Extreme survivor:

Triops – the 300-million-year-old living fossil

The humble tadpole shrimp has outlived mammoths, dinosaurs and trilobites. **Africa Gómez** and **Thorid Zierold** want to know how.

Despite its survival throughout the geological ages *Triops cancriformis* is an endangered species in many European countries, including the UK, mainly because of habitat loss and pollution. In the UK it has special conservation status and just two populations are known, one of them in the Caerlaverock Wetland Centre, Scotland, the other in a pond in the New Forest.

Since life began on Earth, biodiversity has several times been devastated in mass extinction events. Notostracans, or tadpole shrimps, have survived at least three such devastating extinctions maintaining a remarkably stable body shape and way of life. Fossils from *Triops*, a type of Notostracan from over 300 million years ago, are identical to living species today, so they are considered one of the oldest 'living fossils'.

Triops really are extreme survivors: trilobites, dinosaurs and mammoths, to name just a few well-known creatures that have at some point co-existed with the Notostracans, died out while *Triops* lived on unchanged. Ever since a fellow student in the University of Valencia showed me a handful of these ancient-looking creatures frantically swimming in a jam jar I have found them truly fascinating.

A few years ago, an enthusiastic German student, Thorid Zierold, joined our laboratory in the University of Hull to carry out genetic research on the European tadpole shrimp *Triops cancriformis*. This was a great opportunity to shed some light on the mystery: how have these organisms achieved their extraordinary feat of endurance? Do they have a special 'survival kit' or are they just very lucky?

The dozen or so Notostracan species live in seasonal, freshwater ponds, in temperate regions, deserts and even the Arctic, and they are commonly found in large numbers. The most immediate feature that appears to help Notostracans' survival is that they produce eggs that are extremely resistant to hostile conditions. These resistant eggs withstand desiccation; extremely high temperatures, right up to boiling point; freezing; and even digestive enzymes – this lets eggs survive when the adults that carry them are eaten by birds.

Shrimps in suspended animation

These eggs are also dormant, that is, they do not hatch immediately, only when favourable conditions resume, and can survive in this 'suspended animation' state for years, possibly even several centuries. They can build up in numbers in the pond sediments forming 'egg banks' and this allows populations to persist throughout adverse periods. In addition, eggs can disperse between ponds in birds' feet – or guts – or animal hoofs.

Triops populations can 'disappear', giving the impression of population extinction, only to reappear suddenly in great numbers after several decades. In fact, in Scotland's Solway Firth, populations of *Triops cancriformis* have been reported only in 1907, 1948 and 2004, despite regular surveys. Their resistant developmental form, the dormant egg, could be seen as the 'time and space travel' trick in their survival kit.

Another intriguing clue to their survival abilities might lie in their mode of reproduction. Despite their extreme conservatism in body shape, Notostracans show diverse reproductive modes, including separate males and females, hermaphroditism (individuals with both testes and ovaries), and androdioecy. First described by Charles Darwin, androdioecy is an extremely rare reproductive mode, in which populations are made of hermaphrodites and varying proportions of males.

Hermaphrodites can either self-fertilise or mate with males, but in *Triops* hermaphrodites cannot mate with each other. Androdioecy has been described in only a handful of animals: some nematodes (including the model organism *Caenorhabditis elegans*), two crustaceans, *Eulimnadia* and *Triops*, and the mangrove killifish *Kryptolebias marmoratus*. In the European tadpole shrimp *Triops cancriformis* all three described reproductive modes occur in different populations.

Does Triops have a special 'survival kit' – or is it just very lucky?

A crucial advantage of androdioecy and hermaphroditism versus the typical reproductive mode with separate males and females is the dispersal advantage they confer: a single individual – hatching in a new pond from an egg carried there on wildfowl feet or animal hoofs – is able to start a population by itself. One of the hypotheses we addressed in the course of our research is if androdioecy, hermaphroditism or both had been involved in the colonisation of northern Europe after the last Ice Age ended. If a flexible reproductive mode allows for rapid colonisation during favourable periods, this could be a key reason for Notostracan survival.

Hatching hermaphrodites

After contacting dozens of researchers across Europe and carrying out our own sampling, we obtained a wide collection of mud samples containing *Triops* eggs and also preserved *Triops*. We then applied genetic methods to these samples including analysis of DNA sequences. Our analysis on mitochondrial DNA genes revealed that northern European *Triops* populations, which are either hermaphroditic or androdioecious, are closely related and have little genetic variation.

This suggests that such reproductive modes must have evolved relatively recently and then facilitated the expansion of this species after the last Ice Age. In contrast, southern populations in the Iberian Peninsula, which could have survived during the Ice Age due to their more temperate climate, were made of males and females in equal numbers, and also had higher levels of genetic variation. Therefore, it appears that reproductive mode variation could be another element of *Triops* survival kit.

In breeding experiments we used individuals from different *T. cancriformis* populations hatched from our mud samples to test their ability to reproduce in isolation and also investigated how their genetic material is transmitted to the next generation. Our results suggest that only northern populations can reproduce in isolation and that self-fertilisation is substantial in many northern populations, lending support to androdioecy.

Does *Triops* have any other tricks up its sleeve? We carried out analyses of the phylogenetic relationships of Notostracan species and showed that separate males and females seems to be the original reproductive mode, but that hermaphroditism and androdioecy appear to have repeatedly evolved in this group of organisms. We have suggested that the key to this reproductive flexibility may lie in the way maleness/femaleness is genetically determined.

To demonstrate this we will need to delve deeper in *Triops* genomes to understand how the different reproductive modes are genetically determined. Given the speed of development of technological advances to investigate organism genomes, we aim to pursue this fascinating line of research in the future to understand the extraordinary abilities of these extreme survivors. ❖

MORE INFORMATION

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