WHO IS YOUR FATHER?

Universidad de Jaén

M. Alcántara García[1], N. Alcázar Margarito[1], V. Cifuentes Rus[1], M. Coriasso Guerrero[1], L. García Vargas[1], N. Moriana Casado[1], I. Pérez Barrionuevo[1], M. Santiago Gutiérrez[1], V. Navarro Molina[1], A. S. Roco[2], A. Ruiz García[2], M. Bullejos[2]

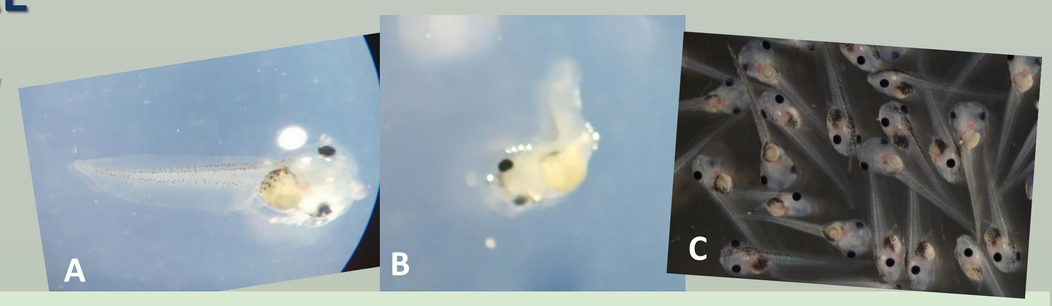
[1]IES Virgen del Carmen ,Paseo de la Estación 44, 23008 Jaén , Jaén, Spain
[2]Departamento de Biología, Universidad de Jaén, Campus Las Lagunillas s/n, 23071 Jaén, Spain

Science IES
Distinctive Education

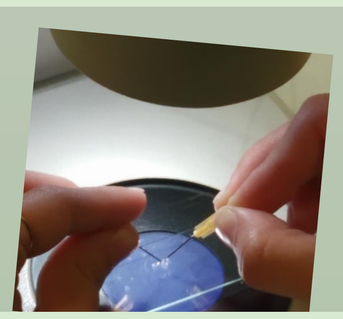
It is possible to obtain offspring from a single parent? In nature, sexual reproduction is associated with the fusion of two different gametes. The aim of this process is to obtain viable offspring, genetically different from the parents. But, does this happen all the time? To answer this question, this project has been carried out based on the reproduction of the amphibian model *Xenopus tropicalis* (Fig. 1), a species that inhabits in West Africa. The characteristics of this anuran species makes it a relevant model for genetic and genomic research.

Xenopus tropicalis

PROCEDURE



Once the eggs have been obtained from the females, *in vitro* fertilization is performed. Fertilization with live sperm produces normal diploid offspring (A), or triploid embryos if a cold shock is performed after fertilization to prevents the extrusion of the second polar body. In contrast, if fertilization is performed with U.V-inactivated sperm, haploid embryos are produced (B), unless ploidy is restored by cold shock, creating gynogenetic diploid embryos (C). One hour after the fertilization of the eggs it will be possible to observe the beginning of the embryonic development. Figures A, B and C show animals with about 3 days of development.



Using 3-day tadpoles, after a 2-hour colchicine treatment, samples are taken from:
- Heads: to obtain metaphase chromosomes and analyse the ploidy of the offspring.
- Tails: to obtain DNA to check if there are paternal markers present in the offspring).

Obtaining metaphase chromosomes

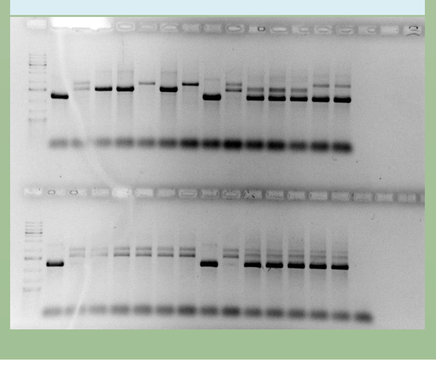
PCR products are analysed by agarose gel electrophoresis. The goal of this process it to determine if it is possible to produce offspring without paternal genetic contribution.

PCR of genetic markers (genetic fingerprint) to determine if paternal DNA is present.

Karyotype analysis allowed us to check the number of chromosomes present in the offspring of the 4 crosses performed.

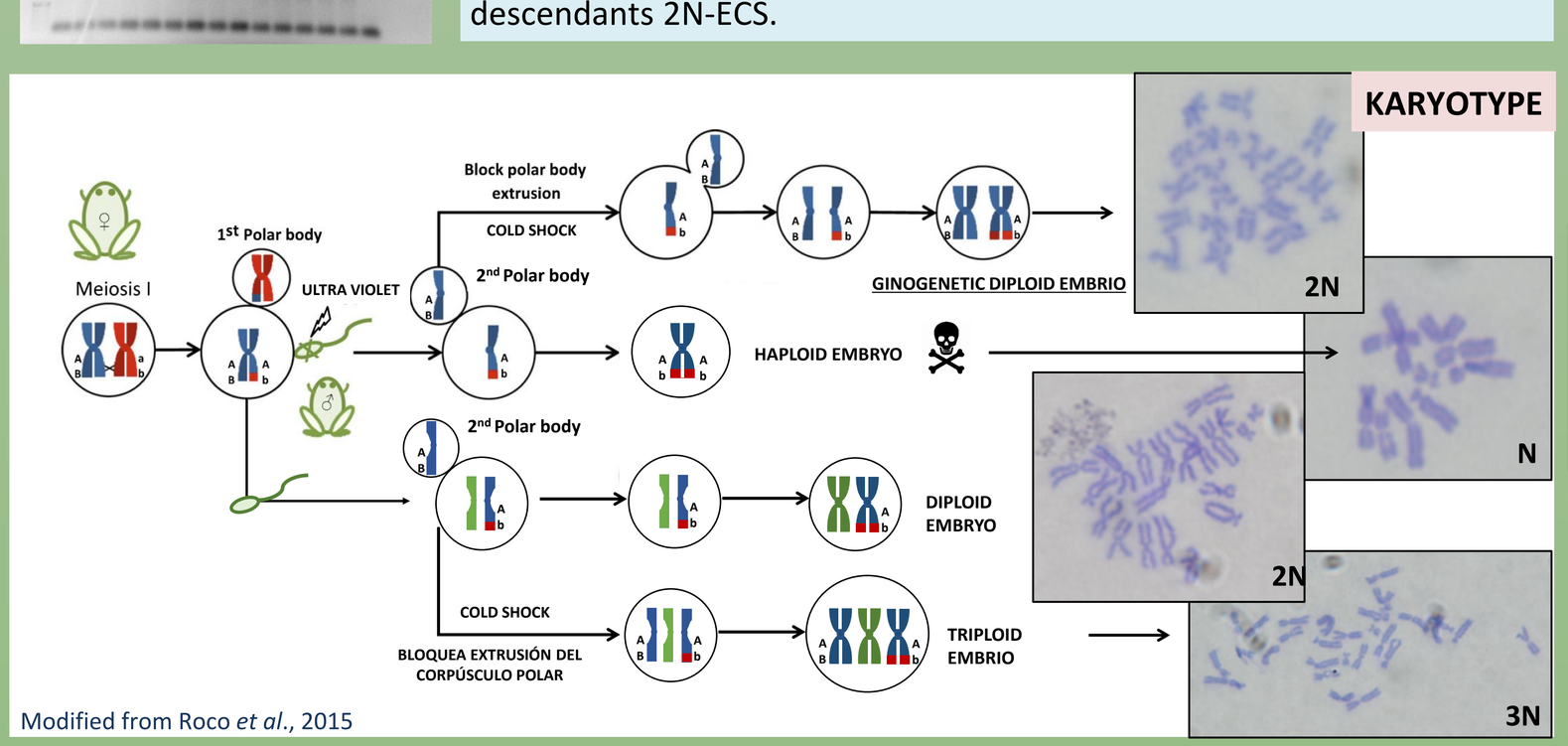
RESULTS

PCR RESULTS



Samples in top row: N and 2N
MWM + Father (Homozygous for the marker) + Mother (Heterozygous with two alleles different to the father's allele) + 5 descendants N + Father + Mother + 5 descendants 2N.

Samples in bottom row : N-ECS and 2N-ECS
MWM + Father + Mother + 5 descendants N-ECS + Father + Mother + 5 descendants 2N-ECS.



Modified from Roco *et al*., 2015

CONCLUSIONS

The knowledge about meiosis allows the development of new techniques that provide different techniques for reproduction. In this project, using *Xenopus tropicalis* as model, we have observed how haploid eggs fertilized with inactivated sperm can produce viable diploid embryos without genetic contribution from the father. The obtain viable offspring an early cold shock is needed to prevent the extrusion of the second polar body.

ACKNOWLEDGMENTS

This activity has been funded by the Ministerio de Industria, Economía y Competitividad, through the project BFU2016-78001-P (cofounded by the European Regional Development Fund), by the Junta de Andalucía through the programme "Ayudas a grupos de investigación" (group RNM-924), and by the University of Jaén, through the Science-IES project.

References:
http://www.xenbase.org/anatomy/intro.do
Álvaro S. Roco, Allen W. Olmstead, Sigmund J.Degitz, Tosikazu Amano, Lyle B. Zimmerman and Mónica Bullejos (2015). Coexistence of Y, W, and Z sex chromosomes in *Xenopus tropicalis*. Proc Natl Acad Sci USA.112:E4752–E4761.