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Zebrafish Strains – A Brief Account of the Experimental Model

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ABSTRACT: Zebrafish, though tiny in size has proven it as an important scientific tool to conduct research and understand the various biological phenomena and system. Several zebrafish strains are available which are contributing differently in deepening our understanding. A number of wild type strains are present which have originated in the various parts of the world with its own history of origin. the most common wild type strains of zebrafish are: AB, WIK and TU. All these wild type strains share same morphological features, but with genetic differences. Gradually, under laboratory conditions different mutant versions with specific morphological and biological features developed and continued as experimental model in different laboratories of the world and gained importance in experimental usages. Among them TL, Absolute, Golden, Nacre etc. are the mutant strains widely used in different biological experiments and studies. Present review will discuss on the origin, morphological, genetic and other specialists of these strains to account a comprehensive idea about the animal model and its usages.

KEYWORDS: absolute, golden, mutant strains, nacre, wild type, zebrafish.

I. INTRODUCTION

Experimental biology always depends on the model organisms or any kind biological system, on which researchers applied their study to establish new findings. In the field of biological research several model organisms are used depending of the characteristics of the research, such as rat, mice, guinea pig and Zebrafish. At the new era of research zebrafish is getting more attention for several reasons. Zebrafish (*Danio rerio*), typically of size 2cm – 3cm is a tropical freshwater fish belonging to the minnow family, which is easy to breed and manage in laboratory condition and has gained much popularity as a model organism. As an aquatic organism it is a good indicator of the aquatic environmental conditions, while different stains of zebrafish behave differently due to their varied habitat. The present study reveals how different strains of zebra fish originated and their importance in different field of biological research. In the recent years this model organism secures a place in the list of most eligible alternative vertebrate model in various research fields to study and understand the various complex process of developmental biology, molecular biology, toxicology, behavioral biology in general; and, to understand human genome, its system, diseases and vaccine research (Bailone *et al.,* 2020; Basheer *et al.,* 2022; Adhish *et al.,* 2023). It was first utilized by George Streisinger for medical research in 1960s.

The zebrafish as model organisms not only broadened our horizon of knowledge and helped in unleashing the mechanism of various processes occurring in our biological system to this date but will continue to do so in future also, and therefore the understanding of different strains of zebrafish becomes important and hence, its origin to determine its suitability and applicability in various research studies (Audira *et al.*, 2020). There are various strains of zebrafish that has originated and developed over time but the most used ones are the wild type strains used for behavioral studies and its related research (Audira *et al.*, 2020). The most popular wild type strains of zebrafish are- AB, WIK (wild Indian karyotype) and TU (Tubingen) which has different lines of origin. However, all the zebrafish are sourced from the wild catches from the Himalayan foothills of south-east Asia including India, Pakistan, Nepal, Bhutan, Bangladesh and Myanmar and then maintained as different stocks in different geographic locations of the world (Séguret *et al*., 2016; Choi *et al*., 2021). Therefore, the primary morphological, functional, genetic and other features are shared between different strains reared and maintained artificially with the wild population found in wide geographic conditions of the sub-Himalayan regions.

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II. PRIMARY STRAINS

AB STRAIN

AB strain of zebrafish was first obtained by Streisinger and Charline walker in Albany, Oregon in 1992. This zebrafish strain was a result of the cross between A line and B line whose origin was probably from a hatchery in Florida and later obtained from pet shops in Oregon by Streisinger in early 1970s. The haploid offspring obtained from this AB line were then crossed with random AB males till 1990s in the Oregon lab and the resultant six diploid progeny each from different haploid females were intercrossed to produce *AB line (read as star AB line) which is presently called as modern AB line (**fig 1**) (Holden and Brown, 2018).

The parental genotype of A line and B line zebrafish are A and B respectively which later gave rise to AB zebrafish with AB genotype. The AB line of zebrafish today is maintained with a help of a "Round Robin" mating technique involving crossing of males and females of different generation of zebrafish to produce new generation of AB line zebrafish. The ABC is also somehow related to *AB lines which is maintained by natural cross or by *in vitro* fertilization like ABC-1, ABC-2 etc. where the number indicates the number of generations it is distant from *AB line (ZDB-GENE-960809-7(2016). AB strain zebrafish besides having certain different morphological characteristics also have a certain genetic (Brown *et al.*, 2011) and behavioral differences (Audira *et al.,* 2020; Seguret *et al.*, 2016) that makes it certainly different from other strains of zebrafishes. Previous studies reveals that various strains acts differently when they are exposed to assorted environmental conditions (Padovani *et al.,* 2022; Benner *et al.,* 2011).

WIK (Wild Indian Karyotype/Kolkata) STRAIN

It is another wild type strain with the genotype WIK originated in the year 1997 in India. This laboratory line strain was established by the mating of single pair of wild Indian zebrafish caught in mid 1990s (**fig 1**) obtained probably from the foothills of Himalayas or western ghats of Indian peninsula and generally reared and the stock was maintained in Kolkata, India (Arunchalam *et al.,* 2013; Audira *et al.*, 2020).

This single pair mating gave rise to several sublines namely WIK2, WIK3, WIK4, WIK9, WIK10, WIK11, WIK20. Out of all these sublines WIK11 was the one with least embryonic and larval lethality and was chosen to test its suitability for its use in genetic mapping and hence, gave rise to the present WIK strain which can also be called as WIK11. Due to the high polymorphic nature of WIK compared to the TU strain, the WIK are widely used to study the genetic map (Rauch *et al.*, 1997).

TU (Tübingen) STRAIN ZEBRAFISH

A wild type strain with the genotype TU originated in the laboratory of Tubingen in Germany in 1994. This strain had its origin from the composite population of fishes which was locally adapted to different geographical locations with different genetic and phenotypic expressions (**fig 2**) (Brown *et al.,* 2011). They were later obtained as well as performed the mating among them by the researchers from the different pet shops in Germany (Mullins *et al.,* 1994). Such composite fish populations were maintained as inbred strain in the lab of Tubingen in Germany (**fig 1**).

Today, the genome of TU has been fully sequenced by Sanger Institute (Howe, 2013) and TU just like AB strain is one of the most common strains evidently used in mutagenesis and gene knockdown experiments (Lin *et al.,* 2016). Due to wide range of geographical distribution throughout Himalaya specifically from Pakistan to Myanmar including Nepal, Bhutan and India, they show varied adaptive capability and genetic sub-structuring. A lot of variation among the different zebrafish strains were known because of the its nature of origin and continuous random mating among composite fish population. TU zebrafish not only shows high genetic recombination and variation compared to parents but also has increased duplicated segments with high CNV (Copy Number Variants) numbers and sizes (Verhoeven *et al.*, 2010).

PET STORE- PURCHASED (PET) STRAIN ZEBRAFISH

PET zebrafish is a wild type zebrafish which consists of genetically different groups of fishes whose genetics is not defined yet, as they were the result of mating between different strains of zebrafishes without defined parental genotype obtained from different pet shops (Audira *et al.,* 2020). This strain of zebrafish also has contributed in various works of behavioral studies and its analysis and to put a light on the preferences and the comparative studies on the various aspects of behavior and growth. This combination of different strains of zebrafishes are now showing increasing scope of its further use in various fields of research (Siregar *et al.,* 2020; Seguin *et al*., 2017; Meyer *et al*., 2013).

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Morphology

While all these wild type strains are genetically different, they all share same morphological features (**fig 3**) and look same with five uniformly distributed pigmented horizontal blue stripes on the side of the body extending to the end of the caudal fin. Male zebrafish have a torpedo body with gold stripes in between blue stripes and females with larger body and silver stripes in between blue stripes. All these features indicate the normal regulation and production of various pigment cells, those are lacking in various mutant strain of zebrafishes which will be discussed further later. The pigment pattern of the zebrafish has its own arrangement and pattern that imparts a typical appearance to the body of fish. All the arrangement of stripes on the body of fish is determined by the three pigment cells derived from the neural crest cells. The three derivatives of neural crest cells as follows, Melanophore- contains black granules called melanosome imparting black color to the body, Xanthophore- contains yellow to orange-colored granules called pterinosomes thus imparts yellow color, and Iridophore- contains reflecting platelets rendering silver, gold or iridescent color to the body (Kirschbanm, 1975; Jhonson *et al*., 1995).These three pigment cells together are arranged in the following way as shown in the **fig 4** to impart the striped appearance of the body.

III. MUTANT STRAINS

Other than the wild types strains of zebrafishes, mutant strains are also the ones which are extensively being used to compare, study and analyze the different strains for behavior in response to stress and thereby understanding behavioral and physiological phenotypes of stress and anxiety in zebrafishes (Egan *et al*., 2009). The most common mutant strain affected due to the mutation in pigment cell of zebrafish are further discussed below.

ABSOLUTE STRAIN ZEBRAFISH

Absolute zebrafish is a genetically engineered double mutant fish with genes *ednrb1a*^{b140} and *mitfa*^{b692} (where ednrb1a and mitfa denotes the affected gene and b140 and b692 indicates the affected allele). The kind of mutation in b140 allele though is unknown, but b692 allele involves a single point mutation and to be precise, the substitution of thymine by guanine results in substitution of isoleucine by serine of the first helix of the helix-loop-helix dimerization domain at position 215 (Lister *et al.,* 2001).

The gene *ednrb1a* is an endothelin receptor Ba probably located in plasma membrane which has a key role to affect the pigmentation of the skin of the fishes in all the parts of the body (**fig 5**). This gene is expressed in areas like pigment cell, trunk, head, gill and iridophores (ZFIN CODE- ZDB-GENE-980526-16). On the other hand, the *mitfa* gene is a melanocyte inducing transcription factor a, probably located in nucleus. This gene plays a role in the regulation of melanocyte differentiation. The mutation on this gene will therefore affect the areas where it is expressed, like the pigment cell, iridoblast cells, immature eye, melanoblast, neural crest cell and in turn affecting the melanophore, xanthophore and most iridophore cell productions imparting transparent body appearance to the absolute zebrafish (Jarret and McCluskey, 2019) (ZFIN CODE- ZDB-GENE-990910-11). Thus, absolute zebrafish in combination of *ednrb1a* and *mitfa* gene mutation results in lack of most iridophores, melanophore and irregular spatial pattern of xanthophore (Patterson *et al*., 2013) leads to lack of any stripes on the body with slight yellowish though transparent body.

GOLDEN ZEBRAFISH

This mutant strain originated due to the mutation in the gene *slc24a5* (Audira *et al*., 2020; Lamason *et al*., 2005; Sturm, 2006) in which the affected allele is b1 imparting light coloured skin phenotype that is the golden colour to the zebrafishes (**fig 6**). The genotype of this mutant strain zebrafish is *slc24a5*$^{b1/+}$ (AB) which is affected by a point mutation. In this point mutation, cytosine is substituted by adenosine resulting in the change of Tyr208 to stop codon (Lamason *et al*., 2005) causing small and irregular shaped melanin granule formation. Thus, lightly pigmented or golden color fish with light-colored lateral stripes on it is observed because of late melanin deposition during the period of embryogenesis.

Slc24a5 gene is basically a cation exchanger that codes for $Na^+/Ca^{2+}/K^+$ exchanger 5 or JSX protein which regulates Ca^{2+} concentration within melanosome, thus melanin deposition also regulated (Lamason *et al*., 2005; Cook *et al*., 2009; Yu *et al*., 2021). Human has an ortholog of this gene and it is important for providing color to the body of not only fish but human also. Prior studies carried out on the golden zebra fish and its gene put a light on the understanding of genetics and molecular basis of the skin color in human (Sturm, 2006; Lamason *et al*., 2005). The characteristic phenotype seen in golden zebrafish is that there are rows of very small, black pigment cell and a lighter shade of black stripes could be seen with xanthophores more prominent on the entire body (Streisinger *et al*., 1986; Tsetskhladze *et al*., 2012)

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NACRE ZEBRAFISH

Nacre mutant is formed by a recessive homozygous mutant caused by a point mutation in the *mitfa* gene (Lister *et al*., 1999) with affected allele named as w2, has a genotype nacre$^{w2/w2}$(AB) and resulted into characteristic strip-loss and depigmentation. Since, this *mitfa* gene plays an important role in melanophore production, the mutation in this affects the melanophore production. Melanophore, a derivative of neural crest cell imparts a black color which basically has a role to play in the stripped pattern of the zebrafish body and affects the pigment pattern of the zebrafish body. While the mutation leads to no melanophore production, compensatory high number of iridophores (silver, gold or iridescent colors) and varying xanthophores are produced rendering body with a very light iridophore stripes but no melanophore stripes (Lister *et al*., 1999). Lister and his colleagues have reported in their studies the effect of this mutation on the neural crest derived pigment cell fate. The mutation caused in the *mitfa* gene is a homology of *mitf* gene found in mouse (Moore, 1995) important for survival and specification of melanocytes. The mechanism of melanophore deformities and the body colour formation against these mutations are summarized in **fig 7**. The morphology of this mutant is characterised by complete loss of melanophores, increased number of iridophore especially in the tail fin and variable xanthophore pattern (Lister *et al*., 1999; Rajpurohit *et al*., 2023) leading to lack of black (melanophore) stripes on the body but a faint iridophore stripes and normal retinal pigmented epithelium could be observed.

IV. SPECIALIZED STRAINS AND FEATURES

Crystal fish-

While nacre has its own use in biological research, this mutant in combination with other pigment mutation has led to the development of crystal fish which has proved to be a boon in the field of biology to deliver an easy access to the eyes and brain of the fish. This strain is the most beautiful and perfect model for *in vivo* imaging of biological phenomena at various cellular level. This crystal overcomes the drawback of *casper* mutant (**fig 8**) (*nacre*$^{w2/w2}$; *roy*$^{a9/a9}$) which even though could cause the body to be transparent but not the transparency of eyes preventing an access to the inside or between the tissues of the eyes of zebrafish. Crystal mutant is developed by combinatorial genetic approach which does not affect the viability of zebrafish but presents a fully transparent body to both the larval and adult zebrafish enabling the study of various biological processes occurring in the tissues especially in and around the eyes of zebrafish (Antinucci and Hindges, 2016).

The three mutation that occurs in the crystal mutant zebrafish are as follows-

i. **Nacre $^{w2/w2}$ mutant**- is a mutation that affects the *mitfa* gene (Lister *et al*., 1999) involving one-point mutation that results in the loss of melanophore production, (one of the derivatives of neural crest cells) important for black color stripes to the body of the zebrafish and hence rendering a stripe-less body to the zebrafish.
ii. **Albino (alb$^{b4/b4}$) mutant-** involves a mutation in the *slc45a2* gene (Streisinger *et al*., 1986) affected by one insertion mutation leading to no melanin production in the retinal pigment epithelium (RPE) formed by pigment cells originating from optic lobe neuroepithelial cells imparting light red eyes to the fishes.
iii. **roy orbison (roy$^{a9/a9}$) mutant-** involves allele with multiple variants resulting in lack of iridophore (one of the derivatives of neural crest cell) production (White *et al*., 2008) in the zebrafishes necessary to imparts silvery or blue coloration to the body.

This combination of three pigment mutations with *nacre*$^{w2/w2}$ and *roy*$^{a9/a9}$ prevent the pigment cells formation and *alb*$^{b4/b4}$ prevents melanin production (Singh and Nusslein-Volhard, 2005) which provide fully transparent crystal zebrafish with clear visibility of the organs and tissues for future studies and research. So, the Crstal strain zebrafish has its genotype as *nacre*$^{w2/w2}$; *alb*$^{b4/b4}$ and *roy*$^{a9/a9}$ (Antinucci and Hindges, 2006).

TL (Tübingen long fin) ZEBRAFISH

Tübingen long fin zebrafish, also known as Tupfel longfin, as the name suggests is long finned zebrafish with its own characteristics body coloration pattern and fin length (**Fig 9**). This zebrafish with the genotype (*leo*$^{t1/t1}$; *lof*$^{t2/t2}$) is a result of double mutation occurring in the two alleles of the zebrafish. While this strain has originally been derived from Tübingen zebrafish population (Draper *et al*., 2020), this mutant has a combination of two mutation, one recessive mutation in *leo* gene i.e., *leo*^{t1} also called as tup or *Brachydanio frankei* (Haffter *et al*., 1996). These mutations impart spotted body pattern like leopard instead of stripes (wild type) in adult and one homozygous dominant mutation in *lof* gene i.e., *lof*^{dt2} imparting strikingly long-fin to the fish compared to TU or

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other strain of zebrafish without affecting development, proportionality or growth rate of the body (Jarret and McCluskey, 2019; Haffter *et al*, 1996; Van-Eden *et al*., 1996).

The *leo*^{t1} involves a non-sense mutation that affects connexin 41.8 gene (Cx41.8), a gene that have a role to play in gap junction, leading to a reduction of Cx41.8 molecular size from 42 to 8 kDa encoded by the leo^{t1} allele (Watanabe *et al*., 2006; Vanden Bos *et al*., 2020). On the other hand, the cause of flowing veil-like tailfin appearance of the TL is the overexpression of *kcnh2a* gene, a K^+ channel encoding gene near *lof* locus that results in the disruption of calcineurin signaling causing delayed fin growth cessation and hence an extraordinary fin growth of the Tübingen long fin zebrafish appears which provides a significantly distinct and distinguishable phenotype to the fish (Stewart *et al*., 2021; Daane *et al*., 2018; Kujawski *et al*., 2014). This *kcnh2a* gene even has been found to be orthologous to ether-a-go-go K^+ channel of humans that can significantly put an insight into the understanding related to arrhythmias and its effect on cardiac action potential in human (Stewart *et al*., 2021; Vandenberg *et al*., 2012; Bohnen *et al*., 2017; Curran *et al*., 1995; Sanguinetti *et al*.,1995).

Leopard gene

The leopard gene which is an orthologue to the mammalian connexin 40 gene is the gene which has a role in the spotting melanophore appearance on the body of zebrafish and these spotting also have their own spatial arrangements depending upon the type *leo* allele affected by the mutation. In zebrafish, connexin41.8 gene (*cx41.8*) is nothing but a leopard gene whose mutation affects the spotting phenotype on the body. There are three alleles that are responsible for different phenotypes i.e., *leo*^{t1}; *leo*^{tq270}; *leo*^{tw28}. Connexin which is a component of intercellular gap junction (Kumar, 1996) contains 2 connexons that allows intercellular passage of various ions, secondary metabolites and secondary messengers (like ATP, cyclic AMP etc.) of size approx. 1500kDa (Saez *et al*., 2005). While each allele has its own characteristics each allele zygosity affects its phenotype differently described in **Table 1**. Watanabe and team also conclude that two genes named as Cx39.4 and Cx41.8 are important for the stripe formation, this two are the founder gene for the production and interaction between melanophores and xanthophores (Watanabe *et al*., 2016). Asai and his colleagues used reaction-diffusion system and depending upon different parameters (c value) used this model to put a light on the role of alleles in different phenotypic pattern formation and hence, enhancing knowledge about its mechanism at a molecular level along with computational analysis. They thus determined that which c value corresponds to which phenotype and thus generating relationship between the two (**fig 10**) (Asai *et al*., 1999). Other than this the "saturation of autocatalysis" has a role to control the shift from stripes to spots according to Meinhardt (Meinhardt,1989).

CONCLUSION

As the various strains of the zebrafish have been developed and increased over time, so does its applicability and use by the researchers in their studies to understand the various mechanisms, processes and developments of various biological phenomena and system functions occurring in our body. A summary of major varieties of zebrafish and their characteristics has been given in **Table 2**. The zebrafish has continued to be one of the favourite models of research because of its various advantages like small size, large number of offspring, easy and cheap maintenance, 70% similarity with human genome, 84% human disease-causing counterpart genes, easy genetic manipulation and transparent embryo are some of the exceptional qualities that alleviates it to one of the most popular scientific tools among researchers (Amatruda *et al*., 2002; Howe *et al*., 2013).

While there are many strains wild and mutant of the zebrafish, the above-mentioned strains are the ones which are widely being used to conduct and demonstrate their experiments. These strains are thus not just different in origin and morphology from one another but they have some specific differences in genetics, behavior and in much more aspect. These features are utilized accordingly as per the demand and suitability to the experiments. Those requirements which could not be fulfilled with wild type are compensated by the mutant strains by knocking out specific genes and hence deepening our knowledge on the role of specific genes. Such models are even used to imitate human diseases like conditions to figure out its potential treatment in future (Adhish and Manjubala, 2023). The transparency of the zebrafish embryo and even genetically engineered crystal mutant of zebrafish that is absolute transparent throughout its life provides us an access to the mechanism occurring inside the fish's body and other biological processes. Therefore, zebrafish are now gaining interest and significantly used in developmental and cell biology research which are dealing with growth, cancer, nervous and immune system disorders and in many more areas of biological research.

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Table 1: Phenotypic characteristics of different Leopard Zebrafish with the corresponding Zygotic differentiation. Three different allele presents in Leopard strain and all of them present in both heterozygote and homozygote conditions with different phenotypic characteristics (adopted from Watanabe et al, 2006)

Allele Name	Allele type	Phenotypic character
leo^{t1}	**Heterozygote (leo^{+}/leo^{t1})**	it is like the wild type, striped body
	Homozygote (leo^{t1}/leo^{t1})	spot pattern of melanophores
leo^{tw28}	**Heterozygote (leo^{+}/leo^{tw28})**	undulating stripes
	Homozygote (leo^{tw28}/leo^{tw28})	interrupted striped pattern with decreased melanophores.
Leo^{tq270}	**Heterozygote (leo^{+}/leo^{tq270})**	spotting pattern like leo^{t1} homozygote
	Homozygote (leo^{tq270}/leo^{tq270})	spot pattern but smaller than leo^{tq270} homozygotes

Table 2: A summarisation of all the prominent zebrafish strains. Each strain is originated differently with differences in their phenotypes and genotypes.

STRAINS FEATURES	AB	WIK	TU	PET	TL	ABSOLUTE	GOLDEN	NACRE
PARENTAL GENOTYPE	A & B	-	-		TU	AB	TU	AB
GENOTYPE	AB	WIK	TU	PET	TL/ $leo^{t1/t1}$; $lof^{t2/t2}$	ednrb1a^{b140}; mitfa^{b692}	Slc24a5$^{b1/+}$ (AB)	Nacre$^{w2/w2}$ (AB)

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ORIGIN YEAR	1992	1997	1994	-	-	-	-	-
PLACE OF ORIGIN	Streisinger lab; US	India	Tubingen, Germany	-	-	Kimmel lab & David Raible lab	Streisinger lab	David Raible lab
LINE	wild	wild	wild	wild	mutant	Mutant	mutant	mutant
TYPE OF MUTATION	-	-	-	-	Non-sense	point mutation	Point mutation	Point mutation
AFFECTED GENE	-	-	-	-	leo & Lof	ednrba & mitfa	Slc24a5	mitfa
AFFECTED ALLELE	-	-	-	-	t1 & t2	b140 & B692	b1	w2
PHENOTYPE	Five blue horizontal stripes (xanthophore, iridophore and melanophore) alternating with light stripes (iridophore and xanthophore) extending to caudal fin.				Black melanophore spots with long fin	Most iridophore and melanophore lacking and abnormal xanthophore spatial distribution, transparent body, normal eye	Rows of very small black pigment cell, xanthphore more prominent, faint melanophre stripes, body colour yellowish orange, normal RPE	All melanocyte absent, increased iridophor, xanthophore variable distribution, body with no melanophore stripes but faint iridophore stripes, normal RPE
REFERENCE	Hirata et al., 2005				Stewart et al., 2021; Van den bos et al., 2020; Watanabe et al., 2006	Audira et al., 2020; Spiewak et al., 2018	Streisinger et al., 1985; Tsetskhaldze et al., 2012	Rajpurohit et al., 2023; lister et al., 1999

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FIGURES:

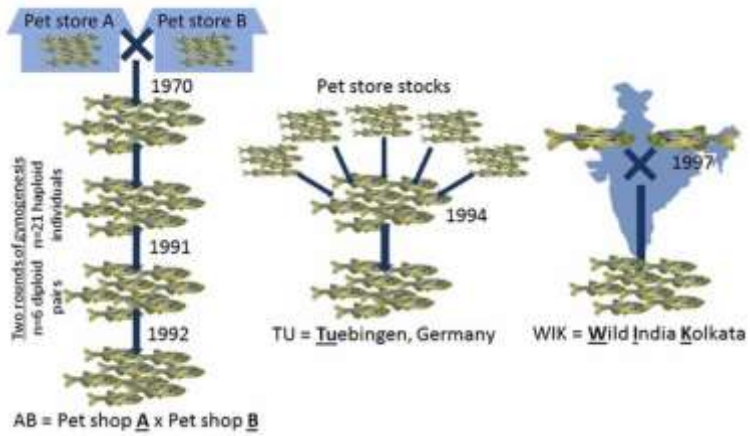

Fig 1: Origin of different wild type strains of AB, TU and WIK zebrafish with its own line of history (Adopted from Holden and Brown, 2018).

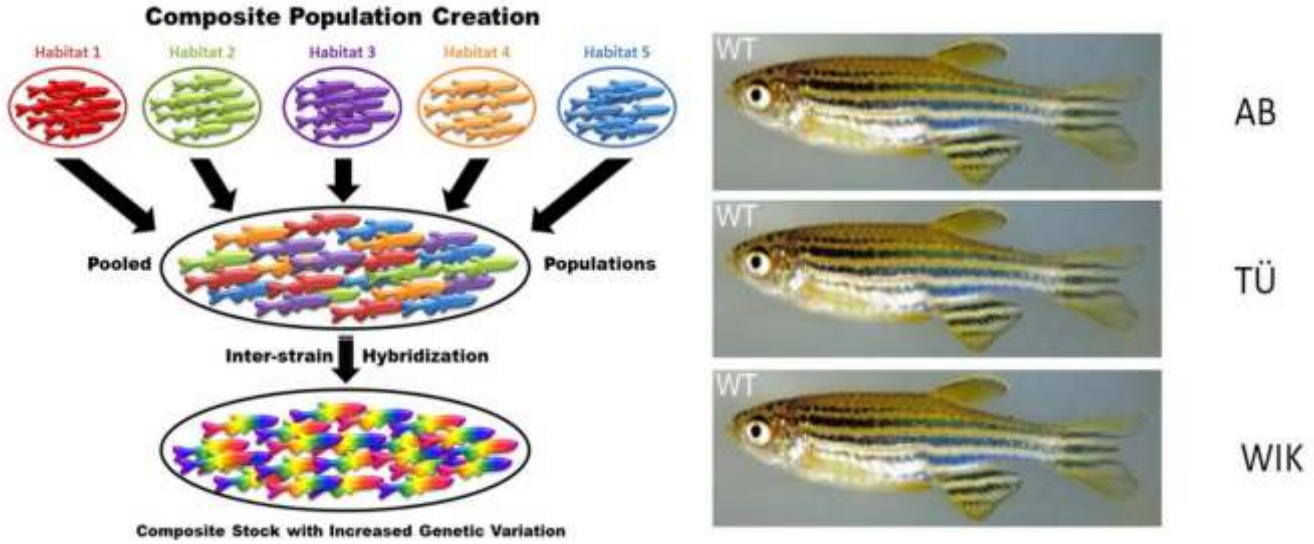

Fig 2: Showing composite population of fish from where TU originated and this population is obtained from different habitat (*Adopted from* Holden and Brown, 2018).

Fig 3: The three common laboratory strains of zebrafish AB, TU, WIK eliciting a common morphological feature though not genetical and hence, difficult to differentiate morphologically. (*Adopted from* elearning.unite.it- ZEBRAFISH (*Danio rerio*).

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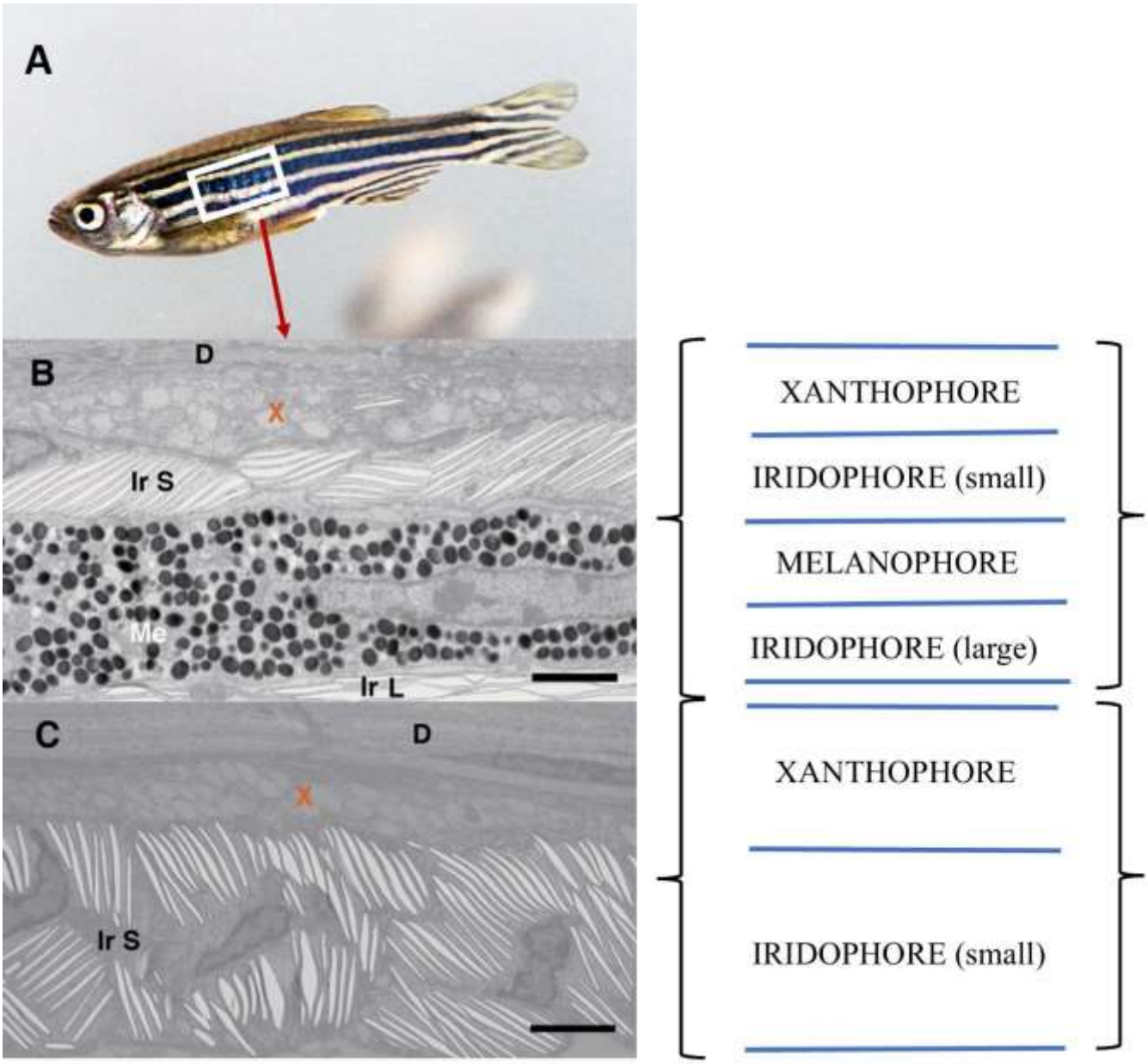

Fig 4: A is the adult Zebrafish, the skin pigmented cell are bordered with white colour box and enlarge as B and C. Both picture B and C showing arrangement of different neural crest derived pigment cell in the different tissue of the zebrafish responsible for the characteristic stripped pattern in the zebrafish where D in the picture A denotes dermis (*Adopted from* Hirata *et al.*, 2005).

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Figure 5

Figure 6

Fig 5: Showing the absolute (above) and wild type (below) zebrafish explicitly exhibiting the stark morphological difference between the two (Image courtesy of Herzberg *et al*., 2016).

Fig 6: Illustrating the comparable phenotypic difference between the wild type (above) and golden mutant (below) with golden exhibiting lighter stripes and body color resembling gold colour with thinner melanophores and fewer melanosomes. (Images courtesy of Dr. Keith Cheng. File name: 3-4_F2_Clark_Ekker in jpg and eps format)

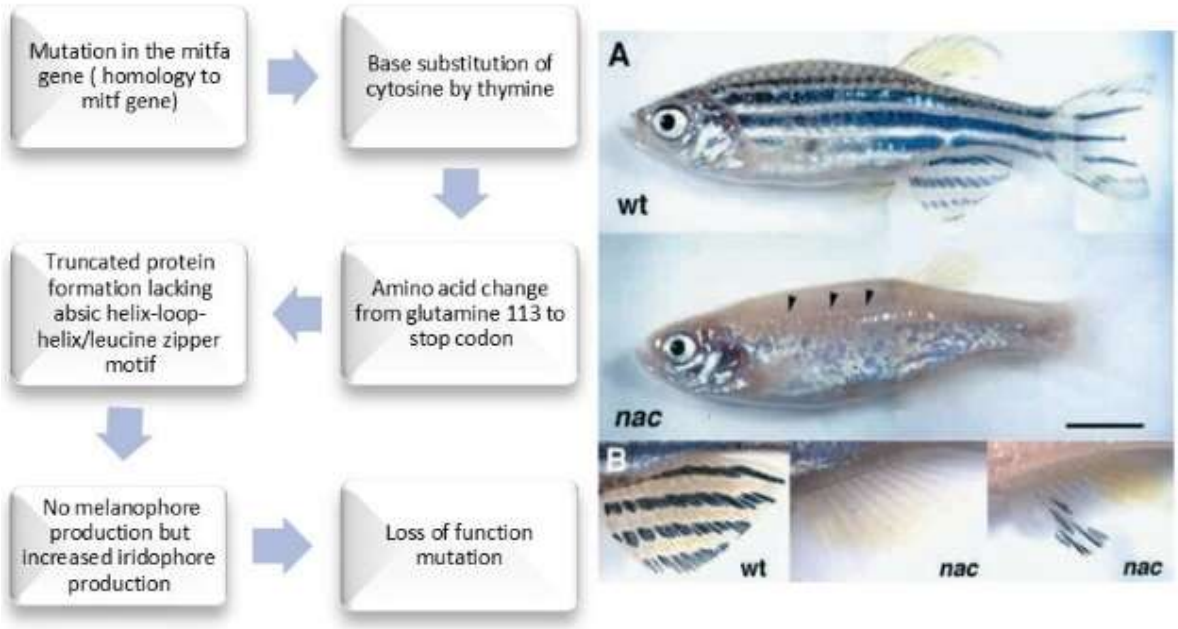

Fig 7: Showing nacre devoid of any stripes due to complete lack of melanophore and body exhibiting iridophore pigmentation and variable xanthophore spatial distribution with arrow heads denoting light iridophore stripes and in B part showing a little melanophore stripes in nacre anal fin. (*Adopted from* Lister, *et al*., 1999).

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Fig 8: Showing casper mutant (left side bottom) result of two recessive pigment mutation with transparent body though not with eyes and the crystal mutant (right side bottom) exhibiting a whole transparent body with a very light red eye. (*Adopted from* White *et al*., 2008).

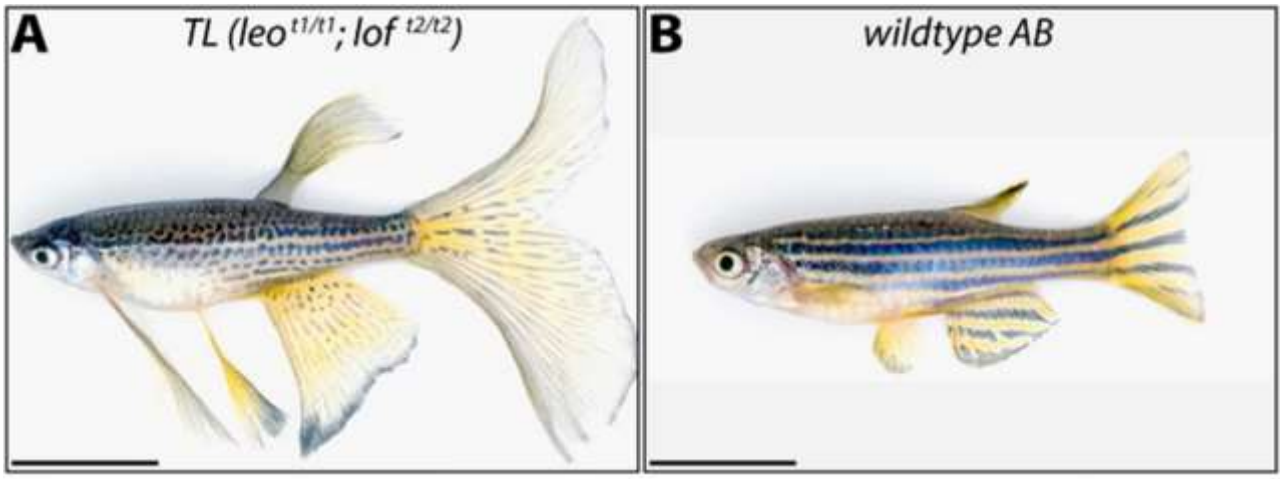

Fig 9: Eliciting Tübingen long fin with spotted like leopard appearance on the body of the zebrafish. (*Adopted from* Stewart *et al*., 2021)

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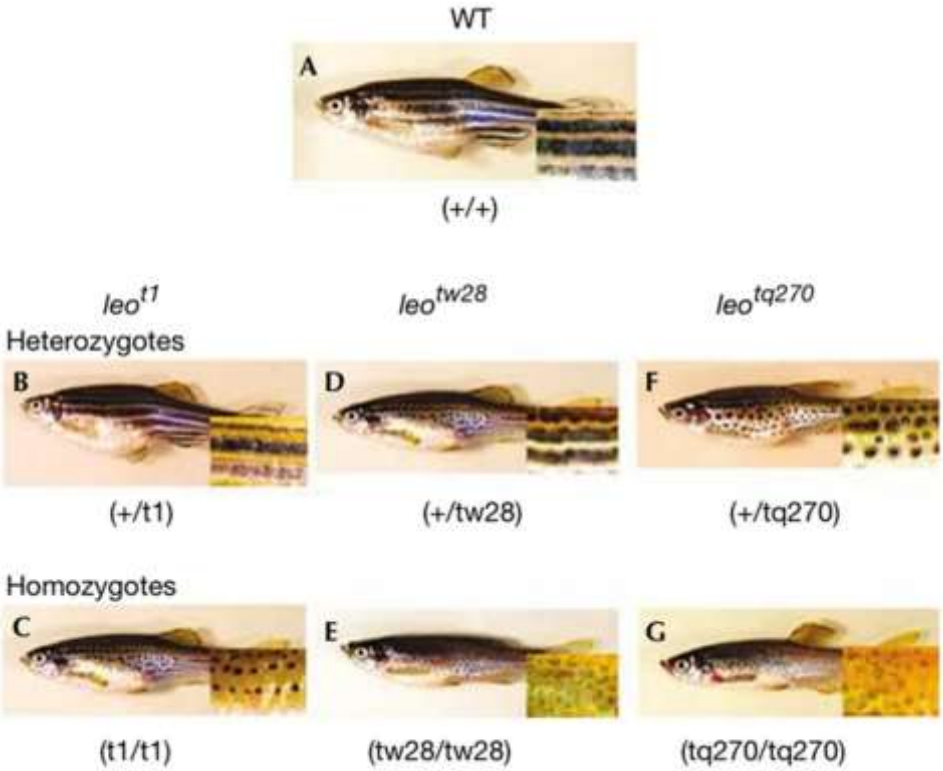

Fig10: The different phenotype with different zygosity of different alleles of leopard gene of zebrafish (*Adopted from* Watanabe *et al.*, 2006).

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