A Self-Fertilizing Lake Victoria Hybrid Cichlid

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A Self-Fertilizing Hermaphroditic Lake Victoria Hybrid Cichlid

*Ola Svensson**

A mouthbrooding cichlid which produced offspring of its own? Is that possible? Obviously it is possible because I and my colleagues just published a report on a hybrid Lake Victoria cichlid held in isolation which spawned with itself and produced offspring. Our detailed analyses showed that she reproduced by self-fertilization or 'selfing' as it usually is called among scientists.

Background

My friend and fellow cichlid researcher, Alan Smith, carried out crosses aimed to unravel the link between the orange blotch (OB) system and sex-determination in Lake Victoria cichlids. I was, at the time, doing a postdoctoral fellowship at Hull University, working on Lake Malawi and Lake Victoria cichlids. Alan photographed all these F1 hybrid fish and therefore he kept them in isolation. One fish which was supposed to be a female spawned. That is not too surprising and happens sometimes, but this fish continued to brood the eggs. Alan and I are both dedicated aquarists and he told me about this strange behavior and we started to observe her while she continued to brood. To our astonishment we later removed three fry from her mouth. How could this be? To answer this question I had to do genetic paternity analyses, and to our surprise 'she' was the father to the offspring too. This is the first case of selfing being genetically confirmed in a vertebrate that otherwise would have sexual reproduction. In 1957 there was a report of a guppy but then there was not genetic paternity analyses available (Spurway, 1957). I will continue to call it 'she' because it had all characteristics of a female.

Reproduction without sex or without a partner or a little bit of both

There are more than 80 known taxa of vertebrates that reproduce without sex and the vast majority of those reproduce by means of parthenogenesis and not selfing. There are two major modes of parthenogenesis. In apomictic (mitotic) parthenogenesis the result is true clones that are genetically identical (barring mutations). With automictic parthenogenesis, meiosis occurs after which diploidy is restored by gamete duplication or fusion of the meiotic (i.e. haploid) products (Stenberg and Saura, 2009). This is the mode of reproduction in these fascinating cases of 'facultative' parthenogenesis sometimes reported in the press. For example, when a single hammerhead shark, sawfish, python snake, or Komodo dragon lizard reproduce without sex.

In well-known parthenogenic teleost fishes, for example the amazon molly *Poecilia formosa*, the parthenogenic lineages have hybrid origin. Parthenogenic hybrid lineages have also been created in the laboratory. Strangely, the egg needs a sperm to initiate development and hence, these fish do have sex but not sexual reproduction (Lamatsch and Stöck, 2009). In contrast, selfing is, strictly speaking, sexual reproduction because it involves the fusion of an egg and a sperm. In vertebrates, the natural occurrence of selfing has only been observed in the clade with at least two species of mangrove killifish. The best known is the killifish *Kryptolebias marmoratus*. Selfing results in inbreeding and a rapid loss of genetic variation and therefore it has been an enigma how these very successful fish avoid going extinct. However, these amphibious fish have a mixed mating system and sometimes males are present which may save the population from the evolutionary cul-de-sac (Avise and Tatarenkov, 2015). To make things more complicated, some bizarre modes of parthenogenic-like

The intersex was produced by a cross between a female *Pundamilia pundamilia* from Python Island and an orange blotch (OB) *Neochromis omnicaeruleus* male from Makobe Island. Photo by A. Smith.

*Ola Svensson is professor at the Department of Biological and Environmental Sciences, Gothenburg University, Sweden

An orange blotch (OB) *Neochromis omnicaeruleus* male from Makobe Island. Photo by A. Smith

reproduction involve paternal leaking of genetic material as well as modes of reproduction which include features of both sperm-dependent parthenogenesis and sexual reproduction (Lamatsch and Stöck, 2009).

The selfing cichlid

Our selfing cichlid was a hybrid between a female *Pundamilia nyererei* from Python Island and an OB-male *Neochromis omnicaeruleus* from Makobe Island. There are, as far as I know, no OB-*Pundamilia* and because turnover of the sex determination system is correlated with OB-polymorphism (Parnell and Streelman, 2013), it is likely that the two species have different sex determination systems. We continued to keep the female in isolation and it continued to spawn on her own. In total she produced forty-six living fry of both sexes in fourteen consecutive broods. Two sons and fifteen daughters survived to adulthood and they were fertile. However, none of her sisters or daughters reproduced when kept in isolation. Mortality was high in all offspring and also in the grandchildren. We also saw other effects of inbreeding such as bad growth and spinal deformations. The genetic analyses showed that 'she' was both father and mother of the offspring and we found no indication of genetic abnormalities. Also, the genetic markers segregated in Mendelian fashion which means that we could rule out apomictic parthenogenesis and most mechanisms of automictic parthenogenesis. After fourteen broods we decided to euthanize the fish and dissect it. The gonads looked like normal ovaries. However, we found a whitish tissue on the gonad. We therefore made thin sections to look further in the microscope. Unfortunately, the results from the histological microscopy were not conclusive although the tissue resembled testicular tissue and small dark dots resembled spermatocytes. With all this information, the most likely mode of reproduction was concluded to be selfing (Svensson et al., 2016).

A male *Pundamilia nyererei* from Python Island. Photo by K. Woodhouse.

How did this happen?

In cichlids, hermaphroditism seems exceedingly rare, or absent which may seem strange given that cichlids belong to the order Perciformes and many of the marine relatives to the cichlids are sequential hermaphrodites (Oldfield, 2005; Sadovy de Mitcheson and Liu, 2008). There is some evidence for sequential hermaphroditism in the South American cichlid *Crenicara punctulata* (Carruth, 2000), and a good description of the intersex (both sex present at the same time) gonads in a spontaneous sex-reversed in the port cichlid *Cichlasoma portalegrense* (Polder, 1971). There have been suggestions of sequential hermaphroditism in *Metriaclima livingstonii* and aquarists sometimes report females of various species changing sex from females to males. However, the latter examples are most often dismissed in the scientific lit-

erature, and it is not generally accepted that hermaphroditism exist in cichlids (Oldfield, 2011).

Relative to birds and mammals, sex determination in teleost fishes is labile both phenotypically and phylogenetically. In cichlids, the heterogametic sex differs between species and sex is environmentally-determined in some species, for example by pH (*Apistogramma*, *Pelvicachromis*) or temperature (*Apistogramma*, *Oreochromis*). Environmental sex determination can sometimes occur even in the presence of genetic sex determination (Oldfield, 2005). Conflicting sex determination genes can also cause intersex and that is what we believed happened in our hybrid female. When she spawned sperm was released and the eggs were fertilized either upon release or in her mouth.

Evolutionary potential

In another article, we referred to the fish as a 'monster' in reference to the use of the word monster for individuals which are very different. The best known use of the word monster in evolutionary biology is probably Goldschmidt's "Hopeful Monster" hypothesis which was ridiculed when he presented it in 1940. However, "monster hypotheses" have recently received interest because mutations with large effect have been documented and also large morphological novelties caused by novel gene combinations in hybrids. These novelties may have evolutionary potential. The most adaptive potential for a selfing fish is its colonizing abilities. Only one individual is needed to establish a population. A selfing lineage is, however, likely to be short -lived. The mangrove killifish (*Kryptolebias marmoratus*) has been hugely successful in terms of numbers – it has an enormous distribution which is likely due to its colonizing potential. Then, why is selfing almost absent in vertebrates? Morphological and hormonal constraints cannot be the answer because obviously it is possible. However, a selfing lineage is expected to be short-lived because recessive deleterious mutations will become expressed in homozygous state which increases the extinction risk. Indeed, in the mangrove killifish fry sometimes develop into males with occasional outcrossing as a result.

***Kryptolebias marmoratus* and its close relatives are the only vertebrates known to be self-fertilizing in nature. Photo by P. Åkervinda.**

A note to aquarists

Aquarists make a lot of observations and many hypotheses that scientists test originate from aquarists – many scientists are indeed aquarists themselves. However, these rare observations are different because they are hard to replicate. Hence, if an aquarist finds something undoubtedly unique or something which is not accepted in the scientific world, it is important to contact the right scientists for a thorough investigation because otherwise it will be dismissed.

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