Species: Sex Graphics: Genetics Explained

By. Mike Sipe

When I first got started with the production of Pure line breeders the two species now known as tilapia mossambica and tilapia horonorum, were known as tilapia mossambica (mossambic) and tilapia mossambica (zanzibar). They were considered such close relatives that they were grouped as two sub species and so the varieties were designated by where they came from. During this time the determination of species was not made by using any DNA markers and only the "science" of meristics was used to determine the species. Since the "science" of meristics was based on such things as gill rakers, which meant counting and typing the gill rakers in each fish. What is more, is that when the number of spines on the Dorsal fin or any other physical characteristics that could be easily counted was used, the job of determining the species of a live fish was difficult because one had to hold the speciman still while the counting was done and needless to say the fish rarely cooperated so the time it took to verify that a group of fish was one speicies or another could be several weeks and even then when a fish looked exactly like another fish, taxonomist tended to group them in the same species.

When I started we had very little to go on except that where the particular tilapia was collected from and that is why it was so important to be sure that the fish you were working with was the same as the original ones that were used to produce the all male hybrid. T. mossambica and t. horonorum were virtually impossible to separate using meristics because so many of the physical characteristics overlapped. Mature males both had a jet black coloration with a red rim around it and white areas that were very similar, and the females were even worse. So any group of researchers who attempted to hold both pure gene lines in containment anywhere where fry or fingerlings could escape and make their way back to any holding area were a constant threat to the purity of the brood stock.

Then in 1981 Giora W. Wohlfarth and Gideon Hulata published "Applied Genetics of Tilapia" where a large number of species and hybrids were compared using information collected from many scientist all over the world. On page 9 of this publication they write "Differences in appearance between species to be hybridized is important in distinguishing between parent species and their hybrids. The sustained production of all-male hybrids between S. (during this period of time tilapia were designated as Sarentherodon instead of tilapia) niloticus females and S. hornorum males , compared to the eventual appearance of varying proportions of females in the crosses between S. mossambicus and s. hornorum, or between s. niloticus and S. aureaus, may be due to the relative ease of distinguishing between S. niloticus and S. hornorum."

What they seem to be saying was that the hybrid between niloticus and hornorum was more reliable and the sustained production over time seemed to be better because the two species were easily separated by looking at them, whereas the hybrid between pure gene lines of mossambicus and hornorum looked just alike which led to increasing percentages of females in the hybrids which was a no, no. This difference is just what I began the color development of the t. mossambica to create.

Below I have presented 5 graphics of the inheritance of t. mossambica (orange), t. horonorum, t. nilotica (red), and of the hybrids of t. mossambica X t. horonorum (called the Pennyfish) and of mossambica X t. nilotica (called Chocolate hybrid)

Five Graphics of sex Chromosome Inheritance and the resulting influence on sex determination.

Graphic One: t. hornorum

This graphic is about the inheritance of sex chromosomes in the pure gene line of tilapia hornorum. The female t. hornorum shown here on the left has one chromosome which is designated W and one chromosome which is designated Z. The W chromosome apparently is loaded with sites that manufacture estrogen to such and extent that the amount of estrogen that shows up in the blood stream of the developing fingerling is great enough to trigger the development of ovaries which then go on to manufacture eggs as the fish matures. The Z chromosome also has some few sites that manufacture estrogen, but many more that manufacture testosterone, however those sites that produce estrogen on the Z chromosome add to the total estrogen level in the bloodstream enough to facilitate the development of ovaries. The Z chromosome also has a number of sites which produce testosterone, but there is not enough testosterone produced in the bloodstream to overcome the development of ovaries and stimulate the development of testicles instead.

So, when sexual reproduction begins in this female the cells that are to become eggs begin a process called meiosis. In the process of meiosis the chromosomes are separated into separate areas where eggs will form and one area separates out the Z chromosome and the other area separates out the W chromosome. Once these two chromosomes are separated along with all of the other paired chromosomes then each area will have half of the total chromosomes present in the original cell. The total number of chromosomes in the cells of t. hornorum is 44 which means when all of the sorting is done during meiosis the are where each of the sex chromosomes ends up will have 21 other chromosomes sharing the space. So when all is said and done and meiosis is complete and eggs are formed each egg will have either an X chromosome and 21 other chromosomes or a W chromosome and 21 other chromosomes. In other words each egg will have 22 total chromosomes.

Meanwhile back at the fish ranch meiosis is also occurring in the male t. hornorum which also has 44 total chromosomes in each of its cells. In the case of the male, meiosis occurs in the testicular tissue where each of the pairs of chromosomes are separated only this time the do not form eggs they form tiny package with a moving tail which we call sperm. Each sperm will now have a Z chromosome and 21 other chromosomes. Since the Z chromosome has a number of sites that produce estrogen and an equal or greater number that produce testosterone, whenever these Z chromosomes come to form a cell which gets the Z from both the mother and the father hornorum then it will have only Z chromosomes in its sex determination package, or in other words it will be ZZ and so will produce enough testosterone in the blood stream of the new fish to determine that the new fish will form testicular tissue or testicles and thus be male.

All of this may seem somewhat difficult to grasp, because what I am saying is that the newly created fish at the time when it is first swimming has not become either sex and the amount of either testosterone or estrogen in its bloodstream will influence whether it produces ovaries or testicles and therefore whether it will be male or female. So the fact that fish that will become male hornorum have two chromosomes to determining sex and they are both Z chromosomes means that the total amount of testosterone produced in the bloodstream of by both of these Z chromosomes influences in a positive way the development of testicular tissue. Just how much testosterone is produced by the Z chromosomes is still a question, but it is enough that when the hornorum males are used to fertilize female fish of another species which carry a different sort of chromosome (generally the X chromosome) almost 100 percent of the fish produced by this breeding are male.

Graphic Two: t. mossambica

The separation out, and the breeding of the pure gene line of Orange t. mossambica is one of the most important achievements made in the development of the process of producing all male hybrids because the fact that we can set them up to breed and tell within a few days whether we have made any mistakes in choosing the breeders. If for instance we sex 200 females to set up as breeders and someone allows a male to slip through the sexing process due to a fold in the sperm papilla or a wrinkle where it does not belong, when fry are created by this male breeding with one of the females the fry will be uniformly orange instead of metallic copper colored and if we get some orange fry then we can go back through the breeders one by one and eliminate the male orange t. mossambica. Also, if we have picked 40 male t. hornorum to breed with the Orange t. mossambica females and one of the fish choosen as male is actually female and she breeds with one of the intended males the color of all of these fry will be a normal grey color and so if normal grey colored fry turn up in the fry we know to go back through each male and find the mistake and get rid of her. This gives us the ability to check for errors and maintain the pure gene lines whereas with the two pure gene lines of the tilapia hornorum and the tilapia mossambica that look alike it is so easy to make mistakes that end up ruining the gene lines for further production of all male hybrids. Evidence suggest that many of the errors made in the dozens of cases where tilapia mossambica and tilapia hornorum were kept at the same facility could have been avoided if they had had this color distinction. In other words it gives us immediate feedback that can be used to preserve the process of producing pure gene lines for future use. This graphic is about the inheritance of sex chromosomes in the pure gene line of tilapia mossambica.

The orange female t. mossambica shown here on the left has one chromosome which is designated X and one chromosome which is designated Y. The X chromosome is loaded with sites that manufacture estrogen to such and extent that when there are two X chromosomes side by side pumping out estrogen the amount of estrogen that shows up in the blood stream of the developing fingerling is great enough to trigger the development of ovaries which then go on to manufacture eggs as the fish matures. The Y chromosome also has some few sites that manufacture estrogen, but many more that manufacture testosterone so that when the Y chromosome shows up in a cell that has an X chromosome it overwhelms the process and produces enough testosterone to cause the development of testicles in the developing tilapia fish. So, when sexual reproduction begins in this female the cells that are to become eggs begin a process called meiosis. In the process of meiosis the chromosomes are seperated into seperate areas where eggs will form and one area seperates out the X chromosomes. Once these two chormosomes are seperated along with all of the other paired chromosomes then each area will have half of the total chromosomes present in the original cell. The total number of chromosmes in each of the cells of t. hornorum is 44 which means when all of the sorting is done during meiosis the area where each of the sex chromosomes ends up will have 21 other chromosomes sharing the space. So when all is said and done and meiosis is complete and eggs are formed each egg will have either an X chromosome and 21 other chromosomes or a W chromosome and 21 other chromosomes. In other words each egg will have 22 total chromosomes.

Meanwhile back at the fish ranch meiosis is also occurring in the male t. mossambica which also has 44 total chromosomes in each of its cells. In the case of the mature male mossambica, meiosis occurs in the testicular tissue where each of the pairs of chromosmes are seperated only this time they do not form eggs they form tiny package with a moving tail which we call sperm. Each sperm will now have either an X chromosome and 21 other chromosomes, or a Y chromosome and 21 other chromosomes. The Y chromosome is a particularly potent producer of testosterone and when combined with most X chromosomes still produces enough total testosterone to switch the balance in the blood stream of the developing fish to stimulate the development of testicles.

It is important to remember that genetics of tilapias is at most a groping tortuous process where we are still forced to guess about what goes on inside the tilapia on a microscopic scale which we cannot as yet see, that results in the creation of a fast growing male, a slow growing male, a slow growing female or a fast growing female.

We know that the development of the reproductive tissue occurs sometime within the first 30 days or so of life and that if we alter the relative amounts of testosterone and estrogen present in the bloodstream of the developing tilapia that we can create the situation where the reproductive tissue becomes a testicle or becomes an ovary. In most tilapias this seems to be an event that is permanent, but in other tilapias it appears to be a temporary situation and can reverse itself after a few months if the right conditions occur.

Several things within each tilapia can appear to have an effect on the developing tissue of the fish. One of these factors appears to be temperature. Some workers have reported major differences in normally bred tilapias as far as the numbers of males and females that occur at different temperature ranges!