ladybird beetle, Harmonia axyridis, *Journal of Insect Biotechnology and Sericology*, DOI: doi.org/10.11416/jibs.87.2_035

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