Poeciliid Research

Melanoma in Poeciliid fishes: the trade-off between beauty and cancer

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Abstract. In this paper we discuss the idea of the aquarium fish genus *Xiphophorus* as a model organism for the study of cancer. Cutaneous malignant melanoma is a form of carcinoma for which both environmental factors and hereditary predisposition are major causative factors. Fish melanoma models have been used in studies of both spontaneous and induced melanoma formation. Genetic hybrids between platyfish (*Xiphophorus maculatus*) and swordtails (*X. hellerii*) have been studied since the 1920s to identify genetic determinants of pigmentation and carcinogenesis. Many *Xiphophorus* varieties are fertile hybrids, obtained following interspecific hybridization and/or artificial selection. Artificial selection led ornamental varieties to exacerbate colors and contrasts, which affected the level of expression of pigments in pigment cells. In this context and considering the polyphyletic/hybrid origin of many varieties of *Xiphophorus*, the premises were created to increase the frequency of carcinogenesis or increase the predisposition to cancer in these fish. Under the simultaneous action of genetic improvement of coloration by artificial selection and natural selection caused by mortality/melanoma, a resultant, a trade-off between death and beauty has been created.
Key Words: *Xiphophorus*, *helleri*, *maculatus*, playfish, swordtail, melanoma, human model.

Introduction. In biomedical research, human models play a special role in deciphering the genetic and physiological mechanisms underlying some diseases (Proorocu et al 2022), including cancer (Petrescu-Mag et al 2021). Some model organisms have become "devoted" to the research of certain diseases. Such is the case of the genus *Xiphophorus* (platyfish and swordtail) for melanoma (Lu et al 2020) (Figure 1). In this paper we discuss the idea of the aquarium fish genus *Xiphophorus* as a model organism for the study of cancer.

Cutaneous malignant melanoma. Cutaneous malignant melanoma is a form of carcinoma for which both environmental factors (such as UV radiation) and hereditary predisposition are major causative factors (Davey et al 2021). Fish melanoma models have been used in studies of both spontaneous and induced melanoma formation (Patton et al 2010). Genetic hybrids between platyfish (*Xiphophorus maculatus*) and swordtails (*X. hellerii*) have been studied since the 1920s to identify genetic determinants of pigmentation and carcinogenesis (Patton et al 2010).

Figure 1. The platyfish, *X. maculatus*. Original picture by Cocan Daniel. Note: In the case of aquarium fish of the genus *Xiphophorus*, it is difficult to identify the species precisely in the absence of knowledge of the genetic origin of the line, because within the genus there are many lines obtained through interspecific hybridization (Petrescu-Mag & Popa 2018).

Why is it easier to use platyfish as a human model than to study by direct observation in humans? When a type of cancer has a strong hereditary component, looking at the entire genealogy of the individual being studied is particularly relevant. In the case of human patients, it would be difficult to investigate the family tree for several reasons: 1) often many of the ancestors are no longer alive, or live in other countries, 2) their medical data is not always available, 3) direct research on human subjects has its ethical limitations, 4) tracing the family tree in humans is difficult due to the long generation time. By contrast, platyfish can have a known ancestry, these data can be stored, they are research organisms and have a generation period of only about 3-4 months. Basically, within a year, 3-4 consecutive generations of platyfish can be obtained (Petrescu-Mag 2008), which can be kept alive for the duration of the study.

Gordon-Kosswig spontaneous melanoma model. In Figure 2, Patton et al (2010) show how a cross within the genus *Xiphophorus* leads to BC1 hybrids with melanoma (skin cancer) în the backcross generation (BC). The sex-linked melanistic pigmentation pattern 'spotted dorsal' (abbreviated, Sd) is shown as the presence of discrete black spots on the dorsal fins of *X. maculatus* individuals; the lines of *X. hellerii* commonly used in this cross (Lancetilla and Sarabia) do not have this color pattern and do not possess the specialized macromelanophores that generate the pattern (Patton et al 2010). To simplify presentation, "Sd" is used in the schematics to denote the sex-linked locus for the pattern of dorsal spot pigmentation. We must emphasize here (according to those presented by Patton et al (2010)) the fact that tissue pigmentation is a complex process in the ontogeny of fish, which involves not only the existence of macromelanophores, but also the differentiation, migration and degree of proliferation of macromelanophores. The abbreviation Mdl, for the macromelanophore-determining locus, was proposed by Wellbrock et al (2002) to designate genetic loci that specify the different sex-linked macromelanophore pigmentation patterns observed in *Xiphophorus*. The Xmrk oncogenic allele is associated with specific Mdl loci, but neither the pigment pattern designation (Sd) nor Xmrk should be considered synonymous with an Mdl; rather, Sd and other loci that determine pigmentation pattern should be considered alleles of Mdl (Patton et al

2010). In the past, the abbreviations Tu and M have been used in this context by Anders (1991) and by Kallman (1975).

As we observe in Figure 2 (A), the F1 generation hybrid from the cross between *X. maculatus* (carrier of the Sd gene) and *X. hellerii* expresses an improved pigmentation pattern on the dorsal fin. In this case, extensive melanocytic hyperplasia or melanosis occurs, denoting a more intense proliferation of macromelanophores (Patton et al 2010). Examination of cells from individuals with enhanced pigment pattern indicates altered cell morphology, with a higher proportion of poorly differentiated cells as well as cells with more active division than observed in pigment pattern cells of the *X. maculatus* parental line (Vielkind & Vielkind 1970; Siciliano et al 1976; Vielkind 1976; Ahuja et al 1980). Tyrosinase activity is also accelerated, as observed by Vielkind & Vielkind (1982). Crossing it with the parental variety *X. hellerii* produces in the backcross generation BC1 progeny of which about half are unpigmented (lower right in Figure 2 (A)). Of the remaining half, there is approximately a 1:1 ratio of BC1 hybrids with enhanced Sd expression, similar to the F1 hybrid (50%), and BC1 hybrids with highly enhanced pigment pattern, which may not be restricted to the dorsal fin (50%). Such intensely pigmented hybrids from BC1, approximately 25% of the total backcross progeny, spontaneously develop nodular, exophytic, invasive melanoma (Patton et al 2010). The apparently Mendelian segregation of these phenotypes is consistent with a two-gene inheritance model of segregation, involving one sex-linked and one autosomal locus, two possible interpretations of which are presented in Figure 2 (B, C) (Patton et al 2010).

In both models of inheritance presented by Patton et al (2010) (Figure 2), the Mdl locus, which is a sex-linked gene complex that determines the spotted dorsal (Sd) pattern, is assumed to be absent in the *X. hellerii* parent. In the model in Figure 2 (B), the autosomal gene is denoted by R, denoting the "regulator" or "repressor" gene (Anders 1967; Ahuja & Anders 1976; Schartl 1995). In this case, there are two copies of the R gene in the highly inbred *X. maculatus* parental strain, resulting in tight regulation of the pigment pattern, with controlled proliferation and a high proportion of terminally differentiated macromelanophores. Hybridization to the inbred parental *X. hellerii* strain, lacking the R loci, results in F1 hybrids heterozygous at each genetic locus; inheritance of a single copy of the R gene leads to some loss of pigment pattern regulation, resulting in increased Sd expression, increased macromelanophore proliferation, and melanosis. This phenotype is frequently referred to in the literature as 'benign melanoma', but Patton et al (2010) suggest using the term 'melanosis' or 'melanocytic hyperplasia' for this phenotype. Crossing F1 generation hybrids with the *X. hellerii* strain produces offspring in which replacement of *X. maculatus* genes occurs in F1 individuals based on random assortment during meiosis (Patton et al 2010).

These descendants of the BC1 generation have pigmentation phenotypes defined by Sd and R inheritance, as we can see in Figure 2 (B). BC1 hybrids inheriting the Sd complex are pigmented on the dorsal fin whether or not there is some regulation of the pigment pattern. That is, there is regulation in pigmented BC1 hybrids that inherit R (Patton et al 2010). Some BC1 individuals lose regulation completely, namely pigmented BC1 hybrids that do not inherit R. The genes at these loci determine whether a BC1 hybrid resembles the F1 phenotype or exhibits severe melanosis and is prone to spontaneous development of primary malignant melanoma (Patton et al 2010). This genetic model clearly lends itself to the interpretation that a gene associated with the Sd-Mdl allele complex behaves as a dominant oncogene and that the non-allelic R gene is a classical tumor suppressor (Ahuja & Anders 1976; Anders 1991; Schartl 1995; Patton et al 2010).

In contrast to the previously shown model (Figure 2 (B)), the inheritance pattern depicted in Figure 2 (C) does not suggest that the autosomal gene that apparently regulates melanoma susceptibility is present only in the *X. maculatus* parent (Patton et al 2010). The Diff notation derives from studies of macromelanophore differentiation in species and hybrids of the genus *Xiphophorus*, as shown in the works of authors such as Vielkind (1976), Ahuja et al (1980), Vielkind & Vielkind (1982).

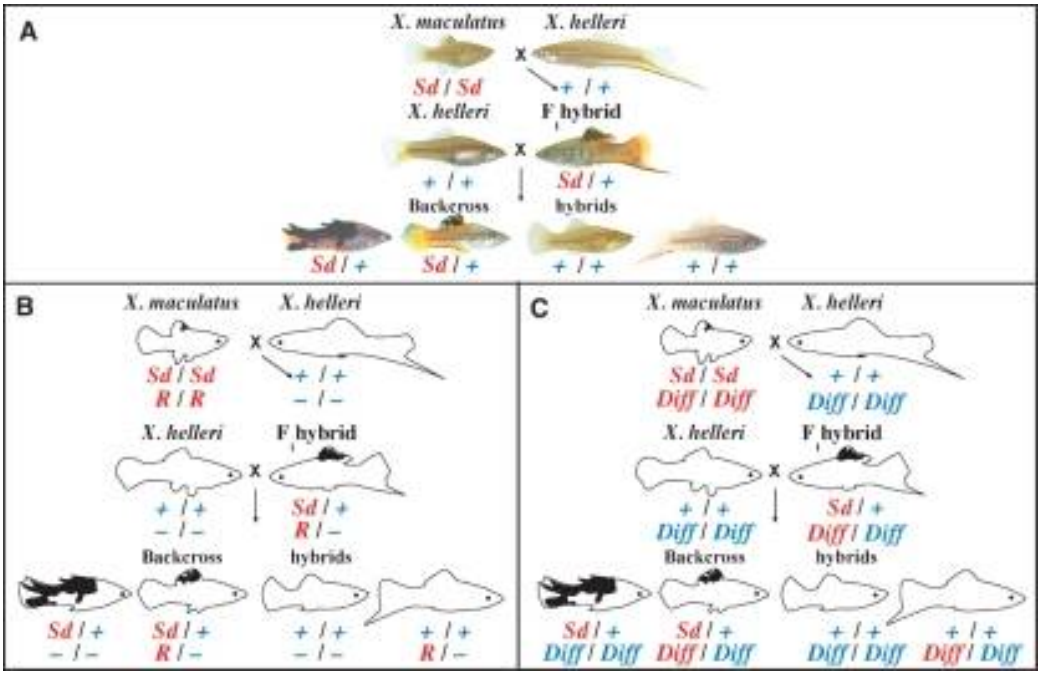

Figure 2. Genetics of the Gordon-Kosswig spontaneous melanoma model (according to Patton et al 2010). (A) Hybridization of the platyfish *X. maculatus*, exhibiting the macromelanophore spotted dorsal (Sd) pigment pattern, to the swordtail X. hellerii generates F1 hybrids with an enhanced Sd pigment pattern on the dorsal fin. Backcrossing F1 hybrids to the *X. hellerii* swordtail species generates first generation backcross hybrids (BC1 hybrids) with three phenotypes, as shown at the bottom of panel A. Approximately one-half of the BC1 hybrids are non-macromelanophore pigmented fish exhibiting no melanistic pigmentation (fish shown at lower right of panel A); these hybrids have not inherited the sex-linked Sd-Mdl allele (designated in the figure as Sd) from the original platyfish parent and therefore are not susceptible to melanoma. Of the remaining approximately one-half of BC1 hybrids, half of these (~25% of total BC1 progeny) are heavily pigmented and develop invasive, exophytic, nodular malignant melanoma (lower left individual in panel A) and the other half (~25% of BC1 progeny) show enhanced Sd pigmentation resembling the F1 hybrid phenotype, but only rarely develop melanoma late in life. (B) Hypothetical two-gene inheritance model explaining the apparently Mendelian inheritance of BC1 phenotypes. In this model, R is a platyfish gene that regulates the expression of the Xmrk oncogene associated with the Sd-Mdl allele, and its total loss in heavily pigmented BC1 hybrids that develop melanoma explains the melanoma susceptibility of these hybrids. Heterozygosity for R in lightly pigmented BC1 hybrids results in some regulation of Xmrk and inhibits melanoma formation. (C) Alternative two-gene inheritance model. In this model, the autosomal locus Diff regulates melanoma susceptibility but is not restricted to the platyfish parent, instead existing as alleles in *Xiphophorus* spp. populations. Mendelian inheritance of melanoma susceptibility in pigmented BC1 hybrids is explained by homozygosity versus heterozygosity for the *X. hellerii* Diff allele.

The trade-off between beauty and cancer. Many *Xiphophorus* varieties are fertile hybrids, obtained following interspecific hybridization and/or artificial selection. Artificial selection led ornamental varieties to exacerbate colors and contrasts, which affected the level of expression of pigments in pigment cells. In this context and considering the polyphyletic/hybrid origin (Petrescu-Mag & Popa 2018) of many varieties of *Xiphophorus*, the premises were created to increase the frequency of carcinogenesis or increase the predisposition to cancer in these fish.

Under the simultaneous action of genetic improvement of coloration by artificial selection and natural selection caused by mortality/melanoma, a resultant, a trade-off between death and beauty has been created.

Conclusions. Fish melanoma models have been used in studies of both spontaneous and induced melanoma formation. Genetic hybrids between platyfish (*Xiphophorus maculatus*) and swordtails (*X. hellerii*) have been studied since the 1920s to identify genetic determinants of pigmentation and carcinogenesis. Many *Xiphophorus* varieties are fertile hybrids, obtained following interspecific hybridization and/or artificial selection. Artificial selection led ornamental varieties to exacerbate colors and contrasts, which affected the level of expression of pigments in pigment cells. In this context and considering the polyphyletic/hybrid origin of many varieties of *Xiphophorus*, the premises

were created to increase the frequency of carcinogenesis or increase the predisposition to cancer in these fish. Under the simultaneous action of genetic improvement of coloration by artificial selection and natural selection caused by mortality/melanoma, a resultant, a trade-off between death and beauty has been created.

Conflict of interest. Authors declare that there is no conflict of interest.

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