Tree frogs with foreign sex chromosomes are less fit

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The Eastern tree frog (Hyla orientalis) recolonized the northern latitudes after the last ice age from a glacial refugium around the Black Sea and meets the other species, the European tree frog, in the region of the Vistula River in Poland. Credit: Christophe Dufresnes

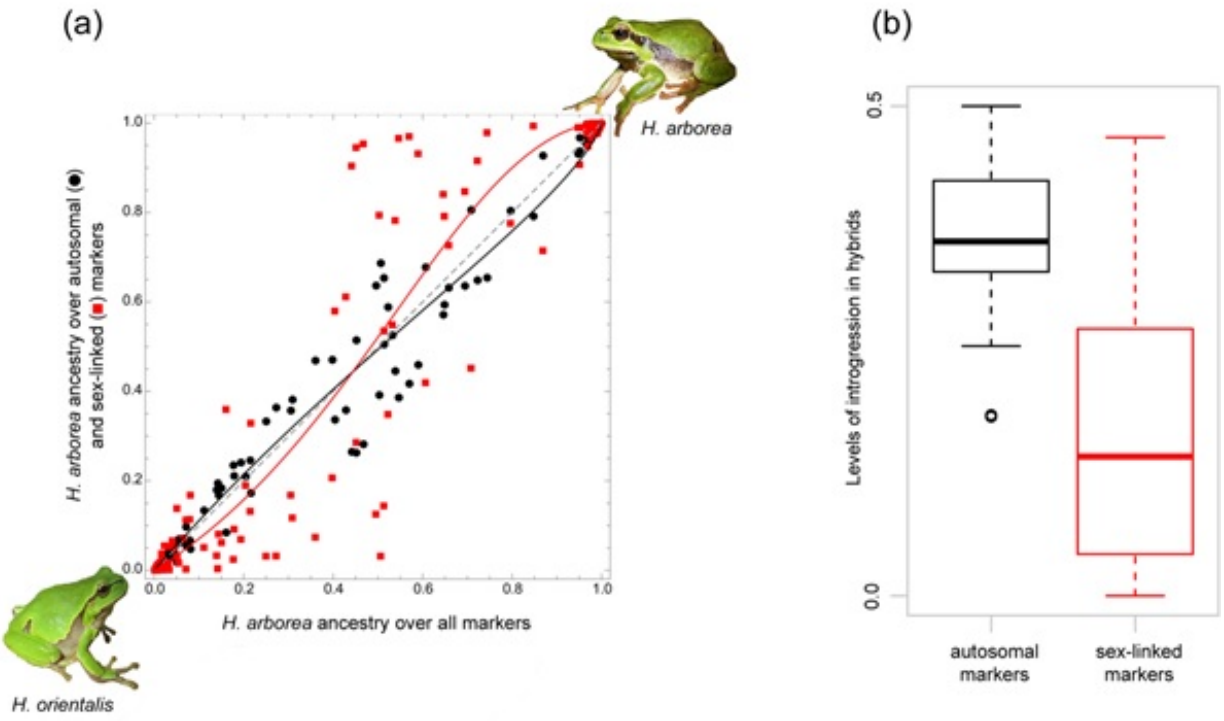
During the last glaciation, a huge ice shield reached up to the region of today's Berlin. By the time it started to melt about 20.000 years ago, it enabled a gradual re-colonization of the northern latitudes by many plant and animal species. Often, they took different colonization routes around the mountain ranges, for example the Carpathians - with astonishing outcomes for a special kind of re-unification that, for instance, happens in Poland: In the region of the Vistula River, two evolutionarily young species of tree frogs meet each other.

Mating of European (Hyla arborea) and Eastern tree frogs (Hyla orientalis) in the lowlands of Poland results in hybrid frogs that carry sex chromosomes from both species. Presumably, they reproduce less successfully, i.e. they are less fit than their ancestral species. This has been found by Dr. Matthias Stöck, Heisenberg-Fellow of the German Science Foundation (DFG) at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), by means of population genetic methods. Together with an international team he investigates the contact of the two tree frog species at the Vistula. For Dr. Christophe Dufresnes from the University of Lausanne, first author of the common study just published in *Scientific Reports*, this "suggests that the undifferentiated sex chromosomes in these tree frogs contribute more to the evolution of new species than other, normal chromosomes".

"We have made great efforts and spent many nights in the field to cover large parts of Poland when sampling saliva from the two tree frog species for genetic analyses", said Tomasz Majtyka, the University of Wroclaw and equal first author of the study.

Amphibian researcher Matthias Stöck refers to the fact that gene flow between these two young tree frog species is not completely interrupted, yet, which is typical of such young species. "The gene exchange is the least between the sex chromosomes in interspecies crosses, the sex chromosomes 'collaborate' the worst with the other chromosomes from

the foreign species", he says. At a certain stage of their evolution, these species have crossed a "point of no return" at which they could no longer completely merge with the other species. Indeed, this point seems to have already been passed in the tree frogs in Poland, their sex chromosomes can no longer easily admix. Later on, there will be no gene flow any more at all. "In the tree frog species studied by us, it is amazing that the mating and hybridizations appear to happen mainly in the Polish lowlands", says Stöck, "while they rarely do so in Greece, where these two species meet each other as well; however, presumably since distinctly longer periods of time". Over there, much less common offspring between Hyla arborea and Hyla orientalis has been found.



Lower introgression at sex-linked compared to autosomal markers. (a) Sex-linked (red squares) and autosomal (black circles) hybrid indices (0: pure H. orientalis, 1: pure H. arborea) for each individual compared to their HI over all markers. (b) Comparison between sex-linked and autosomal introgression of

gene markers in 32 frog hybrids.

Until 2008, the research community assumed that these two tree frog species represented a single one. At that time, Stöck and co-workers investigated the frogs using molecular methods and found differences that justify to consider them distinct species, which are separated as long as about 5 million years. After the ice age, the European tree frog (Hyla arborea) reached Central and Western Europe across the Danube valley. East of the Vistula, in Poland and Ukraine, occurs the other species, Hyla orientalis, that has spread to the north from northern Turkey and Asia Minor around the Black Sea and then colonized east around the Carpathian Arc. The old taxonomists assumed that these could be different species or subspecies. Therefore, an old scientific name was available for the "Eastern tree frog" and could be "reactivated" by Stöck. Since, Hyla orientalis, is back to the scientific world. Both species are hard to distinguish externally, they are so-called "cryptic species".

In the Polish lowlands both species have met less than 14.000 years ago; from an evolutionary point of view a very young contact.

But how do new species evolve? This is one of the great questions of evolutionary biology. "As an amphibian researcher, I would like to find out how long does it take in frogs and toads that a new species arises by geographic isolation and what makes up its genetic identity, we talk about their 'reproductive isolation' " says Stöck.

In the case of the tree frogs, the scientists were lucky to find such natural hybrid zones. The Carpathians form a perfect border between Hyla arborea and Hyla orientalis that are then joined again to the north of the mountains, in the Polish lowlands. When populations are separated (allopatry) over long periods of time, they accumulate adaptations to

their environments and random mutations. Important is also genetic drift which involves random processes on the population level. Both groups develop differently, especially, if only few individuals survive in a population, proceed though a "genetic bottleneck", similarly as it happened once to parts of modern humanity, which presents the offspring of relatively few ancestral individuals.

In the laboratory, such processes are hard to model. Frogs that can be crossed in a terrarium may perhaps not do so in nature and vice versa. "In natural hybrid zones, we find natural species complexes and can examine their genetic constitution", says Stöck. "We estimate that Hyla arborea and Hyla orientalis have been separated for about 5 million years".

During the ice age, Hyla arborea hibernated in southern latitudes, for example in Greece and at the Mediterranean and Hyla orientalis around the Black Sea. After the last glaciation, the two species have spread to the north and finally met each other in the region of the Vistula River.

The popular knowledge on sex chromosomes usually considers the situation in mammals, in which sex chromosomes are easily distinguishable under the microscope. The male Y-chromosome in humans is very small compared to the X-chromosome. These heteromorphic sex chromosomes, i.e. morphologically distinguishable sex chromosomes, are mainly no longer recombining during reproduction. It is assumed that this lack of recombination has led to the degeneration of the mammal Y.

In fish and amphibians, however, various chromosomes can enter the role as a sex chromosome or may lose it again during evolution. As a result, these sex chromosomes have basically no time to degenerate as in mammals. Sex determination in general can be thought as a sex-determination cascade. "At the top, there is a 'master gene' that decides

whether a frog becomes a male or a female", says Stöck. This gene then 'switches on' networks of genes that form the male or the female phenotype. Some genes that form the ova or the sperm production appear amazingly conserved. Strong variation, however, is found on the top of the cascade, meaning different master genes exist and it also varies on which amphibian chromosome the master gene is situated.

At the tree frog sex chromosomes, we can observe "evolution in action", says Stöck. In this way, he and his colleagues can directly have look into the 'laboratory of evolution'.

More information: Christophe Dufresnes et al. Empirical evidence for large X-effects in animals with undifferentiated sex chromosomes, *Scientific Reports* (2016). DOI: 10.1038/srep21029

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