



Oocyte and Sperm from iPSCs

of an Endangered Species

-Sexual flexibility of endangered germ cells-

Key Points

- Derivation of iPSCs from the endangered species *Tokudaia osimensis*.
- Generation of mouse/*T. osimensis* interspecific chimeras.
- Production of oocyte and sperm from *T. osimensis*.
- Discovery of sexual flexibility of XO female *T. osimensis* germ cells.

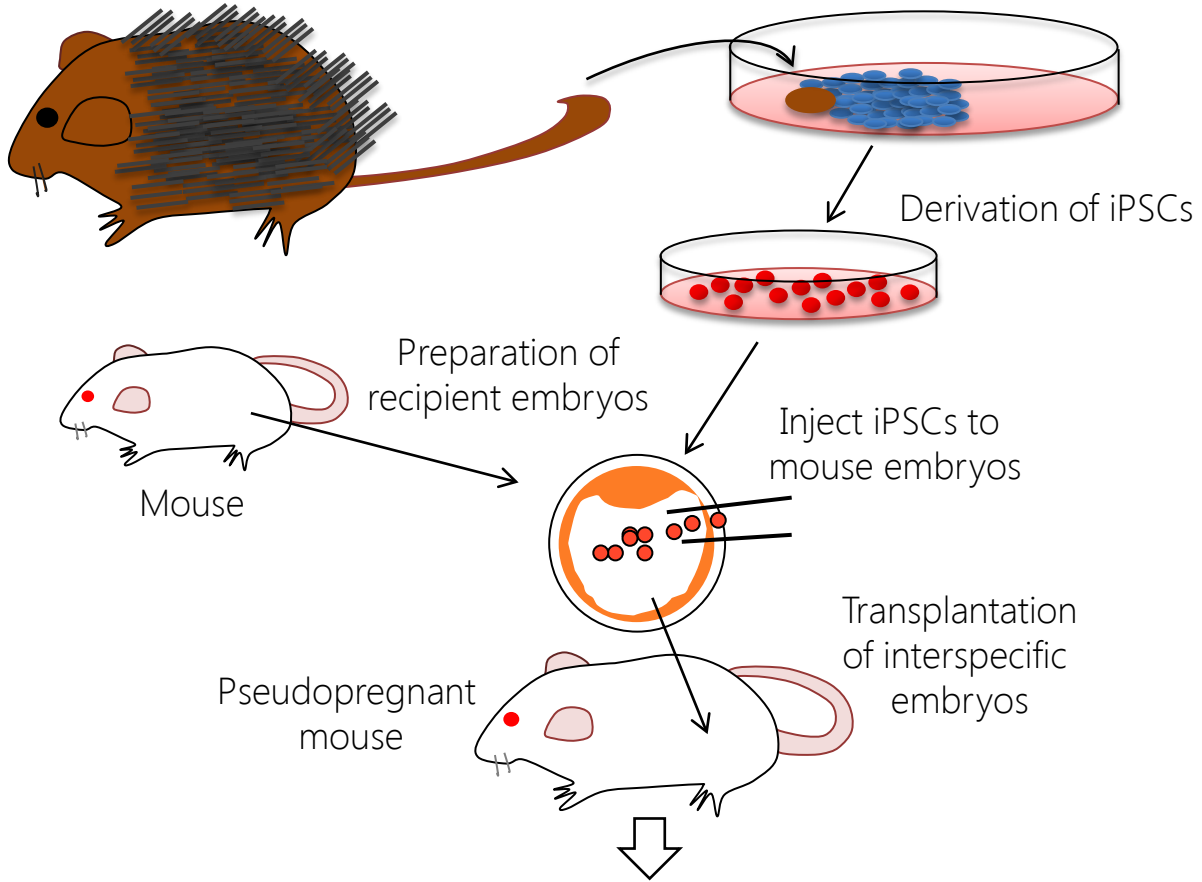
To preserve biodiversity, we must spare no effort to prevent the extinction of endangered species. The derivation of induced pluripotent stem cells (iPSCs) from such species is seen as a potentially effective insurance against extinction. Here, we generate interspecific chimeras that have endangered species cells in their whole body including germ cells.

We have successfully established iPSCs from an endangered species, *Tokudaia osimensis*. Because of a unique sex chromosome composition XO/XO, female *T. osimensis* iPSCs could contribute and survive as germ cells in adult interspecific ovary and testis, which suggests the acquisition of sexual flexibility of germ cells according to their unique evolution. These results provide an attractive research tool to explore the sex determination after disappearance of the Y chromosome according to mammalian evolution. This is the first report to derive live animals and germ cells including endangered species cells, and indicates the potential reduction of extinction risk of an endangered species through the use of iPSCs.

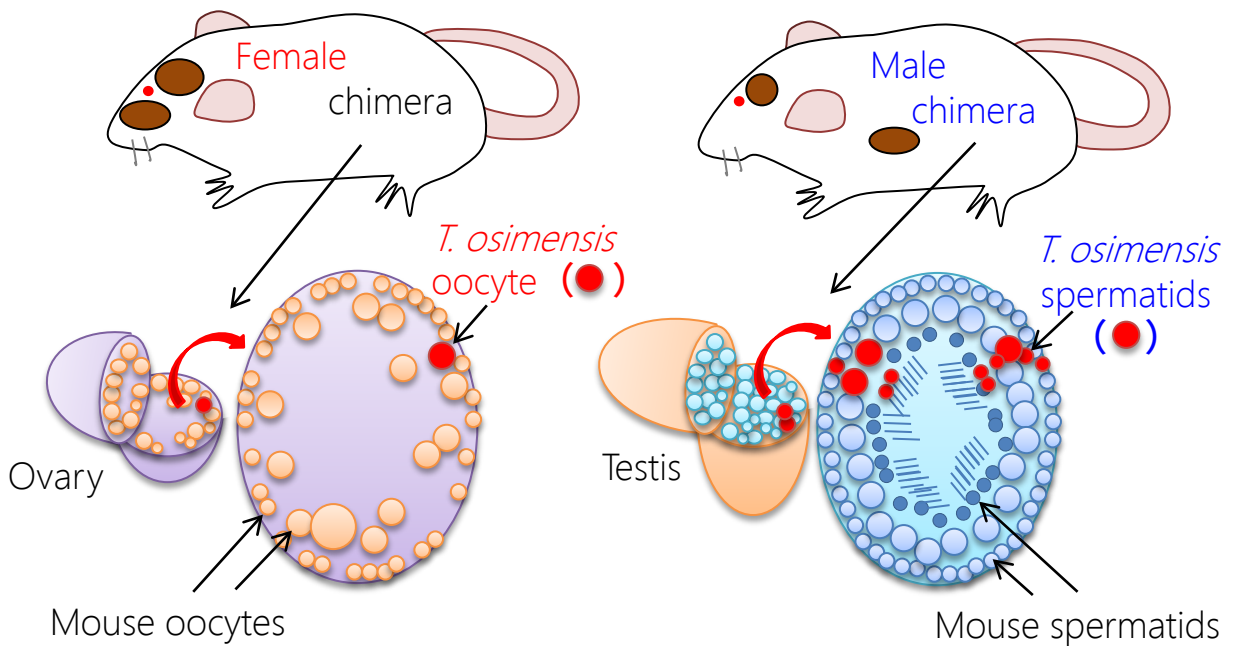
Graphical Summary

T. osimensis (♀: XO)

Fibroblast cells from a tail tip



Interspecific chimeras with endangered cells in their whole body were born



Successful generation of germ cells from an endangered species, *T. osimensis*

Objectives

One in every four animal species is threatened by extinction. Although it is crucial to save animal species by preserving their natural environments, a number of conservation strategies need to be implemented. If an animal species becomes extinct, it is very difficult to rewind such extinction. One of potentially most effective candidates for reducing the risk of extinction of endangered species is the derivation of induced pluripotent stem cells (iPSCs) from a small piece of somatic cell tissue. If iPSCs from threatened endangered species could be derived, it may provide a valuable way to save their genome or cell resource and to produce germ cells. Moreover, their iPSCs provide information about their interesting characteristics without any reduction of the individual.



Photo : Miyazaki City Phoenix Zoo

Tokudaia osimensis

- A small rodent from the Amami-Oshima Island in Japan
- An endangered species (EN)
- Chromosome: $2n=25$, XO/XO

Results

To establish iPSCs from a wild individual *T. osimensis*, a small tip of the tail was obtained from a live female and was used for fibroblast cell culture. We released the captured *T. osimensis* into the field

after recording the individual characteristics and giving first aid to the injured tail. We established iPSCs of *T. osimensis* and successfully detected their specific culture condition (Fig. 1). The derivation of allogeneic preimplantation embryos from an endangered species as a recipient is impractical. We attempted to prepare preimplantation embryos and to generate interspecific chimeras using mice as the host and recipient for embryo transplantation. *T. osimensis* iPSCs effectively contributed to the body of interspecific chimeras (Fig. 2) with germline transmission (Fig. 3). Finally, XO female iPSCs could differentiate into male germ cells in the interspecific mouse testis and survive for more than 7 weeks after birth (Fig. 4).

Fig. 1

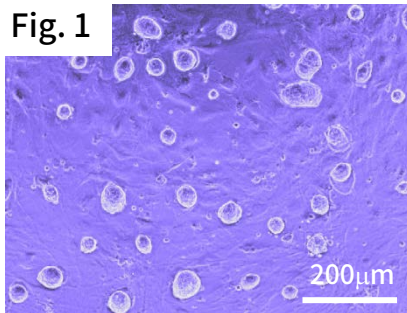


Fig. 2



Fig. 1: *T. osimensis* iPSCs

Fig. 2: Interspecific chimera with brown hair from *T. osimensis* cells

Fig. 3

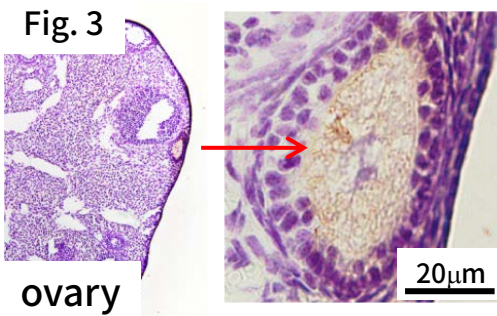


Fig. 4

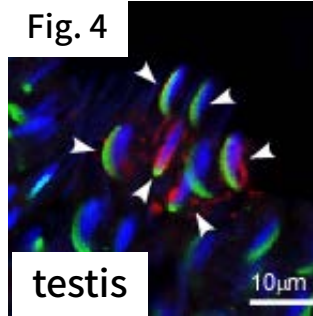


Fig. 3: Detection of *T. osimensis* oocyte in adult interspecific ovary (arrow)

Fig. 4: Detection of *T. osimensis* spermatids in adult interspecific testis (arrowheads)

Significance

Sexual flexibility of endangered *T. osimensis* germ cells

In most mammals, female (XX) cells cannot differentiate and remain

as spermatids in the male reproductive environment. Because Y-related genes have the critical involvements to raise and maintain as male germ cells. However, *T. osimensis* have lost their Y chromosome and evolved to generate males without Y-related genes. In this research, we found that female (XO) *T. osimensis* cells can differentiate and maintain as spermatids according to the testicular contribution, which suggests the acquisition of sexual flexibility of *T. osimensis* germ cells by their evolution. These results raise some interesting questions about mammalian evolution, epigenetic regulation, sex determination, and gamete genesis of the unique animal species.

iPSC technology as insurance against possible extinction

Because many species are now threatened with extinction, it is crucial to preserve biodiversity. We have successfully generated oocyte and spermatids from a small piece of tissue of a wild endangered individual. We demonstrate that iPSC technology is a potentially effective strategy for conserving endangered species from likely extinction.

Note

If endangered species are successfully brought back by iPSC technology in the future, they must be maintained only in strictly managed environments as a precious bioresource.

They should not be released into the wild.

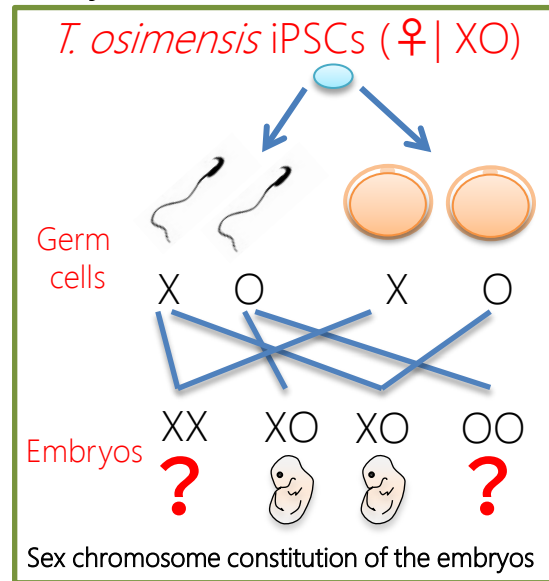
Next aims

Effective generation of sperm and oocytes of *T. osimensis in vitro*.

We aim to generate sperm and oocytes efficiently from this endangered species *in vitro*. If we can produce germ cells *in vitro*, we will try to generate embryos and individuals of this endangered species.

Developmental fate of XX, XO, and OO embryos.

If we can generate *T. osimensis* embryos, their sex chromosome constitution is supposed to consist of XX, XO, and OO. We have been much interested in their developmental fate and their epigenetic regulations.



Gonadal development (Sex determination)

In this research, we have elucidated the sexual flexibility of germ cells of *T. osimensis*. We would like to clarify the mechanism for the sex determination which *T. osimensis* obtained during its evolution.

Generation of iPSCs and germ cells from the other endangered species.

We aim to generate true naïve iPSCs from the other endangered species, such as *Tokudaia tokunoshimensis* and *Tokudaia muenninke*.

Publication

Flexible adaptation of male germ cells from female iPSCs of endangered *Tokudaia osimensis*.

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Science Advances (2017) 3: e1602179

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