Environmental and Hereditary Factors in the Causation of Neoplasia, Based on Studies of the *Xiphophorus* Fish Melanoma System

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ABSTRACT

Neoplasia in *Xiphophorus* can be classified into: a) a large group triggered by carcinogens; b) a large group triggered by promoters; and c) a small group that develops "spontaneously" according to Mendelian law. The process leading to susceptibility for neoplasia is represented by the disintegration of gene systems that normally protect the fish from neoplasia. Interpopulational and interracial hybridization is the most effective process that leads to disintegration of the protective gene systems. Environmental factors may complete disintegration in somatic cells and thus may trigger neoplasia. The applications of the findings on *Xiphophorus* to humans are discussed.

INTRODUCTION

In cancer research emphasis is at present being placed in the impact of carcinogens which mankind receives from our polluted environment. However, the most important factor determining whether or not a particular agent will induce neoplasia in a certain individual is genetic constitution.[1,2] To study experimentally the environmental and hereditary factors involved in carcinogenesis, our research group has used the fish *Xiphophorus* as a model.[2,3] This model was introduced into cancer research 50 years ago by Gordon, Kosswig, and Häusler,[3,4] who independently found that certain hybrids of these animals develop spontaneous melanomas.

Originally the present study was only aimed at an evaluation of the significance of hybridization in the formation of melanomas. In the course of the investigation it turned out that neoplasia in *Xiphophorus* is related to hybridization in general.

INSENSITIVITY AND SENSITIVITY TO MUTAGENIC CARCINOGENS

The different taxonomic groups of *Xiphophorus* known in the literature as platyfish and swordtail species[4,5] can be hybridized in the laboratory without difficulty, and all the hybrids are fertile. This, together with findings on the degree of enzyme polymorphism, chromosome pairing during meiosis, and genome organization, led to the conclusion that the relationship between these groups is at the level of populations and races comparable to the populations and races of the human species.[2]

Tens of thousands of individuals from *wild populations* of *Xiphophorus* have been collected by several investigators (Gordon, Kosswig, Kallman, Siciliano, and ourselves),

but no tumors were detected. In the progeny of the wild populations, which in the case of *X. helleri* from Rio Lancetilla and *X. maculatus* from Rio Jamapa have been bred in the laboratory since 1939 (about 80 and 120 generations, respectively), no tumors occurred. About 10,000 specimens of purebred descendants of the wild populations have been treated with powerful carcinogens such as X-rays, benzo(a)pyrene, and N-methyl-N-nitrosourea (MNU), but none developed neoplasia (see Ref. 2). These animals seem to be protected from neoplasia.

In contrast, animals from laboratory *hybrid populations* derived from crosses between the purebred descendants of wild populations may be sensitive to carcinogens. Eighteen out of 470 F_1-hybrids (3.8%) developed neoplasia following treatment with X-rays or MNU. Tumor incidence increases dramatically in succeeding hybrid generations (F_2-F_{24}). Five hundred eighty-two out of 4,439 (13%) animals developed a large variety of neurogenic, epithelial, and mesenchymal neoplasias following carcinogen treatment (Table 1; Fig. 1a-b; see Ref. 2); 359 of the neoplasms (62%) were melanomas. The same applies to domesticated ornamental xiphophorine fish, which actually are also hybrids.

TABLE 1. MNU- and X-ray-induced neoplasms in an experimental hybrid population of *Xiphophorus*, 1 year after treatment: 4439 survivors

(compiled from Refs. 6–9)

	MNU	X-rays
Melanoma (benign)	128 (2.88%)	93 (2.70%)
Melanoma (malignant)	104 (2.34%)	34 (1.00%)
Neuroblastoma	56 (1.26%)	7 (0.20%)
Squamous cell carcinoma	2 (0.05%)	0
Epithelioma	10 (0.23%)	6 (0.17%)
Carcinoma (low-differentiated)	3 (0.07%)	4 (0.11%)
Carcinoma (high-differentiated)	2 (0.05%)	5 (0.14%)
Adenocarcinoma (kidney)	4 (0.09%)	2 (0,05%)
Adenocarcinoma (thyroid)	2 (0.05%)	3 (0.07%)
Papilloma	5 (0.12%)	0
Hepatoma	3 (0.07%)	1 (0.03%)
Fibrosarcoma	82 (1.85%)	6 (0.17%)
Rhabdomyosarcoma	16 (0.36%)	2 (0.05%)
Lymphosarcoma	1 (0.02%)	0
Reticulosarcoma	1 (0.02%)	0
Total	419	163

582 out of 4439 (13.11%) hybrids developed neoplasia; 87% of the hybrids were sufficently protected.

Studies on the genetic basis for insensitivity and sensitivity to carcinogens have shown that the genetic information coding for neoplastic transformation can be traced in all cases to a particular gene, designated as the "tumor gene" (*Tu*). *Tu* is present in the different cell types of all individuals and is normally under the control of population-specific and cell-type-specific polygenic systems of linked and nonlinked regulating genes (*R*-genes) which suppress the development of the various types of potential neoplasms. Animals containing complete *R*-systems are insensitive to carcinogens because it is unlikely that all *R*-genes will become impaired or deleted by mutation in a somatic cell.[3] Following hybridization the *R*-gene systems become partly disorganized because in the

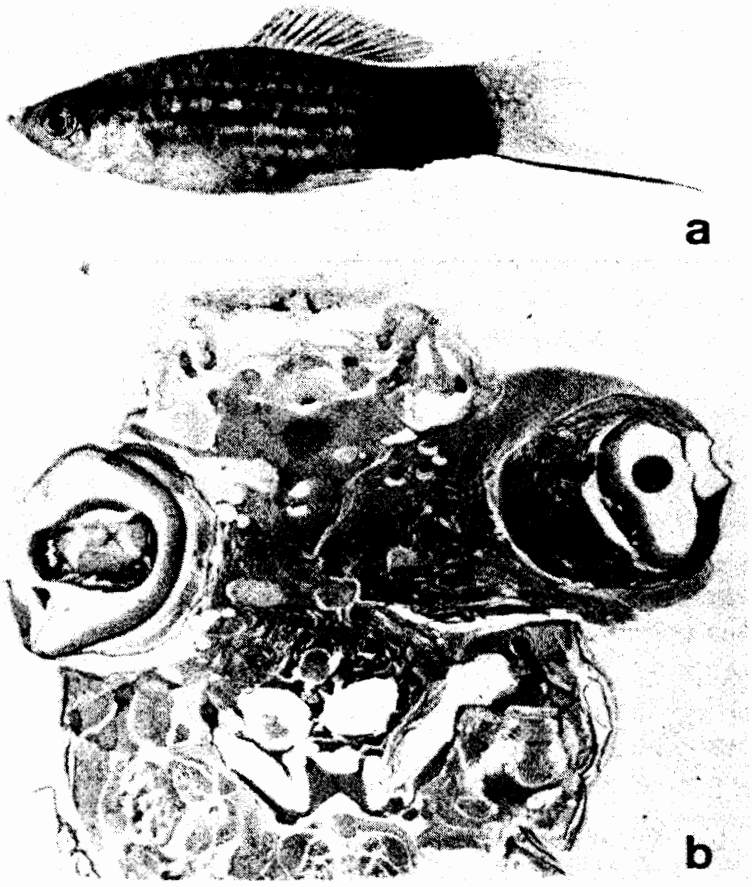

FIG. 1. a) Melanoma triggered by X-rays; and b) Neuroblastoma triggered by MNU in *Xiphophorus* hybrids.

crosses chromosomes carrying *R*-genes may be replaced by homologous chromosomes lacking them.[3] This process leads to sensitivity to carcinogens. If the remaining *R*-genes become impaired or deleted by mutation in a somatic cell, *Tu* may become derepressed and can initiate neoplastic transformation. The majority of neoplasms triggered in the fish by mutagenic carcinogens is due to the mutation of *R*-genes in a particular cell of a particular tissue. This is concluded from the fact that these neoplasms appear as foci of cells in the tissues of the fish. The smallest foci observed so far were found in the pigment cell system. They consisted of four neoplastically transformed pigment cells, indicating that there were two cell divisions between the mutational event and the appearance of these transformed cells (Fig. 2).[8]

SENSITIVITY TO PROMOTERS

In some genotypes hybridization procedures may lead to fish that, although lacking all the *Tu*-specific *R*-genes, do not develop neoplasia spontaneously. In the pigment cell system of these fishes, which is the only cell system studied thoroughly, the differentiation of pigment cells is almost completely arrested at the stage of stem-melanoblasts which are not yet competent for neoplastic transformation.[2,3,10] Those few cells entering the competent stage[11] become neoplastically transformed and immediately afterwards become termi-

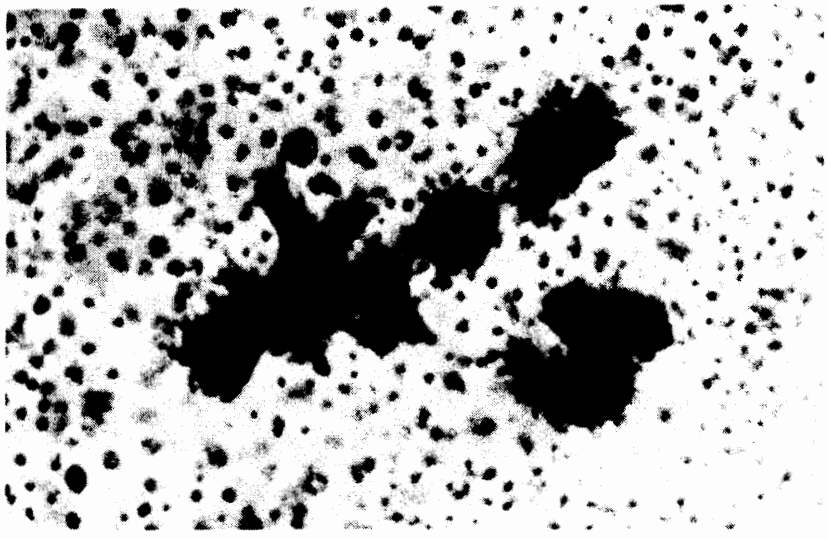

FIG. 2. Development of a somatic mutation-conditioned melanoma. Cell clone consists of 8 T-melanocytes. The small cells are nontransformed pigment cells.

nally differentiated and are removed by macrophages. In these animals the pretransformational delay of cell differentiation represents the main protective mechanism against melanoma formation.

Chemical and biological agents, such as methyl-testosterone,[11,12] cyclic AMP,[13] corticotropin,[13] 12-O-tetradecanoylphorbol-13-acetate (TPA),[14] and BrdUrd,[15] as well as general environmental changes, such as low temperature and an increase in salinity of the water in the tank, promote the differentiation of large numbers of noncompetent cells into the competent stage, and these cells subsequently become neoplastically transformed. Thus promoters of cell differentiation appear as promoters of neoplasia in these hybrids. Carcinogens such as X-rays, UV, and MNU, which are powerful mutagens, may trigger cell differentiation in these animals, and so may methyl-testosterone, cyclic AMP, corticotropin, TPA, etc., which are not mutagenic.

Promoters of differentiation stimulate neoplasia in a large variety of hybrid genotypes.[11] We assume that this is also the case if the promoters are produced endogenously. Evidence for this was obtained from melanomas that develop "spontaneously" during maturity, preferentially in males that are sexually highly active (Fig. 2). Probably these melanomas are triggered by steroid hormones.[12]

THE SIGNIFICANCE OF HYBRIDIZATION FOR SUSCEPTIBILITY TO NEOPLASIA IN PLANTS AND ANIMALS

Interpopulational or interracial hybridization in preceding generations is the main event contributing to the disintegration of genetic protective mechanisms. Germ line mutations that may also disturb the *R*-gene systems are probably less important than hybridization because they are always rare or may become repaired, in contrast to crosses which are easily accomplished. On the other hand, somatic mutations and tumor promotion, which are the majority of carcinogenic triggers, cannot contribute to the disintegra-

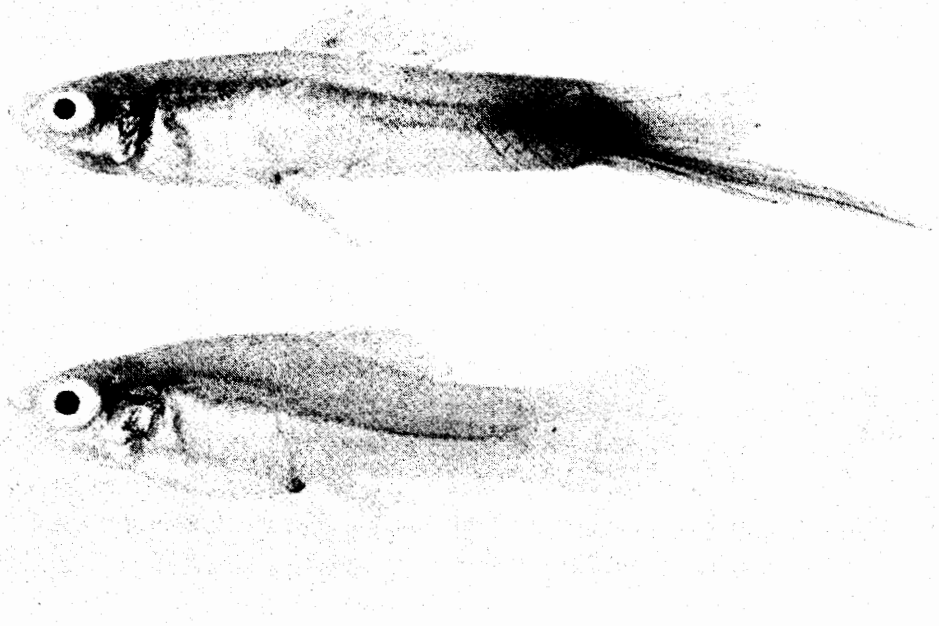

FIG. 3. Amelanotic malignant melanoma triggered by 17-methyltestosterone (2μg per liter aquarium water per day). Top: treated fish; bottom: untreated fish.

tion of the protective mechanisms against cancer in the germ line but can only complete this disintegration in individuals. The majority of the neoplasms of *Xiphophorus* belongs to those types that are triggered by carcinogens or promoters on a competent genetic background like their counterparts in humans, which represent about 90% of all human neoplasms (see Ref. 2). The remaining neoplasms of *Xiphophorus* as well as humans are inherited according to Mendelian law. They appear to be spontaneous and controlled exclusively by genes.

The phenomenon of introducing susceptibility to neoplasia by means of hybridization is not limited to *Xiphophorus*. Susceptibility to neoplasia has been observed in a large variety of plant hybrids, especially in cultivated plants, that are mostly bred by hybridization. Hybrids of cabbage, lilies, tobacco, tomatoes, calanchoe, thorn-apples, poplar, etc., are well-known examples (see Ref. 2). Furthermore, many examples can be cited from the animal kingdom (see Table 2). It appears that: (a) in animals from wild populations neoplasia is difficult to induce and the incidence of "spontaneously" developing neoplasia is low; while (b) in animals of hybrid origin (e.g., domesticated and laboratory animals, naturally-occurring or experimentally-produced interspecific and interpopulational hybrids) neoplasia is easily inducible and the incidence of "spontaneously" developing neoplasms is high.

IS SUSCEPTIBILITY TO NEOPLASIA IN HUMAN BEINGS CONDITIONED BY HYBRIDIZATION?

While we have no data on the relation between hybridization and cancer in human

TABLE 2. Animals exhibiting high tumor incidence (for references, see Ref. 2)

Insects	
Drosophila laboratory stocks	various neoplasms
Solenobia hybrids	various neoplasms
Teleosts	
Xiphophorus hybrids	various neoplasms
Girardinus laboratory stocks	promotor-triggered melanoma
Ornamental guppy strains	carcinogen-triggered hepatoma
Orange medaka	hepatoma
Domesticated trouts	aflatoxin-induced liver tumors
Salvelinus hybrids	fibrosarcoma
Domestic carp	neuroepithelioma
Ornamental hybrid carp	ovarian neoplasia
Lake Ontario hybrid carp	pollution-conditioned gonadal tumors
Goldfish	erythrophoroma
Amphibia	
Bufo calamita / *viridis* hybrids	chordomas
Birds	
Musk duck / mullard hybrids	gonadal tumors
Peacock / guinea fowl hybrids	gonadal tumors
Improved breeds of fowl	leukosis
Mammals	
Mus musculus / *M. bactrianus* hybrids	various neoplasms
Laboratory mice strains	various neoplasms
Hybrids of mice strains	various neoplasms
BALBc/NZB hybrids	50% plasma cell tumors
Blue ribbon mice	100% mammary tumors
Sprague Dawley/Long Evans rat hybrids	increased tumor incidence
Domestic dogs	various neoplasms
Boxers	various neoplasms
Domestic cats	various neoplasms
Sinclair swine	melanoma
Lippizaner horses	100% melanoma

beings comparable to those in plants and animals, it is interesting to speculate whether the many facts on tumor incidence in humans that do not agree with the concept of the primacy of environmental factors in carcinogenesis[16,17] may be explained by inter-populational and interracial hybridization in preceding generations.

Because of the high mobility of human beings as compared to other mammals, one should expect high values of heterogeneity in humans. Various estimates based on enzyme variations show that heterogeneity in humans is comparable to that in domestic animals such as cats, but is about six times higher than that in macaques, about ten times higher than that observed in big wild mammals such as the elk, moose, polar bear, black bear, and elephant seal, and about twice as high as that in most feral rodents studied so far (see reviews and discussions in Refs. 18–20). On the basis of these data and on the assumption that tumor incidence in humans is related to hybridization like that in domestic animals, one could explain why humans have a high incidence of neoplasia as compared to animals in wild populations.

There are also some data on chromosomal heteromorphisms in human populations that might be useful for estimates of heterogeneity within and between different popula-

tions. According to such estimates it appears that, for instance, Japanese populations exhibit a low degree of Q- and C-band chromosome heteromorphisms, whereas Americans have a much higher degree of this heteromorphism, with blacks having more prominent heteromorphisms than whites.[21,22] One is tempted to assume that this chromosomal heteromorphism reflects the differences in the degree of heterogeneity between the Japanese and the white and black U.S. populations.

The same gradation is also reported for the incidence of neoplasia. For example, the ratio of prostatic cancer in Japanese, U.S. whites, and U.S. blacks is reported to be 1 : 30- : 60.[23] These differences cannot be explained by environmental carcinogenic influences, which certainly differ only to a low degree. They also cannot be explained by racial differences: natural selection will not favor one race and discriminate against the other but it will work against susceptibility to cancer in all populations and all races. We suggest that these differences in tumor incidence are due to different degrees of interpopulational and interracial matings in nations favoring genetic heterogeneity, which might destroy the protective mechanisms against cancer as it does in *Xiphophorus*.

Similar explanations might be conceivable, for instance, for the differences in the death rates from cancer between Japan and West Germany, which is about 1 : 2, and tumor incidence between African blacks and American blacks, which is 1 : 3.

On the same basis one could explain the independence of tumor incidence from changes in the environment. For instance, in the area of West Germany, where the environmental conditions have changed dramatically since the beginning of this century, no change in tumor incidence (standardized for age) could be detected.[17] This indicates that the frequency of individuals susceptible or not susceptible to cancer has remained constant, presumably as a consequence of an unchanged interpopulational mating behavior. The unexpectedly low differences in tumor incidence between polluted and nonpolluted areas in the U.S.A. might be also be explainable by an overall constant frequency of susceptible and insusceptible individuals due to a constant degree of heterogeneity. On the other hand, the extremely low tumor incidence of active Mormons and Seventh-Day Adventists, as compared to total U.S. whites,[24] might be due to the biological homogeneity of their populations (which favors insusceptibility to cancer) rather than to environmental factors. The same could apply for the lower tumor incidence in Japan as compared to that of other industrial nations.

It has been argued from changes of cancer incidence in immigrant groups that environmental factors are the predominant cause of neoplasia. For example, in Japan there is an extremely low rate of cancer of the breast, prostate, and colon, whereas in the U.S.A. the reverse is true. When Japanese immigrate to the U.S.A. they adopt the pattern of tumor incidence of their new country.

From our point of view one must, however, also consider that the appearance of the new pattern of tumor incidence does not take place immediately after immigration but in the course of one, two, or more generations. Since matings between members of different Japanese populations (for instance from the north and the south of Japan) are certainly more frequent in the Japanese in the U.S.A. than in the Japanese in Japan itself, it becomes conceivable that the Japanese immigrants represent a more heterogeneous population than the original Japanese populations. Thus, if one assumes that the degree of cancer incidence is correlated with the degree of genetic heterogeneity, it becomes conceivable that the immigrants from Japan, depending on the number of generations after im-

migration, acquire the high rate of individuals susceptible to neoplasia which is typical for the total U.S. population composed of immigrants of different provenance. This depends on the efficiency of the mutagenic carcinogens and promoters which determine the frequency of the type of neoplasia in the total individuals susceptible to cancer. This frequency may be high, as in the case of cancer of the breast, prostate, and colon, or may be low as in the case of cancer of the stomach.

The concept of hybridization-conditioned susceptibility to neoplasia may also lead to a more critical interpretation of some epidemiological data. Breast and colon cancer represent a high percentage of total neoplasias in humans. It has been found that they are highly correlated to animal fat intake in a large variety of nations,[25] and it has been proposed that low animal fat intake is responsible for a low incidence of these neoplasms, while high animal fat intake is responsible for a high incidence. The order of the nations begins with (low fat intake, low tumor rate) Thailand, the Phillippines, Japan, and Taiwan, continues to Czechoslovakia, Austria, France, Switzerland, Poland, the Netherlands, and Finland, and ends with the U.S.A., Canada, Denmark, and New Zealand (high fat intake, high tumor rate). It has been shown, however, that the tumor incidence of the Dutch is twice as high as that of the Finns, though both have the same fat intake. The same is true if we compare the Swiss (high tumor incidence) with the Poles (low tumor incidence, but same fat intake). The Danes have an extremely high animal fat intake and an extremely high incidence of breast cancer. If one compares, however, the population of Copenhagen with that of rural Denmark one finds that fat intake in Copenhagen is much lower than in rural Denmark while urban Danes have a higher tumor incidence than rural Danes.

This is not to say that fat intake will have no influence on the incidence of breast and colon cancer; however, it becomes clear that fat intake alone cannot explain the differences in tumor incidence in different nations. In our opinion it might be extremely valuable to investigate how much effect hybridization in the ancestry may have on the frequency of neoplasia in our highly developed nations, most of which certainly are melting pots of mankind, in contrast with those that consist of genetically more homogeneous populations.

Thus, the *Xiphophorus* fish model, originally restricted to spontaneously developing melanomas in certain hybrids, has opened new perspectives to an understanding of certain phenomena in the distribution of neoplasia in mankind that could not be explained by environmental factors alone.

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