# Melanoma in *Xiphophorus* Interspecies Hybrids


"Will my black pigmented fish develop melanoma?" It is a question asked by many hobbyists and breeders who raise swordtails and platies. Melanoma is a highly invasive and destructive cancer in humans. In *Xiphophorus*, melanoma can "eat away" at the body and fins and ultimately, kill the fish either by metastasizing and disrupting the functioning of vital organs, or enabling infections by opportunistic pathogens. Melanoma occurs spontaneously in wild and domesticated fish at a very low frequency. A more common, heritable form of melanoma is found in *Xiphophorus* interspecies hybrids and is of greater concern for the hobbyist.

## Genetics of Melanoma

The pioneering work of Gordon and Kosswig in the 1920s, established that certain hybrids between *X. maculatus* and *X. helleri* are prone to developing melanoma. They and their collaborators showed that 25% of the backcrossed F2 hybrids carrying the platy sex-linked spotted dorsal (Sd) gene developed melanomas. Other sex-linked genes (e.g. spotted (Sp), nigra (N), etc.) that specify pigment patterns, have subsequently been associated with melanoma in *Xiphophorus* interspecies hybrids. Several models were proposed to explain the genetic interactions that resulted in tumor formation. The most widely accepted view is that platies contain a gene, at the chromosomal locus Tu, that stimulates the abnormal proliferation of specific pigment-containing cells. Additionally, the expression of this gene is normally suppressed in platies by another gene located at the R locus (also known as Diff). Neither of these genes is present in *X. helleri*. In the F2 hybrids, the expression of the Tu locus gene (from the platy) in the absence of the suppressing R locus gene, results in melanoma.

Color and pigment patterns in fish are specified by the localized production of pigment-containing cells called chromatophores. Fish with black markings, either as spots, stripes, or black body coloring, express specialized chromatophores called melanophores, that contain the black/brown pigment, eumelanin. Eumelanin is synthesized from the amino acid tyrosine. Fish and other animals, deficient in the enzyme tyrosinase, cannot make eumelanin and are phenotypically albino. It is important to note that melanophores can be relatively small (micromelanophores) or large (macromelanophores) and that melanoma development in platy-swordtail hybrids involves macromelanophores and the population of proliferating melanocytes from which they are derived. The Sd, Sp and N genes associated with melanoma development, control the production of macromelanophores.

Classically, tumor formation involves the activation of growth-promoting genes (oncogenes) and the inactivation of growth-suppressing genes (tumor suppressors). It was therefore proposed that the Tu and R loci encode an oncogene and tumor suppressor gene, respectively. In 1989, the Tu locus gene was identified by Schartl and his collaborators. The Xiphophorus melanoma receptor tyrosine kinase-2 (Xmrk-2) gene is a member of a growth factor receptor family that is also activated in human breast cancer. The Xmrk-2 gene is located on the X chromosome in close proximity to the genes (e.g. Sd, Sp, N, etc.) that control macromelanophore spotting patterns.

The origin of the Xmrk-2 gene, including its evolutionary persistence in many *Xiphophorus* species, is a fascinating story. All *Xiphophorus* species contain the related Xmrk-1 gene which is not associated with melanoma. During evolution, the Xmrk-1 gene was duplicated and modified, resulting in a new gene, Xmrk-2. In contrast to Xmrk-1, the Xmrk-2 gene is expressed primarily in melanocytes, contains changes to the gene that increase the activity of the Xmrk-2 protein, and is suppressed by the gene located at the R locus. In the wild platy, Xmrk-2 is expressed at relatively low levels because the R locus gene is suppressing its expression. However, in *Xiphophorus* interspecies hybrids lacking the R locus gene, activated Xmrk-2 protein is expressed at very high levels, resulting in uncontrolled melanocyte proliferation and melanoma. The identity of the R locus gene has not yet been determined.

The Xmrk-2 gene is present in various *Xiphophorus* species, including: *X. xiphidium, X. variatus, X. evelynae, X. milleri, X. maculatus, X. montezuma*e, *X. cortezi*, and *X. birchmanni*. Interspecies hybrids between these species and others

lacking the R locus gene, are all prone to developing melanoma. Why certain *Xiphophorus* species contain the Xmrk-2 gene while others do not is under investigation by evolutionary biologists.

As mentioned above, melanomas can also develop spontaneously in wild and domesticated *Xiphophorus* species. Overexpression of Xmrk-2 has been associated with the development of at least some of these spontaneously occurring tumors. However, genetic analyses of human and animal cancers have clearly indicated that not all cancers of a specific type (e.g. lung cancer, melanoma, etc.) are "caused" by the same genes. Therefore it is very likely that genes other than Xmrk-2 will be identified that promote the development of spontaneous melanomas in both wild and domesticated swordtails and platies.

## Melanoma and the Hobbyist

Melanoma in *Xiphophorus* interspecies hybrids is a serious mutilating disease that almost always kills the fish. The majority of colorful, commercially available platies and swordtails were developed by crossing *X. helleri* with either *X. maculatus* or *X. variatus*. Given that both *X. maculatus* and *X. variatus* express the Xmrk-2 gene and *X. helleri* does not express the R locus suppressor gene, the melanoma threat might seem substantial. In reality, black pigmented fish that are commercially available, do not develop melanoma very often. First, pigmented hybrids that look like maculatus or variatus platies (the hybrids have been repeatedly backcrossed to *X. maculatus* or *X. variatus*) are more likely to contain the suppressing R locus gene, therefore reducing melanoma risk. Second, all black and partially black, tuxedo swordtails are not routinely available in most pet stores, but the ones that I have seen, do not contain areas of intense black pigmentation that are associated with increased melanoma risk. Third, many of the available black pigmented varieties of swordtails, such as those exhibiting a moon, comet or wag pattern, have a reduced risk of melanoma because the melanophores responsible for these black pigment patterns are micromelanophores and not the larger macromelanophores associated with melanoma. Finally, fish with melanoma are generally not as healthy or active as unaffected fish and would likely be selected against in large breeding tanks or pools used by commercial

Swordtails with uniformly black dorsal and caudal fins (wag trait) rarely develop melanoma.

breeders. For all of these reasons, melanoma risk for fish purchased at a pet store is relatively low with one caveat.

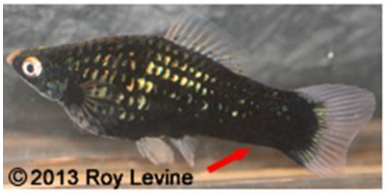

This 5 week old black pigmented swordtail baby has early onset melanoma. The melanoma has spread throughout the posterior region of the body and the caudal fin.

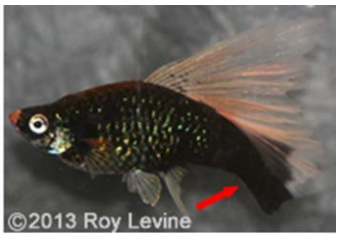
Fish breeders, especially those that are crossing unrelated varieties of fish with the goal of developing new or improved black pigmented platies and swordtails, are more likely to produce fish that develop melanoma. Melanoma can occur in babies, immature fish and in older fish as they age. Melanoma can also develop following sexual maturation in response to the increased production of sex hormones such as testosterone. In general, melanomas that occur in younger fish tend to be more invasive, resulting in rapid spread, fin destruction, and death. Late onset melanomas may or may not be as destructive, but unfortunately, these melanoma-prone fish are often used as breeders before melanoma is detected. This may be the case with fish purchased from a pet store or breeder because they are often young and may develop melanoma as they age, after they have been bred by the hobbyist. It is therefore important to carefully inspect all black pigmented fish before purchasing them and on a regular basis thereafter (see below).

Melanomas commonly arise in stocks of painted and black-bodied (e.g. Berlin/Hamburg) swordtails. Berlin swordtails express the macromelanophore-producing gene, nigra, and genes specifying blue (Bi) or green (Gi) iridescence. A small percentage of my Berlin swordtails develop melanoma as they age, primarily at the base of the caudal fin (caudal peduncle). It is important to identify tumor-prone fish as early as possible to eliminate them as breeders.

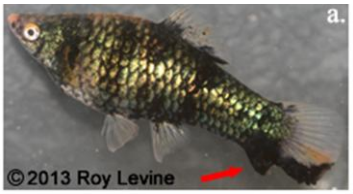


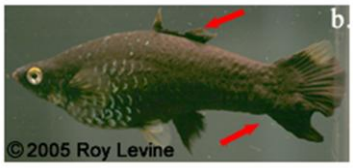
These young Berlin swordtails do not have intense black pigmentation at the base of the caudal fin and therefore have a lower risk of developing melanomas.




These Berlin swordtails have intense black pigmentation at the base of the caudal fin and are at risk of developing melanomas as they age.




These Berlin swordtails have rapidly spreading melanomas. a) This melanoma has destroyed part of the caudal fin and has spread into the dorsal fin. b) This melanoma has spread over the body, obscuring the blue iridescence, destroying the dorsal and caudal fins and producing multiple cancerous nodules throughout the caudal peduncle and caudal fin.

Albino fish can also develop melanomas. This may seem counterintuitive because melanoma is associated with black pigmentation and generally, albinos lack black pigment. As described above, albinos are typically deficient in the enzyme tyrosinase and therefore cannot produce the black/brown pigment eumelanin. However, in some cases, enough active tyrosinase may be produced in albino fish to allow some eumelanin to be made. These fish often contain areas of light black or brown pigmentation if they carry genes for macromelanophore production. If these fish also express Xmrk-2 and are lacking the R locus gene, they will develop melanoma at a high frequency in these pigmented areas. Furthermore, even non-pigmented albinos can develop melanoma. Non-pigmented albinos expressing the N, Sp, or Sd gene still produce melanocytes and macromelanophores, but they will not be black or brown because they do not contain eumelanin. If these fish also express Xmrk-2 and lack the R locus gene, the non-pigmented melanocytes in these white or sometimes yellow areas of the fish can become cancerous, resulting in an amelanotic melanoma.


An albino exhibiting black pigmented (a) and non-pigmented (b) areas that have a high likelihood of becoming cancerous.


An albino swordtail with a melanoma at the base of the caudal fin. The melanoma continues to spread as the fish ages.

Not all black pigmented fish are prone to developing melanoma. The black molly is a striking example of an all black fish containing densely packed macro-melanophores that does not develop melanoma. As you might guess, black mollies do not develop melanomas because they do not have the Xmrk-2 gene. Genetic evidence indicates that while the Xmrk-2 gene and the genes specifying macromelanophore

expression are distinct genes, they are located very close to each other on the X chromosome and consequently, are usually inherited together. However, it may be possible to develop melanoma-free strains of *Xiphophorus* that no longer express the Xmrk-2 gene, by selectively breeding fish that do not develop melanoma during their lifetimes.

## Conclusion

Melanoma in *Xiphophorus* interspecies hybrids is a serious disease and one that should not be taken lightly. All hobbyists should be aware that many varieties of black pigmented platies and swordtails have the potential to develop melanoma. When obtaining a black pigmented fish from a pet store or a breeder, inspect it carefully. Look for areas of intense black pigmentation that are irregularly shaped. Make sure that black pigmented fins are intact and not abnormal in appearance. Inspect the black pigmented body of the fish, especially at the base of the caudal fin, to be sure that it is not thicker than non-pigmented areas and that "bumps" or other surface irregularities are not evident. Regardless of whether you are a casual or serious breeder, inspect your fish on a regular basis and eliminate melanoma-prone fish from your breeding stocks. It may be difficult to completely eliminate melanoma from domesticated platies and swordtails, but by becoming a knowledgeable hobbyist and breeder, you can help to achieve this goal.

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