



Karl Trochu's tribute to Petu Page 5



ISSUE ONE



JUST ASK A SCIENTIST!

Question:

Why are sex ratios off in some of my livebearers? Do temperature or pH skew sex ratios of livebearers like in some other fishes?



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Michi Tobler is a fish biologist who studies the ecology and evolution of livebearers at Kansas State University. In his spare time, he usually has some meat in the smoker and a good book in his hands.

V/e all have experienced this before: we buy a bunch of fry at an auction, and they all turn out to be males. The dreams of a thriving colony ended before it even started. But why does this happen? In most cases, I would wager, it is just bad luck. Even if you buy six fish, you have a 2% chance that they will all be the same sex. That doesn't seem like a lot, but these odds are pretty good compared to winning the lottery! Of course, there can also be biological reasons why sex ratios deviate from our normal expectation of 50:50. Rumors about environmental effects impacting sex determination in livebearers and even sex changes in adult fish have been circulating in the hobby for decades. Interestingly, despite a lot of research in that direction, there is scant or no evidence for either of these phenomena in poeciliids. So, what else is going on then?

Most fishes are gonochoristic, which means that there are distinct sexes, and an individual's sex is determined at an early age and remains fixed throughout the rest of its live. There are certainly many exceptions to this general rule, even in poeciliids. Some fishes are unisexual and only consist of females, including the famous Amazon molly (Poecilia formosa), which I introduced in this column a while ago, and some species of the genus Poeciliopsis. Other fishes are either born as male or female and reverse their sex later in life in response to social cues (for example anemone fish and many wrasses). There are occasional reports that sex reversals are also occurring in green swordtails (Xiphophorus hellerii), both in the scientific literature and in hobbyist journals. However, systematic investigations have indicated that these reports are most likely misinterpretations of two other phenomena that commonly occur in this species. More likely, supposed sex-reversing individuals are either late-maturing males, which look just like adult females for most of their lives, or aging females that develop male characteristics due hormonal changes late in life. To date, we have no solid evidence that swordtails can change from being a reproductive female to a reproductive male, or vice versa.

In gonochoristic species, sex determination—that is the mechanism by which the sex of an individual is decided can happen in many different ways.

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Figure Legend The best-known mechanisms of sex determination are mediated by sex chromosomes (in colors), which are typically smaller than their counterparts.

In XY sex determination males are the heterogametic sex with an Y (blue) and an X (red), while females have two copies of the X chromosomes. In contrast, in ZW sex determination, females are the heterogametic sex with a W (red) and a Z (blue) chromosomes, while males have two copies of the Z chromosomes. However, sex chromosomes may not always be present.

The sex of an organism can also be determined by different genes (represented by circles) that are distributed across different chromosomes, and the sex is simply determined by how many male-making alleles (blue) or female-making alleles (red) are inherited. Such autosomal genes can override sex determination by sex chromosomes in species that have both.

Finally, sex determination may be environmental, where exposure to different conditions–and not chromosomes or genes–determine whether and individual will develop into a male or a female.

In general, we distinguish between genetic and environmental sex determination.

Genetic sex determination happens when males and females have different versions of the same gene or even different genes that influence their development. These genetic differences between the sexes are often reflected in changes in the size and structure of chromosomes, such that the sex is determined based on what chromosomes an individual inherits from its parent. In humans, and indeed some poeciliids, the sex is determined by the X and Y chromosomes, where individuals with two X chromosomes develop into females, and individuals with an X and a Y chromosome develop into males. In this case, we call the male the heterogametic sex, because it has two different versions of the sex chromosomes. However, the pat-

SCIENCE



Amazon Molly

tern can also be reversed. In birds and some other poeciliids, the sex is determined by the Z and W chromosomes. Males have two Z chromosomes, and it is the females that are heterogametic (ZW). Either way, we predict even sex ratios in species with such chromosomal sex determination, because with every mating half of the offspring will receive two copies of the same sex chromosome and the other half a mix (they will be heterogametic).

So, if the chromosomal sex determination that I just described leads to even numbers of males and females, why do some stocks consistently show a skew toward males or females? It is because nature—and our lovely livebearers—are rarely as simple as they appear at first sight. There are multiple reasons why sex ratios can be skewed, and some are genetic and some are environmental.

From a genetic perspective, it is important to highlight that not all species fall into the classic categories for chromosomal sex determination. Some species, like *Limia vittata* and *Xiphophorus signum*, actually have multiple sex chromosomes, where an individual's sex is determined by whether there are more male or female making genes are present. Many other species also have so-called autosomal modifier genes. These are genes that are located outside of the sex chromosomes (the autosomes) and can override the effects of the sex chromosomes. Most confusingly, multiple mechanisms of genetic sex determination can be present in a single species of poeciliid. For example, the Southern platyfish (*Xiphophporus maculatus*) exhibits male heterogamety, female heterogamety, as well as autosomal modifiers. And, green swordtails, depending on the population, have female heterogamety, additional sex chromosomes, and autosomal modifiers. In any of these deviations from the simple chromosomal sex determination, there can be an excess of males or females in any given stock.

Skewed sex ratios can also be a consequence of environmental influences on sex determination. It turns out in some species—most notably turtles and crocodilians sex determination is completely controlled by the environmental conditions an individual experiences during development and not by genes inherited from the parents. In most well-studied species, it is the ambient temperature that has the decisive influence, but other environmental factors, like pH and even the social composition of groups, can also play a role in the sex determination of fishes. So far, we have no solid evidence for strict environmental sex determination in any livebearer, but the environment can potentially act as a modifier of genetic mechanisms of sex determination. For example, temperature has been suggested to impact sex ratios in *Limia melanogaster, Poecilia sphenops*, and *Poeciliopsis lucida*, while pH may contribute to sex determination in *Poecilia sphenops* and *Xiphophorus hellerii*.

Even though there is some evidence for environmental sex determination in livebearers, its actual importance is still really unclear. For one, while it is true that pH may impact sex ratios in *X. hellerii*, only extreme conditions that these fish are not likely to experience in nature appear to have a meaningful effect. In addition, the role of environmental factors has typically been inferred from skews in the adult sex ratio, which doesn't necessarily mean that the environment actually impacted sex determination. Alternatively, males and females may just have different levels of tolerance to stressors (extremes in temperature or pH, or other suboptimal water conditions), and higher mortality in one sex earlier in life could cause skewed sex ratios even if sex determination is not affected.

In summary, besides chance, there are multiple reasons for skewed sex ratios in livebearers. One reason is that sex determination is incredibly complex and diverse in poeciliids, and that is actually why they are a favorite study system for geneticist. But excess of males or females may also have nothing to do with sex determination but just differential survival of the sexes under different environmental conditions. Sadly, determining the actual causes for skewed sex ratios for your particular species or strain is not trivial.



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will hopefully be a regular component of Livebearers in the future. However, this requires your input.

Have you ever wondered about the meaning of observations you made in your fish tank? Do you have questions about the behavior, reproduction, ecology, or evolution of livebearers?

Submit your questions directly to Michi:

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He will do his best to answer your question or find somebody that can.